Androgen biosynthesis in castration-resistant prostate cancer

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Abstract

Prostate cancer is the second leading cause of death in adult males in the USA. Recent advances have revealed that the fatal form of this cancer, known as castration-resistant prostate cancer (CRPC), remains hormonally driven despite castrate levels of circulating androgens. CRPC arises as the tumor undergoes adaptation to low levels of androgens by either synthesizing its own androgens (intratumoral androgens) or altering the androgen receptor (AR). This article reviews the major routes to testosterone and dihydrotestosterone synthesis in CRPC cells and examines the enzyme targets and progress in the development of isoform-specific inhibitors that could block intratumoral androgen biosynthesis. Because redundancy exists in these pathways, it is likely that inhibition of a single pathway will lead to upregulation of another so that drug resistance would be anticipated. Drugs that target multiple pathways or bifunctional agents that block intratumoral androgen biosynthesis and antagonize the AR offer the most promise. Optimal use of enzyme inhibitors or AR antagonists to ensure maximal benefits to CRPC patients will also require application of precision molecular medicine to determine whether a tumor in a particular patient will be responsive to these treatments either alone or in combination.

Key Words

- ▶ intratumoral androgen biosynthesis
- ▶ androgen receptor
- ▶ enzyme inhibitors
- ▶ androgen receptor antagonists

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Introduction

The growth of the normal and abnormal prostate requires a source of androgens. This is evident in advanced prostate cancer as the early studies of Huggins and Hodges showed that surgical castration plus adrenalectomy leads to a remission of the disease (Huggins & Hodges 1941, Huggins 1965). In addition, individuals with an inherited deficiency of steroid 5 α -reductase type 2 have completely atrophied prostates, indicating that 5 α -dihydrotestosterone (DHT) is the most important androgen for the development of prostate (Russell & Wilson 1994). Based on these findings, androgen-deprivation therapy (ADT) has evolved to become a standard treatment of care for patients with localized advanced prostate cancer. ADT now includes the use of a LHRH agonist (e.g. leuprolide or

goserelin) (Sharifi *et al.* 1996) and an androgen receptor (AR) antagonist (e.g. *R*-bicalutamide) (Kennealey & Furr 1991). Following a period of relapse, the cancer often reappears, and this is accompanied by rising levels of serum prostate-specific antigen (PSA). *PSA* (*KLK3*) is an androgen-dependent gene, and increased expression of *PSA* in an environment of castrate levels of circulating androgens indicates that adaptive androgen signaling has emerged in the tumor. This form of the disease is known as castration-resistant prostate cancer (CRPC), which is almost uniformly fatal. The adaptive androgen signaling in CRPC cells can occur due to intratumoral androgen biosynthesis and/or changes in the AR, e.g. gene amplification, AR somatic mutations, and splice variants

that make the AR constitutively active (Knudsen & Penning 2010). This review focuses on the androgen biosynthetic pathways that occur in CRPC cells, the enzyme targets, and their inhibition. It also discusses mechanisms of drug resistance to these agents.

Pathways to testosterone and DHT synthesis

In individuals with an intact testis, circulating testosterone is the immediate precursor of DHT in the prostate. This leads to the proposal that in CRPC cells the dominant route to DHT synthesis is via the classical pathway (Fig. 1). In this pathway, DHEA of adrenal origin is converted to Δ^4 -androstene-3, 17-dione via 3 β -hydroxysteroid dehydrogenase/ketosteroid isomerase (*HSD3B1*), which is then reduced to testosterone by one or more 17 β -HSD isoforms. Testosterone is then reduced to DHT by steroid 5 α -reductase type 1 or 2 (*SRD5A1* or *SRD5A2*); i.e., the route is DHEA $\rightarrow$ Δ^4 -AD $\rightarrow$ testosterone $\rightarrow$ DHT. Recent studies in a variety of prostate cancer cell lines have shown that this pathway may not exist and that Δ^4 -AD is reduced by 5 α -reductase directly to 5 α -androstane-3, 17-dione (Adione), which is subsequently reduced to DHT by 17 β -HSD. As this pathway bypasses testosterone formation altogether, it is known as the alternative pathway; i.e., the route is DHEA $\rightarrow$ Δ^4 -AD $\rightarrow$ Adione $\rightarrow$ DHT (Chang *et al.* 2011). Other studies in the tammar wallaby and on the maturation of genitalia in human neonates have demonstrated the existence of a third pathway to DHT synthesis

(Wilson *et al.* 2003, Auchus 2004, Shaw *et al.* 2006, Fluck *et al.* 2011, Auchus & Miller 2012). In this pathway, reduction of the A-ring of the steroid occurs at the level of C21 steroids. Pregnenolone is converted to progesterone (Prog) by 3 β -HSD, which is subsequently reduced to 5 α -dihydroprogesterone (DHP) by 5 α -reductase. DHP is converted by 3 α -HSD to allopregnanolone (3 α -hydroxy-5 α -pregnane-20-one), which then acts as a substrate for cytochrome P450 17A1 (*CYP17A1*) (17 α -hydroxylase/17,20-lyase) to produce androsterone. Androsterone is then reduced by 17 β -HSD to yield 3 α -androstenediol (3 α -Adiol), which is oxidized to yield DHT by 3 α -HSD; i.e., the route is Prog $\rightarrow$ DHP $\rightarrow$ allopregnanolone $\rightarrow$ androsterone $\rightarrow$ 3 α -Adiol $\rightarrow$ DHT. This pathway is known as the 'backdoor pathway' (Wilson *et al.* 2003, Auchus 2004, Shaw *et al.* 2006, Fluck *et al.* 2011, Auchus & Miller 2012). The field has now advanced so that the discrete 17 β -HSD and 3 α -HSD isoforms involved in these interconversions are known, and these are discussed below.

Enzyme targets and their inhibition

P450 17 α -hydroxylase/17, 20-lyase, P450 17A1 (*CYP17A1*)

In pathways to intratumoral androgen synthesis in castrated males, there could either be a dependency on adrenal steroids (DHEA and DHEA-SO₄) or a dependency on *de novo* synthesis of androgens from cholesterol within the prostate. The major precursors for androgen

Figure 1

Androgen biosynthesis in prostate cancer cells. Reactions in the blue rectangle are known to occur in the prostate. Names of genes (in italics) that code for the various enzymes are shown. The classical pathway to

DHT synthesis is indicated by black arrows, the alternative pathway to DHT synthesis is indicated by green arrows, and the backdoor pathway to DHT synthesis is indicated by blue arrows.

biosynthesis in CRPC patients are likely to be DHEA and DHEA-SO₄ of adrenal origin, in these patients circulating levels are of the order of 200 and 200 000 ng/dl respectively. In contrast, the circulating levels of testosterone are less than 10 ng/dl (Tamae *et al.* 2013). It is difficult to imagine that prostate tumors could achieve this level of DHEA synthesis. The conversion of pregnenolone to DHEA in the adrenal is catalyzed by P450 17A1, which is a bifunctional enzyme catalyzing 17 α -hydroxylation of the steroid side chain followed by acyl-carbon-bond cleavage via its 17, 20-lyase activity (Nakajin & Hall 1981, Nakajin *et al.* 1981, Chung *et al.* 1987). This enzyme is inhibited by abiraterone acetate (Zytiga[®]), which has recently been approved by the FDA for the treatment of CRPC patients, who have been shown to be unresponsive to the taxanes (e.g. docetaxel) (Attard *et al.* 2009, 2012; Table 1). The disadvantage of this approach is that in the absence of P450 17A1 the adrenal can no longer synthesize cortisol. This leads to compensatory increases in the production of ACTH in the anterior pituitary and elevated production of the potent mineralocorticoid desoxycorticosterone in the adrenal, which can lead to life-threatening hypertension (Attard *et al.* 2012). This side effect is eliminated by the co-administration of a synthetic glucocorticoid, e.g. prednisone. Because prednisone suppresses the adrenal-pituitary axes, the issue of whether prednisone by itself would be effective in limiting adrenal DHEA production arises. Due to this, second-generation P450 17A1 inhibitors exhibiting a preference for inhibiting only the 17, 20-lyase VT-464 have been developed (Abbott *et al.* 2012). In addition, other P450 17A1 inhibitors that also enhance AR degradation, e.g. galeterone (TOK001), have been developed (Njar & Brodie 1999, Bruno *et al.* 2008). Each of these P450 17A1 inhibitors is steroid-based and possesses a heterocycle at the 17 β -position, which

creates a sixth ligand for the heme moiety of the P450. The availability of the P450 17A1 crystal structure will aid in the development of superior agents that may only inhibit the lyase step (DeVore & Scott 2012). Currently, abiraterone acetate is used in the standard treatment of care for CRPC patients.

The clinical success of abiraterone has also led to its use in clinical trials in the neoadjuvant setting as a possible first-line agent in ADT. In a recent clinical trial that compared leuprolide alone with leuprolide plus abiraterone, only the abiraterone arm was found to lead to a significant >90% reduction in serum testosterone, DHEA, and DHEA-SO₄ levels as might be expected (Taplin *et al.* 2014). However, the levels of DHEA-SO₄ (20 000 ng/dl) that still remained represented a significant reservoir of androgen precursor, assuming that prostate cancer patients have appropriate levels of the organic anionic transporters and steroid sulfatase (STS). If this reservoir of DHEA-SO₄ is bioavailable, it can be assumed that other agents targeting P450 17A1 would have the same profile.

3 β -Hydroxysteroid dehydrogenase, 3 β -HSD (HSD3B)

3 β -HSD is a bifunctional NAD⁺-dependent enzyme that converts DHEA to Δ^4 -AD in the prostate (Luu The *et al.* 1989, Rheume *et al.* 1991, Labrie *et al.* 1992, Lachance *et al.* 1992). There are two isoforms of this enzyme that are encoded by distinct genes, but these are closely related, structurally, functionally, and kinetically (HSD3B1 and HSD3B2) (Thomas *et al.* 2002a). The 3 β -HSD type 1 enzyme is the isoform that is dominantly expressed in prostate cancer cells. Traditionally, the 3 β -HSD type 2 enzyme (found in the gonads and adrenals) was thought to be a poor drug target as it introduces the

Table 1 Drugs that block intratumoral androgen biosynthesis

Target enzyme	Gene	Drug	Properties
P450 17A1	CYP17A1	Abiraterone acetate Galeterone (TOK001) Orteronel	Competitive inhibitors that may require co-administration with prednisone
Steroid 5 α -reductase	SRD5A1 and SRD5A2	Finasteride Dutasteride	Mechanism-based inhibitor Mechanism-based inhibitor
3 β -HSD type 1	HSD3B1	Epristeride Trilostane Epostane	Non-competitive inhibitor Competitive inhibitor Competitive inhibitor
AKR1C3 (type 5 17 β -HSD)	AKR1C3	Indomethacin GTX-560 Astellas ASP9521	Competitive inhibitor Competitive inhibitor Competitive inhibitor
Steroid sulfatase	STS	667-Coumate (STX64, Irosustat)	Irreversible inhibitor

Δ^4 -3-ketosteroid functionality, present in androgens, progestins, glucocorticoids, and mineralocorticoids, and thus the inhibition of this enzyme would block the production of most steroid hormones. Inherited deficiencies of 3β -HSD type 2 provide a view of the phenotype that enzyme inhibition would cause, and this can lead to low, moderate, and severe salt wasting (Rheaume *et al.* 1992, 1994). This raises the issue of whether it is possible to discriminate between the two enzyme forms with inhibitors (Thomas *et al.* 2002b). Compounds such as epostane and trilostane exhibit a 13- to 16-fold preference for the inhibition of the type 1 enzyme, but this difference is unlikely to be sufficient to be of clinical utility (Thomas *et al.* 2008). Interestingly, abiraterone is a substrate for 3β -HSD type 1 and the enzyme-generated product containing the Δ^4 -3-ketosteroid functionality is a reasonably potent inhibitor (Li *et al.* 2012). It is unclear whether this mode of action contributes to the clinical efficacy of abiraterone. Recently, a unique gain-in-function somatic mutation has been identified in 3β -HSD type 1 in CRPC patients (Chang *et al.* 2013). This gain-in-function mutation did not alter the steady-state kinetic parameters (K_m and V_{max}) of the enzyme, but it increased its stability by preventing ubiquitination and proteasome degradation of the enzyme. These intriguing observations indicate that somatic mutations in other enzymes involved in the androgen metabolic pathway may be selected as an adaptation to androgen deprivation, but these are yet to be identified.

5 α -Reductase types 1, 2, and 3 (SRD5A1, SRD5A2, and SRD5A3)

Deficiency of 5 α -reductase type 2 (SRD5A2) results in an atrophied prostate (Russell & Wilson 1994), and this has led to the development of inhibitors of the enzyme, such as finasteride, which is effective in the treatment of benign prostatic hyperplasia (BPH) patients (Bartsch *et al.* 2002). However, treatment of BPH patients with finasteride does not result in complete reduction of DHT levels in the prostate, and this has led to the development of bifunctional agents that could target 5 α -reductase type 1 and type 2, e.g. dutasteride (Frye 2006). Transcript profiling of CRPC patients revealed that there was a change in the ratio of expression of the two isoforms that favored 5 α -reductase type 1 (Stanbrough *et al.* 2006), indicating that compounds such as dutasteride might be preferred to block intratumoral androgens in patients with advanced prostate cancer. Clinical trials on the chemoprevention properties of finasteride or dutasteride yielded

the same outcome (Thompson *et al.* 2003, Andriole *et al.* 2010). The trials demonstrated that although there was a reduction in tumor incidence in a subset of patients, a more aggressive form of the cancer appeared. These results have led to controversy as to whether the detection of the more aggressive tumors was due to the frequency of biopsy and biopsy bias in the trials. Because of these data, both finasteride and dutasteride are not approved by the FDA for the treatment of prostate cancer patients and there is a warning label for this indication (U.S. Food and Drug Administration 2011). Explanations for these data could also include accumulation of testosterone in the presence of 5 α -reductase inhibition, accumulation of DHT precursors that might activate a mutant AR, or the appearance of a third form of 5 α -reductase (SRD5A3) (Uemura *et al.* 2008, Titus *et al.* 2014). 5 α -Reductase type 3 may not play such an important role in intratumoral androgen biosynthesis, as it is predominately involved in polyprenol biosynthesis (Cantagrel *et al.* 2010).

Finasteride and dutasteride are steroid-based competitive inhibitors of 5 α -reductase that were developed out of consideration of the transition-state analogs for the enzyme. Each of these compounds can give rise to the enolate intermediate proposed for the enzyme-catalyzed reaction. Subsequently, finasteride has been found to be a mechanism-based inactivator of 5 α -reductase type 2. In this mechanism, finasteride is reduced to dihydrofinasteride, which reacts with NADP⁺ to form a bisubstrate analog with a K_i of 10^{-13} M (Bull *et al.* 1996). Slow hydrolysis of the analog releases dihydrofinasteride into solution. It is likely that dutasteride works via the same mechanism.

Other 5 α -reductase inhibitors have been developed, e.g. epristeride from Smith, Kline and French. These compounds are acrylate-based, mimic the enolate reaction intermediate, and act as uncompetitive inhibitors by forming E.NADP⁺ inhibitor complexes in the ordered bi-bi kinetic mechanism (Levy *et al.* 1994).

Aldo-ketoreductases (AKRs): AKR1C1, AKR1C2, and AKR1C3

AKR1C1, AKR1C2, and AKR1C3 are NADPH-dependent monomeric cytosolic ketosteroid reductases (Penning *et al.* 2000) that play pivotal roles in steroidogenesis in the human prostate (Table 2; Rizner *et al.* 2003, Lin *et al.* 2004, Bauman *et al.* 2006a, Fung *et al.* 2006). These enzymes have different ratios of 3-, 17-, and 20-ketosteroid reductase activity (Rizner & Penning 2013). *In vitro*, AKR enzymes act as hydroxysteroid dehydrogenases. However, in mammalian cell transfection studies, in which the

prevailing concentration of NAD(P)(H) must be used, these enzymes function only as NADPH-dependent reductases (Rizner *et al.* 2003). This is due to their nanomolar potency for NADPH and the inhibition of the NAD^+ -dependent oxidation reactions by low micromolar concentrations of NADPH (Rizner *et al.* 2003). In androgen metabolism, AKR1C1 acts predominately as a 3β -HSD and reduces DHT to 5α -androstane- 3β , 17β -diol (3β -Adiol) (Steckelbroeck *et al.* 2004). 3β -Adiol is an inactive androgen, but a potent agonist of ER β , which would be an anti-proliferative signal in prostate cancer cells (Guerini *et al.* 2005). AKR1C2 acts predominately as a 3α -HSD and converts DHT ($K_d = 10^{-11}$ M for the AR) to 5α -androstane- 3α , 17β -diol (3α -Adiol, $K_d = 10^{-6}$ M for the AR) effectively terminating androgen action (Jin & Penning 2006). The role of AKR1C2 was recapitulated in transfection studies in LNCaP and PC-3 cells (Rizner *et al.* 2003).

AKR1C3 (also known as 17β -HSD type 5) is perhaps the most important AKR1C isoform in CRPC patients (Fig. 1). AKR1C3 was originally cloned from a human prostate cDNA library and was found to be overexpressed in prostate cancer cells (Lin *et al.* 1997, Fung *et al.* 2006). AKR1C3 catalyzes the NADPH-dependent reduction of Δ^4 -AD to testosterone, the reduction of Adione to DHT, and the reduction of androsterone to 3α -Adiol (Lin *et al.* 1997, Penning *et al.* 2000). Thus, all key steps in the classical, alternative, and backdoor pathways to DHT synthesis proceed through AKR1C3. In LNCaP-AKR1C3 cells, Δ^4 -AD is robustly converted to testosterone- 17β -glucuronide

(Byrns *et al.* 2012). AKR1C3 is necessary and sufficient to cause androgen-dependent gene expression in prostate cancer cell lines and in xenografts. For example, the gene expression of *PSA* and *TMPSSERG* is increased in the presence of the AKR1C3 substrate Δ^4 -AD, and this effect is blocked by *AKR1C3* short hairpin RNA (shRNA) and the AKR1C3 isoform-specific inhibitor indomethacin in VCaP cells (Cai *et al.* 2011). AKR1C3 is also overexpressed in response to ADT in prostate cancer patients. This is observed in prostate cancer cell lines maintained in androgen-deprived media, in xenograft models of CRPC, and in CRPC patients (Hofland *et al.* 2010, Pfeiffer *et al.* 2011, Hamid *et al.* 2012, Mitsiades *et al.* 2012). Compelling evidence that AKR1C3 is overexpressed in CRPC patients has been obtained using Affymetrix microarray, qPCR, and immunohistochemistry (Stanbrough *et al.* 2006). It is also overexpressed in soft-tissue metastasis in CRPC patients (Stanbrough *et al.* 2006). Measurements of intratumoral androgen levels also support the involvement of AKR1C3 in prostate cancer steroidogenesis. A 30-fold increase in the ratio of testosterone:DHT over that observed in prostate cancer cells was found in soft-tissue metastasis, indicating that the tumor is more dependent on testosterone than on DHT and that the source of androgen is probably AKR1C3 (Montgomery *et al.* 2008). The buildup of testosterone in these patients would be contrary to the findings of Chang *et al.* (2011), who showed that the pathway to DHT synthesis bypasses testosterone formation altogether. These findings may be reconciled

Table 2 Hydroxysteroid dehydrogenases expressed in the human prostate

Enzyme	Gene	Chromosome	Reaction catalyzed in the prostate
3β -HSD type 1	<i>HSD3B1</i>	1p13.1	DHEA to Δ^4 -AD Δ^5 -Androstene- 3β , 17β -diol to testosterone DHT to 3β -Adiol
AKR1C1 3α -HSD type 1 $20\alpha(3\alpha)$ -HSD	<i>AKR1C1</i>	10p15-p14	
AKR1C2 3α -HSD type 3	<i>AKR1C2</i>	10p15-p14	DHT to 3α -Adiol
AKR1C3 17β -HSD type 5 and 3α -HSD type 2	<i>AKR1C3</i>	10p15-p14	Δ^4 -AD to testosterone 5α -Adione to DHT Androsterone to 3α -Adiol
17β -HSD type 2	<i>HSD17B2</i>	16q24.1–q24.2	Testosterone to Δ^4 -AD DHT to Adione
17β -HSD type 3	<i>HSD17B3</i>	9q22	Δ^4 -AD to testosterone DHT to Adione
17β -HSD type 4	<i>HSD17B4</i>	5q21	Testosterone to Δ^4 -AD DHT to Adione
17β -HSD type 6 RL-HSD	<i>HSD17B6</i>	12q13	3α -Adiol to DHT

Δ^4 -AD, Δ^4 -androstene- $3,17$ -dione; DHT, 5α -dihydrotestosterone; 3β -Adiol, 5α -androstane- 3β , 17β -diol; 3α -Adiol, 5α -androstane- 3α , 17β -diol; Adione, 5α -androstane- $3,17$ -dione.

if the pathway to DHT synthesis that dominates in CRPC patients is patient-dependent. Interestingly, in the studies of [Chang *et al.* \(2011\)](#) in which Δ^4 -AD was used as a precursor, a large buildup of Adione was observed, indicating that AKR1C3 may catalyze the rate-determining step in the conversion of Adione to DHT. Thus, AKR1C3 appears to be involved in androgen biosynthesis in the prostate irrespective of the pathway involved and may also be rate-determining. These findings have led to a spectrum of AKR1C3 inhibitors being developed both in academia and in industry. Astellas Pharmaceuticals and GTx have both developed AKR1C3 inhibitors ([Astellas Pharmaceuticals 2013](#), [Watanabe *et al.* 2013](#), [Yepuru *et al.* 2013](#)).

The key to the development of AKR1C3 inhibitors is specificity and potency. AKR1C3 inhibitors should not inhibit AKR1C1 and AKR1C2 as these inactivate DHT and this would be counterproductive (see above). This creates an immediate challenge as AKR1C1, AKR1C2, and AKR1C3 have over 86% sequence identity and share similar (α/β)₈-barrel crystal structures ([Jin *et al.* 2001](#), [Lovering *et al.* 2004](#)). Nevertheless, our laboratory has been successful in developing AKR1C3 inhibitors based on three different chemical scaffolds that have mid-nanomolar inhibitory potency and the desired isoform selectivity. Each of these scaffolds was developed based on a compound screen that identified classes of non-steroidal anti-inflammatory drugs as potential AKR1C3 inhibitors. Class I agents are based on *N*-phenylaminobenzoates, which are derivatives of *N*-phenylanthranilates (e.g. flufenamic acid) ([Adeniji *et al.* 2012](#)). Class 2 agents are based on *N*-naphthylaminobenzoates, which have the unusual property of inhibiting AKR1C3 with nanomolar potency, having >200-fold selectivity, but also act as AR antagonists ([Chen *et al.* 2012](#)). Class 3 agents are based on indomethacin. Within the indomethacin series, three types of compounds were developed, one set based on indomethacin; another set in which the 2'-methyl group of indomethacin was removed, the compounds of which are referred to as *des*-methyl indomethacin analogs; and the last set in which the carboxylic acid side chain at the 3'-position of the indole ring was switched with 2'-alkyl group, the compounds of which are referred to as 3'-alkyl analogs ([Liedtke *et al.* 2013](#)). Lead agents from all the three classes were counterscreened against COX1 and COX2 and were found to be devoid of inhibitory properties against these enzymes.

Crystal structures of the AKR1C3 inhibitor complexes provide a rationale as to why the agents developed do not inhibit AKR1C1 or AKR1C2 ([Jin *et al.* 2001](#), [Lovering](#)

et al. 2004, [Chen *et al.* 2012](#), [Flanagan *et al.* 2012](#), [Liedtke *et al.* 2013](#)). Unique subpockets (SP1, SP2, and SP3) into which different portions of the drug molecules bind were identified. These subpockets are either absent or of a smaller size in AKR1C1 and AKR1C2 ([Byrns *et al.* 2011](#)).

Further proof-of-principle studies have been carried out by GTx and Astellas Pharmaceuticals, both of which have their own AKR1C3 inhibitor programs. GTx-560 was found not only to inhibit AKR1C3 competitively, but also to be built out of a selective AR modulator program. Interestingly, GTx-560 was used as a tool to show that AKR1C3 also worked as a coactivator of the AR in HEK293 cells and this function was blocked by competitive inhibition of the enzyme by GTx-560 ([Yepuru *et al.* 2013](#)). Thus, AKR1C3 competitive inhibitors may have the hidden property of blocking AR coactivation. GTx-560 and the Astellas compound ASP9521 were found to inhibit the growth of CRPC tumors in xenograft models. Importantly, ASP9521 was the first AKR1C3-specific inhibitor to be used in human trials, but the phase I/II clinical trial was halted because despite being well tolerated, the drug was not clinically beneficial to the small number of CRPC patients in the trial. Importantly, no molecular pathology was performed on tumor biopsies of these patients to determine that AKR1C3 was over-expressed in the first place ([Loriot *et al.* 2014](#)).

Other 17 β -HSD isoforms (HSD17B2, HSD17B3, HSD17B4, and HSD17B6)

17 β -HSD isoforms that belong to the short-chain dehydrogenase/reductase (SDR) family also play important roles in androgen biosynthesis and metabolism in the prostate ([Castagnetta *et al.* 1997](#)). 17 β -HSD type 2 (HSD17B2) ([Andersson *et al.* 1995](#)) and 17 β -HSD type 4 (HSD17B4) ([Adamski *et al.* 1995](#), [Carstensen *et al.* 1996](#)) are predominately NAD⁺-dependent dehydrogenases that inactivate testosterone and DHT by forming Δ^4 -AD and Adione respectively. Both are expressed in the human prostate and in prostate cancer patients ([Castagnetta *et al.* 1997](#)). 17 β -HSD type 3 (HSD17B3) converts Δ^4 -AD to testosterone; however, this enzyme is predominately Leydig cell-specific and mutations in this enzyme are associated with pseudohermaphroditism ([Geissler *et al.* 1994](#)). Although present in the prostate, its expression level is 1000- to 10 000-fold less than that of AKR1C3 and it is unlikely to play an important role in testosterone production, unless upregulated (R Mindnich & TM Penning, 2012, unpublished observations). Inhibitors of

17 β -HSD type 3 have been developed with the promise that they may be superior agents to leuprolide for causing chemical castration (Day *et al.* 2009, Vicker *et al.* 2009). 17 β -HSD type 6 (*HSD17B6*) is in fact predominately a 3 α -HSD that converts 3 α -Adiol back to DHT (Bauman *et al.* 2006b). Earlier work carried out in this laboratory revealed all human 3 α -HSD isoforms to be capable of catalyzing this oxidation reaction using IRES-transfection constructs so that activity could be normalized against β -galactosidase in COS-1 cells. This was coupled with expression studies and AR reporter gene assays to determine which isoform could activate the AR, starting with 3 α -Adiol. Data unequivocally revealed 17 β -HSD type 6 to be the major oxidative 3 α -HSD required for the conversion of 3 α -Adiol to DHT when compared with all the other candidate 3 α -HSD genes (Bauman *et al.* 2006a). This work was validated by results from subsequent studies, which demonstrated that 17 β -HSD type 6 is the major oxidative enzyme in the 'backdoor pathway' in prostate cancer cell lines and xenografts (Mohler *et al.* 2011). These findings will probably lead to the emergence of inhibitor programs for 17 β -HSD type 6.

Steroid sulfatase

There is only one STS enzyme, formerly known as arylsulfatase C, and it is encoded by the STS gene (Yen *et al.* 1987). This enzyme is required to cleave DHEA-SO₄ of adrenal origin to produce free DHEA for prostate steroidogenesis. STS has been detected in prostate tissues by a number of investigators, and it can be inhibited by aryl sulfamates including 667COUMATE (STX64, Irosustat) and other compounds (Purohit *et al.* 1995, 1996). However, it should not be forgotten that STS deficiency is associated with X-linked ichthyosis, a scaly-skin disease affecting roughly 1 in every 2000–6000 males (Shapiro 1979, 1985), and would be an expected phenotype of chronic sulfatase inhibition in which cholesterol can no longer be mobilized from cholesterol sulfate.

Uridine glucuronosyl transferases

Uridine glucuronosyl transferases (UGTs) are major enzymes that catalyze the conjugation of hydroxyandrogens with UDP-glucuronic acid for elimination from the prostate. Some prostate cancer cell lines have very high UGT activity; e.g. in LNCaP cells, the major isoforms involved appear to be UGT2B15 and UGT2B17 (Guillemette *et al.* 1997). These isoforms are upregulated by ADT and by AR antagonists (Grosse *et al.* 2013). Studies

carried out by the Plymate group revealed that during the progression to CRPC, there is not only increased expression of constitutively active AR splice variants but also overexpression of UGTs (Hu *et al.* 2012). This indicated that increased UGT expression is part of an adaptive response of the tumor to become androgen-independent. In other studies, 526 Caucasian and 320 Asian men who underwent radical prostatectomy for clinically localized prostate cancer were examined for deletions in *UGT2B* genes. The relationship between genotypes and biochemical recurrence was assessed with multivariate Cox proportional hazard models. Plasma steroid levels were measured using specific and sensitive mass-spectrometry-based methods. These studies demonstrated that at least two deleted copies of *UGT2B17* and *UGT2B28* genes resulted in a hazard ratio of 2.26 for increased prostate cancer incidence (Nadeau *et al.* 2011).

Clinical efficacy and mechanisms of drug resistance

The clinical efficacy of drugs that target steroidogenic enzymes to disrupt the androgen axis can be determined by measuring the reduction in serum and intraprostatic androgen levels; measuring the reduction in serum PSA levels; measuring the intraprostatic levels of androgen-regulated genes in tissue biopsies; and by correlation of serum drug levels with tissue drug levels. Stable-isotope dilution liquid chromatography–tandem mass spectrometry represents the state-of-the-art approach to measure serum and tissue androgen levels (Tamae *et al.* 2013). No single method can measure the levels of all the androgens of interest as the required sensitivity in the 0.1–1.0 pg range requires steroid derivatization. Ketoandrogens can be derivatized as oximes with Girard-T (Tamae *et al.* 2013), while hydroxyandrogens have to be measured as picolinates (Higashi *et al.* 2005, 2006). Even with these methods, there is a need to measure both conjugated and free steroids, which requires additional digestion steps with β -glucuronidase and sulfatase. Measurement of a declining PSA level and its rebound could represent a measurement of drug response and relapse in the tumor respectively. However, if there is an intact prostate with underlying BPH, this could be an important confounder that will influence PSA measurements. Measurement of drug levels in serum and tissue will help in the determination of whether effective drug concentrations have been attained, but could be confounded if drug metabolites that are also enzyme

inhibitors are present. With at least three different pathways to DHT synthesis, this built-in redundancy could be blocked with dual steroid 5 α -reductase type 1 and type 2 inhibitors. However, the classical pathway to DHT synthesis could compensate, leading to a build-up of testosterone. With this redundancy, it is predicted that acquired drug resistance may occur. The one exception may be in the targeting of AKR1C3, which will not only inhibit all the pathways to DHT synthesis but also block testosterone formation.

Abiraterone effectively causes tumor remission in CRPC patients and prolongs survival by 3–4 months. Reasons for therapeutic failure could be the following: i) incomplete blockade of DHEA and DHEA-SO₄ formation in the adrenal; ii) overexpression of P450 17A1; iii) overexpression of AKR1C3; iv) AR gene amplification, and v) the emergence of AR splice variants that are constitutively active without hormone (Cai *et al.* 2011, Mostaghel *et al.* 2011). These observations indicate that molecular profiling of the tumor should be performed before a drug is administered so that the correct target is blocked. For example, if constitutively active AR splice variants are present, the administration of agents to block P450 17A1 would be futile.

Eradication of the androgen axis

Because of the adaptive responses that can occur with ADT, there has been excitement about the introduction of the AR superantagonist enzalutamide (Xtandi[®]) (Tran *et al.* 2009, Scher *et al.* 2010, 2012). As might be predicted, enzalutamide also causes remission followed by the emergence of resistance. Thus, there are now clinical trials planned in which combination therapy of abiraterone and enzalutamide will be given together. Instead of combination therapy, there is hope that some of the new agents will be bifunctional and block intraprostatic androgen synthesis and the AR. Galeterone has this property; it is both a P450 17A1 inhibitor and will cause degradation of the AR simultaneously (Vasaitis *et al.* 2008). The N-naphthylaminobenzoate BMT4-158 is a 'first-in-class' bifunctional AKR1C3 inhibitor and an AR antagonist. BMT4-158 will inhibit AKR1C3 with an IC₅₀ value of 50 nM, and it will block the conversion of Δ^4 -AD to testosterone and Δ^4 -AD-stimulated PSA production in LNCaP-AKR1C3 transfected cells. BMT4-158 will also block DHT-driven reporter gene activity with an IC₅₀ value of 5.0 μ M and displace [³H]-R1881 binding to the AR competitively.

Importantly, it will cause degradation of the AR in the presence and absence of DHT (Chen *et al.* 2012).

Future considerations

Advanced prostate cancer and CRPC remain hormonally driven, and inhibitors that block enzymes involved in intratumoral androgen biosynthesis in the castrate environment offer a therapeutic strategy. Redundancy in the pathways to DHT synthesis indicates that in many cases drug resistance may occur through bypass of one pathway. This can be surmounted by targeting enzymes required for all the pathways to testosterone and DHT synthesis. One enzyme of particular promise in this regard is AKR1C3. However, the tumor can also undergo adaptive AR signaling, indicating that eradication of the androgen axis may require bifunctional agents that block both enzymatic synthesis of androgens and AR signaling.

Declaration of interest

The author is the founder of Penzymes, LLC, which develops selective intracrine modulators to block the local synthesis of hormones in prostate, breast, and endometrial cancers.

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