REVIEW

The role of 11-oxygenated androgens in prostate cancer

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Abstract

11-oxygenated androgens are a class of steroids capable of activating the androgen receptor (AR) at physiologically relevant concentrations. In view of the AR as a key driver of prostate cancer (PC), these steroids are potential drivers of disease and progression. The 11-oxygenated androgens are adrenal-derived, and persist after androgen deprivation therapy (ADT), the mainstay treatment for advanced PC. Consequently, these steroids are of particular interest in the castration-resistant prostate cancer (CRPC) setting. The principal androgen of the pathway, 11-ketotestosterone (11KT), is a potent AR agonist and the predominant circulating active androgen in CRPC patients. Additionally, several precursor steroids are present in the circulation which can be converted into active androgens by steroidogenic enzymes present in PC cells. *In vitro* evidence suggests that adaptations frequently observed in CRPC favour the intratumoral accumulation of 11-oxygenated androgens in particular. Still, apparent gaps in our understanding of the physiology and role of the 11-oxygenated androgens remain. In particular, *in vivo* and clinical evidence supporting these *in vitro* findings is limited. Despite recent advances, a comprehensive assessment of intratumoral concentrations has not yet been performed. The exact contribution of the 11-oxygenated androgens to CRPC progression therefore remains unclear. This review will focus on the current evidence linking the 11-oxygenated androgens to PC, will highlight current gaps in our knowledge, and will provide insight into the potential clinical importance of the 11-oxygenated androgens in the CRPC setting based on the current evidence.

Key Words

- androgen
- androgen receptor
- prostate
- steroid
- testosterone

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Introduction

Prostate cancer (PC) is one of the most common malignancies in men and represents a major challenge to our healthcare systems with over 1.4 million new cases and over 375,000 deaths worldwide in 2020 (Sung *et al.* 2021). PC typically develops later in life and is rare before the age of 50 (Howlader *et al.* 2019, Rawla 2019). Localized, non-advanced PC can be treated effectively (5-year survival: >99%) and does not always necessitate active treatment when the risk of progression is low (Howlader *et al.* 2019). Metastatic disease, however, carries a significantly worse prognosis (5-year survival: 31%) and is considered incurable (Howlader *et al.* 2019). The androgen receptor (AR) has long been recognized as a major driver of PC pathogenesis and progression (Zhou *et al.* 2015, Fujita & Nonomura 2019).

Physiologically, the AR is a key regulator of various important processes, including male sexual development, muscle growth and bone health.

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To accomplish its physiological role, the AR must be activated by steroid hormones known as androgens. The testes are the primary source of testosterone, the predominant circulating active androgen in adult men. In the Leydig cells, cholesterol is converted into pregnenolone, 17α-hydroxypregnenolone and subsequently to dehydroepiandrosterone (DHEA) and androstenedione (A4) (Zirkin & Papadopoulos 2018). Finally, A4 is converted into testosterone, which is then released into circulation. The production of testosterone is regulated by the luteinizing hormone (LH) through the hypothalamic–pituitary–gonadal axis. Testosterone can activate the AR directly at physiological concentrations (de Launoit *et al.* 1991, Campana *et al.* 2016) but can also be converted into the more potent 5α-dihydrotestosterone (DHT) by steroid 5α-reductase, an enzyme present in many AR target tissues.

In PC cells, the AR drives the activation of genes that promote cell growth and survival (Buchanan *et al.* 2001*b*, Heinlein & Chang 2004). Consequently, suppression of the AR pathway through androgen deprivation therapy (ADT) by either gonadotropin-releasing hormone analogues/antagonists, which inhibit the release of LH from the pituitary, or bilateral orchiectomy forms the mainstay of advanced PC treatment (Perlmutter & Lepor 2007, Narayanan 2020). The serum testosterone concentration typically exceeds 10 nmol/L in healthy men but falls below 1 nmol/L in men who receive ADT (Snaterse *et al.* 2017). Following ADT, the adrenal glands are the principal source of residual testosterone, in addition to several androgen precursor steroids such as DHEA, DHEA-sulphate (DHEAS) and A4 (Rege *et al.* 2013, Turcu *et al.* 2014).

Under castrate conditions, intratumoral androgen levels fall and the AR pathway is unstimulated, resulting in the inhibition of tumour growth. ADT can effectively control the disease for months or even years, but ultimately, castration-resistant prostate cancer (CRPC) emerges, often paired with metastatic disease (Ross *et al.* 2008). Although this stage of the disease was initially considered to be AR-independent, the central role of AR in CRPC pathophysiology in a majority of patients is now recognized (Chen *et al.* 2004, Mohler *et al.* 2004, Barnard *et al.* 2020*b*). Several AR-dependent mechanisms conferring castration resistance have been discovered over the past decades. Upregulation of the AR sensitizes cancer cells to low androgen concentrations, allowing AR pathway activation even under castrate androgen concentrations (Donovan *et al.* 2010, Taylor *et al.* 2010, van Dessel *et al.* 2019). Changes to the intratumoral expression of steroidogenic enzymes such as aldo-keto reductase family 1 member c3 (AKR1C3) and steroid 5-alpha reductase 1 (SRD5A1) that enhance the conversion of androgen precursors to active androgens similarly allow CRPC cells to escape systemic androgen deprivation through local DHT production (Chen *et al.* 2004, Stanbrough *et al.* 2006, Montgomery *et al.* 2008, Chang *et al.* 2011). Alternatively, mutations in the AR ligand-binding domain (LBD) occur in up to 20% of CRPC patients (Buchanan *et al.* 2001*a*, Romanel *et al.* 2015, Lallous *et al.* 2016, Wyatt *et al.* 2016, Snaterse *et al.* 2022). These mutations confer ligand promiscuity, enabling activation of the AR by steroid hormones that normally have no androgenic properties, such as progesterone and cortisol (Veldscholte *et al.* 1990, Zhao *et al.* 1999, 2000, Duff & McEwan 2005, van de Wijngaart *et al.* 2010, Lallous *et al.* 2016, Prekovic *et al.* 2016, Snaterse *et al.* 2022). Furthermore, splice variants of the AR have been discovered that are constitutively active and drive AR pathway activation even in the absence of androgens (Antonarakis *et al.* 2014, Kohli *et al.* 2017, Tagawa *et al.* 2019). In other patients, the glucocorticoid receptor (GR) takes over as the dominant driver of pathogenesis (Arora *et al.* 2013).

A novel class of androgenic steroids, known as the 11-oxygenated androgens was shown to be present in humans in recent years (Rege *et al.* 2013, Storbeck *et al.* 2013, Pretorius *et al.* 2016, Turcu *et al.* 2020). The primary bioactive androgen of this pathway, 11-ketotestosterone (11KT), is capable of activating the AR at concentrations similar to testosterone (Rege *et al.* 2013, Storbeck *et al.* 2013, Pretorius *et al.* 2016, Snaterse *et al.* 2022). Physiologically relevant concentrations of 11KT have since been observed in the circulation of CRPC patients (Wright *et al.* 2020, Snaterse *et al.* 2021*b*). These new androgens are of significant interest to the CRPC field as potential drivers of AR activation.

This review focuses on the role of 11-oxygenated androgens in PC and their (potential) involvement as drivers of castration resistance. This review contains a comprehensive summary of our current understanding of 11-oxygenated androgen actions, regulation and metabolism within patients with (CR)PC. Finally, this review discusses limitations and gaps in our knowledge as well as key focus areas for future research.

11-oxygenated androgens

The presence of potent androgens other than testosterone and DHT in humans was discovered in 2013

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(Rege *et al.* 2013, Storbeck *et al.* 2013). Since then, studies have sought to elucidate the 11-oxygenated androgen pathway, the affinity of these steroids for the AR and their circulating concentrations under physiological and pathophysiological conditions (Rege *et al.* 2013, Storbeck *et al.* 2013, Swart *et al.* 2013, Swart & Storbeck 2015, Pretorius *et al.* 2016, Barnard *et al.* 2018, Turcu *et al.* 2020, 2021*a*,*b*, Snaterse *et al.* 2021*b*). Already, the 11-oxygenated androgens have been implicated in several hyperandrogenic disorders, including congenital adrenal hyperplasia, polycystic ovarian syndrome and premature adrenarche (Turcu *et al.* 2016, 2018, 2020, O'Reilly *et al.* 2017, Turcu & Auchus 2017, Kamrath *et al.* 2018, Rege *et al.* 2018).

The 11-oxygenated androgen pathway consists of several potent androgens, adrenal precursors and downstream metabolites. The production of 11-oxygenated androgens appears to be independent of gonadal steroidogenesis and is instead subject to hypothalamic–pituitary–adrenal (HPA) axis regulation (Rege *et al.* 2013). Indeed, 11-oxygenated androgen production increases upon stimulation with adrenocorticotropic hormone (ACTH) (Rege *et al.* 2013) and decreases upon treatment with glucocorticoids, such as prednisone or dexamethasone (Snaterse *et al.* 2021b). The pathway commences with the conversion of A4 into 11β-hydroxyandrostenedione (11OHA4) by steroid 11β-hydroxylase (CYP11B1), which is expressed exclusively in the adrenal gland (Storbeck *et al.* 2013, Swart *et al.* 2013). 11OHA4 is the most abundant 11-oxygenated androgen, circulating at concentrations of 3.1–6.1 nmol/L (interquartile range) in CRPC patients who did not receive glucocorticoids or abiraterone (Snaterse *et al.* 2021*b*). Similarly, testosterone can be 11-hydroxylated to 11β-hydroxytestosterone (11OHT) (Swart *et al.* 2013). The circulating concentrations of 11OHT are substantially lower than 11KT in CRPC patients, at approximately 0.1–0.4 nmol/L (Snaterse *et al.* 2021*b*). Both 11OHA4 and 11OHT can be converted by 11β-hydroxysteroid dehydrogenase type 2 (HSD11B2) into 11-ketoandrostenedione (11KA4) and 11KT, respectively (Storbeck *et al.* 2013, Swart *et al.* 2013, Pretorius *et al.* 2017). Both 11KA4 and 11KT circulate at concentrations between 0.4 and 1.3 nmol/L in CRPC patients who did not receive adrenal suppression (Snaterse *et al.* 2021*b*). These concentrations are comparable to those observed in healthy men, although for 11KT specifically, concentrations in elderly men (aged 60–80 years) are lower compared to younger men (age 18–30 years) (Turcu *et al.* 2021*b*). HSD11B2 is primarily expressed in peripheral tissues, with especially high expression in the kidneys. This suggests that the production of 11KA4 and 11KT occurs outside of the adrenal gland. The reverse reaction is catalysed by 11β-hydroxysteroid dehydrogenase type 1 (HSD11B1), which is expressed primarily in the liver, adipose tissue and muscle (Morgan *et al.* 2014, Gent *et al.* 2019, Amai *et al.* 2020). Considering the circulating concentrations and reported enzymatic activities, the conversion of 11OHA4 > 11KA4 > 11KT appears to be the predominant 11-oxygenated androgen pathway (Storbeck *et al.* 2013, Swart *et al.* 2013, Barnard *et al.* 2018). Much of the circulating 11OHT may derive from 11KT through the actions of peripheral HSD11B1, rather than from the direct conversion of testosterone in the adrenal.

Androgenic activity and serum concentrations

The steroids of the 11-oxygenated androgen pathway have varying degrees of androgenic activity. The precursor steroids 11OHA4 and 11KA4 have no intrinsic androgenic activity and must first be converted before they can affect the AR signalling pathway (Storbeck *et al.* 2013). In contrast, 11KT activates the AR at concentrations comparable to or slightly higher than testosterone, depending on the experimental design (Pretorius *et al.* 2016, Rege *et al.* 2018, Snaterse *et al.* 2022). Using an AR reporter assay in non-PC cells, a recent study found that EC50 of 11KT for wild-type AR was around 0.74 nmol/L compared to the 0.22 nmol/L for testosterone (Snaterse *et al.* 2022). Activation of the AR at even lower concentrations (0.1 nmol/L and below), measured using proliferation assays and qPCR analysis, has been reported in the PC cell lines PC346C, LNCaP and VCaP (Storbeck *et al.* 2013, Snaterse *et al.* 2022). 11OHT is a relatively weaker AR agonist, although its exact potency is still a matter of debate (Rege *et al.* 2013, Storbeck *et al.* 2013, Pretorius *et al.* 2016, Handelsman *et al.* 2022, Snaterse *et al.* 2022). Given its low circulating concentrations and relatively low potency, 11OHT does not appear to be a major direct contributor to AR pathway reactivation in CRPC.

Mirroring the classical androgen pathway, steroids of the 11-oxygenated androgen pathway can be converted by steroid 5α- and 5β-reductases (Barnard *et al.* 2020*a*). The 5α-reduced product of 11KT, known as 11-ketodihydrotestosterone (11KDHT) is a potent androgen comparable to DHT (Storbeck *et al.* 2013, Pretorius *et al.* 2016, Snaterse *et al.* 2022). Thus far, most studies have failed to reliably quantify 11KDHT in human serum. In part, this may be due to the technical

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challenge of quantifying 5α-reduced steroids compared to their Δ4 counterparts. Moreover, circulating 11KDHT concentrations are estimated to be below 20 pmol/L based on studies using derivatization approaches (Häkkinen *et al.* 2019, Caron *et al.* 2021). These concentrations are well beyond the capabilities of the majority of liquid chromatography tandem mass spectrometry (LC-MS/MS) setups. At these concentrations, circulating 11KDHT is also unlikely to be a major contributor to AR pathway reactivation. Low circulating 11KDHT concentrations may in part be due to the less efficient conversion of 11KT by steroid 5α-reductases, especially SRD5A1 (Barnard *et al.* 2020*a*). Despite this, the intratumoral production of 11KDHT may still be relevant (although this remains to be proven), as *in vitro* studies indicate that both 11KT and 11KDHT may be metabolized less efficiently than DHT, which may contribute to intratumoral build-up (du Toit & Swart 2018, Barnard *et al.* 2020*a*). 11KT and 11KDHT appear to be less susceptible to conjugation, a metabolic process that leads to the inactivation of steroids through the addition of a sulphate or glucuronide moiety (du Toit & Swart 2018).

Several other downstream metabolites of the 11-oxygenated androgen pathway exist, although their physiological or pathophysiological importance is still poorly understood. 11KDHT can be metabolized in a way that mirrors the classical androgen pathway, yielding steroids such as 11-ketoandrosterone and 11-keto-3α-androstanediol (Storbeck *et al.* 2013, van Rooyen *et al.* 2018, 2020). It has been proposed that these steroids can be converted back to 11KDHT in a way similar to the backdoor DHT pathway (van Rooyen *et al.* 2018). 11β-hydroxy-DHT (11OHDHT) is the 5α-reduced product of 11OHT and has androgenic activity, albeit less than 11KT (Storbeck *et al.* 2013). *In vivo* concentrations are unknown, and due to the relatively low circulating 11OHT concentration, 11OHDHT is probably not a major bioactive androgen.

11-oxygenated androgen metabolism within the prostate cancer cell

The intratumoral conversion of inactive precursor steroids to active androgens has been identified as a major contributor to intratumoral androgen accumulation, and thereby AR pathway reactivation (Chen *et al.* 2004, Stanbrough *et al.* 2006, Hofland *et al.* 2010, Kumagai *et al.* 2013, Barnard *et al.* 2020*b*, Moll *et al.* 2022). A full overview of the intratumoral classical and 11-oxygenated androgen pathways is shown in Figure 1.

Changes in the expression of steroidogenic enzymes are a frequent adaptation in CRPC tumours and have been highlighted in Figure 1 (Mohler *et al.* 2004, Titus *et al.* 2005, Stanbrough *et al.* 2006, Pfeiffer *et al.* 2011, Mitsiades *et al.* 2012). The adaptations contribute to intratumoral androgen levels comparable to pre-castrate conditions (Mohler *et al.* 2004). Frequently observed changes include the upregulation of the androgen-activating enzyme AKR1C3 (Stanbrough *et al.* 2006, Pfeiffer *et al.* 2011, Mitsiades *et al.* 2012) and the downregulation or silencing of the androgen-inactivating enzyme 17β-hydroxysteroid dehydrogenase 2 (HSD17B2) (Friedlander *et al.* 2012, Gao *et al.* 2019). These changes contribute to an enhanced flux from A4 to T, thereby leading to androgen accumulation. These same enzymes also catalyse the conversions between 11KA4 and 11KT (Storbeck *et al.* 2013, Swart & Storbeck 2015, Pretorius *et al.* 2017).

AKR1C3 is expressed in CRPC tissue, and *in vitro* studies show that PC cells are capable of producing 11KT from 11KA4 (Pretorius *et al.* 2016, Barnard *et al.* 2018). Interestingly, AKR1C3 appears to convert 11KA4 more efficiently than A4 (Barnard *et al.* 2018). Increased expression of AKR1C3, common in CRPC (Stanbrough *et al.* 2006, Pfeiffer *et al.* 2011), may therefore cause 11KT to accumulate intratumorally at a much faster rate compared to DHT. In contrast, 11OHA4 appears to be a poor substrate for AKR1C3, and conversion by HSD11B2 into 11KA4 is necessary for the conversion into active 11KT by AKR1C3 (Pretorius *et al.* 2017, Barnard *et al.* 2018, Paulukinas *et al.* 2022). *HSD11B2* mRNA expression was higher than that of *HSD11B1* in CRPC tumours (Snaterse *et al.* 2021*b*), and Li *et al.* detected significant HSD11B2 activity in PC cells by studying the conversion of cortisol into cortisone (Li *et al.* 2017). Indeed, *in vitro* studies confirm the formation of 11KT from both 11OHA4 and 11OHT in PC cells (Storbeck *et al.* 2013, Swart *et al.* 2013). Gent and colleagues similarly report that the actions of HSD11B2 are dominant in PC cells *in vitro* (Gent *et al.* 2019). Considering the conversions rates reported by Storbeck and colleagues and the low circulating concentrations of 11OHT in CRPC patients (Snaterse *et al.* 2021*b*), it is likely that the conversion of 11OHA4 > 11KA4 > 11KT is also the main intratumoral 11-oxygenated androgen metabolic pathway.

Given the absence of intratumoral CYP11B1 expression, circulating DHEA, DHEAS and A4 likely do not fuel the intratumoral 11-oxygenated androgen pathway. Nevertheless, these precursors are still substrates for the local conversion to testosterone and DHT, thereby contributing to AR pathway activation.

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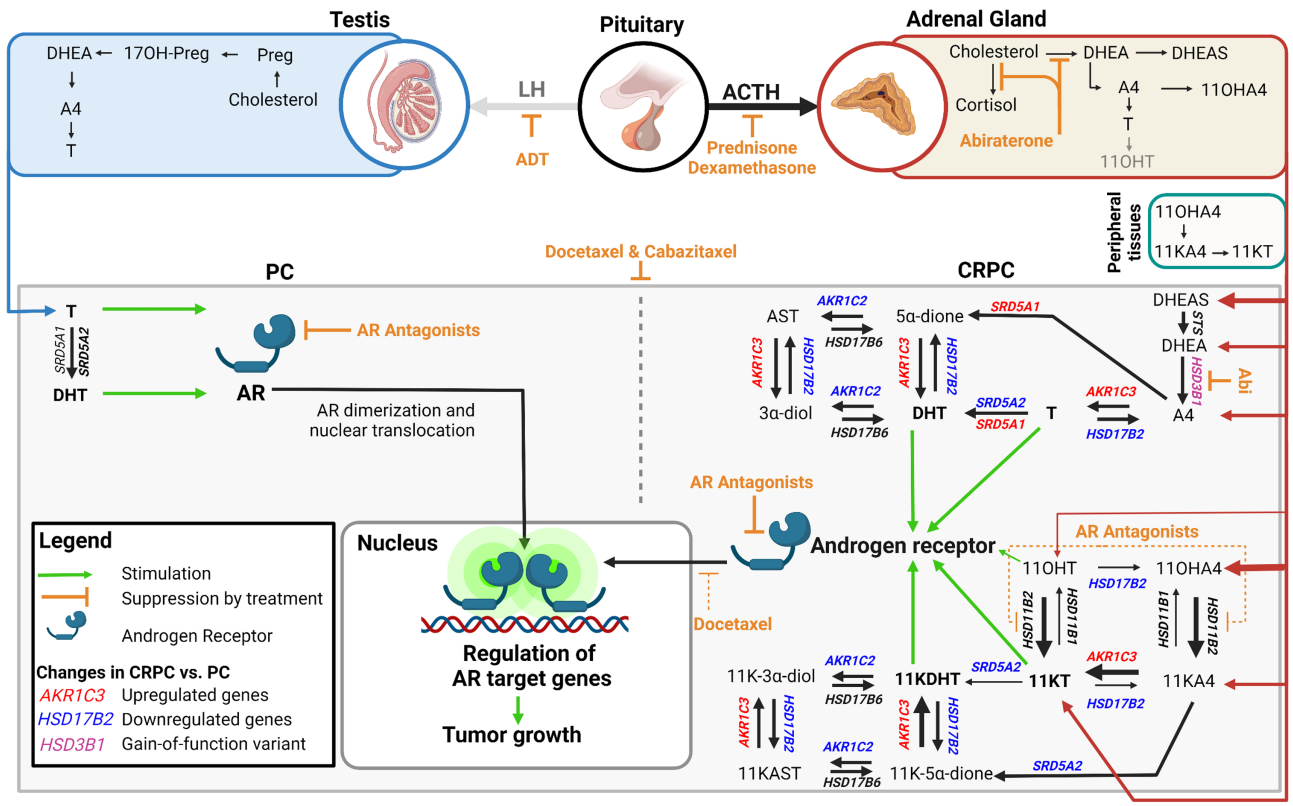

Figure 1

An overview of the major androgen and 11-oxygenated androgen pathways in the testis, adrenal gland, prostate cancer (PC) and castration-resistance prostate cancer (CRPC). Steroidogenic enzymes that are differentially regulated in CRPC compared to PC are highlighted in blue (downregulation) and red (upregulation). An increased or decreased arrow size in the 11-oxygenated androgen pathway indicates if the reaction is known to be substantially more or less efficient compared to the classical androgen pathway. Enzymes or proteins known to be affected by clinically relevant gain-of-functions mutations are highlighted in purple. The inhibitory actions of frequently used treatments in CRPC are shown in orange. This figure was prepared using https://www.biorender.com/.

The SRD5A enzymes are the key mediators of intratumoral DHT accumulation. Interestingly, whereas testosterone is rapidly converted by SRD5A1 and SRD5A2, 11KT is converted with lower efficiency by SRD5A2 and is not converted at all by SRD5A1 (Barnard *et al.* 2020*a*). These enzymes can catalyse the conversion of 11-oxygenated precursors, and 11OHA4 and 11KA4 appear to be the preferred substrates, yielding 11β-hydroxy-5α-androstanedione and 11-keto-5α-androstanedione (11K-5α-dione), respectively (Barnard *et al.* 2020*a*).

The efficiency of the conversion of 11KT by aldo-keto reductase family 1 member D1 (AKR1D1), the sole 5β-reductase, is more comparable to the efficiency observed for testosterone (Barnard *et al.* 2020*a*). AKR1D1 is primarily expressed in the liver where it regulates steroid action (Chen *et al.* 2011, Nikolaou *et al.* 2019), and it is unclear to what extent it affects intratumoral 11KT metabolism, as mRNA expression was low in CRPC tissue (Snaterse *et al.* 2021*b*). The differences in the conversion of classical and 11-oxygenated androgens by 5α and 5β-reductases have two-fold implications: first, the production of the potent AR agonist 11KDHT is lower compared to DHT in tissues expressing high levels of 5α-reductase, and especially in tissues expressing SRD5A1. These tissues may be more responsive to A4, testosterone and DHT as a result. Based on the substrate preferences of AKR1C3 (Barnard *et al.* 2018) and the steroid 5α-reductases (Barnard *et al.* 2020*a*), 11KDHT may be produced mainly through the conversion of 11KA4 > 11K-5α-dione > 11KDHT rather than through 11KT > 11KDHT.

Secondly, testosterone and DHT are metabolized by PC cells at a much faster rate compared to the respective 11-oxygenated analogues (Pretorius *et al.* 2016). Differences in substrate affinity of 3α- and 5α-reducing enzymes may play an important role here, as well as the differential conversion by UGT-family conjugating enzymes (du Toit & Swart 2018). In CRPC tissues, the reduced metabolism by both steroid 5α/β-reductases and conjugating enzymes may be responsible for the

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reduced clearance of 11KT and could potentially lead to higher intratumoral concentrations of 11KT compared to testosterone and DHT.

Based on these *in vitro* data, the adaptations seen in CRPC tissues – for example, AKR1C3 upregulation (Mohler *et al.* 2004, Montgomery *et al.* 2008), HSD17B2 downregulation (Koh *et al.* 2002) or silencing (Friedlander *et al.* 2012, Gao *et al.* 2019), downregulation of AKR1C2 (Ji *et al.* 2003, 2007) and the shift from SRD5A2 to SRD5A1 (Titus *et al.* 2005) – may all contribute to increased intratumoral 11KT (and to some extent, 11KDHT) accumulation.

Conversion of 11-oxy C21 steroids

Recent studies have identified 11β-hydroxyprogesterone (11OHP4) and 11-ketoprogesterone (11KP4) as potential upstream precursors of the 11-oxygenated androgen pathway (Barnard *et al.* 2017, van Rooyen *et al.* 2018, 2020). Van Rooyen and colleagues showed *in vitro* that these steroids can be converted by enzymes such as HSD11B1/2, SRD5A and Cytochrome P450 17A1 (CYP17A1) to ultimately yield 11KDHT (van Rooyen *et al.* 2018). The *in vivo* importance of these steroids as potential contributors to intratumoral androgen accumulation is currently unknown. Turcu *et al.* reported circulating concentrations of 11OHP4 in healthy volunteers to be below their limits of quantification, which was 30 ng/dL (0.9 nmol/L) (Turcu *et al.* 2015). Secondly, *in vitro* experiments in LNCaP cells show that only a very small fraction (<1%) of 11OHP4 and 11KP4 was converted to 11KDHT (van Rooyen *et al.* 2018). These conversions require CYP17A1, and to date, there is little quantitative evidence of significant CYP17A1 activity in CRPC tumours (Hofland *et al.* 2010, Kumagai *et al.* 2013, Moll *et al.* 2022). Together, these data do not suggest an important contribution of 11OHP4 and 11KP4 towards intratumoral androgen accumulation in CRPC patients.

It is important to realize, however, that research into the intratumoral actions and metabolism of 11KT and the 11-oxygenated androgen is still in its infancy. While 11-oxygenated androgens have been shown to persist after ADT and *in vitro* study displays the steroidogenic potential of PC cells, studies have yet to show to what extent 11KT accumulates in CRPC tissues. A single study reported 11-oxygenated androgen tissue concentrations in tumours obtained from two patients, in which the 11KT concentration was equal to or higher than the testosterone concentration (du Toit *et al.* 2017). However, these results are difficult to interpret, as plasma 11-oxygenated androgen concentrations reported in this study (11OHA4 > 200 nmol/L, 11KT > 150 nmol/L, 11KDHT > 10 nmol/L) (du Toit *et al.* 2017) far exceed the concentrations ranges reported in other studies (11OHA4 0.9–19.8 nmol/L, 11KT 0.12–2.4 nmol/L, 11KDHT ~10–30 pmol/L) (Häkkinen *et al.* 2019, Snaterse *et al.* 2021*b*). Determining the intratumoral concentrations and metabolism in patient tissues is key to increasing our understanding of the actions and importance of the 11-oxygenated androgen pathway.

Interactions with treatment

Androgen deprivation has been the central pillar of advanced PC treatment for several decades. Due to their adrenal origin, 11-oxygenated androgens persist after ADT, with 11KT becoming the predominant active androgen in the circulation (Snaterse *et al.* 2021*b*). Indeed, 11-oxygenated androgen levels are comparable between CRPC patients (before glucocorticoid or abiraterone treatment) and untreated men aged 60–80 (Snaterse *et al.* 2021*b*, Turcu *et al.* 2021*b*). Other treatments do, however, directly affect 11-oxygenated androgen production, metabolism or action (also shown in Fig. 1).

Abiraterone

Abiraterone acetate is the most potent inhibitor of adrenal androgen production that is currently used to treat CRPC patients (James *et al.* 2017). Specifically, abiraterone is an inhibitor of CYP17A1, which catalyses the conversion of pregnenolone into 17α-hydroxypregnenolone and subsequently DHEA. In addition, abiraterone can be converted by 3β-hydroxysteroid dehydrogenase 1 (HSD3B1) into Δ4-abiraterone, which inhibits CYP17A1, HSD3B1 and acts as an AR inhibitor (Li *et al.* 2015). Since the 11-oxygenated androgen pathway originates from A4, downstream of CYP17A1, abiraterone was predicted to be a potent inhibitor of 11-oxygenated androgen production. Wright *et al.* show that abiraterone indeed suppresses the various classical (69–90%) and 11-oxygenated androgens (64–94%) in CRPC patients (Wright *et al.* 2020). It should be noted that patients who are treated with abiraterone show some residual DHEAS production, which could fuel local testosterone or DHT production (Attard *et al.* 2009, McKay *et al.* 2017).

Glucocorticoids

Exogenous glucocorticoids such as prednisone, prednisolone and dexamethasone are frequently used in

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CRPC patients. Prednisone or prednisolone (5–10 mg/day) are often prescribed together with abiraterone in order to limit abiraterone-induced mineralocorticoid excess. Prednisone or prednisolone are also frequently combined with docetaxel or cabazitaxel chemotherapy (5–10 mg/day), possibly with the addition of dexamethasone, which is given briefly before the start of each chemotherapy cycle (1–3 × 8 mg). In some patients, dexamethasone (0.5 mg/day) is used instead of prednisone/prednisolone. Exogenous glucocorticoids potently activate the GR and thereby cause suppression of the HPA axis through negative feedback, leading to ACTH suppression and decreased cortisol production. Early studies have shown that the production of 11-oxygenated androgens is under the control of ACTH (Rege *et al.* 2013). Indeed, exogenous glucocorticoid treatment decreased the circulating 11KT in CRPC patients by a median of 84% (Snaterse *et al.* 2021*b*). Precursor steroids such as 11OHA4 and 11KA4 were similarly suppressed, while glucocorticoid treatment lowered testosterone by a median of 68%. It is therefore important to recognize that exogenous glucocorticoids effectively provide AR pathway inhibition by lowering androgen levels. These effects will be especially apparent in the absence of abiraterone. This could in part explain the beneficial effects of glucocorticoid treatment in the CRPC setting, which have long been recognized (Tannock *et al.* 1989, Venkitaraman *et al.* 2008). These effects should also be considered when evaluating optimal treatment strategies, especially in the third or fourth line, as cross-resistance between AR-targeting treatments is known to occur (Loriot *et al.* 2013, van Soest *et al.* 2015).

Androgen receptor inhibitors

Second-generation AR inhibitors have proven to be greatly effective in the treatment of CRPC. Currently, three AR antagonists have been approved for the treatment of high-risk PC and CRPC: enzalutamide, apalutamide and darolutamide (Scher *et al.* 2012, Chi *et al.* 2019, Fizazi *et al.* 2019). These drugs are considerably more effective at suppressing the AR than first-generation antiandrogens, such as flutamide or bicalutamide. Unlike abiraterone, these drugs are pure AR antagonists and were not intended to directly target steroidogenic enzymes. However, an interesting mechanism was recently uncovered that suggests that AR inhibitors may in fact modulate steroid metabolism, including the 11-oxygenated androgen pathway. Li and colleagues showed that enzalutamide treatment resulted in an AR-mediated loss HSD11B2 in cell line models and CRPC patient tissue (Li *et al.* 2017). The loss of HSD11B2 resulted in decreased cortisol metabolism (Li *et al.* 2017). While the 11-oxygenated androgens were not investigated in this study, the decreased HSD11B2 activity likely limits the intratumoral conversion of 11OHA4 and 11OHT into 11KA4 and 11KT, thereby reducing intratumoral 11KT levels. A second study showed that enzalutamide not only affects intratumoral HSD11B2 activity but also suppressed renal HSD11B2 activity in men, leading to changes in the circulating cortisol levels and cortisol/cortisone ratio (Alyamani *et al.* 2020). Again, though not specifically investigated, this will most likely have affected serum 11KT levels as well.

Role of 11-oxygenated androgens in castration-resistance

While intratumoral conversion of precursor steroids is an important mechanism by which CRPC cells become resistant to androgen deprivation, there are several other mechanisms that similarly contribute to castration resistance. To date, there is limited data on how the 11-oxygenated androgens are involved in these other mechanisms.

Androgen receptor overexpression

AR genomic amplification and overexpression are among the most frequently observed adaptations in CRPC patients (Donovan *et al.* 2010, Taylor *et al.* 2010, van Dessel *et al.* 2019). AR overexpression typically sensitizes CRPC cells by increasing the likelihood of AR–ligand interaction, thereby allowing AR pathway activation even at castrate androgen levels. Since the physical interaction between receptor and ligand itself is not altered, AR amplification likely sensitizes CRPC tumours to both classical and 11-oxygenated androgens. The relatively abundant 11-oxygenated androgens are likely contributors to AR activation under these conditions, although this remains to be confirmed in patients.

Androgen receptor mutations

Although AR mutations affecting the LBD are rare in treatment-naïve patients, they are observed much more frequently in CRPC patients (Romanel *et al.* 2015, Lallous *et al.* 2016, Wyatt *et al.* 2016, Snaterse *et al.* 2022). These mutations typically offer one or more selective advantages: (i) the AR becomes highly sensitized to its canonical ligands, (ii) they confer promiscuity, enabling

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activation by non-canonical ligands that are abundant in CRPC patients or (iii) they alter the interaction between the AR and AR inhibitors, which causes antagonists to activate the AR instead. Although various mutations have been detected in CRPC patients, three mutations (p.L702H, p.H875Y, p.T878A) are particularly abundant, with each mutation present in approximately 3–5% of all CRPC patients (Snaterse *et al.* 2022). The presence of AR-LBD mutations is associated with a significantly worse prognosis (Lallous *et al.* 2016, Jernberg *et al.* 2017) and early progression on treatment (Prekovic *et al.* 2016, Conteduca *et al.* 2017).

Two of these common mutations – p.L702H and p.H875Y – drastically alter the interaction between the AR and the 11-oxygenated androgens (Snaterse *et al.* 2022). The p.H875Y mutation confers broad ligand promiscuity, sensitizing the AR to various steroids including androgen precursors, androgen metabolites and progesterone (Snaterse *et al.* 2022). It also lowers the EC50 for 11KT by almost five-fold compared to wildtype from 0.74 nmol/L to 0.15 nmol/L. Similarly, the sensitivity for 11OHT is greatly increased (116 nmol/L to 0.4 nmol/L) (Snaterse *et al.* 2022). In contrast, the p.L702H mutation greatly desensitizes the AR for both classical and 11-oxygenated androgens, resulting in EC50 values for testosterone (3.9 nmol/L) and 11KT (35.8 nmol/L) that are well above the respective circulating concentrations in CRPC patients (Snaterse *et al.* 2022). Instead, AR_{L702H} can be activated by cortisol (EC50 = 29.1 nmol/L) and prednisolone (48 nmol/L) (Snaterse *et al.* 2022) at physiological/pharmacological concentrations (van de Wijngaart *et al.* 2010). This mutant has been detected more frequently in patients receiving prednisone/prednisolone treatment (Carreira *et al.* 2014, Romanel *et al.* 2015). The p.T878A mutant slightly decreased sensitivity for both testosterone and 11KT, while increasing sensitivity for 11OHT. This mutation also confers resistance to many types of antiandrogens (Brinkmann *et al.* 1999, Zhao *et al.* 1999, Lallous *et al.* 2016). Although evidence on the promiscuity of AR mutants is plentiful, it is important to note that the *in vitro* findings still await *in vivo* confirmation. The effect of these mutations on classical and 11-oxygenated androgen sensitivity and the implications for the mechanism of AR pathway activation highlight the importance of considering both steroid levels and AR for the development of personalized treatment strategies.

There are several other mechanisms that can contribute to castration resistance, including the expression of constitutively active AR splice variants (Hörnberg *et al.* 2011, Antonarakis *et al.* 2014), GR (Arora *et al.* 2013, Li *et al.* 2017, Moll *et al.* 2022) mediated resistance and neuroendocrine prostate cancer (Beltran *et al.* 2016, Aggarwal *et al.* 2018). However, these are not ligand- and/or androgen-dependent, and it is unlikely that the 11-oxygenated androgens play an important role in patients affected by these resistance mechanisms.

Future perspectives

Studies on the AR activating potential of the 11-oxygenated androgens consistently show that 11KT and 11KDHT are potent androgens, capable of activating the AR concentrations comparable to testosterone and DHT. 11-oxygenated androgens have been shown to drive the expression of AR target genes and PC cell growth *in vitro* (Storbeck *et al.* 2013, Pretorius *et al.* 2016, Snaterse *et al.* 2022). Still, our current understanding of the role and importance of 11-oxygenated androgens in CRPC is limited. Circulating concentrations in CRPC patients have been reported, but intratumoral concentrations and direct evidence of 11-oxygenated androgen-mediated resistance are still lacking. Determining how and when these steroids exactly contribute to castration-resistance in patients should be one of the main future objectives. Additionally, little is known about the actions of the 11-oxygenated androgens in patients who have not received ADT. In these patients, the testosterone concentration greatly exceeds 11KT, and it is presumed that 11KT is not a major contributor to AR activation. However, lacking formal evidence, it is possible that 11-oxygenated androgens are involved to some extent if intratumoral steroid metabolism is proven to favour the formation of 11KT.

11-oxygenated androgen quantification

While the number of laboratories that measure the 11-oxygenated androgens has grown in recent years, the total number is still low, hampering progress. LC-MS/MS equipment capable of reliably measuring these steroids is becoming more widely available, however, and increased awareness about the proven and potential clinical significance is, therefore, necessary for more widespread adoption. Appropriate internal standards are also necessary to accurately measure the different intermediates and metabolites of the pathway.

While several analytical methods to measure the 11-oxygenated androgens in serum and plasma have now been published, the number of studies describing

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methods for intratissue measurement is still very limited. Intratissue measurements are more challenging in general, but they are essential in order to elucidate the tissue-specific actions of the 11-oxygenated androgens, including in prostate cancer. The need for deeper insight into tissue-specific actions was recently highlighted by Schiffer and colleagues who showed the preferential activation of 11-oxygenated androgens in peripheral blood monocytes (Schiffer *et al.* 2021). Our understanding of the actions of the classical androgens may not directly translate to the 11-oxygenated androgen pathway due to the different ways both pathways are affected by steroidogenic enzymes. The tissue-specific expression of steroidogenic enzymes (SRD5A, AKR1D1, AKR1C3, HSD17B2, UGT-family) may therefore be key determinants of tissue-specific sensitivity to classical and 11-oxygenated androgens.

So far, methods for intratissue quantification of 11-oxygenated androgen have been only described in two studies (du Toit *et al.* 2017, Häkkinen *et al.* 2019). These may serve as a basis for future studies investigating intraprostatic 11-oxygenated androgen concentrations. Additionally, a method for the intratumoral quantification of DHEAS concentrations was recently reported (Mostaghel *et al.* 2021). A more in-depth analysis of tissue steroid profiling methods was provided in a recent review (Šimková *et al.* 2021).

Another key limitation in the analysis of intratumoral 11-oxygenated androgen action is the scarcity of suitable biopsy material. Most CRPC metastases are located in bone or lymph tissues, and these tumours are often small in size and provide little usable material. Circulating tumour cells or circulating tumour DNA (sometimes also referred to as liquid biopsies) can provide insight into tumour genetics but are not well suited to provide insight into intratumoral steroid concentrations.

11-oxygenated androgen bioavailability

Testosterone local bioavailability is regulated through steroidogenic enzyme expression. However, access to circulating testosterone is also regulated by steroid-binding proteins sex-hormone binding globulin (SHBG) and albumin. Together, these proteins bind approximately 98% of the total serum testosterone. The non-protein bound fraction, known as free testosterone, appears to best represent biological activity and local androgen exposure (Mendel 1989), for example, in hypogonadal men (Antonio *et al.* 2016). Similarly, cortisol bioavailability is in part regulated by corticosteroid-binding globulin (CBG) and albumin (Ousova *et al.* 2004, Bae & Kratzsch 2015, Verbeeten & Ahmet 2018). To date, there is no information on the bioavailability of the 11-oxygenated androgens. While these steroids are likely bound to albumin, it is unclear if these steroids also bind to SHBG and/or CBG. As the concentrations of these binding proteins are subject to intra- and interindividual variation, the bioavailability of the 11-oxygenated androgens may be affected. Insight into the binding properties and bioavailability of steroids such as 11OHA4 and 11KT may help us better understand how tissue exposure to these steroids is regulated.

11-oxygenated androgens as potential biomarkers

There is an urgent need for suitable biomarkers to guide the treatment of CRPC. Recently, two meta-analyses have investigated the prognostic value of the circulating testosterone concentration in the PC and CRPC settings (Claps *et al.* 2018, Miura *et al.* 2020). Unsurprisingly, higher testosterone levels during ADT were associated with early progression (Claps *et al.* 2018). However, high testosterone levels in CRPC patients treated with AR-targeting treatment were associated with a *longer* progression-free survival (PFS). This is in line with clinical studies in abiraterone- and enzalutamide-treated patients (Attard *et al.* 2009, Sakamoto *et al.* 2019). Patients with higher serum DHEAS also appear to respond well to AR-targeting treatment (Mostaghel *et al.* 2021). Similarly, high 11KT and total active androgen (testosterone + 11KT + DHT) levels were associated with longer PFS (Snaterse *et al.* 2021*b*). Here, 11KT showed a stronger association with PFS than testosterone, but it should be noted that the sample size of this study was limited.

High androgen levels being associated with longer PFS may seem counterintuitive at first, but there may be a logical (albeit still theoretical) explanation. In the presence of relatively high residual androgens and 11-oxygenated androgens levels, adaptations that confer resistance through ligand-mediated AR pathway activation (AR upregulation, conversion of adrenal precursors) may provide the most selective advantages. These tumours are theoretically still responsive to competitive inhibitors (e.g. enzalutamide) or adrenal suppression (abiraterone). In the absence of adrenal androgens and precursor steroids, androgen-dependent adaptations provide little selective advantage. Instead, androgen-independent mechanisms such as promiscuous AR mutants, AR-V and GR offer a stronger selective advantage. These adaptations have been shown to be less likely to respond to AR-targeted treatment (Antonarakis *et al.* 2014, Jernberg *et al.* 2017).

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Consequently, under low androgen conditions, there is selective pressure for resistance mechanisms associated with poorer outcomes. High circulating androgen and 11-oxygenated androgen concentrations may therefore have prognostic potential as biomarkers used to identify patients who are more likely to respond well to enzalutamide or abiraterone treatment. In patients with very low androgen concentrations an alternative strategy, for example, involving chemotherapy, may be more suitable. It has even been proposed that cycling between castrate and supraphysiological androgen concentrations, known as bipolar androgen therapy, may be beneficial against tumours that have adapted to low androgen conditions (Schweizer *et al.* 2015). It is clear that additional research is necessary in order to determine the prognostic potential of the androgens and 11-oxygenated androgens in the CPRC setting. To our knowledge, there have been no prospective studies yet that use androgen status to guide CRPC treatment.

Optimal treatment strategies

Several large-scale clinical studies have provided insight into ways to combine or sequence CRPC treatments in order to achieve optimal responses. A combination of docetaxel, abiraterone or AR inhibitors with ADT in the first line greatly increases patient survival (Sweeney *et al.* 2015, Fizazi *et al.* 2017, James *et al.* 2017, Chi *et al.* 2019, Davis *et al.* 2019). Additionally, cabazitaxel with prednisone was shown to be a more effective treatment option in subsequent lines of treatment, as AR-targeting treatments develop cross-resistance (van Soest *et al.* 2013, de Wit *et al.* 2019). As a result of these novel developments, the landscape of CRPC treatment continues to evolve. Yet, despite these advances and despite increased insight into resistance mechanisms, true personalized treatment approaches have not yet been developed.

Based on our current understanding of resistance mechanisms and the actions of circulating steroid hormones, there certainly is potential. Future personalized approaches may use high serum 11KT, testosterone or precursor concentrations to help identify patients that are most likely to respond to AR-targeted therapies. High AKR1C3 expression may be indicative of increased intratumoral 11KT production and a reliance on adrenal precursors, implying the need for inhibition of adrenal steroidogenesis. The presence of AR mutants, AR-Vs or GR expression may instead indicate that AR-targeting therapies are likely to fail and that chemotherapy may be the preferred treatment modality. Liquid biopsies may become a key tool in guiding treatment strategies, as they can be used to gain insight into both tumour genomics, epigenomics and transcriptomics (van Dessel *et al.* 2020), while also being suitable for steroid hormone measurement (Snaterse *et al.* 2021*a*).

Conclusion

In conclusion, 11KT has been identified as the predominant active androgen in CRPC patients, and 11-oxygenated androgen precursors persist after ADT. There is strong and consistent evidence supporting the androgenic potential of 11KT. *In vitro* data also suggest that 11-oxygenated androgens are converted in 11KT by (CR)PC cells, and 11KT should accumulate intratumorally. The 11-oxygenated androgens may therefore be important in AR-mediated castration resistance. However, *in vivo* evidence to support these findings remains scarce, as few studies have investigated this in CPRC tumour tissue. Determining the intratumoral concentrations and precise actions of the 11-oxygenated androgens and how these actions are regulated should be a main goal for future studies. Finally, the potential of androgen status as a biomarker for risk of progression and AR-targeting treatment selection is worth exploring.

Declaration of interest
The author has no interest to declare

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