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New Hormonal Therapies for Castration Resistant Prostate Cancer

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Introduction

Androgen deprivation therapy (ADT) remains the primary treatment modality for patients with metastatic prostate cancer, but is uniformly marked by progression to castration-resistant disease over a period of 18 to 20 months with an ensuing median survival of 1 to 2 years. The development of castration-resistant prostate cancer (CRPC) has been the subject of intensive investigation, and continued activation of androgen receptor (AR) signaling despite castration appears to remain a critical driving force in tumor progression.¹ Accumulating data emphasize that ‘androgen independent’ or ‘hormone refractory’ tumors retain a clinically relevant degree of hormone sensitivity and highlight the continued importance of AR axis activation in advanced tumors.² Accordingly, therapeutic strategies designed to more effectively ablate tumoral androgen activity are required to improve clinical efficacy and prevent disease progression. This paper reviews AR-dependent mechanisms underlying CRPC progression and the status of novel hormonal therapies targeting the AR axis that are currently in clinical and pre-clinical development.

Ligand-dependent mechanisms mediating AR transactivation in CRPC

Recent evidence suggests that castration resistant tumors are not, in fact, androgen-independent but develop in a setting of continued AR-mediated signaling potentiated by residual tumoral androgens. We and others have demonstrated that castration resistant tumors are characterized by elevated tumor androgens and by steroid enzyme alterations which may potentiate *de novo* androgen synthesis or the utilization of circulating adrenal androgens.^{3–5} These observations suggest that tissue based alterations in steroid metabolism facilitate the development of CRPC and underscore these metabolic pathways as critical targets of therapy.

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1. Persistence of intratumoral androgens despite castration

The efficacy of ADT is routinely based on achieving castrate levels of serum testosterone (T) defined as $T \leq 20$ ng/dl. However, tissue androgen measurements in men with either locally recurrent or metastatic CRPC clearly demonstrate that castration does not eliminate androgens from the prostate tumor microenvironment, and that residual androgen levels are well within the range capable of mediating continued AR signaling and AR-mediated gene expression.^{6–9} Compared to untreated tissue, prostatic dihydrotestosterone (DHT) levels were decreased approximately 80% in castrate patients with locally recurrent prostate cancer, while T levels were actually equivalent to prostate tissue from untreated men.⁷ Moreover, we have demonstrated that T levels in metastatic tumors obtained via rapid autopsy from men with CRPC are approximately 3-fold higher than levels within primary prostate tumors from untreated (eugonadal) patients.⁵

Adrenal androgens have also been detected at significant levels in the prostate tissue of castrate men. Prostatic levels of dehydroepiandrosterone (DHEA), dehydroepiandrosterone-sulfate (DHEA-S), and **androstenedione (AED)** were decreased by about 50% in castrate patients with recurrent prostate cancer and far exceeded the values of testosterone and DHT in the recurrent tumor tissue.⁷ A separate study found no decrease in prostatic levels of 5-androstenediol (a primary metabolite of DHEA and a direct precursor of testosterone, Figure 1) after castration,¹⁰ which is of particular significance as this androgen has been shown to bind the wild type AR without being inhibited by flutamide or bicalutamide.¹¹

2. Source of intratumoral androgens despite castration

Alterations in key enzymes involved in sterol bio-synthesis and androgen metabolism have been identified in prostate tumor samples from castrate patients, and the dependence of CRPC on intratumoral androgen metabolism has been modeled *in vitro* and *in vivo*.^{12–14} These data suggest the uptake and conversion of adrenal androgens as well as the *de novo* synthesis of androgens (from progestins, cholesterol, or earlier precursors) may contribute to the residual tissue androgens present after castration.

In the classical pathway of androgen synthesis, C21 steroids generated from cholesterol such as pregnenolone and progesterone are first converted to the C19 steroids DHEA and AED via the sequential hydroxylase and lyase activity of CYP17A. These adrenal steroids are then acted on by HSD3B, HSD17B3, and SRD5A to generate T and DHT. In steroidogenic tissues in which both CYP17A and SRD5A are expressed, an alternate route to DHT is possible wherein C21 steroids are first acted upon by HSD3B and SRD5A followed by CYP17A and HSD17B3.¹⁵ This ‘backdoor pathway,’ wherein steroid flux to DHT bypasses the conventional intermediates of AED and testosterone, has also been postulated to be operative in prostate tumors, Figure 1.¹⁴

The enhanced expression of transcripts encoding key enzymes in the cholesterol biosynthetic pathway has been demonstrated in castration resistant prostate tumors, including expression of squalene epoxidase (SQLE), the rate-limiting enzyme in cholesterol synthesis.³ We and others have reported the altered expression of genes encoding many steroidogenic enzymes including the upregulated expression of FASN, CYP17A1, HSD3B1, HSD17B3, CYP19A1, and UBT2B17 in CRPC metastases, suggesting that castration resistant tumors have the ability to utilize progesterone as androgenic precursors.^{4, 5} Differential expression of several 17 β -hydroxysteroid dehydrogenase family members (HSD17B) has been observed in prostate cancer, suggesting a shift in tumoral androgen metabolism towards the formation of testosterone and DHT with increased expression of reductive enzymes catalyzing conversion to active androgens (HSD17B3 and HSD17B5—also known as aldo-keto reductase AKR1C3), and decreased expression of oxidative

enzymes catalyzing the reverse reaction (HSD17B2) (reviewed in¹⁶). Differential expression of AKR1C1 and AKR1C2, which mediate the catabolism of DHT, has also been observed in prostate tumors, including a selective loss of AKR1C2 and AKR1C1 in primary prostate tumors accompanied by a reduced capacity to catabolize DHT and an increased level of tumoral DHT.

Thus, alterations in a number of critical enzymes responsible for DHT synthesis and catabolism provide mechanistic support for the role of intracrine androgen production in maintaining the tumor androgen microenvironment in CRPC and underscore these metabolic pathways as critical therapeutic targets.

AR-based alterations mediating AR transactivation in CRPC

Numerous molecular alterations mediating AR signaling at low or absent androgen levels have been described in CRPC, demonstrating that AR activation in CRPC may occur via both ligand-dependent and independent mechanisms. Here we review AR-based alterations that promote CRPC progression by facilitating AR transactivation at low or absent androgen levels.

1. Overexpression/amplification of wild type AR

AR overexpression is a well-recognized feature of CRPC and believed to be a critical driver of CRPC progression. In preclinical prostate cancer models, Chen et al identified AR as the most common gene up-regulated following androgen deprivation. AR overexpression supported *in vitro* proliferation of transfected cells at 5 fold lower androgen levels than untransfected cells, and it was both necessary and sufficient to induce tumor formation when placed in castrate SCID mice compared to un-transfected controls.¹ Importantly, AR overexpression not only mediated sensitivity to low ligand concentrations but converted anti-androgens such as bicalutamide and flutamide from antagonists to agonists via alteration of co-activators recruited to the AR promoter. While rarely identified in primary prostate tumors, AR gene amplification leading to AR overexpression is present in approximately 30% of clinical CRPC specimens.¹⁷ Additional mechanisms that mediate increased AR transcription and/or AR stability are likely operative as increased AR expression is frequently observed in the absence of AR amplification. Recent data suggest that dimerization of AR with ligand-independent AR splice variants (discussed below) may increase AR levels by preventing AR protein degradation.¹⁸

2. AR mutations

Mutations in the AR have been identified following castration or ADT in approximately 20–40% of CRPC tumors and are rarely found in hormone naïve prostate tumors.¹⁹ Multiple mutations are frequently isolated from the same tumor, demonstrating the high degree of heterogeneity present in prostate cancers.²⁰ Several hundred AR mutations have been described following androgen ablation, but >90% are nonsense or missense in nature and result in a non-functional AR. A number of important AR mutations occur in the ligand binding domain (LDB) and it is notable that none have been identified in this region in the absence of castration. The most common mutation occurs at or around amino acid 877. The Thr877Ala mutation was originally described in the LNCaP human prostate cancer cell line and permits binding of an expanded repertoire of steroid ligands such as progestins and estradiol as well as the anti-androgen flutamide, converting its activity as an anti-androgen to an agonist.²¹ Gain of function mutations also occur in both the N- and C- termini, which can alter the N/C interaction involved in cofactor recruitment. Although AR mutations are associated with castration resistance, none of them occur with a frequency that would suggest they are responsible for the development of castration resistance. However, the

potential agonist activity of steroidal anti-androgens in the setting of AR amplification and/or AR mutation has spurred the development of AR antagonists without agonist properties.

3. Alterations in AR co-regulators

Literally several hundred AR co-regulators have been described which influence AR activation via multiple mechanisms, including recruitment of transcriptional machinery, modulation of chromatin remodeling enzymes, and initiation of RNA polymerase activity.²² A number of AR co-activators are increased in CRPC including TIF-1, MAGE-II, SRB-1, NFkB, and ARA70, while co-repressors such as SMRT are down-regulated. Whether alterations in the balance between AR and its co-regulators can activate AR in the absence of ligand in CRPC is not clear. However, altered co-regulator expression may sensitize the AR for activation under low androgen conditions, as well as converting AR antagonists into agonists via co-repressor down-regulation and/or co-repressor dismissal from the AR complex.²³ Inhibition of AR co-regulators has been proposed as a target for suppressing AR activity in CRPC.²⁴

4. Activation of AR by peptide ligands

Several studies have demonstrated that peptide molecules may transactivate the AR in the absence of ligand, including insulin like growth factors (IGF-I and -II), epidermal growth factor (EGF), keratinocyte growth factor (KGF), and cytokines such as interleukin 6 (IL-6) (reviewed in²⁵). The impact of these factors *in vivo* is not known, although we have shown that inhibition of IGF-IR by the IGF-IR inhibitory antibody A12 affects AR translocation and transactivation *in vivo*.²⁶ Probably the most convincing of these potential AR peptide ligands is IL-6, which has been shown to bind the LBD and transactivate the AR as determined by ARE-luciferase reporter constructs or by increased expression of androgen-regulated genes. Additionally, nuclear translocation of AR by IL-6 has also been described. However, the clinical relevance of IL-6 in prostate cancer is not clear. While IL-6 is significantly elevated in the serum and bone metastases of patients with advanced prostate cancer, a recent clinical trial in which men with CRPC were treated with an IL-6 inhibitory antibody showed no evidence of benefit.²⁷

5. AR splice variants

We and others have recently described the generation of post-transcriptional AR splice variants following castration. Approximately 25 variants have been identified in human tissues and human prostate cell lines.^{18, 28–32} Some of these variants have no function while others appear to enhance the effect of the full-length wild-type receptor. Most significant among these splice variants are those in which the AR ligand binding domain (LBD) is lost, resulting in ligand-independent constitutive AR activation (Figure 2). Mechanisms responsible for generation of the AR splice rearrangements have not been determined, but the consistency with which ARV7/AR3 and AR^{v567es} are found in human tumors from castrate men suggests that production of these variants may occur in response to castration, and it is notable that the variants most prevalent in human tissues are those most consistently found following androgen deprivation *in vitro*. We have found that the generation of AR^{v567es} is especially sensitive to suppression of intratumoral androgens,^{18, 32} suggesting growth of these tumors is dependent on AR variants in the presence of castrate levels of androgen.

While AR variants lacking the LBD are transcriptionally active when placed in AR negative cells lines, the expression of AR variants has generally been reported to occur in conjunction with ongoing expression of full length AR. Interestingly, Sun et al have demonstrated that in the absence of ligand the AR^{v567es} AR variant forms a heterodimer with full length AR, causing it to be translocated to the nucleus, and that in the presence of ligand AR^{v567es}

enhances the activity of wtAR. These data suggest that AR variants can activate an AR transcriptional program by themselves, but that they can also potentiate the activity of full-length AR under low ligand conditions by essentially functioning as AR ligands themselves. In this respect, Watson et al recently demonstrated that in the presence of both truncated and full length AR variants, targeting the full length AR with the anti-androgen MDV3100 suppressed AR activity and cell growth as efficiently as when only full length AR was present, suggesting activity of the AR splice variants is mediated through the full-length AR.³²

Novel agents targeting intra-tumoral androgens

Since many castration resistant tumors are not truly androgen-independent, therapeutic strategies that more effectively target residual tumoral androgens are necessary. Androgen metabolism within primary prostate tumors is characterized by steroid enzyme alterations, which may potentiate the intracrine conversion of adrenal androgens to T and DHT and/or inhibit the conversion of DHT to inactive metabolites. Enzymes mediating the metabolism of adrenal androgens to T, DHT and subsequent metabolites are also increased in studies of castration resistant metastatic tumors, further enhancing the potential utilization of adrenal androgens in the castrate microenvironment.^{4, 5} These observations suggest that tissue based alterations in steroid metabolism may facilitate prostatic tumor development and underscore these metabolic pathways as critical therapeutic targets.

CYP17A inhibitors

CYP17A is a single enzyme that catalyzes sequential steps in the conversion of C21 progesterone precursors to the C19 adrenal androgens, DHEA and AED. While ketoconazole (a weak inhibitor of CYP11A and CYP17A) has been utilized for suppression of residual adrenal androgens in men with CRPC, limited efficacy and significant treatment related side effects have prompted the development of more potent inhibitors of androgen biosynthesis. A number of CYP17A inhibitors have been reported, including a series of novel agents exhibiting both CYP17A inhibition and anti-androgen activity.³³

1. Abiraterone—Abiraterone, a pregnenolone derivative, is a selective irreversible inhibitor of both the 17 α -hydroxylase and C17,20-lyase activity of CYP17, and has shown promising efficacy in reducing circulating androgen levels and achieving PSA responses in men with CRPC. In eugonadal men, abiraterone suppressed testosterone levels by >50% accompanied by a corresponding rise in luteinizing hormone (LH) levels, while in castrate men, abiraterone further suppressed serum testosterone levels by >75%.³⁴ Phase I/II studies have demonstrated PSA responses and clinical activity in chemotherapy naïve and post-docetaxel treated CRPC patients (including patients who have progressed on ketoconazole) and Phase III studies in these areas are underway.

In men with chemotherapy naïve metastatic CRPC, Phase I/II studies have demonstrated durable PSA declines $\geq 50\%$ in approximately two-thirds of patients treated with abiraterone, with partial radiographic responses (by RECIST criteria) in 37.5% and a median time to progression of 32 weeks.^{35, 36} PSA responses $\geq 50\%$ were observed in 47% of patients with prior ketoconazole treatment compared to 64% of patients without.³⁷ Abiraterone generally suppressed DHEA levels by approximately 75% and suppressed DHEA-S, AED, and testosterone levels to essentially undetectable.^{36, 37} As observed in studies of ketoconazole, patients achieving $\geq 50\%$ PSA declines had higher baseline levels of DHEA-S, DHEA, and AED and, in contrast to progression on ketoconazole, increases in testosterone, AED, or DHEA levels were not observed on progression with abiraterone.^{35, 38}

In a Phase II study of post-docetaxel treated CRPC patients, PSA declines $\geq 50\%$ were observed in 51% of patients treated with abiraterone alone, with a median time to progression of 24 weeks.³⁹ In a second study of abiraterone in the post chemotherapy setting, in which 41% of patients had received prior ketoconazole, abiraterone (in combination with prednisone, 5mg twice daily) achieved PSA declines $\geq 50\%$ in 45% of ketoconazole-naïve patients and 26% of ketoconazole treated patients, with a median time to progression of 28 and 14 weeks respectively.⁴⁰ These findings, together with those in the chemotherapy naïve setting, demonstrate that abiraterone maintains a reasonable degree of clinical efficacy in CRPC patients previously treated with docetaxel and/or ketoconazole. Two Phase III randomized, placebo-controlled studies of abiraterone in combination with prednisone versus prednisone alone have been ongoing, one in the chemotherapy naïve setting and one in CRPC patients after docetaxel treatment, both with a primary endpoint of overall survival. Early results of the Phase III COU-AA-301 study in the post-chemotherapy setting were recently released, demonstrating an overall survival of 14.8 months compared to 10.9 months in the placebo-treated group (hazard ratio 0.646, $p < 0.0001$), representing a 35% reduction in risk of death with abiraterone treatment.⁴¹

Abiraterone was associated with expected increases in the C21 steroids upstream of CYP17A (including a 10 fold increase in deoxycorticosterone and 40 fold increase in corticosterone) accounting for the treatment related side effects. These were generally mild and primarily related to symptoms of mineralocorticoid excess (including grade 1 and 2 hypertension, hypokalemia, edema, and fatigue) and responded to treatment with eplerenone or low dose glucocorticoids (spironolactone should be avoided as it may act as an AR agonist). Decreases in serum cortisol (2 fold) with concomitant elevations in ACTH (5 fold) were also observed. Interestingly, 4 of 15 patients progressing on abiraterone responded to the addition of dexamethasone, which decreased ACTH and deoxycorticosterone levels to below baseline,³⁶ consistent with prior reports that steroids upstream of CYP17, including progestins and corticosteroids, can stimulate the AR. At present, use of abiraterone in combination with low dose prednisone or dexamethasone is recommended to prevent the treatment related rise in ACTH and attendant side effects.

2. Tak-700—TAK-700 (Millenium, Takeda Oncology Company) is a novel non-steroidal CYP17 inhibitor with high selectivity against the C17,20-lyase over 17-alpha hydroxylase activity of CYP17. In a Phase I/II dose-escalation study in men with metastatic CRPC, 11 of 20 patient receiving ≥ 300 mg twice daily showed PSA declines $> 50\%$ and 4 had reductions $> 90\%$. Median testosterone and DHEA-S levels at 4 weeks decreased from 4.9 to 0.6 ng/dL and 53.8 ug/dl to undetectable, respectively. The most common adverse effects included fatigue, nausea, constipation, and anorexia, without a significant incidence of hypertension, consistent with the agent's selective inhibition of 17,20-lyase over 17 alpha-hydroxylase activity.⁴² The Phase II portion, including an arm evaluating the concomitant use of prednisone, is ongoing.

3. VN-124—VN/124-1, a novel heteroaryl steroid, is a potent CYP17 and AR inhibitor currently being evaluated in a Phase I/II study under the trade name TOK-001 (Tokai Pharmaceuticals). VN/124-1 exhibits 3 and 4 fold stronger inhibition of CYP17 activity than abiraterone and ketoconazole, respectively, and is also a potent inhibitor of the AR, both as a competitive antagonist (with a binding affinity comparable to bicalutamide) and as a dose-dependent inhibitor of AR protein expression, mediated in part via an increase in AR degradation.⁴³ Notably, VN/124-1 has similar AR inhibitory activity against the T877A AR mutant. In preclinical studies VN/124-1 was significantly more effective than castration or bicalutamide in suppressing growth of the androgen sensitive LAPC4 xenograft. Moreover, VN/124-1 maintained its potent down-regulation of the AR *in vivo*, leading to a 10 fold reduction in tumor AR levels compared to castration or bicalutamide treated tumors (both of

which demonstrated a 2–3 fold increase in AR expression). Interestingly, this agent had also been demonstrated to inhibit the growth of AR negative prostate cancer cells via induction of the endoplasmic reticulum stress response.⁴⁴ Phase I studies evaluating this agent in CRPC are in development.

Other steroidogenic enzyme inhibitors

1. Inhibitors of SRD5A—SRD5A1 and SRD5A2 catalyze the intraprostatic conversion of testosterone to the more potent androgen DHT. SRD5A2 is the primary isoform expressed in benign prostate tissue, while a relative increase in SRD5A1 expression and activity is observed in prostate cancer. Finasteride (a specific inhibitor of SRD5A2) and dutasteride (a dual SRD5A inhibitor) are 4-azasteroids extensively used in the treatment of BPH and have been explored for the prevention and treatment of prostate cancer. While dutasteride alone has limited activity in men with CRPC, a Phase II study of ketoconazole, hydrocortisone, and dutasteride (KHAD) demonstrated PSA responses $\geq 50\%$ in 56% of men and a median time to progression of 14.5 months, nearly twice that observed in the Phase II studies of abiraterone and leading the authors to postulate that intratumoral DHT synthesis may contribute to abiraterone resistance.⁴⁵

2. Inhibitors of AKR1C3 and HSD17B3—HSD17B3 and AKR1C3 catalyze the final steps in testosterone and DHT biosynthesis: the reduction of the adrenal androgens, AED and androstenedione, to testosterone and DHT. HSD17B3 is primarily expressed in Leydig cells, while AKR1C3 mediates production of testosterone and DHT in peripheral tissues. Several studies have demonstrated increased expression of these enzymes in castration resistant prostate tumors, suggesting they may be important targets for inhibition.^{4, 5, 46} Conversely, the AKR1C family members AKR1C1 and AKR1C2 mediate the catabolism of DHT (to 3β and 3α -diol, respectively), and a selective loss of these enzymes (accompanied by a reduced capacity to metabolize DHT and an increase in tumoral DHT levels) has been reported in prostate tumors.⁴⁷ Therefore, agents which selectively target AKR1C3, but not the highly related AKR1C1 and AKR1C2, are under development

AKR1C family members are inhibited by non-steroidal anti-inflammatory drugs (NSAIDs) and the COX-2 selective inhibitor, celecoxib.⁴⁹ Indole acetic acids (e.g., indomethacin) are among the most potent agents targeting the AKR1C family, and indomethacin analogs that selectively target AKR1C3, but do not inhibit COX-1, COX-2, AKR1C1 and AKR1C2 have been reported.⁵⁰ Small molecule inhibitors of HSD17B3 have been developed and tested using *in vitro* and *in vivo* systems and have been shown to reduce systemic androgen levels (reviewed in⁴⁸). However, to date no studies in prostate cancer have been reported.

3. Inhibitors of 3BHSD—3BHSD mediates conversion of delta4 steroids such as DHEA and androstenediol to the delta5 steroids AED and testosterone, respectively. The activity of 3BHSD is required for the *de novo* biosynthesis of androgens from cholesterol (via either the classical or backdoor pathway) as well as for pathways converting adrenal androgens to T and DHT. The type 1 isoform is expressed in the adrenal, ovary, and testis, and type 2 in peripheral tissues such as the prostate, although transcripts encoding both isoforms have been observed in CRPC metastases. While several studies have demonstrated that DHEA or androstenediol can directly activate wild type and mutated AR,¹⁰ others have clearly demonstrated a requirement for 3BHSD mediated conversion to downstream metabolites first,⁵¹ implicating 3BHSD as a therapeutic target for CRPC. Epostane, a competitive inhibitor of 3BHSD1, has already been used in human studies for the medical termination of pregnancy via inhibition of progesterone synthesis and has been shown to inhibit the DHEA-induced proliferation of breast cancer MCF-7 cells,⁵² suggesting its study in prostate cancer may be warranted.

4. Inhibitors of steroid sulfatase—Steroid sulfatase (STS) is the enzyme responsible for hydrolyzing inactive sulfates of estrogen and DHEA to biologically active steroids. Prostate cancer cell lines have demonstrated both STS expression and activity, as demonstrated by hydrolysis of estrone-S and DHEA-S to unconjugated forms, and immunohistochemical expression of STS in prostatic tumors has been reported (reviewed in¹⁶). Although not yet tested in prostate cancer, STS inhibitors have been evaluated in patients with breast cancer and may have efficacy in preventing prostatic utilization of the adrenal androgen DHEA, which primarily circulates as the inactive sulfate, DHEA-S. Given the proposed importance of adrenal androgens in maintaining prostate tumor androgen metabolism in anorchid patients, the clinical efficacy of preventing tumoral DHEA-S utilization by STS inhibitors warrants examination.

5. Apoptone—Apoptone (HE3235, Hollis Eden Pharmaceuticals) is a synthetic analog of 3-beta androstanediol, a naturally occurring metabolite of DHT formed in prostate tissue, that has been shown to suppress tumor growth, decrease AR expression and nuclear localization, and suppress levels of intratumoral androgens in castration resistant prostate cancer xenografts.^{53, 54} Although its mechanism of activity has not been fully elucidated, HE3235 appears to inhibit conversion of d-cholesterol to d-pregnenolone, without inhibition of CYP17. HE3235 is currently under study in a Phase I/II clinical trial of men with CRPC, with early results demonstrating promising PSA responses.⁵⁵

6. LH and LHRH antagonists—Although GnRH analogues are used to achieve medical castration via suppression of the pituitary-testicular axis, variants of the GnRH and LHRH receptor are also present in prostate epithelium.⁵⁶ GnRH antagonist therapy may have direct anti-tumorigenic effects,⁵⁷ and LHRH receptors on prostate tumors may serve as targets for LHRH analogs hybridized to cytotoxic moieties.⁵⁸ A cytotoxic analog of LHRH conjugated to doxorubicin has been clinically tested in women with gynecologic tumors expressing LHRH receptors,⁵⁹ and studies in prostate cancer may be of interest.

Interestingly, receptors for LH itself have also been described in prostate cancer specimens. A recent study demonstrated that exposure of both androgen-sensitive (LNCaP) and androgen-independent (22RV1 and C4-2B) prostate cancer cell lines to LH increased the protein expression of steroidogenic enzymes including STAR, CYB5B, CYP11A, and 3BHSD, and a 2.5 fold increase in progesterone synthesis was observed in LH treated C4-2B cells compared to controls.⁶⁰ These data suggest that LH may have a role in the regulation of steroid biosynthesis in prostate cancer cells and identify the LH receptor as a potential therapeutic target.

Novel agents targeting the AR and downstream AR signaling

The AR, a member of the nuclear receptor superfamily, is a ligand-dependent transcription factor with a four-domain structure consisting of an N-terminal activation function domain involved in co-activator binding (AF-1), a central DNA binding domain, a hinge region with a nuclear localization signal, and a C-terminal ligand binding domain involved in receptor dimerization as well as ligand-dependent co-activator binding (AF-2). In the absence of ligand, the AR is bound to cytoplasmic chaperones or heat shock proteins (such as Hsp-90) which maintain receptor conformation and prevent degradation. Ligand binding results in conformational activation, Hsp-90 dissociation, receptor dimerization, phosphorylation, and nuclear translocation. The dimerized receptor binds to target DNA sequences known as androgen response elements (ARE's) in promotor and enhancer regions of androgen-responsive genes, with subsequent recruitment of coregulator proteins that dictate activation or repression of gene expression.

Anti-Androgens

AR agonists elicit a structural conformation in the AF-2 domain promoting N-terminal/C-terminal interactions that stabilize the AR in the agonist conformation, as well as mediating recruitment of co-activator complexes and enhancing stable DNA association. In contrast, AR antagonists prevent the AR from achieving the transcriptionally active conformation required for stable DNA binding. Different antagonists can engage and modulate the AR by distinct mechanisms, including inhibition of chaperone dissociation, alterations in subcellular AR localization, recruitment of nuclear corepressor complexes, and ineffective recruitment of co-activator proteins (reviewed in ⁶¹). Several mechanisms by which non-steroidal anti-androgens function as AR agonists have been described, including AR mutations and/or alterations in co-factor recruitment. This has been a critical impetus in the development of novel, more potent AR inhibitors without agonist activity against either wild-type or mutant ARs.

1. MDV3100—MDV3100 is a second generation, diarylthiohydantoin competitive AR antagonist which binds to the AR with 5–8 fold greater affinity than bicalutamide and only 2–3 fold lower affinity than DHT. Preclinical studies have demonstrated that, compared to bicalutamide, MDV3100 potently decreased the nuclear translocation of AR, markedly reduces chromatin occupancy at canonical ARE's, and is significantly more effective in suppressing the growth of LNCaP tumors.⁶² Importantly, MDV3100 did not elicit agonist activity against LNCaP tumors overexpressing the AR, or against the T877A or W741C AR mutations, situations in which bicalutamide demonstrates agonist activity. Of note, treatment with androgen was able to overcome the AR inhibitory effects of MDV3100 *in vitro*, suggesting this agent will not have efficacy in the eugonadal setting.

Clinical evaluation of MDV3100 in a Phase I/II study of 140 men with CRPC has recently been reported. MDV3100 led to >50% PSA declines in 62% of chemo-therapy naïve patients and 51% of CRPC patients previously treated with docetaxel, with a median time to PSA progression of 41 and 21 weeks, respectively.⁶³ Two thirds of patients had partial remissions or stable disease in soft-tissue and bone metastases and all patients who were assessed by [18F]-FDHT PET scans showed declines in FDHT accumulation. Thirty-seven percent of patients with prior ketoconazole treatment achieved a PSA decline >50% vs. 71% of those without. The most common adverse events were fatigue, nausea, dyspnea, anorexia, and back pain. A Phase III, randomized placebo-controlled trial of MDV3100 in docetaxel-treated men with metastatic CRPC is currently ongoing.

While pre-clinical studies show that MDV3100 is highly effective in tumors driven by an amplified AR, the observation that ligand can overcome the effect of MDV3100 raises a question as to whether MDV3100 will be equally effective in the setting of a non-amplified AR, particularly if residual tumor androgens are present. AR is amplified in about 20–25% of CRPC cases,¹⁷ although AR amplification has been demonstrated in up to 50% of CRPC cases when circulating tumor cells (CTC) are evaluated,⁶⁴ and these may represent cases in which MDV3100 will have most efficacy.

2. N terminal AR inhibitors—An alternative approach to targeting the AR LBD is the development of N terminal domain (NTD) AR inhibitors, as the NTD is essential for both ligand dependent and independent AR activation. Agents such as MDV3100 or non-steroidal anti-androgens do not inhibit ligand independent transactivation of the AR NTD (mediated by bypass mechanisms such as IL-6 and other peptide growth factors), nor do they directly target constitutively active AR splice variants lacking the LBD. At present the most promising compound that has been published is EPI-001, which is a degradation product of bisphenyl-A and was found by testing a library of products isolated from marine

sponges.⁶⁵ EPI-001 binds to the amino terminus of the AR and inhibits AR transactivation. EPI-001 does not alter AR nuclear translocation or prevent ligand binding, but disrupts the AR N/C interaction thereby inhibiting co-factor recruitment. When given to castrate mice, EPI-001 decreased the size of human prostate cancer xenografts. No apparent toxicity has been noted in the animals and it has 85% bioavailability after oral administration. Combined use with LBD and ligand targeting agents would be of potential benefit by more robustly knocking-out AR activity.

Modulators of AR expression, stability, and downstream signaling

A number of agents under investigation for prostate cancer therapy do not target the AR directly, but alter cellular pathways involved in maintaining the expression, stability, and downstream signaling components of the AR axis. While many of these agents are still in development, several have been tested in clinical studies of men with CRPC. The most studied pathways include the heat shock protein (HSP) chaperones, histone deacetylases (HDACs), and mammalian target of rapamycin (mTOR).

1. HSP inhibitors—HSP90 is an ATP-dependent chaperone protein involved in maintaining the stability, localization, and activity of the AR as well as other oncogenic client proteins such as Her2 and AKT. The ansamycin antibiotic, geldenamycin, binds the ATP-binding pocket of HSP90 leading to the degradation of client proteins. In pre-clinical studies, tanespimycin (17-AA-geldenamycin) inhibited the growth of AR positive prostate cancer xenografts accompanied by an 80% decrease in AR expression.⁶⁶ Phase I studies have not shown significant clinical activity in men with CRPC, and agents with improved solubility characteristics are currently being evaluated.

2. Histone deacetylase inhibitors—Chromatin remodeling determined by the balance of histone acetylation versus deacetylation regulates the transcriptional activity of numerous genes involved in cell survival and differentiation. HDAC inhibitors have been shown to decrease transcription of the AR, to inhibit AR-mediated transcription (by blocking recruitment of RNA polymerase to the promoter of HDAC dependent AR-target genes), and to promote AR degradation (via acetylation induced inhibition of HSP90 ATP-binding).⁶⁷ The HDAC inhibitor voronistat (SAHA) has shown synergistic activity in suppressing prostate cancer cell proliferation *in vitro* when combined with the AR antagonist bicalutamide.⁶⁸ A Phase I study of voronistat in combination with docetaxel and a Phase II study of the single agent voronistat in the post-chemotherapy setting were both associated with minimal clinical response and significant dose limiting toxicity, suggesting alternative agents in this class with a more favorable toxicity profile will be required to test the therapeutic efficacy of this approach. A Phase I/II study of panobinostat (LBH589) in combination with bicalutamide in men with CRPC is ongoing.

3. mTOR inhibitors—The PI3K/Akt/mTOR pathway is central to a number of signaling cascades mediating cell growth and survival, and alterations in this pathway (including loss or mutation of the negative regulator PTEN) are present in 30–50% of prostate tumors. Moreover, the Akt/mTOR pathway can activate AR in the absence of androgen. Many agents targeting PI3K, Akt, and mTOR have been evaluated in both *in vitro* and *in vivo* models of prostate cancer. Clinically, the mTOR inhibitors rapamycin and its analogs everolimus (RAD-001) and temsirolimus (CCI-779) have shown the most promise and multiple Phase I and II clinical trials with these agents are currently ongoing.⁶⁹

4. Src-kinase inhibitors—Src-kinases are non-receptor protein tyrosine kinases involved in signal transduction downstream of multiple cell surface receptors including EGFR, PDGFR, and VEGFR, and have been implicated in the androgen-induced proliferation of

CRPC cells. Dasatinib, a dual Abl and Src family kinase inhibitor, has been shown to inhibit AR phosphorylation and activation *in vitro*,⁷⁰ as well as targeting osteoclast and osteoblast activity⁷¹ and has recently been evaluated in a phase II study of chemotherapy naïve men with CRPC. Although progression occurred in 60% and 80% of patients at 12 and 24 weeks respectively, nearly half the patients showed a decrease in markers of bone metabolism.⁷² A randomized Phase III study of dasatinib in combination with docetaxel (with skeletal-related events as one end point) is ongoing.

Conclusions

Hormonal therapy remains the primary treatment modality for patients with advanced prostate cancer and is associated with significant anti-tumoral efficacy and clinical benefit. However, many studies demonstrate heterogeneity in the pathologic response to ADT and the ultimate failure of androgen suppression to control disease progression is well recognized. Data regarding the molecular response of prostate cancer to hormone therapy continues to emerge, providing critical insight into cellular growth and signaling pathways that may be exploited as therapeutic targets. The presence of residual androgens and persistent activation of the AR signaling axis in CRPC suggest that a multi-targeted treatment approach to ablate all contributions to AR signaling within the prostate tumor will be required for optimal anti-tumor efficacy. Such a strategy would include targeting all potential contributors to the prostatic androgen milieu, including testicular, adrenal, and intratumoral sources, as well as novel agents targeting the stability, degradation, and activity of the AR. While historic studies of combined androgen blockade have yielded small gains in overall survival compared to ADT alone, the introduction of potent steroidogenic inhibitors such as abiraterone and the dual CYP17A/AR inhibitor VN/124-1, in combination with novel AR inhibitors such as MDV3100 hold significant promise for improving the treatment of men with CRPC.

Synopsis

Continued activation of the androgen receptor (AR) axis despite castration remains a critical force in the development of castration resistant prostate cancer (CRPC). Therapeutic strategies designed to more effectively ablate tumoral androgen activity are required to improve clinical efficacy and prevent disease progression. Tumor based alterations in expression and activity of the AR and in steroidogenic pathways mediating ligand generation facilitate the development of CRPC. This paper reviews AR and ligand dependent mechanisms underlying CRPC progression and the status of novel hormonal therapies targeting the AR axis that are currently in clinical and preclinical development. The introduction of potent steroidogenic inhibitors such as abiraterone and the dual CYP17A/AR inhibitor VN/124-1, in combination with novel AR inhibitors such as MDV3100 hold significant promise for improving the treatment of men with CRPC.

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Figure 1. The classical and backdoor pathways of androgen biosynthesis

In the classical pathway (solid gray arrow), C21 precursors (pregnenolone and progesterone) are converted to the C19 adrenal androgens DHEA and androstenedione (AED) by the sequential hydroxylase and lyase activity of CYP17A1. Circulating adrenal androgens (including the sulfated form of DHEA, DHEA-S), enter the prostate and can be converted to testosterone by a series of reactions involving the activity of HSD3B, HSD17B and AKR1C enzymes. Testosterone is then converted to the potent androgen DHT by the activity of SRD5A. In the backdoor pathway to DHT synthesis (short gray arrows), C21 precursors are first acted upon by SRD5A and the reductive 3 α -HSD activity of the AKR1C family member, AKR1C2, followed by conversion to C19 androgens via the lyase activity of CYP17A. DHT is subsequently generated by the action of HSD17B3 and an oxidative 3 α -HSD enzyme, including HSD17B6 (also called RL-HSD) or HSD17B10 (as well as RODH4, RODH5 and NT 3 α -HSD, not shown). *Adapted from* Mostaghel EA, Nelson PS. Intracrine androgen metabolism in prostate cancer progression: mechanisms of castration resistance and therapeutic implications. *Best Pract Res Clin Endocrinol Metab.* 2008;22:243, with permission.

Figure 2. Androgen receptor splice variants

Domains of the full length androgen receptor (AR^{FL}) include the N-terminal domain (NTD), the DNA binding domain (DBD), the hinge region (HR) and the C-terminal ligand binding domain (LBD), involved in mediating the indicated functions. The major AR splice variants identified in human prostate cancer cell lines and specimens include the exon-skipping variant, AR^{V567es}, in which exons 5–7 of the LBD are deleted, as well as the indicated truncated AR variants in which exons 2 or 3 become spliced to cryptic exon (CE) sequences. AR^{V7} and AR^{V3} were published separately but later shown to encode the same protein product.