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Antiandrogens and androgen depleting therapies in prostate cancer: novel agents for an established target

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Summary

Activation of the androgen receptor is critical for prostate cancer growth at all points in the illness. Currently therapies targeting the androgen receptor, including androgen depletion approaches and antiandrogens, do not completely inhibit androgen receptor activity. Prostate cancer cells develop resistance to castration by acquiring changes such as AR overexpression that result in reactivation of the receptor. Based on understanding of these resistance mechanisms and androgen synthesis pathways, novel antiandrogens and androgen depleting agents have been tested. Notably, MDV3100, a novel antiandrogen designed for activity in prostate cancer model systems with overexpressed AR and, abiraterone acetate, a 17-α-hydroxylase/17,20 lyase inhibitor that blocks steroid biosynthesis in the adrenal gland and in the tumor, have demonstrated significant activity in early phase trials and are being tested in the phase III setting.

Introduction

Prostate cancer is an androgen dependent malignancy, first demonstrated in 1941 by the Nobel Prize-winning research of Huggins and Hodges showing that reducing serum androgen levels by orchiectomy or exogenous estrogen administration induced tumor regressions and palliation of symptoms (Figure 1). Subsequently gonadotropin-releasing hormone (GnRH) analogs and antiandrogens were added to the armamentarium, but overall outcomes were essentially the same: responses that were often dramatic but rarely complete, a period of quiescence in which the disease does not proliferate, and eventual relapse despite castrate levels of testosterone in the blood. It is this point in the illness that represents a transition to the lethal phenotype of the disease to which most patients eventually succumb. More important is that despite the availability of palliative options, only one treatment, docetaxel, has been shown to prolong life (Figure 2) (1-3).

Through the convergence of basic research, molecular profiling studies of prostate cancers representing different points in the disease spectrum, and clinical insights, the outlook is changing. Studies over the last decade have shown that castration-resistant prostate cancers (CRPC) remain dependent on AR function for growth by evolving multiple mechanisms to activate receptor signaling. The mechanisms of AR reactivation include overexpression of the

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Conflicts of interest

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receptor, mutations in AR that allow activation by antiandrogens or other endogenous steroids such as progesterone or hydrocortisone, ligand independent activation by growth factor signaling pathways, changes in levels of AR transcriptional cofactors, and upregulation of the enzymes involved in androgen biosynthesis which have been shown recently to produce higher levels of androgen in tumor relative to those in the blood (4-6).

That radiographic and symptomatic disease progression is preceded by a rise in serum prostate specific antigen levels illustrates the clinical significance of these findings. Transcription of this secreted protein is AR dependent and indicative of AR reactivation. While it is likely that several mechanisms contribute to CRPC progression in an individual patient, the most commonly observed oncogenic change is overexpression of AR to levels that are significantly higher than those documented in non-castrate diseases (7-9). In prostate cancer xenograft models, forced overexpression of AR is both necessary and sufficient for tumor growth in castrate mice, sensitizes tumors to lower androgen levels, and surprisingly, is associated with conversion of the antiandrogens bicalutamide and flutamide into AR agonists (10).

Most encouraging is the recent results reported in clinical trials of two novel compounds targeting specific alterations in AR signaling: MDV3100, a novel antiandrogen specifically engineered for activity in prostate cancer model systems with overexpressed AR and, abiraterone acetate, a 17-α-hydroxylase/17,20 lyase (CYP17) inhibitor the blocks steroid biosynthesis in the adrenal gland and in the tumor (Figure 1, 3). Both have shown sufficient promise to justify definitive testing in phase 3 randomized registration trials in patients with CRPC who have progressed on docetaxel-based chemotherapy where there is no standard of care (Figure 2). This review will place the development of these and other agents in the context of our understanding of the biology and current management.

Antiandrogens

For patients with non-castrate levels of testosterone in the blood, traditional AR-targeted therapies include the gonadotropin releasing hormone agonists and antagonists, estrogens or surgical orchiectomy which reduce androgen levels and antiandrogens which do not. As a class, antiandrogens compete with endogenous androgens for binding in the ligand binding pocket of AR, inducing conformational changes that prevent optimal transcriptional activity (i.e., antiandrogens). There are two general classes of antiandrogens: steroidal and non-steroidal. The steroidal compounds include progesterone analogs RU-486 (mifepristone, Mifeprex®), cyproterone acetate (CPA, Androcur®, Procur®, Siterone®), and the mineralocorticoid analog spironolactone (Aldactone®, Spirotone®) (Figure 3A). CPA is non-specific and can activate the glucocorticoid, mineralocorticoid and progesterone receptor. A meta-analysis of randomized trials comparing ADT with ADT plus CPA in patients with non-castrate disease showed inferior survival in the cyproterone acetate group (11), while the response to spironolactone and RU-486 is limited (12). As these agents have partial agonist activity, neither is used as first-line therapy (13).

Non-steroidal antiandrogens were originally developed in the 1970s to circumvent the off-target effects of the steroidal agents, and have no significant interaction with nuclear receptors other than AR. Historically, they have been considered “pure antagonists” because they neither activate AR-dependent reporters in tissue culture nor activate AR-dependent genes in prostate cancer cell lines. Three are currently approved for use based on specific indications (Figure 3A). Flutamide (Eulexin®), the first generation compound was approved to block the exacerbation of disease that can occur following the rise in serum testosterone that occurs with the initial administration of GnRH agonists. Bicalutamide (Casodex®) (50mg daily) was approved in combination with an androgen depletion approach based on a more favorable safety relative to flutamide. Nilutamide (Anandron®, Nilandron®) was approved following the

demonstration of an improve disease free and overall survival relative to placebo in combination with orchiectomy. Given as monotherapy to patients with non-castrate levels of testosterone, nonsteroidal anti-androgens as a class are better tolerated than androgen lowering approaches producing less impotence, hot-flashes, anemia, and bone loss, at the expense of gynecomastia and breast tenderness. Side-effects of flutamide and nilutamide include diarrhea, alcohol intolerance, interstitial lung disease, and hepatotoxicity. Nilutamide has a unique ocular toxicity that affects the perception of color and the ability to adapt to light.

Initial treatment in combination with androgen deprivation therapy

In addition to trials as monotherapy, antiandrogens have been combined with androgen lowering approaches with the objective of achieving a "more complete" androgen blockade (so called, combined or maximal androgen blockade) (14) (Figure 2). The concept has been tested in multiple phase III trials. One, a phase III randomized trial of 600 patients with metastatic prostate cancer receiving daily subcutaneous injections of the GnRH agonist leuprolide plus flutamide or placebo showed a 25% improvement in survival in favor of the flutamide treated patients.(15). Unfortunately, the results were not confirmed in a second randomized trial of nearly 1400 patients using orchiectomy as the androgen depleting approach ($p=0.14$, risk ratio 0.91 (0.81–1.01)), raising the question whether the additional benefit of flutamide in the first trial may have been due to an unblocked flare related to GnRH monotherapy in the control area and / or patient non compliance with daily self-administered leuprolide injections.

Nilutamide is a derivative of flutamide (Figure 3A) with pharmacokinetic properties that permit once daily dosing. Nilutamide was approved in 1996 based on a phase III trial of 450 patients showing that the addition of once daily oral nilutamide to orchiectomy resulted in improved response rates, survival and progression-free survival, in comparison with orchiectomy alone (16).

Bicalutamide was derived from flutamide by addition of a bulky 4-fluorophenylsulfonyl moiety (Figure 3A). It has a 2-fold increased affinity for AR compared to flutamide and nilutamide, a longer half-life of 1 week, and significantly decreased toxicities, notably hepatotoxicity. It was approved in 1995 based on a phase III non-inferiority trial of 831 patients randomized to long-acting GnRH agonists, plus either flutamide (250 mg three times daily) or bicalutamide (50 mg once daily). In the final analysis, not only was non-inferiority met by bicalutamide, but there was a non-significant trend towards improved overall survival as well as decreased toxicity and withdrawal from treatment (mainly due to diarrhea) (17). Due to the improved side effect profiles and the convenience of once daily dosing, bicalutamide is the most commonly employed antiandrogen.

Antiandrogens in castration-resistant disease

In meta-analyses of combined androgen blockade trials, the survival benefits have been modest at best (~2% at 5 years) (11). In addition, most patients randomized to the ADT monotherapy arms were not treated with antiandrogens after progression, so it is not known if patients would derive the same benefit if an antiandrogen were added later. In phase 2 trials, the addition of an antiandrogen to patients progressing on ADT monotherapy is modest, and in part a function of the number of prior therapies a patient has received (18-20). In small series, patients previously treated with flutamide responded to bicalutamide, while those receiving multiple hormonal interventions did not (18). This fact, together with the cost and inconvenience of prolonged antiandrogen treatment, has led many physicians to use a short course of antiandrogens to block the flare associated with GnRH administration, and restart an anti-androgen the castration-resistant phenotype is documented. (Figure 2, "TYPICAL").

Bicalutamide monotherapy

When given as monotherapy, bicalutamide is better tolerated than ADT with improved sexual function, decreased hot flashes, less weight gain, less muscle loss, less fatigue, preserved bone strength, and better sense of well-being, at the expense of increased gynecomastia and breast tenderness. Consequently, bicalutamide monotherapy has been extensively studied as an alternative to watchful waiting in localized disease, and to ADT in advanced disease. Several trials of more than 8000 individuals in different disease states (Figure 2) have suggested the only benefit of bicalutamide monotherapy may be in patients treated with radiotherapy for locally advanced disease (T3/T4). Here, high-dose bicalutamide monotherapy (150 mg daily) showed similar efficacy and better tolerance relative to ADT and was associated with a survival advantage when compared to placebo (21). In metastatic disease, bicalutamide monotherapy is inferior to ADT (22,23) and in early localized disease (T1/T2) bicalutamide monotherapy shows a trend towards decreased survival compared to placebo (21). At present data, bicalutamide (150mg daily) is approved for locally advanced disease in the EU but not in the US.

A deficiency of all the currently approved anti-androgen is that following long-term use in combination with testosterone lowering treatment, a proportion of patients will respond to the selective discontinuation of the drug. This phenomenon of declines in PSA and regression of tumor upon stopping the drug has been termed the anti-androgen withdrawal syndrome,(24, 25) indicating that these antiandrogens can serve as agonists under the right circumstances. These observations along with recent results from cellular and mouse model systems suggest reclassification as partial agonists (10,26).

Novel Antiandrogens

More recently, investigators utilized mechanistic understandings of the current antiandrogens as well as structure function discoveries of the androgen receptor to rationally design and test novel compounds. Here, we will review some of these insights in order to understand the design of novel antiandrogens.

Mechanism of AR activation

AR is a 110 kD member of the steroid receptor family. It shares a common fold with other nuclear receptors and contains modular functional domains that include an N-terminal domain (NTD) capable of ligand-independent transcriptional activation, a DNA-binding domain (DBD), a hinge region important for nuclear localization and a ligand-binding domain (LBD) which also mediates dimerization and ligand-dependent transcriptional activation (Figure 4, inset). In the inactive “apo” (unbound) state, AR is an unstable protein predominately located in the cytoplasm in complex with heat shock proteins. In particular, HSP90 binds to the LBD to stabilize AR and maintain the LBD in a conformation easily accessible to ligands (27). Indeed, HSP90 inhibitors have been shown to destabilize AR and inhibit transcriptional activation (28). Upon androgen binding, AR activation can be conceptualized in several steps (Figure 4,Table 1) (29-31).: **1)** A conformational change is induced in the 12 α-helices of the LBD, with helix-12 forming a lid over the ligand-binding pocket. This repositioning creates a hydrophobic surface on the LBD that is capable of binding LxxLL or (LXX(H/I)XXXI) consensus motifs found in AR coactivators (eg steroid-receptor coactivator-1 (SRC-1)) and corepressors (eg nuclear-receptor corepressor (NCoR)), respectively **2)** The AR LBD dissociates from HSP90 and binds intramolecularly to a similar FxxLF motif in the NTD. This N- to C-terminal folding further stabilizes AR and perhaps serves to prevent coactivator binding until AR is stably docked onto DNA (32). **3)** The nuclear localization sequence of AR is exposed and allows translocation into the nucleus. **4)** AR binds to the androgen response elements in promoter and enhancer elements of the DNA and **5)** AR recruits co-activators

required to activate transcription of target genes, as well as co-repressors that modulate this activity (Figure 4, left) (33).

Antiandrogen antagonism

While the mechanistic details of AR antagonism by bicalutamide are most studied, the structurally similar toluidides flutamide and nilutamide are thought to behave similarly. Crystal structures of AR in an antagonist conformation have not been solved; however, structural information can be inferred from structural studies of the estrogen receptor (ER) in complex with anti-estrogens. The anti-estrogen 4-hydroxytamoxifen (4OHT) competes with estradiol for binding to the LBD of ERα. Antagonism occurs, in part, due to steric clash of bulky aryl side-chains in 4OHT with helix 12, preventing its repositioning over the ligand binding pocket (34). Co-crystal structures of bicalutamide have been solved with an AR mutant variant (Tryptophan 741 → Leucine) where CPA and bicalutamide serve as agonists. The structures suggest that when bicalutamide is instead complexed to wild-type AR, a similar steric clash mechanism may result, due to the bulky phenyl ring of bicalutamide, leading to partial unfolding of the AR LBD (Figure 4, middle) (35).

As with agonists, currently available antiandrogens cause AR to translocate efficiently into the nucleus and bind DNA at androgen response elements, though more transiently and with less affinity than agonist-bound AR (Figure 5A) (36). This weak but detectable DNA binding is supported by three sets of experiments: 1) When AR is fused to the strong transcriptional activator of herpesvirus VP16, which obviates the need for coactivator recruitment or nuclear translocation and requires only DNA-binding for activity, bicalutamide activates transcription indicating that AR has bound DNA (36); 2) chromatin immunoprecipitation of AR indicates that while DHT-bound AR binds to both PSA enhancer and promoters, bicalutamide-bound AR binds only to the PSA promoter (33); 3) Fluorescence recovery after photobleaching studies using fluorescently-tagged AR indicates that agonist-bound AR is relatively immobile due to DNA binding, but bicalutamide-bound AR is more mobile, suggesting the AR pool is mostly unbound or transiently bound to DNA (37). Despite DNA binding, bicalutamide-bound AR neither recruits coactivators nor activates transcription in LNCaP cells or reporter assays. Bicalutamide-bound AR is still able to recruit corepressors but this does not seem to be critical for its ability to serve as an antagonist (33, 38).

Antagonist to agonist conversion

One mechanism whereby antiandrogens can be converted to agonists in the cell is through mutation of AR. AR mutation is very uncommon in primary tumors but may occur in up to 30% of metastatic samples (39). For example, a threonine to alanine mutation of amino acid 877 (T877A), recapitulated in the LNCaP prostate cancer cell line, confers agonist properties to flutamide, cyproterone acetate, progesterone, and estrogens. Mutation of amino acid 741 from tryptophan to leucine or cysteine confers agonist properties to bicalutamide. Current evidence indicates that the frequency of AR mutation cannot account for the majority of clinically observed resistance or for the antiandrogen withdrawal response.

The AR protein is overexpressed in the majority of CRPC (7,40). Forced overexpression of AR in prostate cancer cell lines converts bicalutamide to an agonist, causing it to activate the same AR-regulated genes as do agonists. In the setting of AR overexpression, bicalutamide binding results in both enhancer binding and co-activator recruitment, processes that are inhibited by bicalutamide in cells without AR overexpression (Figure 5B,Table 1) (10,26). To isolate the effects of bicalutamide on the recruitment of coactivators, an *in vitro* assay to assess binding of purified AR LBD to coactivator peptides containing the LxxL motif showed that bicalutamide can induce coactivator binding, albeit less efficiently than the agonist dihydrotestosterone (DHT) (26). These data indicate that currently available antiandrogens

induce changes in AR that continue to allow nuclear translocation, DNA binding, and coactivator recruitment at variable efficiencies, suggesting that these compounds should be classified as partial agonists. In the setting of overexpressed AR as occurs in CRPC, this partial agonistic activity may be sufficient to activate AR and maintain prostate cancer growth and survival.

Development of novel antiandrogens

Due to the drawbacks of currently available antiandrogens, there has been extensive research into development of novel and more effective agents. Several groups have designed antagonists by adding chemical bulk to agonist scaffolds, on the basis of structural studies suggesting that steric displacement of helix 12 can inhibit co-activator. Compounds have been synthesized with higher affinity for AR than bicalutamide, and that more potently antagonize transcriptional activation via the W741C and T877A mutant ARs (26,41-44). Another strategy has been to incorporate steric hindrance by linking a potent AR agonist to a ligand of the ubiquitous FK506-binding proteins (FKBPs) (45). A third strategy has utilized a fluorescence based screen to identify compounds that inhibit AR nuclear translocation and the interaction between the N- and C-terminus (46). Following, we will expand on two antiandrogens that are furthest along in clinical development: BMS-641988 and MDV3100.

BMS-641988

BMS-641988 was discovered in a structure-assisted drug screen using nilutamide as the parent compound (Figure 3). Compounds with a hydantoin ring showed increased affinity for AR and combinatorial chemistry produced derivatives that were screened for AR affinity and for inhibition of transcriptional activation in the AR-positive MDA-MB-453 breast cancer cell line. Promising leads were tested for ability to reduce prostate weight in immature rats, for optimal pharmacokinetics, and for antitumor activity in xenografts of the CWR22LD1 human prostate cancer cell line. The lead compound, BMS-641988 (Figure 3), binds AR with approximately 20-fold increased affinity compared to bicalutamide, decreases prostate weight of rats more than bicalutamide, and is more effective in treatment of CWR-22LD1 xenografts. Gene expression profiling of CWR-22LD1 xenografts indicates that the transcriptional effects of BMS-641988 treatment are more similar to the effects that occur with castration than with bicalutamide treatment. In additional, BMS-641988 treatment and castration of mice induced a similar serum proteomic profile (43,47). Based on these preclinical data, BMS-641988 is currently in a phase I clinical trial in patients with CRPC.

MDV3100

Preclinical development—Increased AR levels are implicated as a molecular cause of drug resistance and currently available antiandrogens have AR agonist properties when AR is overexpressed. This suggests that third-generation antiandrogens might be identified by their lack of AR agonism coupled with retention of antagonism in cells expressing excess AR. The laboratories of Charles Sawyers (MSKCC) and Michael Jung (UCLA) selected the non-steroidal thiohydantoin agonist RU59063 (Figure 3) as a starting chemical scaffold, based on its high affinity and selectivity for AR. A panel of nearly 200 derivatives were iteratively synthesized and screened for AR agonism and antagonism in human prostate cancer cells engineered to express increased levels of AR. Extensive development of the structure activity relationships (SAR) followed by optimization of pharmacokinetic properties resulted in the selection of the diarylthiohydantoin MDV3100 for further preclinical and clinical studies (Ouk et. al., submitted).

MDV3100 binds AR with 8-fold higher affinity compared to bicalutamide. MDV3100 does not activate either wildtype AR, or the T877A or W741C mutants. In two AR overexpressing cell lines, LNCaP/AR and VCaP, MDV3100 inhibits AR-mediated transcription and cell

growth *in vitro* while bicalutamide does not. MDV3100 treatment induces tumor regression in established LNCaP/AR xenograft tumors growing in castrate male mice, while bicalutamide treatment merely slows tumor growth compared to vehicle-treated controls (26). The effects of MDV3100 on AR function have been extensively characterized are distinct from those of bicalutamide (Table 1, Figure 4, right). When bound to MDV3100 rather than bicalutamide, AR translocates into the nucleus far less efficiently and a significant AR fraction remains in the cytosol (Figure 5A). Furthermore, MDV3100-bound AR does not bind DNA as evidenced by two separate assays: 1) in AR-overexpressing LNCaP/AR cells, bicalutamide treatment causes AR binding to the PSA and TMPRSS2 enhancers, whereas MDV3100 treatment does not (Figure 5B); 2) When AR is fused to the VP16 transactivation domain, bicalutamide activates transcription indicative of DNA binding, whereas MDV3100 does not. Lastly, using an *in vitro* assay of coactivator peptide recruitment by the purified AR LBD, MDV3100 binding does not cause peptide recruitment, whereas bicalutamide binding does. These data indicate that MDV3100 may be a true AR antagonist without partial agonist properties.

Phase I/II data—The phase I/II trial accrued patients with chemotherapy-naïve CRPC (Figure 2, "TYPICAL") as well as patients who have progressed on docetaxel-based chemotherapy for whom there is no standard of care (Figure 2, "ATYPICAL"). The latest update at the 2009 ASCO Genitourinary Cancers Symposium reported safety data on the first 140 patients and initial efficacy data on the first 114 patients who were enrolled at five dose levels between 30 mg and 360 mg per day. The treatment was generally well tolerated with a dose-limiting toxicity of fatigue and 240 mg per day was selected as the maximum tolerated dose. Plasma half-life was approximately one week and plasma levels ranged from ~2 μg/mL in the 30 mg per day cohort to ~20 μg/mL in the 240 mg per day cohort - these levels correlate to effective drug concentrations in the preclinical mouse models.

Prior to MDV3100 treatment, seventy-five patients had disease progression following two or more lines of prior hormone therapy, while 50% of patients had progressed on chemotherapy. After 12 weeks of MDV3100 treatment, 37 of 65 (57%) chemotherapy-naïve patients and 22 of 49 (45%) post-chemotherapy patients attained a PSA > 50% decline in PSA from baseline. The median duration of therapy for these two patient groups was 9 and 5 months, respectively. Circulating tumor cells were obtained before and after MDV3100 treatment and a cutoff of 5 cells per 7.5 mL blood was use to define the number of circulating cells as either favorable (< 5 cells) or unfavorable (> 5 cells) (48). Ninety-two percent of patients with favorable CTC counts pre-treatment maintained a favorable count post treatment and importantly, 53% of patient with initially unfavorable counts converted to favorable counts after treatment (49). New data suggests that changes in CTC count following treatment is more predictive of survival than PSA levels, with a favorable CTC count after treatment associated with a 21 month median survival (50).

Of the 16 patients who were evaluated by 18F-FDG and 18F-FDHT PET scans after 12 weeks of MDV3100 therapy, 14 patients showed declines in 18F-FDG accumulation while all 16 showed declines in 18F-FDHT accumulation (Figure 4C, example). The 18F-FDHT PET serves as a pharmacodynamic marker for AR binding by MDV3100, since increased binding by MDV3100 results in reduced accumulation of 18F-FDHT due to competition for the same AR binding site (49). A phase III trial of patients with CRPC who have progressed on docetaxel based chemotherapy (Figure 2, "ATYPCAL") is currently under regulatory review is planned.

Resistance—While MDV3100 is a highly promising therapy for CRPC, not all patients respond to MDV3100 treatment and resistance develops in many initial responders. Just as with any other AR directed therapy, disease progression most frequently correlates with a rise in PSA, indicating reactivation of AR. In the patients who received 18F-FDHT PET scans and who were progressing on MDV3100 therapy, subsequent scans indicated continued inhibition

of 18F-FDHT accumulation in tumors, indicating that MDV3100 binding to AR is preserved (Figure 4D). Therefore, while preclinical data indicates that MDV3100-bound AR cannot bind DNA to activate transcription, clinical evidence indicates that escape must be possible. The discovery of resistance mechanisms will aid not only in the design of next generation antiandrogens, but also in further elucidation of AR function. One possible escape mechanism may be the emergence of drug-resistant AR mutants, while another could arise from the recent discovery of alternate AR splice forms that can result in constitutively active receptors that are truncated right before the LBD. These truncated transcripts and proteins are upregulated in CRPC tumors compared to localized disease (51, 52).

Androgen synthesis inhibitors

The prior discussion in this review has focused on drugs that block androgen signaling via competition for binding to the androgen receptor. However, agents that target the androgen signaling axis in CRPC by interfering with androgen biosynthesis have also met with recent success in clinical trials.

The testis is not the sole source of androgens, borne out by the fact that ADT through surgical castration or treatment with GnRH agonists does not completely eliminate serum or intratumoral androgens. ADT reduces serum testosterone from a normal range of >200 ng/ml to ~10 ng/ml; however, ADT does not affect levels of adrenal androgens such as dehydroepiandrosterone (DHEA) (Figure 1, 3B). The remaining androgens are synthesized by the adrenal gland, and complete inhibition of adrenal gland function decreases serum testosterone and adrenal androgens to undetectable levels.

An additional source of androgens may be the tumors themselves. Expression profiling studies found that multiple enzymes involved in steroid synthesis are upregulated in CRPC compared to localized prostate cancer (Figure 3B) (4, 7, 40). Significant gene upregulation was observed for the CYP17 that converts progestins to androgens, for 17 keto-reductase enzyme which converts the weakly potent adrenal androgen androstenedione to testosterone, and for 5α-reductase 1 (SRD5A1), which converts testosterone into the potent androgen dihydrotestosterone (DHT). In addition, CRPC specimens isolated from men on ADT contain higher levels of intratumoral testosterone than primary prostate tumors of untreated men, even though the latter group has higher circulating testosterone levels. Similarly, in a mouse xenograft model, CRPC tumors grown in castrate mice maintained similar intratumoral testosterone levels to those measured in hormone sensitive tumors grown in intact mice (4).

There is evidence going back to the 1950s that suppression of adrenal androgen biosynthesis is effective in patients with CRPC. Hypophysectomy to abrogate ACTH-mediated stimulation of the adrenals, and bilateral adrenalectomy both induce clinical responses in up to 50% of patients with disease progression following surgical castration (53). Given the morbidity and mortality rates of these procedures, pharmacological agents that inhibit androgen biosynthesis have been attractive targets. In addition to the adrenal gland, such agents should also inhibit any intratumoral androgen synthesis.

First Generation Androgen Synthesis Inhibitors

Glucocorticoids inhibit CRH and ACTH secretion via a negative feedback mechanism (Fig. 1), thereby decreasing production of adrenal androgens. Prednisone and hydrocortisone lower serum testosterone and adrenal androgen levels, displaying modest efficacy in CRPC (20,54, 55) and in addition, reducing pain and raising energy levels. Currently, glucocorticoids are commonly administered adjunct to chemotherapy in CRPC.

Aminoglutethimide (AGT, Cytadren®) was initially discovered as an aromatase inhibitor but blocks multiple cytochrome P450 enzymes involved in adrenal corticosteroid synthesis. AGT was studied in several phase II trials in CRPC. While serum levels of the adrenal androgen dehydroepiandrostenedione (DHEA) was suppressed, neither mean testosterone nor mean DHT levels were significantly changed and remained at ~10 ng/dL and 7 ng/dL respectively (Figure 3B). In addition, when compared to hydrocortisone alone, addition of AGT does not further decrease adrenal androgen levels. Approximately 25-50% of patients are reported to have attained stable disease, although the duration of therapy was short (55, 56).

Ketoconazole, an imidazole antifungal agent, is another non-specific inhibitor of P450 enzymes. In the phase III CALGB 9583 trial, comparing AAWD (60% bicalutamide, 35% flutamide, 5% nilutamide) with AAWD plus ketoconazole and hydrocortisone, the PSA declines of > 50% rates were 11% and 27%, respectively. However, there was no statistically significant difference in overall survival (57). As seen with AGT, ketoconazole significantly decreased serum levels of the adrenal androgens DHEA and androsteinedione by ~50%; however, it did not significantly affect testosterone levels. Surprisingly, a rebound of adrenal androgen levels occurs upon disease progression, as compared to the levels after just 1 month of treatment, indicating tachyphylaxis to ketoconazole inhibition (57). These non-selective p450 enzyme inhibitors have significant dose limiting toxicities including fatigue, neurotoxicity, hepatotoxicity and nausea, limiting quality of life and treatment duration. Additional complications can arise from interference of these agents with the metabolism of multiple drugs.

CYP17A Inhibitors

CYP17 is a key enzyme in the androgen biosynthesis pathway that functions in the testes and adrenal glands to catalyze the respective conversion of pregnenolone and progesterone into the weak androgens DHEA and androstenedione (Fig. 5). These weak androgens are further converted into testosterone and DHT, a process that may occur in peripheral tissues, including prostate cancer tumors.

Abiraterone acetate—Abiraterone is a pregnenolone derivative that is a selective, high affinity (IC_{50}=2 nM), irreversible inhibitor of CYP17 (Figure 3A, B). Oral Abiraterone acetate was developed to improve oral bioavailability, and undergoes rapid deacetylation in serum. Preclinical studies in intact mice showed that abiraterone acetate treatment at tolerable doses lowered serum testosterone concentrations to castrate levels without significantly affecting serum hydrocortisone levels, whereas ketoconazole treatment suppressed hydrocortisone production more than testosterone production (58). Abiraterone acetate was initially developed as an oral alternative to GnRH agonists. However, when administered as a single agent, testosterone levels partially recover due to a feedback increase in GnRH levels. Importantly, addition of abiraterone to GnRH treatment results in a substantial decrease in both testosterone and adrenal androgen levels (59).

In a dose-finding phase I trial of abiraterone acetate in patients with CRPC, serum levels of testosterone, DHEA, and androstenedione dropped from their respective pretreatment levels of 7ng/dL, 280 ng/dL and 34 ng/dL, to <1 ng/dL, 84 ng/dL, and <2 ng/dL, respectively. This decrease was sustained for more than 4 months of treatment and there was no evidence of tachyphylaxis. Thus, not only is the suppression of andrenal androgens by abiraterone more profound than that obtained with ketoconazole or AGT, but also abiraterone is the first drug that significantly suppresses testosterone levels. Because there were no grade III or IV toxicities, the phase II dose of 1000 mg per day was selected, based on maximal androgen inhibition (60).

Extensive phase I and phase II experience with abiraterone in CRPC has now been accumulated. Separate phase II trials were conducted in chemotherapy-naïve and post-chemotherapy CRPC patients (Figure 2). The drug is very well tolerated. The most recent data from treatment of >100 chemotherapy-naïve patients and >100 post-chemotherapy patients, reported at the 2009 ASCO Genitourinary Cancers Conference, showed significant efficacy with PSA declines of 50% or more in chemotherapy-naïve and ~40% in post-chemotherapy patients. The medium time to progression in these two patient groups was 8 and 5.5 months, respectively (48,61,62). This data has led to an ongoing multinational phase III trial of abiraterone vs placebo with a primary endpoint of overall survival, randomized in a 2:1 manner and targeted to accrue almost 1200 patients whose disease has progressed after docetaxel-based chemotherapy.

Resistance

Despite promising therapeutic activity, most patients treated on the phase I/II abiraterone trials have progressed. As with other therapies, the progression usually corresponds to an increase in serum PSA levels, suggesting reactivation of AR. One possible mechanism for AR reactivation is the expression of a truncated, constitutively active form of AR, as discussed in the previous section. In addition, abiraterone treatment results in a significant increase in concentrations of steroidal compounds in the biosynthetic pathway upstream of the CYP17 blockade – these include corticosterone, aldosterone and progesterone. These steroids have been shown to activate certain AR mutant proteins isolated in CRPC and AR mutation thus may represent another mechanism for escape. In fact, during the phase I portion of the trial, the addition of 0.5 mg/dL dexamethasone to suppress ACTH mediated stimulation of the adrenal glands resulted in a clinical response in 4 of 15 patients with disease progression while on abiraterone acetate alone. We have observed a similar salvage response in a patient progressing on abiraterone alone where spironolactone was given as a diuretic after withdrawal of spironolactone. Indeed, given its steroidal backbone and structural similarity to progesterone, it would not be unexpected if abiraterone emerged as an AR agonist in certain subsets of CRPC (Figure 2). In fact, a structurally similar compound, VN/124-1, binds AR with high affinity (see below). Nevertheless, an abiraterone withdrawal response has not yet been reported.

Novel CYP17 inhibitors

A number of groups have developed novel inhibitors of CYP17, but none have yet reached clinical testing. Given the excellent potency and specificity of abiraterone, novel agents would likely require mechanism-based advantages. One such compound may be the steroid VN/124-1 (3β-hydroxy-17-[1H-benzimidazole-1-yl]androsta-5,16-diene). In addition to inhibition of CYP17, VN/124-1 binds and inhibits AR with 10-fold increased affinity compared to bicalutamide. Upon binding, it causes degradation of AR in both cell lines and xenograft models (63). Given the possible disadvantage of the steroidal structure of both abiraterone and VN/124-1, several groups have developed nonsteroidal CYP17 inhibitors. After several iterations, agents have been synthesized with potencies and pharmacological properties comparable to aberaterone (64).

5α Reductase Inhibitors

Testosterone in peripheral androgen dependent tissues is converted to the more potent DHT by two isoforms of 5α-reductase, SRD5A1 and SRD5A2 (Figure 1, 3B). SRD5A2 is the predominant isoform in the benign prostate. Finasteride, a specific inhibitor of SRD5A2, lowers PSA levels and reduces prostate weights and has been approved for use in benign prostatic hyperplasia. However, progressive castration resistant prostate cancer is characterized by increased SRD5A1 and decreased SRD5A2 levels(7,65). Dutasteride, a potent inhibitor of

both SRD5A1 and SRD5A2, inhibits tumor growth in the androgen-sensitive R-3327H rat prostatic adenocarcinoma model and in the probasin large-T antigen (TRAMP) mouse prostate tumor model (66,67). In a LNCaP xenograft model, addition of dutasteride but not finasteride treatment to surgical castration, inhibits tumor growth more than the effects of castration alone (67). In clinical testing, dutasteride has limited activity in CRPC. However, in a phase II trial of combination therapy with ketoconazole, hydrocortisone and dutasteride (KHAD) in 57 patients with CRPC, 30 patients (53%) attained a PSA response and the median time to progression was 13.7 months (68). How these results compare to abiraterone would require prospective testing.

Conclusions

AR is a validated target in all clinical states of prostate cancer. ADT remains the standard first line approach for patients with advanced disease and non-castrate levels of testosterone in the blood. Underappreciated are the responses to AR signaling directed approaches in patients with progressive CRPC including those previously treated with cytotoxic drugs where the use of "hormonal" agents is generally not considered. Novel agents developed specifically to target specific molecular alterations identified in tumor samples from patients with CRPC, including MDV3100 that directly binds the receptor and abiraterone acetate that selectively block CYP17 to inhibit residual androgen synthesis in the adrenal gland and tumor cells, have shown promising antitumor effects in the clinic. Both are under study in phase III registration trials in CRPC patients for whom there is no standard of care.

Further research will address several issues. First, can we identify predicative factor of clinical benefit? To address this, both phase III trials incorporate multiple correlative studies including isolation and characterization of circulating tumor cells as a "liquid biopsy" of tumor tissue, and measurement of serum levels of adrenal androgens and other steroids. A second related issue is to identify mechanisms of resistance. Just as bicalutamide resistance was critical to the development of MDV3100, future AR directed drug development depends on an understanding of how AR can be activated despite binding to MDV3100 or in ultralow androgen environment of an abiraterone treated patient. The current excitement in the field lies not only with novel compounds at hand, but future agents designed on our increasing understanding of AR biology.

Search strategy and selection criteria

Data for this Review were identified by searches of PubMed, ASCO abstracts, and references from relevant articles using the search terms "androgen receptor, "abiraterone", "prostate cancer", "ketoconazole", "bicalutamide", "CYP17", "flutamide", "nilutamide". Abstracts and reports from meetings were included only when they related directly to previously published work.

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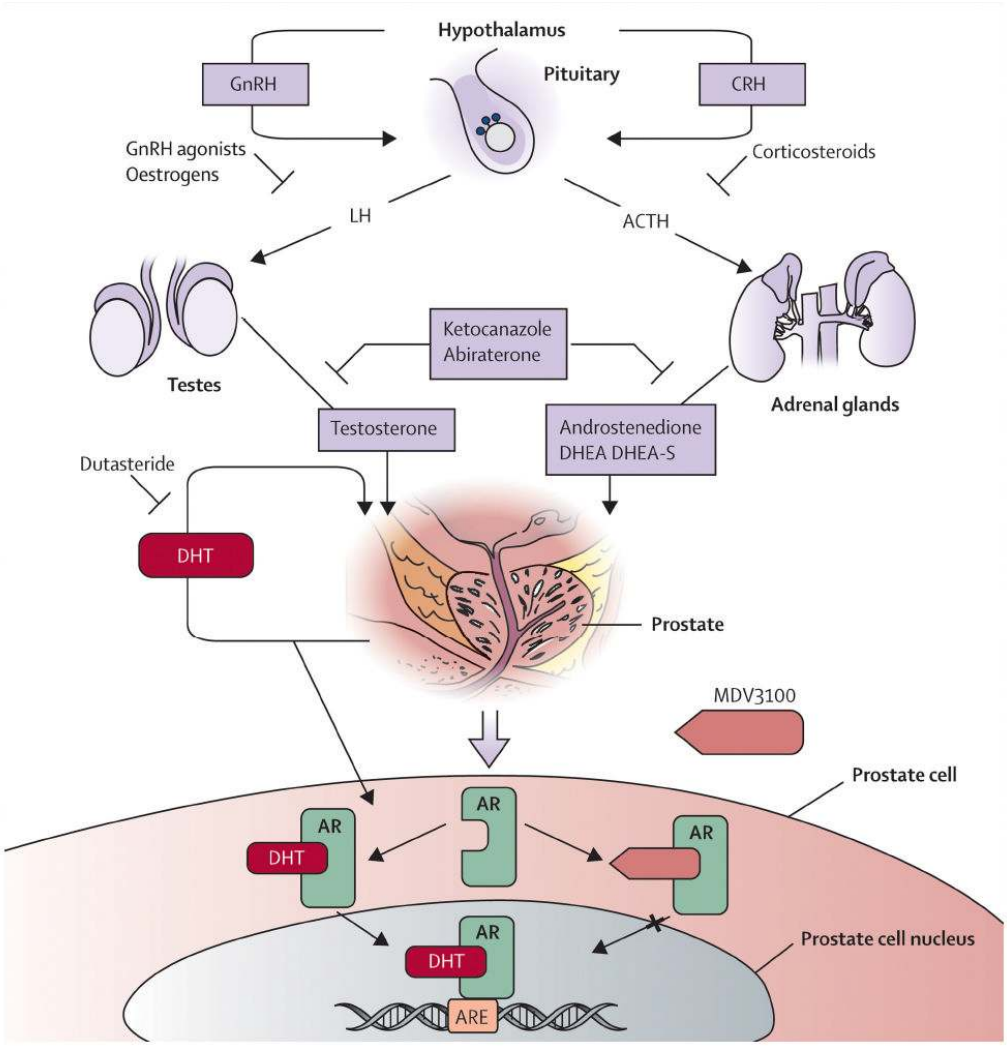

Figure 1. Schematic of androgens axis and inhibitors

(Top) In men, androgens are mainly synthesized mainly by the testes as testosterone, but also in the adrenal glands as androsteinedione and dehydroepiandrosterone (DHEA). These two endocrine organs are stimulated by anterior pituitary secretion of luteinizing hormone (LH) and adrenocorticotropic hormone (ACTH) respectively. The anterior pituitary secretion of these two hormones is themselves regulated by hypothalamic secretion of gonadotropin-releasing hormone (GnRH) and corticotropin-releasing hormone (CRH) respectively. Surgical castration, adrenectomy, and hypophysectomy have been performed to inhibit androgen production. Treatment with GnRH agonists and estrogens inhibits pituitary stimulation of the testes while treatment with corticosteroids inhibits pituitary stimulation of the adrenals. Abiraterone and other CYP17 inhibitors inhibit a key step in androgen synthesis, decreasing both testicular and adrenal androgen production. Circulating testosterone can be converted to the more potent dihydrotestosterone (DHT) in prostate cancer by 5α-reductase, a process that is inhibited by dutasteride. **(Bottom)** Androgens exert their effects on prostate cancer cells by binding and activating the androgen receptor (AR). MDV3100 and other antiandrogens competitively inhibit the binding of the AR by agonists. When bound to MDV3100, AR does not bind DNA required to activate transcription.

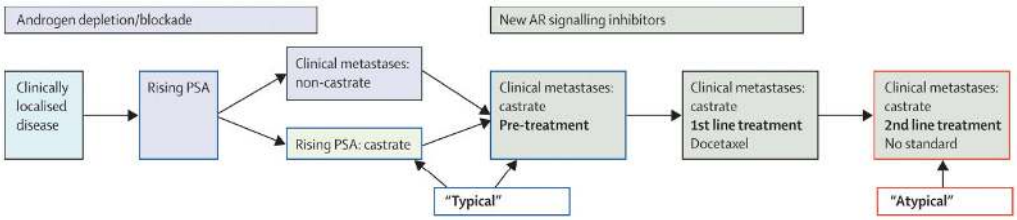

Figure 2. Clinical states where AR directed therapies are used

In patients with rising PSA or metastatic disease, initial treatment usually consists of ADT, usually by GnRH agonists. Antiandrogens can be added to ADT during initial treatment, often termed "Combined Androgen Blockade" or "Maximum Androgen Blockade". More commonly antiandrogens or ketoconazole is added when patients progress on ADT into CRPC ("TYPICAL"). Docetaxel is the only treatment shown to prolong survival in CRPC. After progression on doctaxel, there is no effective treatement. MDV3100 and abiraterone have shown promising activity in both chemotherapy-naïve "TYPICAL" and post-docetaxel "ATYPICAL" CRPC patients. Currently, both agents are in phase III trials of post-docetaxel patients randomized to placebo with overall survival as the primary endpoint.

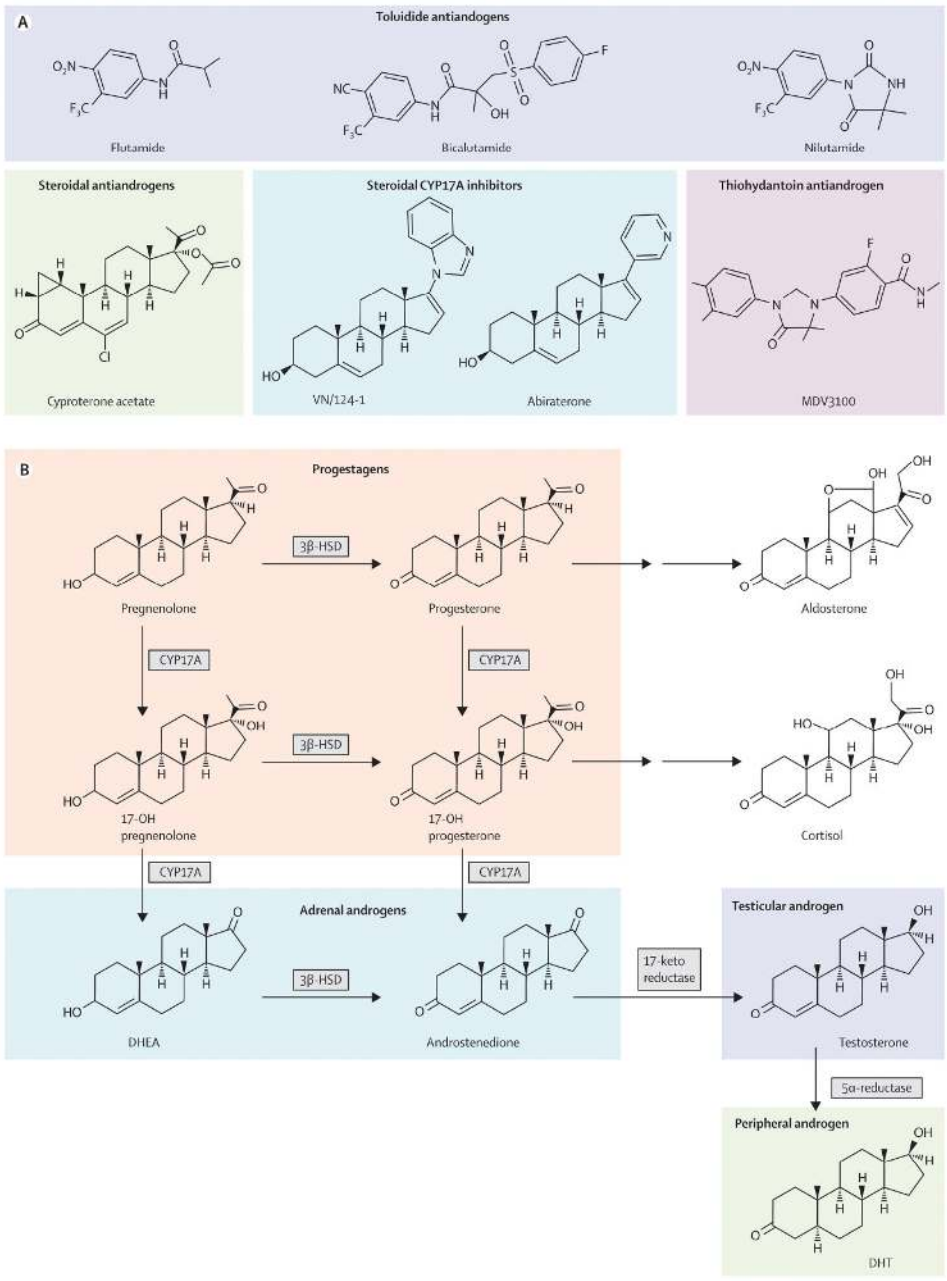

Figure 3. Chemical Structures of antiandrogens and CYP17 inhibitors

A) First and second generation non-steroidal antiandrogens as well as the novel compound BMS 641988 share a toliudide backbone with increasing bulk. MDV3100 is a non-steroidal antiandrogen with a thiohydantoin backbone. Cyproterone actetate (CPA), abiraterone, and VN/124-1 are all steroidal compounds similar to progesterone. CPA is an antiandrogen with partial agonist properties; abiraterone is an irreversible inhibitor of CYP17, and VN/124-1 both inhibits both CYP17 and binds AR and causes its degradation. B) The steroid synthesis pathway starts with the generation of pregnenolone from cholesterol. CYP17 and 3β-HSD are present in both adrenal glands and testes and generates weak androgens androsteinedione and DEAS. 17-ketoreductase in the testes further generates testosterone. In peripheral tissues, testosterone is converted to the more potent DHT by 5α-reductase. In CRPC, the enzymes involved in

androgen synthesis (CYP17, 3β-HSD, 17-ketoreductase, 5α-reductase) have been found to be cancer cells (4,7,40).

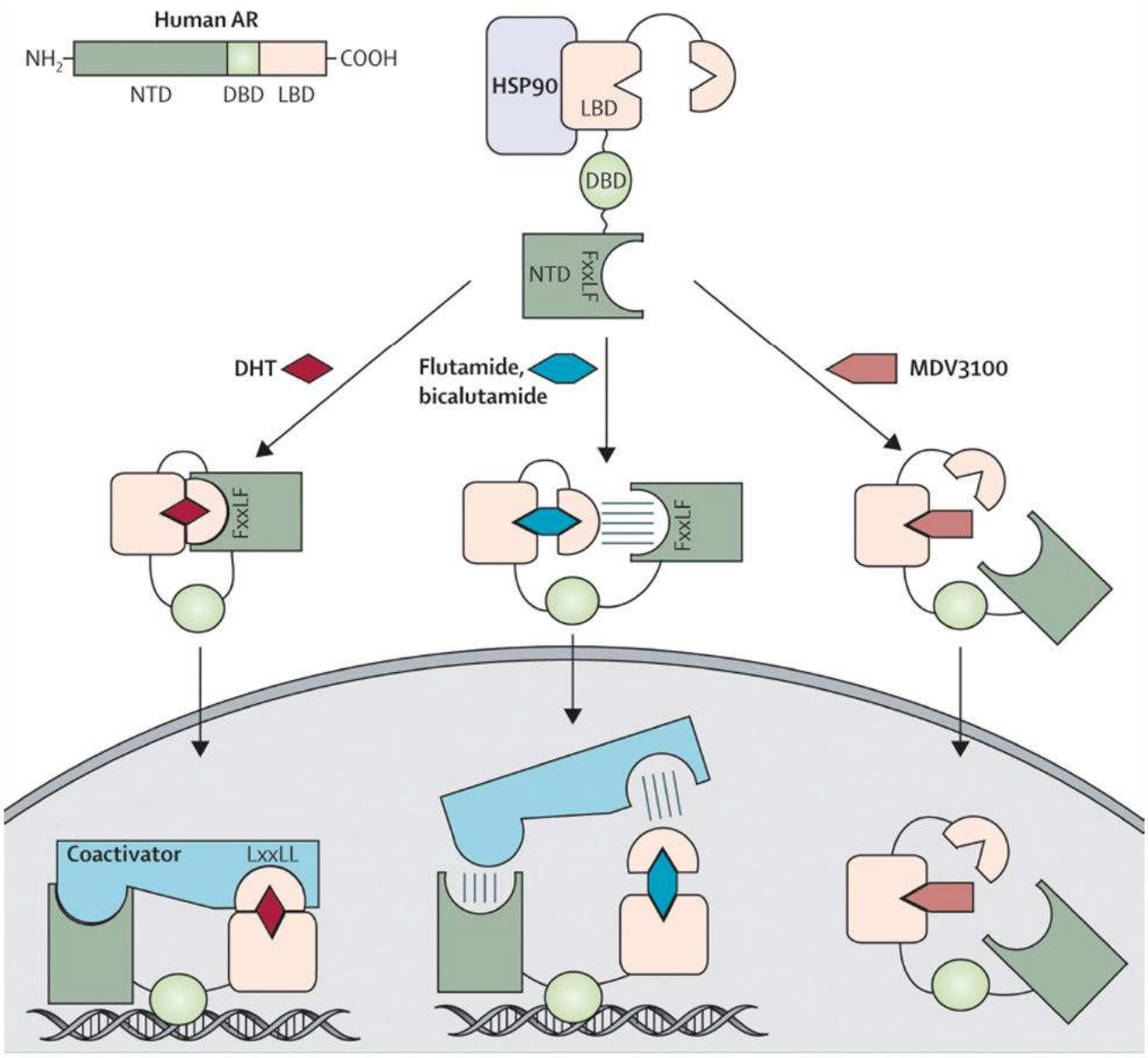

Figure 4. Schematic of AR activation and antiandrogen mechanism

AR consists of N-terminal domain with ligand-independent activation functions (NTD), a DNA binding domain (DBD), followed by ligand binding domain (LBD). HSP90 binds to LBD of Apo-AR and stabilizes the protein. Androgen binding causes dissociation from HSP90, conformation change in the LBD that allows intramolecular binding to the NTD and intermolecular binding to coactivators, nuclear translocation, and DNA binding. When bicalutamide and other available antiandrogens bind AR, nuclear translocation is preserved and DNA binding and coactivators recruitment occurs when AR is overexpressed. When MDV3100 binds AR, nuclear translocation is inefficient and DNA binding and coactivators recruitment is completely inhibited.

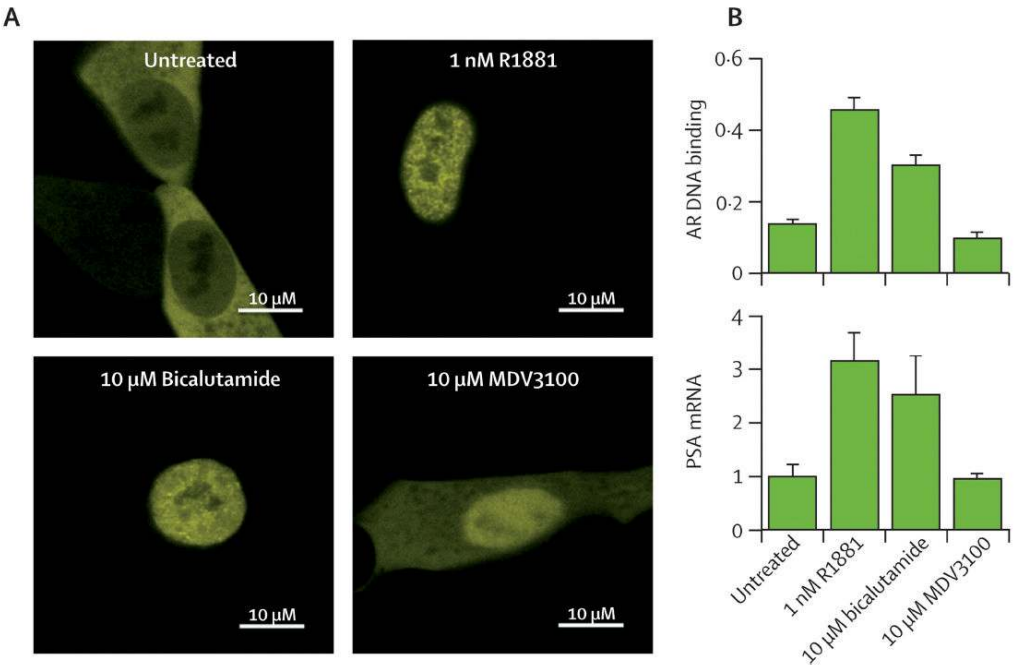

Figure 5. Activity of MDV3100

A) AR localizes in the cytoplasm in the absence of ligand and almost completely translocates to the nucleus when bound to the synthetic androgen R1881 or bicalutamide. When bound to MDV3100, nuclear translocation is incomplete (adopted from Tran et al, permission from Science).

B) In prostate cancer cells that overexpress AR, R1881 and bicalutamide stimulates AR binding to the PSA enhancer region PSA production whereas MDV3100 does not (adopted from Tran et al, permission from Science).

Table 1

Effect of DHT, bicalutamide, and MDV3100 on AR activities

	DHT	Bicalutamide		MDV3100	
		AR normal	AR overexpressed	AR normal	AR overexpressed
Target Gene Induction	++++	-	++	-	-
AR affinity	++++	++		+++	
AR nuclear translocation	++++	++++		++	
AR DNA binding	++++	++	+++	-	-
AR coactivator recruitment	++++	-	+++	-	-

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