Androgen Receptor Rediscovered: The New Biology and Targeting the Androgen Receptor Therapeutically

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ABSTRACT

Discoveries over the past decade suggest that castration-resistant prostate cancer (CRPC) is sensitive, but not resistant to, further manipulation of the androgen–androgen receptor (AR) axis. Several new therapies that target this axis have demonstrated clinical activity. In this article, preclinical and clinical findings occurring in the field of AR-targeted therapies are reviewed. Reviews of scientific and clinical development are divided into those occurring prereceptor (androgen production and conversion) and at the level of the receptor (AR aberrations and therapies targeting AR directly). Intracrine androgen production and AR amplification, among others, are among the principal aberrancies driving CRPC growth. Phase III data with abiraterone acetate and phase II data with MDV-3100, along with other similar therapies, confirm for the clinician that the scientific findings related to persistent AR signaling in a castrate milieu can be harnessed to produce significant clinical benefit for patients with the disease. Studies aimed at optimizing the timing of their use and exploring the mechanisms of resistance to these therapies are under way. The clinical success of therapies that directly target androgen synthesis as well as the most common aberrancies of the AR confirm that prostate cancer retains dependence on AR signaling, even in the castrate state.

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INTRODUCTION

Androgen stimulation is fundamental to prostate cancer growth and ultimately to its lethality. Interest in impeding androgen and androgen receptor (AR) interactions in the disease has been the foundation of its systemic treatment for more than six decades. Recently, however, interest has been accelerated by the development of active secondary hormonal therapies that are reaching the clinic concurrently with an understanding of the mechanisms of resistance to androgen deprivation therapy (ADT). These developments not only promise to change the standard therapies in use clinically but also are likely to enhance our perspective on the persistent role of androgens and the AR in advanced prostate cancer.

As conventionally described, ADT involves the use of leutinizing hormone–releasing hormone (LHRH) drugs (agonists or antagonists) or orchiectomy, consistently resulting in a 90% to 95% reduction in circulating levels of testosterone, as measured by commercially available immunoassays. Selection pressure induced by this treatment ultimately leads to the emergence of a tumor phenotype characterized by disease progression despite castrate levels of testosterone (typically defined as ≤ 50 ng/dL), which, coupled with metastatic spread, renders the disease lethal. Over the course of the past 5 to 8 years, the term castration-resistant prostate cancer (CRPC) has surpassed the use of the term hormone refractory or androgen independent. This is based predominantly on the implications of recent findings suggesting that advancing prostate cancer is not uniformly refractory to further hormonal manipulation and that androgens and the progression of disease are frequently dependent on—not independent of—androgen-AR interactions. Although the importance of treatment-mediated selection pressure has been appreciated for some time, it is unclear whether the emergence of the lethal phenotype is a function of ADT itself (ie, given time, all tumors will develop castration resistance) or a function of factors initiated at the time of carcinogenesis. Numerous studies demonstrate an advantage to early, as opposed to deferred, androgen deprivation, a topic that has been reviewed previously. However, the question of whether earlier-onset ADT, through this selection pressure, leads to earlier CRPC has not been fully addressed in clinical or experimental models. Suffice it to say, toxicities related to ADT (including cardiac, metabolic, and psychologic toxicities) and the hypothetical question of potentiating castration resistance underlie the argument against early and indiscriminate use of ADT in patients with
a low disease burden. Further clinical study of this topic is warranted, although it is challenged by the expense and length of study required to answer the question.

Interestingly, metastasis and CRPC growth may occur separately, resulting in the development of nonmetastatic CRPC, a clinical state that is now the target of a therapeutic approach. Because early data suggest that androgens can be detected in the microenvironment of metastatic tumors, it is provocative to consider that AR-targeted therapies initiated at the time of progression to nonmetastatic CRPC may be one strategy to prevent or delay the development of metastatic disease. Many such studies are under way.

The hypothesized dependence of prostate cancer on androgen stimulation for survival and proliferation even in the castrate state underpins the rationale for drug development strategies that target androgenic ligands as well as the AR itself. These two approaches form the centerpiece for the treatment of advanced disease. Myriad aberrations in these steps, from androgen synthesis to receptor binding on DNA, suggest mechanisms that contribute to disease progression in CRPC. For the purposes of this review aimed at focusing on these events, the events will be referred to as those that occur before ligand and receptor interaction (pre–receptor-ligand binding events), those that involve receptor activity itself (receptor events), and those that follow the binding of ligand to receptor (post–receptor-ligand binding events). We describe them sequentially in detail (Fig 1).

PRERECEPTOR-LIGAND BINDING EVENTS

Pre receptor events—those pertaining to the production, transport, and conversion of steroid ligand—have generated significant enthusiasm in recent years because of the development of inhibitors of enzymes critical for androgen synthesis. Critical pre receptor steps include the conversion of pregnenolone-like steroids into androgens, mediated largely by the CYP17 enzyme complex, and the conversion of testosterone and other steroids into 5α-dihydrotestosterone (DHT) by 5α-reductase. Both steps are implicated in the emergence of the resistant tumor, and both can be targeted by therapeutic intervention.

Although ADT is capable of inducing significant reductions in serum androgens, often below the limits of detection, this therapy does not uniformly lead to suppression of the entire androgen signal in target tissues. Demonstration that the expression of androgen-regulated genes is often retained in the prostate gland after ADT suggests that current therapies incompletely suppress testicular androgen production or, as has been recently described, that the prostate tissue itself is capable of androgen synthesis. Indeed, recent data have demonstrated that mRNA associated with androgen-converting enzymes as well as the AR itself is elevated in metastatic deposits taken from patients with CRPC. Taken together, these data suggest that even in the castrate state, the presence of androgen is clinically relevant and may be useful as a therapeutic target.

With the initiation of endocrine therapy for prostate cancer in the 1940s, it was assumed that all nontesticular androgens arose from the adrenal gland, and attempts at surgical adrenal ablation as a therapeutic strategy were investigated as clinical approaches, often with deleterious results. More recently, selective targeting of androgen synthesis, particularly dehydroepiandrosterone and androstenedione in nontesticular tissues, has been the focus of drug development. The clinical use of aminoglutethemide and, more importantly, ketoconazole and abiraterone has demonstrated that targeting androgen biosynthesis can lead to disease remissions, delays in progression of disease, and improvements in survival of patients. Ketoconazole inhibits the cholesterol side chain cleavage enzyme 11-beta hydroxylase as well as CYP17. Abiraterone is more specific to the CYP17 enzyme and is capable of irreversible CYP17 inhibition and thus more durable androgen suppression (Fig 2). Clinical studies with ketoconazole serve as important primers on the interaction of pharmacology and disease biology.

Early studies with ketoconazole demonstrated prostate-specific antigen (PSA) declines and clinical improvements in approximately 35% to 65% of patients. It was also discovered that high doses of...
Ketoconazole led to clinical adrenal insufficiency and that hydrocortisone supplementation was required to offset this. Subsequent work by the Cancer and Leukemia Group B demonstrated that approximately 35% of unselected patients experienced a 50% decline in PSA when treated with the combination of ketoconazole and hydrocortisone. This work further demonstrated that median levels of androgens (dehydroepiandrosterone sulfate (DHEAS), DHEA, and androstenedione) declined significantly with this therapy and that progression while receiving ketoconazole was associated with a rise in DHEAS and androstenedione but not testosterone levels.12 Further analysis suggested that higher levels of baseline adrenal androgens may be more predictive of response to this therapy and better overall survival.13 Taken together, these data suggest that in CRPC, a divergence of disease biology may occur in which certain patients develop CRPC and progression despite low levels of androgens (ie, true hormone-refractory prostate cancer), whereas others may develop a form of the disease that continues to rely to some extent on androgen-AR signaling and are thus amenable to therapies that target androgen synthesis or receptor activation.

Genetic variation across the spectrum of androgen production and conversion is likely to play a role in castration-resistant growth, as has been shown in castration-sensitive tumors.14 Among the expressed genes, CYP17 itself is worthy of review because of its dual links to prostate cancer epidemiology and to therapeutics under development. A series of studies have associated polymorphisms of CYP17 with risk of prostate cancer incidence and mortality. In a population-based study of prostate cancer incidence, Stanford et al15 demonstrated that men with a family history of prostate cancer who are homozygous for the A2 allele of CYP17 have a 19-fold increased risk of prostate cancer. Hamada et al16 demonstrated that the presence of a polymorphism in the promoter region of the CYP17 gene is associated with survival in patients with CRPC regardless of the therapy. In this analysis, median survival from time of diagnosis in patients with the variant allele (n = 126) was 8.9 years versus 6.7 years in patients with the A1 (reference) allele, an observation worthy of prospective validation.

Genetic lesions that lead to increased intracellular androgen production are diverse but are likely to involve amplification or polymorphisms in the production of the enzymes CYP17 and AKR1c3, the latter of which catalyzes the conversion of androstenedione to testosterone. Recognition of this phenomenon was delayed by a lack of

Fig 2. Androgen synthesis cascade and sites of inhibition of ketoconazole (keto) and abiraterone (abi). Abi selectively inhibits CYP17 and is a more potent inhibitor of CYP17 than keto. 3BHSD, 3-β-hydroxysteroid dehydrogenase; ACTH, adrenocorticotropic hormone; DHEA, dehydroepiandrosterone sulfate; DHT, 5α-dihydrotestosterone; DOC, deoxycorticosterone.

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Multiple investigators have demonstrated that prostate and metastatic tumor tissue from patients with CRPC contain higher levels of androgen than that in the serum, suggesting that the tumor could serve as a so-called androgen sink or that the tumor itself produces androgen. These tissues may also contain higher levels of type 1 5-alpha reductase, the enzyme critical to the conversion of testosterone to DHT, the ligand with the highest affinity for the AR. These findings change the perspective from what has long been considered a pure endocrine disease to one in which autocrine/paracrine must be considered and suggest a mechanism to explain why the postreceptor androgen signal is frequently active in neoplastic tissue after ADT. Thus, a common path in prereceptor androgen metabolism involves conversion of testosterone to DHT by 5-alpha reductase. This enzyme is the target of pharmacologic inhibition in patients with benign prostate hypertrophy. Aberrations in this pathway, occurring via upregulation of enzyme levels, may account for some of the effects in which prostate cancer is capable of growth in the androgen-deprived milieu.

Clinical Results: Next-Generation Androgen Synthesis Inhibitors

The limitations of ketoconazole (toxicity, duration of efficacy, and nondurable androgen suppression) and the desire to optimize ADT have motivated research toward the development of more potent and selective CYP17 inhibitors. Abiraterone is the result of testing ADT have motivated research toward the development of more potent and irreversible inhibition of CYP17,19-21 and suggest a mechanism to explain why the postreceptor androgen signal is frequently active in neoplastic tissue after ADT.

First-in-human studies include a series of three dose-escalation studies carried out in men with histologically confirmed prostate cancer, both castrate and noncastrate. Pharmacokinetic studies have suggested good bioavailability; mean elimination half-life of abiraterone in these studies was 27.6 hours, thus supporting the use of once-daily dosing. In the men with noncastrate testosterone levels, an LH surge occurred during therapy, presumably a physiologic response by the hypothalamic pituitary axis in response to lowered testosterone levels, which could be overcome with doses of abiraterone acetate of 500 mg/d or greater. In contrast, when an LHRH analog was used to suppress the LH surge, abiraterone resulted in more durable androgen suppression, thus forming the basis for combining abiraterone with LHRH therapies in the clinical setting.

Two parallel phase I dose-escalation studies were initiated in 2006 to evaluate abiraterone acetate as a novel secondary hormonal therapeutic in patients with CRPC (one conducted at the Royal Marsden Hospital in London, United Kingdom, and the other at the Dana-Farber Cancer Institute, Boston, MA, through the Department of Defense Prostate Cancer Clinical Trials Consortium). In each study, declines in PSA by 50% or more were observed in a majority of patients (51% and 55%, respectively), and no maximum-tolerated dose was reached. The toxicities observed in this study were predominantly the result of secondary mineralocorticoid excess (eg, hypertension, hypokalemia, and lower extremity edema), and no patient developed clinical adrenocortical insufficiency.22 However, on the basis of the development of mineralocorticoid-induced hypertension and hypokalemia, phase I investigators suggested that in subsequent studies abiraterone be administered in combination with prednisone 10 mg daily to suppress an adrenocorticotropic hormone surge that could occur in the context of a partially blocked adrenal gland. Moreover, the phase I studies demonstrated that nine (47%) of 19 patients who received prior ketoconazole therapy also responded to abiraterone, suggesting that this is a more potent therapy as well as the notion that certain patients are CYP17-pathway addicted. These data suggest that patients with prior ketoconazole exposure do not have an increased risk of toxicity to abiraterone and that a high proportion of these patients respond to this therapy.

Phase II data also support the high rate of response to abiraterone in both pre- and postchemotherapy settings. In turn, pivotal phase III studies have been launched in both of these settings as well. In one phase III study (COU-301), patients with disease progression after docetaxel-based therapy were randomly assigned to receive abiraterone plus prednisone or prednisone plus placebo, with treatment continuing until disease progression or death. The results from the study demonstrated a significant improvement in overall survival in favor of abiraterone from 10.9 to 14.8 months, with a hazard ratio of 0.646. The PSA response proportion to abiraterone/prednisone was 38% versus 10% in this study, and grade 3 to 4 adverse events leading to discontinuation occurred in 10% of those treated with abiraterone and 13% of those treated with placebo. A second phase III study is under way, exploring the effect of abiraterone versus placebo on overall and progression-free survival in patients not yet treated with chemotherapy.

The optimal timing of usage of abiraterone remains to be defined. Although approval by the US Food and Drug Administration will be based on the survival benefit seen in patients who received prior docetaxel chemotherapy, it is unclear whether this is the optimal pathophysiologic space for this therapy. Phase III data do confirm the activity of abiraterone and targeting of androgen synthesis in this setting. However, it is possible that in this more advanced setting, the disease is dependent on multiple aberrant pathways (AR mediated and non–AR mediated), suggesting that targeting CYP17 alone may only benefit a fraction of patients. In the COU-301 study, only 40% of patients experienced a decline in PSA of 50% or more, whereas in earlier states of CRPC, such as the nonmetastatic CRPC population as well as those individuals with metastatic who have not yet been treated with chemotherapy, the degree and duration of response may be greater. In the phase II setting of patients treated with abiraterone before chemotherapy, more than 60% to 70% of patients experienced a decline in PSA of 50% or more, and median progression-free survival (by PSA criteria) was 63 weeks, suggesting that more patients harbor disease that is more fully dependent on androgen production as the principal mode of progression. Data are lacking in patients with
earlier disease states (eg, nonmetastatic CRPC); however, these studies are of interest.

Although CYP17 inhibition results in a state of super castration by reducing testosterone and its precursors to nearly undetectable levels, it is unlikely that CYP17 inhibition will replace the widespread use of LHRH agonists for several reasons. One reason is that adherence cannot be assured with a daily oral agent, as it can be with the LHRH agonist, which is typically injected in a depot form every 3 or 4 months. Second, the need for prednisone complicates further the possibility of prolonged abiraterone therapy in the primary setting. Thus, next-generation CYP17 inhibitors—particularly those with more C17-20 lyase activity relative to inhibition of 17-hydroxylase, which do not require prednisone—are needed for long-term therapy. Finally, a trial designed to determine efficacy of CYP17 inhibitors as compared with LHRH agonists would require many years to complete, given the high response proportion to ADT, a task that might not be pursued either by industry or by cooperative group sponsors.

Marote et al25 found the incidence of breakthrough testosterone levels (>50 ng/dL) in as many as 25% of patients treated with LHRH agonists. This observation and others like it suggest that much is to be gained through the integration of a second-line ADT such as a CYP17 inhibitor. It also raises the question of whether a degree of the success of abiraterone, and drugs in this class, can be attributed to merely preventing such rises.

Another potential application of CYP17 inhibition may come with the inclusion of these drugs in patients treated with finite-duration ADT (eg, coadministration with radiation or as part of intermittent ADT regimen). Given that LHRH agonists fail to attain castrate levels of testosterone in a certain proportion of patients, it is reasonable to hypothesize that inclusion of CYP17 inhibition during the on phase of the intermittent blockade may enhance the clinical benefit of this approach.

Androgen deprivation, however achieved and to whatever degree, does not really constitute targeted cancer therapy; it is more correctly termed ligand-reduction therapy. Thus, standard practice in prostate cancer is for ADT to be continued even in patients with CRPC, the logic being that a return of stimulatory ligand after discontinuation of ADT would exacerbate tumor growth. However, it is reasonable to consider the continued use of abiraterone in patients with CRPC beyond the standard definition of progression, although the safety of the combined use of abiraterone plus subsequent therapy (eg, docetaxel and cabazetaxel) has yet to be determined.

Assuming regulatory approval of this therapy, consideration of the following treatment questions will be required in the postapproval setting. First, is the mechanism of resistance to abiraterone via AR- or non-AR–mediated mechanisms? Second, what is the effect of CYP17 inhibition on intracrine androgen production? Third, should this therapy be continued beyond the time of clinical progression, as is the case with LHRH-based androgen deprivation (and can it be combined with chemotherapy and other drugs)? Next-generation CYP17 inhibitors such as Tak-700 (Millenium Pharmaceuticals, Cambridge, MA/ Takeda, Osaka, Japan), which has little inhibition of 17-hydroxylase activity and therefore may not require concomitant steroid replacement, and Tok-001 (Tokai Pharmaceuticals, Cambridge, MA), which is a combined CYP17 and AR inhibitor, are in development. Tak-700 is currently being studied in a phase III trial and has demonstrated activity in a phase II study showing dose-dependent reductions in androgen levels and PSA declines of >50% in a majority of patients.25a

The enzyme 5-alpha reductase is required for the conversion of numerous androgen precursors to dihydrotestosterone, the androgen with the most potent direct agonist effect on the AR. Inhibitors of 5-alpha reductase are clinically available and widely used in the treatment of benign prostatic hypertrophy. The mechanism of action of these drugs lends itself to consideration as a component of androgen blockade in prostate cancer. Studies in nonmetastatic serologic relapse of prostate cancer suggest that these agents are capable of lowering PSA at standard doses, and in combination with AR inhibitors, these agents have demonstrated prolonged reductions in PSA. Dutasteride has also been added to ketoconazole in phase II studies.26 There are few definitive data, however, to support the use of these agents in a regimen for CRPC. Ongoing studies with dutasteride seek to determine whether this agent contributes to the management of CRPC. It is likely that doses above that used for benign prostatic hypertrophy will be required.

Taken together, these data paint a compelling portrait of androgen production playing a critical role in the growth of tumors in the castrate state. They suggest that targeting androgen production is a viable way of inhibiting disease progression.
the capability of androgen-independent growth, suggesting that this relationship is one shaped by a misallocation in the balance between AR coactivators and corepressors because of high levels of the receptor. Their work has also lead to a plausible biologic explanation for the antiandrogen withdrawal phenomenon, in which bicalutamide and related therapies are agonists in an amplified AR milieu where corepressors may be saturated, thus resulting in a net proactivator state.

Given these mechanistic considerations, two new antiandrogens, MDV3100 (Medivation, San Francisco, CA) and ARN-509 (Aragon, San Diego, CA), have been developed and are currently in clinical trials. MDV3100, which is a small-molecule antagonist of the AR, binds to the AR with significantly higher affinity than current antiandrogens, inhibits AR translocation to the nucleus, and blocks AR-DNA binding. The compound was selected from a screening process in which it was found to inhibit the growth of bicalutamide-resistant, AR-overexpressing LnCAP cells.34 MDV-3100 entered into phase I/II testing in July 2007. Of the 42 patients with progressive disease who were treated with MDV3100 in the phase I study, 55% experienced a reduction in PSA of more than 50%, with durable responses seen in a subset.35 This effect included 13 (52%) of 31 of patients who had received prior chemotherapy. The treatment was well tolerated in a majority of patients.35 However, a lingering concern with respect to highly potent direct AR antagonists is the possibility that they may lead to tonic-clonic seizure activity, as was witnessed in two of 140 patients in the phase I/II study (even without prior history of seizure); this could be a limiting factor in their development, as has been reported with similar agents in this class.36 The mechanism of action of these seizures is not well known, although it is related to CNS penetration of the drug and/or a drug-drug interaction. MDV-3100 is currently being tested in two phase III clinical trials in the pre- and postchemotherapy settings.34-36 Phase II studies with ARN-509 are currently being initiated, and preliminary efficacy is anticipated within the coming 1 to 2 years.

Expression profiling consistently shows that the expression of AR-regulated genes is a predominant feature of tumors that have survived or progressed during ADT. Thus, a formidable challenge to both the basic researcher and clinician remains the ability to interrogate a tumor to ascertain whether the AR signal is turned on. It is not clear whether selection of patients will be required or even necessary before the use of a highly potent AR inhibitor such as MDV-3100 or a ligand-depriving therapy such as abiraterone. A strategy aimed at optimizing the duration of exposure to such drugs may be best centered on determining the mechanisms of resistance to them, specifically whether AR-mediated mechanisms drive resistance to these therapies. MDV-3100 and related compounds have been developed to target tumors harboring an amplified AR. The emergence of resistance to this therapy may be the result of alternative signaling mechanisms or the emergence of tumors harboring mutated, not amplified, ARs.

**POSTRECEPTOR EVENTS: THE AR SIGNAL**

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Procurement and study of tumors resistant to these therapies will be necessary and should be prioritized. Optimally, pretherapy tissue collection and assessment of AR activity via transcriptional or other analysis, as has recently been proposed, may serve to identify and select patients for AR-targeted therapy.

Future studies will determine if highly potent AR-targeted therapy can replace castration-based therapy. Prior studies of ADT, with or without an oral antiandrogen, have not demonstrated consistent benefits from these agents in untreated androgen-dependent metastatic disease. Nevertheless, a meta-analysis of the bicalutamide in or without an oral antiandrogen, have not demonstrated consistent intraprostatic androgen concentrations after androgen withdrawal and treatment with ketoconazole and hydrocortisone in patients with advanced prostate carcinoma. Cancer 80:1755-1759, 1997

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Inhibitors and AR-targeted therapies may lead to alterations in the appearance of disease progression. Resistance to CYP17 inhibitors, for example, may occur downstream of CYP17 at the level of the receptor, and it is possible, even probable, that in certain patients, amplification of the AR will be part of the mechanism behind this resistance. Given this, combined therapy, particularly with abiraterone plus MD-3100 and/or related compounds, will be a particularly interesting area of study.

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