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Enzalutamide: Development from bench to bedside

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Abstract

Prostate tissue, whether benign or malignant, is heavily dependent on androgen receptor (AR) signaling for growth and proliferation. Androgen deprivation therapy has been standard of care for management of metastatic prostate cancer for the past 70 years. AR antagonists were developed to further abrogate signaling through this pathway by competitive inhibition of the receptor. First-generation compounds such as bicalutamide had modest efficacy, and in the setting of AR overexpression or specific mutations in the AR ligand–binding domain, these early compounds had partial agonist properties that could stimulate tumor growth. Enzalutamide was developed to overcome these deficiencies, and here, we present the story of its preclinical discovery, clinical development, and ultimate approval as a standard-of-care therapy for castration-resistant prostate cancer. Also discussed are ongoing efforts to elucidate mechanisms of resistance to this agent as well as studies that are investigating its role in other prostate cancer disease states and other cancer types.

Keywords: Enzalutamide; Prostate cancer; MDV3100; castration resistant prostate cancer; AR targeted therapies

Introduction

Prostate tissue, both benign and malignant, is heavily dependent on androgen receptor (AR) signaling for growth and proliferation [1]. The therapeutic benefit of targeting this pathway has been apparent since 1941, when Huggins and Hodges [2] reported that reducing androgen levels through surgical castration or exogenous estrogen administration decreased prostate cancer proliferation (as measured by serum acid phosphatase levels) and that exogenous testosterone increased its activity. Palliation of symptoms was also documented in the primary and distant sites [3]. Later, the demonstration in the 1980s that gonadotrophin-releasing hormone analogues produced a medical castration and allowed patients to avoid surgical orchiectomy, positioned these compounds as the first-line standard of care for the management of advanced disease: a strategy termed androgen deprivation therapy (ADT) [4].

The biology of prostate cancers progressing on ADT (i.e., castration-resistant prostate cancer [CRPC]) is notable for a number of features that contribute to continued AR signaling despite castrate levels of serum testosterone (<50 ng/dl). These tumors harbor amplification of the *AR* gene in 30% of cases and activating *AR* point mutations in others (the specific types and frequency vary across reports) [5,6]. AR protein is expressed at higher levels in CRPC relative to benign prostate tissue and treatment-naïve prostate cancer [7]. AR splice variants, truncated forms of the AR protein lacking the C-terminal ligand-binding domain, can also emerge, which can activate signaling in the absence of the ligand [8,9]. Furthermore, CRPC can evolve mechanisms that result in high intratumoral androgen levels despite serum levels in the castrate range. Contributing to the high levels are continued production of androgens in the adrenal glands, increased tumor uptake of available circulating androgens, and up-regulation of the androgen biosynthetic machinery in the tumor itself [10,11]. Reciprocal feedback between the AR and PI3K pathways, the latter altered in upwards of 70% of CRPC cases, also contributes to resistance [12,13]. These and other molecular alterations can sensitize the tumor to lower levels of circulating androgens or enable growth independent of them.

The result is that, in most cases, CRPC remains dependent on AR signaling. Clinical evidence of this includes the fact that the overwhelming majority of CRPCs continue to secrete

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prostate-specific antigen (PSA), an AR response gene, and that continuation of ADT in CRPC improves median overall survival by 2 to 6 months [14]. First-generation AR antagonists were developed to further abrogate signaling through this pathway by competitive inhibition of the AR molecule. Beginning in the 1970s, a series of compounds were brought to the clinic including cyproterone acetate, flutamide, bicalutamide, and nilutamide, each with activity as single agents in the non–castrate disease setting. Subsequently, each was evaluated in combination with standard ADT (orchiectomy or gonadotrophin-releasing hormone agonist/antagonist therapy) and ultimately shown to provide, at best, modest improvement in overall outcome relative to ADT alone [15]. Their effects in CRPC were also modest, with $\geq$50% PSA decline seen in only 25% of cases and few radiographic tumor regressions [16–18]. Nevertheless, these agents provided further proof that AR signaling could be targeted in CRPC, although more potent molecules were needed. An additional concern was that, over time, these agents could become agonists, as evidenced by the observation of withdrawal responses when they were discontinued [19]. This was subsequently shown in laboratory models to be due, in some cases, to mutations in the ligand binding domain of the AR or, in other cases, agonist action of the drugs in the setting of AR protein overexpression [20]. The focus of clinical investigation then shifted toward the development of cytotoxic agents, and in 1996, the first cytotoxic—mitoxantrone—was shown to provide palliation of symptoms in CRPC and was FDA approved [21]. This was followed by the tubulin targeting agent docetaxel in 2004 based on a survival benefit in two landmark phase 3 trials [22,23]. With this approval, drug development efforts focused on the pre-chemotherapy space, post-chemotherapy space, or on combining new drugs with docetaxel seeking to improve on the activity of the single agent. Studies of AR directed therapies in CRPC were limited due to the central belief, reinforced by nomenclature, that these tumors were "hormone refractory" and the role for "hormonal agents" in this disease state was limited at best. Against this view, in addition to the clinical findings, were the results of molecular profiling studies showing that in many CRPC cases the AR was overexpressed at the mRNA and protein levels, and that the androgen biosynthetic machinery itself was upregulated leading to increased intratumoral androgens. As a result interest remained in identifying new and more potent AR signaling blockade strategies and in particular next generation anti-androgens with greater AR binding affinity and without agonist effects in tumors that overexpressed AR [20].

Enzalutamide discovery and development

Preclinical discovery

A mechanistic approach to developing a next generation AR directed therapy in CRPC began with an experiment in which 7 matched isogenic castration-sensitive and castration-resistant prostate cancer cell lines were profiled to identify differences in gene expression [20]. The result was that *AR* was the only gene consistently overexpressed in all 7 lines. Subsequently, Jung and Sawyers used derivatives of the nonsteroidal thiohydantoin AR agonist RU59063, selected for its high affinity and selectivity for AR over other steroid receptors, in a screen for activity against LNCaP-AR cells with overexpressed AR protein [24]. Nearly 200 derivatives of the compound were evaluated for their ability to inhibit growth and PSA secretion, of which RD162, later modified to become MDV3100 (now enzalutamide), was chosen for further study based on oral bioavailability and longer serum half-life. Both compounds produced tumor regressions in xenograft models of prostate cancer with overpressed AR, a model in which bicalutamide showed little or no antitumor activity and in some cases showed agonist effects. Mechanistically, both enzalutamide and bicalutamide bind AR at its ligand-binding domain, however, enzalutamide has 4-fold greater binding affinity than bicalutamide and, unique from bicalutamide, inhibits AR translocation to the nucleus and inhibits binding of the ligand-bound receptor complex to DNA (Fig. 1) [24]. Enzalutamide is also active against prostate cancer cell lines bearing the W741C *AR* point mutation that is known to confer resistance to bicalutamide [24].

Clinical testing

Phase I/II clinical trial

The first in-human study of enzalutamide enrolled 140 men with metastatic CRPC across 5 US centers from July 2007 to December 2008 [25]. Importantly, this and subsequent trials incorporated the Prostate Cancer Working Group 2 (PCWG2) recommendations for evaluating systemic treatment approaches in CRPC [26]. The recommendations are based on several key principles: abandon grouped categorizations of response that consider all sites of disease together in favor of reporting outcomes for each manifestation of disease (e.g., changes in PSA levels, osseous disease and soft tissue disease - nodal or visceral) independently; when evaluating bone scans, interpret apparent worsening with new lesions on a first follow-up scan carefully by requiring the documentation of new lesions on a second follow-up before considering a patient to have progressed (in the absence of other signs of progression); ensure a drug is no longer working before stopping therapy; and continue treatment despite signs of progression (e.g., slow rises in PSA levels) that are not clinically meaningful.

The trial was initially designed as a single-arm phase I study to assess safety, tolerability, and maximum-tolerated dose using a 3 + 3 rule. However, when PSA level declines were observed in all the first 6 patients, the trial was modified and expanded to include 12 pre- and 12 post-chemotherapy patients per dose level to enable an assessment of treatment efficacy. Doses ranging from 30 to