H2AX activation and H2AX-mediated angiogenesis will aid in the development of novel angiogenic inhibitors.

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# Development of Androgen Receptor Antagonists with Promising Activity in Castration-Resistant Prostate Cancer

**Howard C. Shen**[1] **and Steven P. Balk**[1,*]
[1]Hematology-Oncology Division, Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA 02115, USA
*Correspondence: sbalk@bidmc.harvard.edu
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**Androgen receptor (AR) continues to play a central role in prostate cancers that relapse after androgen deprivation therapy, but these tumors are refractory to available AR antagonists. In a recent issue of *Science*, Tran et al. describe an antagonist that prevents AR recruitment to chromatin and shows efficacy in relapsed prostate cancer.**

Androgen receptor (AR) plays a central role in prostate cancer (PC) development and progression. The unliganded AR is inactive and associates with an Hsp90 chaperone complex, which maintains AR in a conformation competent to bind androgen (testosterone or the higher-affinity dihydrotestosterone, DHT). Androgen binding moves helix 12 in the ligand-binding domain (LBD) to the proximity of helices 3, 4, and 5 and generates a hydrophobic cleft that initially binds a peptide sequence (FQNLF) in the AR N terminus (Figure 1). AR subsequently homodimerizes and binds to androgen-responsive elements (AREs) in androgen-regulated genes, where it initiates recruitment of multiple transcriptional coactivator proteins that interact both with the N-terminal domain and the hydrophobic cleft generated by helices 3, 4, 5, and 12. This latter interaction is mediated by the LxxLL motif found in many coactivator proteins, which presumably displace the FQNLF peptide. AR antagonists currently in clinical use (bicalutamide, flutamide, and nilutamide) compete with androgens for the ligand-binding pocket but displace helix 12 and prevent formation of the coactivator-binding cleft. Significantly, these antagonist-liganded ARs still weakly bind to AREs but fail to effectively recruit coactivator proteins via the LxxLL motif (Masiello et al., 2002). Moreover, they may have enhanced recruitment of corepressor proteins (NCoR and SMRT), which contain extended LxxLL-like motifs (CoRNR boxes) that interact with helices 3, 4, and 5.

Suppression of testicular androgen production by surgical castration or administration of LHRH agonists (medical castration), termed androgen deprivation therapy (ADT), has been the standard systemic treatment for recurrent and/or metastatic PC since 1941. Unfortunately, though most patients initially respond, they invariably relapse with aggressive PC that has been termed hormone-refractory, androgen-independent, or castration-resistant/recurrent PC (CRPC). Subsequent studies indicated that these tumors were still, to some extent, androgen responsive, as further suppression of residual androgen by adrenalectomy or hypophysectomy led to objective responses (tumor shrinkage) in about one-third of CRPC patients and symptomatic improvement in the majority of patients (Mahoney and Harrison, 1972). These responses suggested that more complete AR blockade by castration in combination with AR antagonists (combined androgen blockade, CAB) may be more effective than castration alone and led to a series of clinical trials that combined castration with available AR antagonists, but these showed only a very small survival advantage over castration monotherapy.

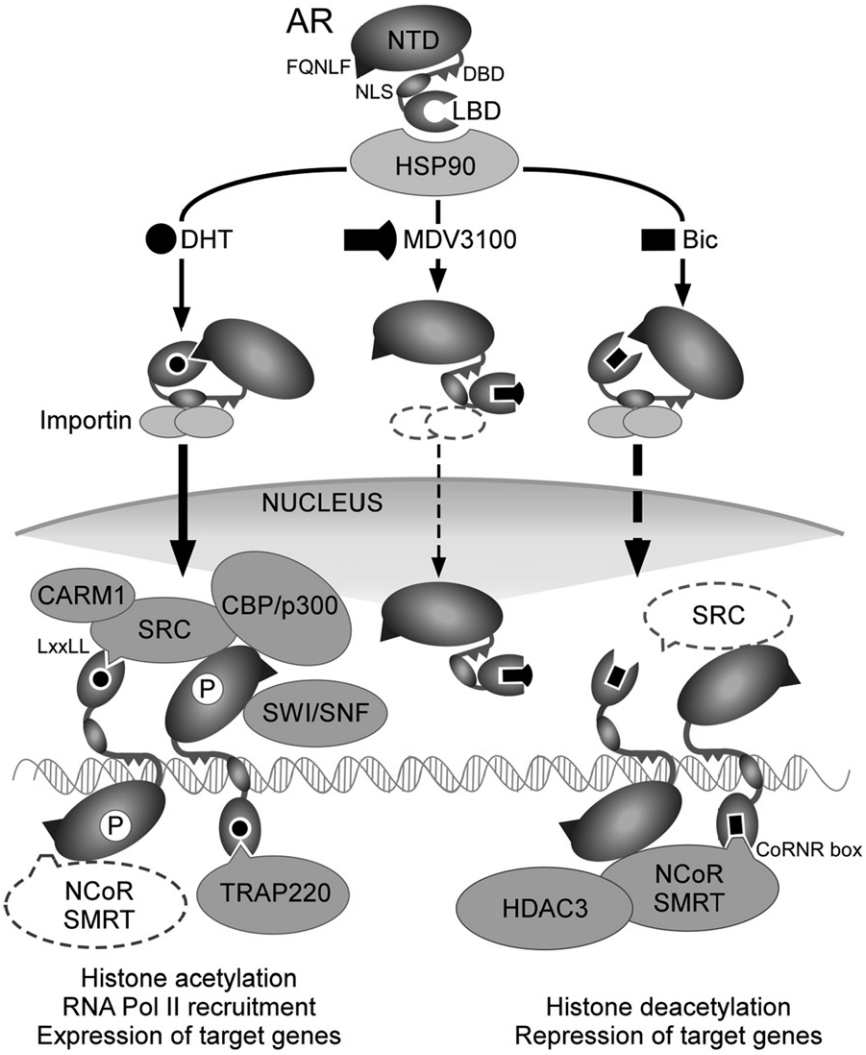


**Figure 1. Agonist and Antagonist Modulation of AR Transcriptional Activity**

Unliganded AR, comprised of an N-terminal transactivation domain (NTD), a central DNA-binding domain (DBD), a hinge region containing a nuclear localization signal (NLS), and a C-terminal ligand-binding domain (LBD), associates with an HSP90 chaperone complex. Binding of agonist (DHT) alters LBD structure to form the coactivator-binding site, which initially binds an FQNLF peptide in the NTD (represented by triangle). The resulting intramolecular N/C-terminal interaction may function to expose the NLS and enhance AR nuclear translocation, chromatin binding, and/or the initial recruitment of coactivator/chromatin-modifying proteins by the NTD. Direct interaction between closely positioned DBDs and possibly intermolecular N/C interactions (not shown) stabilizes the AR homodimer on DNA. The LBD and NTD then cooperatively recruit transcriptional coactivators such as TRAP220 and steroid receptor coactivators (SRC) that interact with the LBD coactivator-binding site through LxxLL motifs (displacing the NTD FQNLF peptide). SRC recruitment of the SWI/SNF complex, histone acetyltransferases (CBP/p300), protein methyltransferases (CARM1), and additional factors results in the AR-targeted relaxation of chromatin and propagation of a transcriptionally active gene locus. AR antagonists such as bicalutamide (Bic) still enhance nuclear translocation and chromatin binding but fail to induce optimal LBD helix 12 repositioning for generation of the coactivator-binding site, resulting in an AR that lacks transcriptional activity due to ineffective coactivator recruitment and enhanced recruitment of corepressors (NCoR and SMRT) that bind weakly to the NTD and are stabilized by binding of extended LxxLL-like motifs (CoRNR boxes) to the LBD. However, in CRPC, high-level AR expression and/or other mechanisms may enhance the recruitment of coactivators versus corepressors, resulting in agonist activity. In contrast to bicalutamide, MDV3100 more effectively impairs nuclear translocation and appears to completely prevent chromatin binding, which may reflect further displacement of helix 12 and abrogation of the FQNLF/LxxLL coactivator-binding site.

Interest in the AR as a therapeutic target in CRPC subsequently waned due to the failure of these CAB trials and due to modest responses to even high-dose AR antagonists in patients who relapsed after castration monotherapy. Nonetheless, immunohistochemical studies in the early 1990s showed that AR protein was highly expressed in CRPC, and a correlation between CRPC and increasing levels of serum prostate-specific antigen (PSA, encoded by the AR-regulated *KLK3* gene), a marker for AR activity, indicated that AR transcriptional activity was reactivated in CRPC. Moreover, *AR* gene amplification in about one-third of CRPC patients (Visakorpi et al., 1995) and emergence of mutant ARs that were strongly stimulated by AR antagonists in antagonist-treated patients (Taplin et al., 1995) indicated that there was strong selective pressure to retain AR activity in CRPC. Studies in PC xenograft models similarly showed that AR was active in tumors that recurred after castration, and AR depletion studies in CRPC-derived cell lines confirmed that AR was required for tumor growth (Zegarra-Moro et al., 2002). More recent studies indicated that increased intratumoral androgen synthesis is a mechanism for AR reactivation in CRPC (Mohler et al., 2004; Stanbrough et al., 2006; Montgomery et al., 2008), and the efficacy of abiraterone, a selective inhibitor of CYP17 that markedly decreases residual androgen synthesis, now in phase III clinical trials, has refocused interest on AR in CRPC.

Although it now appears clear that androgens are contributing to AR activation in CRPC, it has been unclear why available AR antagonists that can effectively block AR in patients prior to castration are relatively ineffective in CRPC. Sawyers and colleagues have addressed this issue with a report on a novel AR antagonist, MDV3100, and its promising activity in CRPC (Tran et al., 2009). In a previous study, the Sawyers lab found that overexpression of exogenous AR alone could render PC xenografts resistant to castration and to bicalutamide, which gained partial agonist activity in AR-overexpressing cells (Chen et al., 2004). Based on these observations, derivatives of the nonsteroidal AR antagonist RU59063 were screened for antagonist activity and lack of agonist activity in LNCaP PC cells overexpressing wild-type AR (LNCaP/AR cells). Subsequent modifications for improved pharmacokinetics led to RD162 and MDV3100. Both drugs bound AR with 5- to 8-fold greater affinity than bicalutamide and, in contrast to bicalutamide, lacked agonist activity in LNCaP/AR cells and could suppress

the growth of VCaP PC cells, which have an amplified wild-type AR. Moreover, RD162 induced regression of LNCaP/AR xenografts in castrated mice, whereas bicalutamide at the same dose (yielding ~2-fold higher serum concentrations) was much less effective, although it suppressed growth relative to vehicle control in most xenografts. Importantly, results from an ongoing phase I/II clinical trial of daily oral MDV3100 showed that 22 out of 30 CRPC patients had declines in serum PSA levels, with > 50% declines in 13 patients, substantially greater than previous results with other antagonists.

Though higher affinity for AR may contribute to this in vivo efficacy, Tran et al. further show that RD162/MDV3100, in contrast to bicalutamide, only weakly stimulates AR nuclear translocation and fails to stimulate AR binding to chromatin (Tran et al., 2009). Finally, FRET experiments showed that the RD162/MDV3100-liganded AR was unable to bind an FxxLF motif-containing peptide, whereas weak binding could be induced by bicalutamide. Taken together, these results indicate that the efficacy of RD162/MDV3100 reflects a unique antagonist conformation of the AR LBD, presumably with further displacement of helix 12, which more effectively prevents protein:protein (or protein:DNA) interactions required for chromatin binding and subsequent enhancement of agonist activity in CRPC.

As with any major advance, these results raise new questions. Most important is, of course, the molecular basis for MDV3100 resistance/relapse, which appears to reflect continued AR activity based on rising serum PSA. Interestingly, PET scanning using 16β-[$^{18}$F]fluoro-5α-DHT indicates that MDV3100 is still effectively blocking ligand binding in these patients, but the mechanistic implications of this finding are not yet clear. Additional questions include the molecular basis for bicalutamide's agonist activity in cells overexpressing AR and whether this agonist activity is the basis for its lack of efficacy in CRPC. Though a small fraction of patients who are treated long term with an AR antagonist respond to discontinuation of the drug (antiandrogen withdrawal response), bicalutamide does not stimulate tumor growth as assessed by serum PSA when given to patients who have relapsed after castration (although this could reflect precise balancing of a mixed agonist/antagonist response). Further questions ask how the ligand-induced conformation of LBD dictates nuclear accumulation and chromatin binding of AR and whether this correlates with (or is dependent upon) AR LBD binding to the N-terminal FQNLF peptide or other LxxLL motif peptides. In any case, this work has provided critical new insights into the molecular basis of AR antagonist resistance in CRPC and has established that efficacious antagonists with novel mechanisms of action can be developed, which will hopefully provide agents to more effectively treat and possibly prevent CRPC.

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