Prostate cancers become “addicted” to male hormones during the pathogenesis of the disease. In doing so, the cancer cells co-opt androgen receptor (AR) signaling, a driver of secretory cell differentiation in normal prostate cells, for maintenance of a malignant phenotype. Somatic chromosomal translocations and deletions, creating fusions between androgen-regulated differentiation genes and cancer genes, may enable this addiction (1). For more than 70 years, androgen signaling has been targeted for prostate cancer treatment. Initially accomplished via removal of the testes, therapeutic reduction in circulating androgens in men with advanced prostate cancer almost always leads to improvement in disease-related symptoms, to diminution in blood biomarkers of disease activity, and to improvement in radiographic images of disease sites. Unfortunately, for most men, this benefit is short-lived. The disease inevitably progresses despite low levels of circulating androgens to “castration-resistant prostate cancer” (CRPC). CRPC cells often remain addicted to AR signaling, fomenting a recent flurry of new drug discovery and development, already yielding two new approved agents targeting androgen action: the androgen biosynthesis inhibitor abiraterone and the AR antagonist enzalutamide (2).

In the current issue of Cancer Discovery, both Joseph and colleagues (3) and Korpahl and colleagues (4) report the detection of a missense mutation generating an F876L change in the ligand binding domain (LBD) of the androgen binding protein (ABP) in the LNCaP prostate cancer cell line and of an LNCaP/ABP cell subline engineered to overexpress AR via chronic exposure to the second-generation antiandrogens androgens to “castration-resistant prostate cancer” (CRPC). CRPC cells often remain addicted to AR signaling, fomenting a recent flurry of new drug discovery and development, already yielding two new approved agents targeting androgen action: the androgen biosynthesis inhibitor abiraterone and the AR antagonist enzalutamide (2).

In the current issue of Cancer Discovery, both Joseph and colleagues (3) and Korpahl and colleagues (4) report the detection of a missense AR mutation, leading to an amino acid substitution (F876L) in the ligand binding domain (LBD), that conferred resistance to enzalutamide. A similar finding has also been described by Balbas and colleagues (5). AR is a ligand-activated transcription factor normally responsive to testosterone and dihydrotestosterone. AR mutations had been previously described as emerging in response to prostate cancer treatment with “first-generation” receptor antagonists, including flutamide and bicalutamide, resulting in changes in the LBD such that the ligand specificity for AR transcriptional activation was broadened, even to include the receptor antagonists themselves (6). Such mutations may have accounted for some cases of “antiandrogen withdrawal” syndrome, where men with progressive prostate cancer despite receptor antagonist treatment seemed to benefit from cessation of therapy (7). However, these mutations did not explain the majority of CRPC cases. Rather, increased AR expression levels, sometimes associated with AR amplification, were found to heighten ligand sensitivity and increase ligand promiscuity to drive CRPC progression (8). This CRPC phenotype motivated the pursuit of “second-generation” AR antagonists, such as enzalutamide and ARN-509, identified using screens for AR inhibition despite high-level AR expression.

To explore mechanisms of resistance to enzalutamide and ARN-509, Joseph and colleagues (3) selected variants of the LNCaP prostate cancer cell line and of an LNCaP/AR cell subline engineered to overexpress AR via chronic exposure to the second-generation antiandrogens in vitro. In three of 10 resistant variant sublines, both enzalutamide and ARN-509 exhibited partial AR agonist activity, stimulating both cell proliferation and target gene expression. AR sequencing revealed a missense mutation generating an F876L change in the LBD in each of these sublines. AR F876L bound enzalutamide and ARN-509 with 48-fold and 30-fold greater affinity than wild-type AR. Forced expression of this AR mutant in LNCaP cells was sufficient to confer agonist activity to the second-generation AR antagonists in vitro and in vivo, likely by permitting a homodimeric association of one N-terminus with helix 12 at the other C-terminus known to form an agonist conformation at AR DNA-binding sites.

Joseph and colleagues (3) then analyzed plasma DNA from a phase I clinical trial of ARN-509 for metastatic CRPC. Although more than 40% of men receiving ARN-509 showed declines in serum prostate-specific antigen (PSA), indicating response to treatment, of 29 men available for molecular analysis, 18 ultimately exhibited PSA increases, hinting at intrinsic or acquired resistance to the drug. Using a PCR-based BEAMing (Beads, Emulsions, Amplifications, and Magets) method to detect F876L-encoding mutant AR variants, mutant AR sequences (C to A change at nucleotide 2628) were found in plasma.
Mechanisms of resistance to AR-directed therapies. Despite treatment with androgen-deprivation therapy (ADT) or first-generation antiandrogens, prostate cancers progress to castration resistance, often with emergence of an AR-dependent resistant phenotype that is sensitive to treatment with second-generation antiandrogens such as enzalutamide or ARN-509. However, AR-dependent resistance emerges again, this time driven by mutant AR. This tendency to maintain AR addiction will permit treatment with next-generation AR antagonists. If prostate cancer clones that have escaped AR addiction appear at any time during disease progression, such treatments will prove ineffective.

**Figure 1.** Mechanisms of resistance to AR-directed therapies. Despite treatment with androgen-deprivation therapy (ADT) or first-generation antiandrogens, prostate cancers progress to castration resistance, often with emergence of an AR-dependent resistant phenotype that is sensitive to treatment with second-generation antiandrogens such as enzalutamide or ARN-509. However, AR-dependent resistance emerges again, this time driven by mutant AR. This tendency to maintain AR addiction will permit treatment with next-generation AR antagonists. If prostate cancer clones that have escaped AR addiction appear at any time during disease progression, such treatments will prove ineffective.
DNA from three of the men with progressive cancer despite ARN-509 treatment, whereas no such variants were present in any of the men before treatment (3). When this association of AR\(_{F876L}\) with prostate cancer progression despite ARN-509 treatment was considered in the context of the agonist activity of ARN-509 in prostate cancer cells expressing AR\(_{F876L}\), a compelling case for AR\(_{F876L}\) mediating clinical resistance to second-generation antiandrogens could be made.

Using a similar approach, Korpal and colleagues (4) also generated LNCaP variant sublines using prolonged exposure to enzalutamide \textit{in vitro}, isolating four sublines exhibiting resistance to the drug. For these sublines, enzalutamide was unable to prevent AR trafficking to the cell nucleus or to abolish expression of AR-regulated genes. Whole-transcriptome sequencing disclosed an F876L-encoding AR mutation in each subline, and transient transfection of cDNA for AR\(_{F876L}\) and AR-dependent reporter constructs showed a switch from antagonist to agonist activity upon exposure to enzalutamide. Predictably, three of four LNCaP tumor xenographs with acquired resistance to enzalutamide also showed AR\(_{F876L}\) expression. Forced stable expression of AR\(_{F876L}\) conferred enzalutamide-resistant growth to LNCaP, VCaP, and Myc-CaP cells \textit{in vitro}. Curiously, LNCaP variant sublines carrying AR\(_{F876L}\) grew poorly, if at all, as xenograft tumors in castrated mice \textit{in vivo}. The growth of such xenograft tumors was nonetheless stimulated by enzalutamide. Examining gene expression data for enzalutamide-resistant sublines, Korpal and colleagues (4) speculated that persistent expression of “cell cycle” and “E2F1 activation” gene sets might nominate CDK4/cyclin D1 assembly as a candidate therapeutic target for prostate cancers progressing despite second-generation antiandrogen treatment. In support of this notion, the enzalutamide-resistant LNCaP sublines appeared sensitive to the CDK4/6 inhibitors LEE011 and PD033299.

The consistent finding of AR\(_{F876L}\) in LNCaP sublines selected for resistance to second-generation antiandrogens in both reports, along with the propensity for AR\(_{F876L}\) to mediate agonist responses to enzalutamide and ARN-509, strongly indicates this mutant receptor as a likely mediator of clinical resistance to this class of drugs. The detection of mutations encoding AR\(_{F876L}\) in men progressing despite treatment with ARN-509 further supports this contention. Ready emergence of treatment resistance has long bedeviled inhibitory or toxic therapy of microorganisms and of human cancers. Luria and Delbrück (9) distinguished between spontaneous and induced mutations as a source of resistance using fluctuation analysis to study phage lysis of bacteria, a formalism recapitulated for cancer by Goldie and Coldman (10). For most acquired antineoplastic drug resistance, spontaneous mutation seems to account for gen- resistance. However, most men with CRPC have not been exposed to such drugs. Of interest, to activate transcription of target genes, AR recruits TOP2B to its DNA binding sequences, increasing chromosomal translocations triggered by TOP2B double-strand breaks (13). Although AR-induced translocations could conceivably drive AR amplification, this mechanism seems unlikely to generate missense mutant AR forms like AR\(_{F876L}\).

Often new mutations in critical genes subject to selective pressures arise at a significant fitness cost. As an example, although mutant forms of the BCR–ABL fusion gene product can mediate resistance to imatinib in chronic myelogenous leukemia (CML), when imatinib is discontinued, imatinib-sensitive CML cells can reappear (14). Like BCR–ABL for CML, prostate cancer cells exhibit marked addiction to AR. The emergence of CRPC cells with AR\(_{F876L}\) during second-generation antiandrogen treatment underscores this addiction. Nonetheless, the poor growth of AR\(_{F876L}\) expressing cells as xenograft tumors in castrate mice seen by Korpal and colleagues (4) hints at a potential fitness cost of the mutation. If this finding anticipates the clinical behavior of CRPC treated with second-generation antiandrogens, then discontinuation of treatment might lead to a “second-generation antiandrogen withdrawal” syndrome. Of note, using a similar approach, Balbas and colleagues (5) did not observe attenuated growth of AR\(_{F876L}\) prostate cancer cells in castrate mice. Instead, Balbas and colleagues (5) used a combination of molecular modeling, medicinal chemistry, and cell-based screening to define pharmacophores for “next-generation antiandrogens” capable of antagonizing AR\(_{F876L}\) function. The promising drug candidates that have been identified by this approach suggest that as long as prostate cancer cells remain addicted to AR signaling, AR can be therapeutically targeted (Fig. 1). The more concerning clinical challenge on the horizon will be the tendency for CRPC to end its AR addiction, adopting a more neuroendocrine phenotype unresponsive to AR signaling disruptors, a condition presenting few attractive treatment options (Fig. 1; ref. 15).

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

Published online September 9, 2013.

**REFERENCES**

# Resistance Emerges to Second-Generation Antiandrogens in Prostate Cancer

William G. Nelson and Srinivasan Yegnasubramanian


<table>
<thead>
<tr>
<th>Updated version</th>
<th>Access the most recent version of this article at: <a href="http://cancerdiscovery.aacrjournals.org/content/3/9/971">http://cancerdiscovery.aacrjournals.org/content/3/9/971</a></th>
</tr>
</thead>
</table>

### Cited articles

This article cites 15 articles, 11 of which you can access for free at: [http://cancerdiscovery.aacrjournals.org/content/3/9/971.full.html#ref-list-1](http://cancerdiscovery.aacrjournals.org/content/3/9/971.full.html#ref-list-1)

### Citing articles

This article has been cited by 3 HighWire-hosted articles. Access the articles at: [http://cancerdiscovery.aacrjournals.org/content/3/9/971.full.html#related-urls](http://cancerdiscovery.aacrjournals.org/content/3/9/971.full.html#related-urls)

### E-mail alerts

Sign up to receive free email-alerts related to this article or journal.

### Reprints and Subscriptions

To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

### Permissions

To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.