Resistance Emerges to Second-Generation Antiandrogens in Prostate Cancer

William G. Nelson and Srinivasan Yegnasubramanian

Summary: The appearance of a mutant androgen receptor, ARF876L, in prostate cancer cells chronically exposed to enzalutamide or ARN-509 promotes a switch from antagonist to agonist receptor function, undermining the potential long-term effectiveness of these second-generation antiandrogen drugs. Cancer Discov; 3(9): 971–4. ©2013 AACR.

See related article by Joseph et al. p. 1020 (3).
See related article by Karpal et al., p. 1030 (4).

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 this addiction (1). For more than 70 years, androgen signaling has been targeted for prostate cancer treatment. Initially this addiction (1). For more than 70 years, androgen signaling has been targeted for prostate cancer treatment. Initially this addiction (1).

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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 ARF876L 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 ARF876L, a compelling case for ARF876L 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 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 ARF876L and AR-dependent reporter constructs showed a switch from antagonist to agonist activity upon exposure to enzalutamide. Predictably, three of four LNCaP tumor xenografts with acquired resistance to enzalutamide also showed ARF876L expression. Forced stable expression of ARF876L conferred enzalutamide-resistant growth to LNCaP, VCaP, and Myc-CaP cells in vitro. Curiously, LNCaP variant sublines carrying ARF876L grew poorly, if at all, as xenograft tumors in castrated mice 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 ARF876L in LNCaP sublines selected for resistance to second-generation antiandrogens in both reports, along with the propensity for ARF876L to mediate agonist responses to enzalutamide and ARN-509, strongly inducts this mutant receptor as a likely mediator of clinical resistance to this class of drugs. The detection of mutations encoding ARF876L 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 Crawford (10). For most acquired antineoplastic drug resistance, spontaneous mutation seems to account for generating variant cells capable of growth after initial treatment responses. With spontaneous mutation rates in some cancer cells reported as high as 1 in 10^5 per cell/generation, drug-resistant variants are nearly certain to be present at the time of advanced cancer treatment (11). Such a scenario is likely to apply to AR variants appearing in response to second-generation anti-androgen treatment, where cancer cell burdens are likely in the order of 10^10 or higher. This may be especially true for prostate cancers with DNA mismatch repair defects, as the LNCaP cells used both by Joseph and colleagues (3) and Korpal and colleagues (4) fail to express MSH2 or MSH6 (12). Cytotoxic chemotherapy may also induce copious mutations, which could contribute to anticancer drug 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 ARF876L.

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 ARF876L during second-generation antiandrogen treatment underscores this addiction. Nonetheless, the poor growth of ARF876L 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 ARF876L 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 ARF876L 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.

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REFERENCES


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