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Predicting treatment response in castration-resistant prostate cancer: could androgen receptor variant-7 hold the key?

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Abstract

There is an emerging interest in understanding mechanisms of response and resistance to next-generation hormonal therapies: abiraterone and enzalutamide. While many explanations for resistance to these agents have been postulated, the importance of androgen receptor splice variants is gaining momentum. Androgen receptor (AR) splice variants are constitutively active isoforms of the AR that lack the ligand-binding domain yet retain their transcriptional activity in a ligand-independent fashion. Of these, AR variant-7 may be the most important, and has been implicated in primary resistance to abiraterone and enzalutamide in men with advanced prostate cancer. In this editorial, the clinical relevance of AR splice variant-7 (AR-V7) will be reviewed within the context of AR-directed therapies, and next steps for the analytical and clinical validation of this potential biomarker will be proposed.

Keywords

abiraterone; androgen receptor; AR-V7; enzalutamide; splice variant

Although it was once thought that castration-resistant prostate cancer (CRPC) was androgen-independent, an abundance of preclinical and clinical data have shown that this entity often remains driven by both androgens and the androgen receptor (AR) [1]. Exploiting this fact, two next-generation AR-targeting therapies have recently been approved by the US FDA for the treatment of patients with metastatic CRPC. The first of these drugs, abiraterone, inhibits the androgen synthesis enzyme CYP17 that is responsible for converting steroid precursors into androgens both in the testes and the adrenal glands, as well as in the tumor itself [2]. The second drug, enzalutamide, is a potent AR signaling inhibitor that has at least three functions: it blocks the ligand-binding domain of AR, it inhibits translocation of the AR from the cytoplasm into the nucleus and it impairs the interaction between the AR and the DNA promoter/enhancer regions thereby decreasing transcription of androgen-responsive genes [3]. Both abiraterone and enzalutamide have

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been shown to improve survival in men with metastatic CRPC [4,5], and are among the most widely used agents for this condition.

However, a significant minority of patients receiving either abiraterone or enzalutamide does not respond at all to these agents [4,5]; this phenomenon is called primary (or innate) resistance. Furthermore, the vast majority of patients who do initially respond to abiraterone and enzalutamide invariably develop secondary (acquired) resistance over time. Understanding the underlying mechanisms of primary and secondary resistance to next-generation AR-directed therapies is an area of intense laboratory and clinical investigation. Although there are many postulated mechanisms of resistance to AR-directed therapies (many of which may be overlapping) [6–8], our group has focused on investigating AR splice variants (AR-Vs) as a putative resistance mechanism [9]. AR-Vs are abnormally truncated variants of the AR that are generated through abnormal splicing of the AR mRNA or (more rarely) may be the result of genomic rearrangements at the AR locus. Most AR-Vs are comprised of the first 3 (of 8) exons of the AR gene followed by a premature stop codon, resulting in the translation of a truncated AR protein that usually lacks the ligand-binding domain (the direct target of enzalutamide and the indirect target of abiraterone).

Although there are at least 20 described AR-Vs [10], we have focused our attention on AR splice variant-7 (AR-V7) for several reasons [11]. First, AR-V7 is the most abundant AR splice variant, and is compatible with detection in human clinical samples from patients with CRPC. Second, AR-V7 is a constitutively active variant that is capable of stimulating transcription of androgen-responsive genes even in the absence of ligand (i.e., testosterone and dihydrotestosterone); it cannot be inhibited *in vitro* or *in vivo* by drugs that target the AR ligand-binding domain. Third, AR-V7 produces a proven protein product and is not affected by the process of nonsense-mediated mRNA decay. Finally, the relevance of AR-V7 is supported by the fact that its expression is increased at least 20-fold in CRPC tissues compared with hormone-sensitive prostate cancer tissues [11].

For these reasons, and because AR-V7 lacks the ligand-binding domain, which is the target of both enzalutamide and abiraterone, we hypothesized that the detection of AR-V7 in tumor cells from men with CRPC undergoing therapy with either enzalutamide or abiraterone would be associated with resistance to both drugs. In addition, because blood-based detection of AR-V7 would be a less invasive way to determine AR-V7 status than through a tumor biopsy, we developed a circulating tumor cell (CTC) assay to reliably detect AR-V7 at the mRNA level [12]. This CTC-based AR-V7 assay was prospectively tested in 62 patients with CRPC undergoing therapy with either enzalutamide (n = 31) or abiraterone (n = 31). CTC samples for AR-V7 testing were collected before initiating therapy with enzalutamide/abiraterone, during the course of therapy, and at the time of disease progression.

Among the 62 total patients, AR-V7 was detected in baseline CTC samples from 18 men (29%) [12]. All clinical outcomes were inferior in men with detectable AR-V7 compared with those with undetectable AR-V7. For example, in AR-V7-negative patients, the prostate-specific antigen (PSA) response rate (50% reduction in PSA from baseline) was 61% (27/44 men; 95% CI: 45–76%), whereas in AR-V7-positive patients, the PSA response

rate was 0% (0/18 men; 95% CI: 0–19%) ($p < 0.001$). In addition, median progression-free survival was 6.4 months (95% CI: 6.1–not reached) in AR-V7-negative men and 2.1 months (95% CI: 1.9–3.1) in AR-V7-positive men (hazard ratio: 12.7; 95% CI: 5.1–31.9; $p < 0.001$) [12]. Finally, median overall survival was >16.0 months (95% CI: 16.0–not reached) in AR-V7-negative men and 9.9 months (95% CI: 4.5–13.8) in AR-V7-positive men (hazard ratio: 5.5; 95% CI: 2.5–12.0; $p < 0.001$) [13]. These clinical data strongly suggest that detection of AR-V7 is associated with primary resistance to enzalutamide and abiraterone in men with CRPC. Importantly, a separate study exploring AR-V7 detection (at the protein level) using immunohistochemistry on formalin-fixed paraffinembedded bone marrow biopsies obtained from patients undergoing treatment with enzalutamide also demonstrated an association between AR-V7 staining and primary resistance to enzalutamide [14]. In that study, the AR-V7 protein was detected in bone marrow specimens from 8/14 patients (57%), who progressed within 4 months of starting enzalutamide treatment compared with 0/7 patients (0%), who responded to enzalutamide for longer than 6 months ($p = 0.018$).

Interestingly, 14% of the patients in our study, who were AR-V7-negative in their baseline CTC samples, subsequently converted to AR-V7-positive during the course of therapy with enzalutamide/abiraterone or at the time of progression [12]. In univariate analyses, these patients had clinical outcomes (PSA response rates, progression-free survival) that were intermediate between those men who were AR-V7-negative at baseline and remained negative during the course of treatment, and those who were AR-V7 positive at baseline (all of whom remained positive during treatment). In addition, the prevalence of AR-V7 was higher after treatment with either enzalutamide or abiraterone (compared with enzalutamide/abiraterone-naïve men), and increased even further in patients who received both enzalutamide and abiraterone. These data perhaps suggest that emergence of AR-V7 during the course of therapy may be associated with secondary (acquired) resistance to enzalutamide and abiraterone. However, the small numbers of patients that converted from AR-V7 negative to AR-V7 positive in our study (as well as the inability to adjust for other prognostic factors in multivariable analyses) limits any definitive conclusions, and these results should be viewed only as hypothesis generating.

Although the findings from our study are intriguing, and represent the first clinical evidence that AR variants may be associated with resistance to enzalutamide and abiraterone, AR-V7 testing should not yet be recommended routinely in clinical practice. This is for several reasons. First, it must be recognized that the CTC-based AR-V7 assay performed in this study was conducted in a research laboratory. Clinical testing would require that the assay be performed in a Clinical Laboratory Improvement Amendments (CLIA) certified setting. In addition, the optimal readout for the assay would need to be determined (i.e., should the results be reported as ‘positive’ or ‘negative’ based on a certain detection threshold, or as a continuous variable based on absolute transcript copy numbers?). Second, our preliminary findings would have to be validated in larger scale clinical studies involving multiple sites across the country. This, in turn, would require standardization of the assay itself and analytical validation of the methodology. It would also require uniform protocols for collecting, processing and shipping samples from one institution to another. Third, the utility of the AR-V7 assay would have to be clinically validated within the context of at least one additional prospective clinical trial involving patients receiving enzalutamide and/or

abiraterone. The clinical utility of AR-V7 testing would be further strengthened if the prognostic ability of AR-V7 status was maintained when using different detection assays developed in different laboratories.

In addition, it is currently unclear if AR-V7 status is simply a prognostic marker of poor outcomes in all patients with CRPC, if the test provides additional information beyond what can be obtained by routine clinical measures, and whether or not it may be used as a treatment-selection marker to inform clinical decision-making. To this end, our group and others are now testing the clinical utility of AR-V7 status in the context of other therapies, such as taxane chemotherapies. If it can be demonstrated, for example, that taxanes retain their activity in AR-V7-positive patients, then it could be argued that AR-V7 status may serve as a predictive marker to guide treatment decisions (i.e., to steer AR-V7-positive patients away from AR-directed therapies and toward taxane chemotherapies). However, such clinical data are still being generated and it remains to be seen if this hypothesis will be supported. Clearly, such a hypothesis would have to be tested prospectively within the context of a biomarker-enriched or biomarker-stratified randomized trial (in which patients would undergo AR-V7 testing at baseline and would then be randomized to AR-directed therapy or taxane chemotherapy).

In conclusion, the detection of AR-V7 may be the first actionable biomarker for men with metastatic CRPC. However, before this assertion can be justified, much work needs to be done both in terms of validating the assay performance (i.e., analytical validation) and confirming the clinical relevance of AR-V7 status in predicting response/resistance to treatment in a context-specific manner (i.e., clinical validation). These next steps are essential and critical in validating the importance (or lack thereof) of AR-V7 as a treatment-selection marker in CRPC. These validation steps will also be key for drug development and clinical testing of new compounds that may have activity in AR-V7-positive CRPC [15,16], which will likely become an area of intense clinical investigation over the next several years.

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