Androgen Receptor Splice Variant, AR-V7, as a Biomarker of Resistance to Androgen Axis-Targeted Therapies in Advanced Prostate Cancer

Tian Zhang,1 Lawrence I. Karsh,2 Michael J. Nissenblatt,3 Steven E. Canfield4

Abstract

Many therapeutic options are now available for men with metastatic castration-resistant prostate cancer (mCRPC), including next-generation androgen receptor axis-targeted therapies (AATTs), immunotherapy, chemotherapy, and radioisotope therapies. No clear consensus has been reached for the optimal sequencing of treatments for patients with mCRPC, and few well-validated molecular markers exist to guide the treatment decisions for individual patients. The androgen receptor splice variant 7 (AR-V7), a splice variant of the androgen receptor mRNA resulting in the truncation of the ligand-binding domain, has emerged as a biomarker for resistance to AATT. AR-V7 expression in circulating tumor cells has been associated with poor outcomes in patients treated with second- and third-line AATTs. Clinically validated assays are now commercially available for the AR-V7 biomarker. In the present review of the current literature, we have summarized the biology of resistance to AATT, with a focus on the AR-V7; and the clinical studies that have validated AR-V7 expression as a strong independent predictor of a lack of clinical benefit from AATTs. Existing evidence has indicated that patients with AR-V7-positive mCRPC will have better outcomes if treated with taxane chemotherapy regimens rather than additional AATTs.

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Introduction

Prostate cancer remains the second most common cause of cancer deaths in men in the United States, with 31,620 estimated deaths in 2019.1 The median overall survival (OS) for metastatic castration-resistant prostate cancer (mCRPC) has remained <3 years.2,3 Conventional androgen-deprivation therapy (ADT)—gonadotropin-releasing hormone analogs or surgical castration—has been the mainstay treatment for men with advanced prostate cancer for many years and is highly effective in patients who experience biochemical recurrence after therapy for localized prostate cancer (surgery or radiation therapy). However, disease progression and resistance to conventional ADT is inevitable, and, until 2010, docetaxel was the only US Food and Drug Administration (FDA)—approved therapy to show improvement in OS in mCRPC.4,5 Since then, several new therapeutic agents have emerged (Table 1), including next-generation androgen receptor (AR) axis-targeted therapy (AATT), such as the androgen biosynthesis inhibitor abiraterone acetate2,6; next-generation AR inhibitors, such as enzalutamide and apalutamide3,7,8; next-generation taxanes (cabazitaxel)9 and immunotherapy (sipuleucel-T)10; and the γ-emitting radiopharmaceutical radium-223.11 Each of these newer agents has been demonstrated to improve median OS, leading to FDA approval for their use in mCRPC. Some have also now been approved for use earlier in the continuum of care of men with microscopic disease (ie, nonmetastatic CRPC [M0 CRPC]); eg, enzalutamide and apalutamide).8,12 An additional new class of drugs, the poly(ADP-ribose) polymerase (PARP) inhibitors (olaparib and rucaparib), although not yet FDA approved, has been granted FDA breakthrough therapy status as treatment of mCRPC with BRCA 1/2 or ATM mutations, and is likely to become a part of our treatment armamentarium in the near future.13,14 At present, men with advanced prostate cancer will typically receive multiple lines of therapy after disease progression during standard...
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ADT. Thus, a critical problem for treating physicians has been determining the optimal treatment sequence for an individual patient. No randomized trials have been performed of second-line AATT versus taxane in this setting. However, several studies have reported on the real-world clinical practice of second-line therapy for mCRPC. In 4 US studies totaling nearly 5000 patients, most patients (57%-77%) had received second-line AATT, presumably because of the ease of oral administration and a perceived lower incidence of side effects compared with chemotherapy.15-18 Only a few patients (15%-43%) had received taxane chemotherapy as second-line treatment, and these patients tended to be younger and to have higher risk disease. Although 3 clinical studies observed improved outcomes in patients with high-risk mCRPC treated with taxanes,15,17,19 the largest clinical studies reporting on clinical outcomes showed no significant difference in OS between the AATT- and taxane-treated patients, with a hazard ratio (HR) of 1.04 (P = .694).16

Thus, the existing clinical data have not provided clear guidance regarding which therapy will be the best choice for second-line treatment. In addition, an empiric approach to treatment sequencing will result in patients receiving potentially toxic and expensive treatments that might not be effective for them individually and delays in the patients receiving potentially beneficial treatment. Ultimately, the cumulative lifetime costs of a patient’s systemic therapies can be substantial, potentially dwarfing the cost of their original definitive local therapy.20 The ideal of personalized medicine is to individualize care by identifying and targeting the unique molecular characteristics of a given patient or tumor; however, prostate cancer has historically been bereft of such targetable markers of response and resistance.5,21

A validated and commercially available panel of biomarkers to guide the sequencing of subsequent treatments would be ideal, such that therapies that are likely to be ineffective could be avoided. However, at present, there are no well-validated, commercially available biomarkers that can predict for resistance or sensitivity to chemotherapy, immunotherapy, or radiopharmaceutical agents. Thus, no clinically applicable “standard” approach has been determined for the selection or sequencing of these agents. Interest in identifying those men whose tumors have defects in DNA repair genes, such as BRCA1, BRCA2, and others, making such tumors more susceptible to DNA-damaging therapies such as PARP inhibitors has been increasing.22 As more therapeutic options become available, it will be critical to identify markers of effectiveness for each.

In contrast to identifying markers to evaluate the treatment response, a wide variety of molecular resistance mechanisms to AATT have been identified. One of these, the AR splice variant 7 (AR-V7), has been extensively evaluated as a predictor of the unlikelihood of a response to AATT. Predicting a lack of response will be equally useful for treatment sequencing. The focus of the present review was to summarize the development of the AR-V7 biomarker and the clinical studies reported to date.

Mechanisms of Resistance to AATTs

CRPC is a genomically unstable disease and has been associated with a wide array of molecular alterations that can lead to resistance to pharmacologic blockade of the AR pathway.23 During the past 20 years, many resistance mechanisms have been identified. Most of them have involved alterations in the AR axis. Thus, although mCRPC will inevitably recur after primary ADT, a large proportion of prostate tumors will remain dependent on signaling through the AR for much of their natural history. Although a detailed discussion of those mechanisms was beyond the scope of our review (a more detailed review has been reported by Davies et al24), they include the following: (1) upregulation of steroidogenesis within prostate tumors, enabling them to synthesize endogenous androgens25-27; (2) increased AR expression within prostate tumor cells, usually due to AR gene amplification28; (3) point mutations in the ligand-binding domain of the AR gene29-31; (4) functional silencing via methylation of the androgen inactivation enzyme gene HSD17B232; (5) HSD3B1 variants33; (6) glucocorticoid receptor upregulation34, and (7) emergence of AR splice variants. The emergence of AR splice variants was the focus of our review.

AR Splice Variants as Novel Mediators of Resistance to AR-Targeted Therapies

During the past 10 years, several important mechanistic studies of androgen resistance have implicated AR splice variants as a mechanism for short-circuiting androgen signaling. Beginning with the pioneering work by Dehm et al,35 and separately by Hu et al,36 several investigators have identified the presence of AR splice variants in prostate cancers, which encode a truncated AR protein that lacks the ligand-binding domain and, thus, is constitutively active in the absence of androgens.37 The AR-V7 variant emerged as 1 variant of particular clinical interest because of its frequent expression in advanced prostate cancer (Figure 1).

Detection of the AR-V7 Splice Variant in Clinical Prostate Tumor Specimens

Several studies have examined AR-V7 expression in normal prostate tissue and clinical tumor specimens. Taken together, these studies have shown AR-V7 to be the most abundant of the AR splice variants. Hu et al36 showed that AR-V7 mRNA levels were detectable in normal prostate tissue but were more highly expressed in prostate tumors, with greater expression in CRPC samples than in hormone-naive samples. Subsequently, Guo et al38 used an AR-V7-specific antibody to show minimal expression of AR-V7 protein in normal-appearing prostate tissues but strong expression in tumor cells. The AR-V7 protein was localized to the cytoplasm in hormone-naive tumor cells and partially localized to the nucleus in CRPC tumor cells.

Recently, Sharp et al39 assessed AR-V7 expression using immunohistochemistry (IHC) in 358 primary prostate tumor specimens and 293 metastatic biopsy specimens after ADT. Sharp et al39 observed that AR-V7 protein expression was very rare in primary tumors (<1%) but common in metastatic tumors (75%), suggesting that AR-V7 expression adaptively increases under the selective pressure of AR-directed therapies. In addition, AR-V7 protein expression was predominantly localized to the nucleus in tumor cells from patients with mCRPC. In some cases, different metastases from the same patient were heterogeneous with regard to AR-V7 expression. Finally, AR-V7-negative patients showed significantly better prostate-specific antigen (PSA) responses (100% vs. 54%, respectively; P = .03) and markedly improved OS compared with AR-V7-positive patients after AATT (74 vs. 25 months, respectively; HR, 0.23; P = .02).
## Table 1  New Therapies for Advanced Prostate Cancer

<table>
<thead>
<tr>
<th>Therapeutic Agent</th>
<th>First US FDA Approval Date</th>
<th>MOA</th>
<th>Indication</th>
<th>Administration Route</th>
<th>Doses per Month</th>
<th>Dosage</th>
<th>Cost by Price Index (USD)</th>
<th>Estimated Cost (USD)</th>
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<td></td>
<td></td>
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<td>AWP&lt;sup&gt;c&lt;/sup&gt;</td>
<td>WAC&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>Abiraterone</td>
<td>2011</td>
<td>CYP17 inhibitor</td>
<td>mCRPC; mCSPC</td>
<td>Oral</td>
<td>120</td>
<td>250 mg</td>
<td>11,050</td>
<td>3316</td>
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<td>AR inhibitor</td>
<td>mCRPC; nmCRPC</td>
<td>Oral</td>
<td>120</td>
<td>40 mg</td>
<td>13,858</td>
<td>11,549</td>
</tr>
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<td>Apalutamide</td>
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<td>AR inhibitor</td>
<td>nmCRPC</td>
<td>Oral</td>
<td>120</td>
<td>60 mg</td>
<td>14,008</td>
<td>11,673</td>
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<td>Sipuleucel-T</td>
<td>2010</td>
<td>Immunotherapy</td>
<td>amCRPC</td>
<td>IV</td>
<td>q2w × 3</td>
<td>250 mL of &gt;50 M cells</td>
<td>66,985</td>
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<tr>
<td>Radium-223</td>
<td>2013</td>
<td>Radioisotope</td>
<td>smCRPC</td>
<td>IV</td>
<td>q4w × 6</td>
<td>1.49 μCi/kg</td>
<td>28,727</td>
<td>23,939</td>
</tr>
<tr>
<td>Cabazitaxel</td>
<td>2010</td>
<td>Mitotic inhibitor</td>
<td>mCRPC</td>
<td>IV</td>
<td>q3w × 6 (10 maximum)</td>
<td>20 mg/m²</td>
<td>12,976</td>
<td>10,813</td>
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<td>Olaparib</td>
<td>BTD, not approved</td>
<td>PARP inhibitor</td>
<td>BRCA 1/2 mCRPC</td>
<td>Oral</td>
<td>120</td>
<td>150 mg</td>
<td>16,663</td>
<td>13,866</td>
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<tr>
<td>Rucaparib</td>
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<td>BRCA 1/2 mCRPC</td>
<td>Oral</td>
<td>120</td>
<td>300 mg</td>
<td>7961</td>
<td>9553</td>
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</tbody>
</table>

Abbreviations: amCRPC = asymptomatic metastatic castration-resistant prostate cancer; AR = androgen receptor; AWP = average wholesale price; BRCA 1/2 = breast cancer type 1/2, early onset breast cancer gene mutation; BTD = breakthrough therapy designation; CYP17 = cytochrome P450 17A1A; IV = intravenous; FDA = Food and Drug Administration; mCRPC = metastatic castration-resistant prostate cancer; mCSPC = metastatic castration-sensitive prostate cancer; MOA = mechanism of action; NA = not available; nmCRPC = nonmetastatic castration-resistant prostate cancer; PARP = poly(ADP-ribose) polymerase; q2w = every 2 weeks; q3w = every 3 weeks; q4w = every 4 weeks; smCRPC = symptomatic metastatic castration-sensitive prostate cancer; USD = US dollars; FDA = Food and Drug Administration; VAFSS = Veterans Affairs Federal Supply Schedule; WAC = wholesale acquisition price.

<sup>a</sup>Package price shown for AWP and WAC; VAFSS price given as unit price (per tablet for oral agents, per μCi for radium-233, and per mL for sipuleucel-T).

<sup>b</sup>Cost source: Micromedex (for AWP/WAC); Micromedex (electronic version; IBM Watson Health, Greenwood Village, CO; available at: https://www-micromedexsolutions-com; accessed March 27, 2019; accessed April 26, 2019 for cabazitaxel).

<sup>c</sup>Cost source: VAFSS (available at: https://www.fss.va.gov; accessed March 27, 2019; accessed April 26, 2019 for cabazitaxel).
Noninvasive Detection Methods ("Liquid Biopsy") of AR-V7 Splice Variants in Peripheral Blood

Given the practical and technical challenges of metastatic lesion biopsy in patients with advanced prostate cancer, the successful development of methods to detect AR-V7 mRNA and protein in the peripheral blood and identify circulating tumor cells (CTCs) has greatly facilitated the clinical use of AR-V7 testing in these patients. Given the heterogeneity of AR-V7 expression at different metastatic sites, the testing of peripheral blood for CTCs permits potential sampling of all parts of all tumors. This overcomes the sampling errors of a focal biopsy and offers a more balanced assessment of the AR-V7 status in a patient’s disease in its entirety. Furthermore, the relative ease of peripheral blood procurement and testing facilitates monitoring of AR-V7 changes during and after treatment.

Several peripheral blood CTC capture and analysis assays have been developed. The enumeration of CTCs has been shown to be prognostic for multiple tumor types, including lung, breast, colorectal, and prostate cancer. In metastatic prostate cancer, > 5 CTCs are prognostic for poor overall outcomes, and changes in CTC enumeration can serve as an early measure of treatment response. The CellSearch CTC test has been approved by the FDA for use in this setting. In addition, the ability to identify CTCs using IHC staining has facilitated the detection of nuclear-localized AR-V7 protein and real-time polymerase chain reaction (RT-PCR)–based detection of AR-V7 RNA in these cells (see the next section).

Clinical Studies of AR-V7 Detection in CTCs as a Predictive Biomarker in mCRPC

Several recent studies have reported the association of AR-V7 expression in CTCs using either IHC-based testing or quantitative RT-PCR (qRT-PCR)–based testing and outcomes for men with mCRPC treated with second- and third-line therapies (Table 2).

Clinical Studies of qRT-PCR–Based AR-V7 Assays

The landmark study by Antonarakis et al first demonstrated that the presence of AR-V7 transcripts in the CTCs of men with progressive mCRPC was associated with resistance to second-line AATT. In their study, of the 62 enrolled patients, one half had received abiraterone acetate and one half enzalutamide. Of the abiraterone-treated patients and enzalutamide-treated patients, 19% and 39%, respectively, had had AR-V7–positive CTCs. The AR-V7–positive cells had been detected using qRT-PCR of mRNA from pooled epithelial cell adhesion molecule–positive CTCs. The AR-V7–positive patients had significantly worse outcomes than AR-V7–negative patients, including lower PSA response rates, a shorter interval to progression, and worse OS. In a follow-up study of AR-V7 testing in 202 prospectively enrolled patients starting either abiraterone or enzalutamide therapy at the same institution, the investigators observed that AR-V7 positivity was significantly associated with other adverse prognostic factors, including Gleason score of ≥ 8, the presence of metastatic disease, and previous treatment with second-line AATT and taxanes. On multivariable
analysis, AR-V7—positive patients had significantly worse progression-free survival (PFS) and OS compared with AR-V7—negative patients and patients with no detectable CTCs. This same group also examined AR-V7 mRNA expression as a predictor of outcomes in patients with mCRPC who received taxanes (docetaxel or cabazitaxel). This study enrolled 37 patients, 46% of whom had AR-V7—positive CTCs. The AR-V7—positive patients in their cohort had had outcomes similar to those of AR-V7—negative patients (including PSA response rates, progression times, and OS), suggesting that AR-V7 status was not predictive of clinical resistance to taxanes. However, the AR-V7—positive patients who had received taxanes had had significantly better outcomes than the AR-V7—positive patients who had received abiraterone or enzalutamide, suggesting that taxane therapy is preferable to secondary hormone therapies among AR-V7—positive patients.

A second group had studied 29 patients with mCRPC who had received cabazitaxel. Of these 29 patients, 55% were AR-V7 positive, using a similar RT-PCR–based assay. In their study, AR-V7 status was not associated with progression-free survival (PFS) or OS. The findings from these 3 studies, although involving relatively small numbers of patients, suggest that AR-V7 expression in CTCs is a marker of resistance to AATT but not to taxanes.

Clinical Studies of Intracellular AR-V7 Protein in CTC as a Predictive Biomarker in mCRPC

Scher et al examined an automated method of detecting intranuclear AR-V7 protein using immunofluorescent staining of CTCs that was also predictive of the clinical response to AATT. This detection assay involves dispensing of nucleated blood cells from fresh blood samples onto glass slides; automated immunofluorescent staining of the slides for DNA (to identify nuclei), cytokeratin (epithelial cell marker), CD45 (lymphocyte marker), and a rabbit-monoclonal antibody specific for the AR-V7 protein; and immunofluorescent scanners and morphology algorithms to identify CTCs using a combination of epithelial expression and/or malignant morphologic features that are CD45-negative with AR-V7 localized to the nucleus (Figure 2 shows representative photomicrographs). This assay was analytically validated in both prostate cancer tissue microarrays and patient specimens to ensure the specificity of AR-V7 protein detection.

In a single-center clinical study of this novel automated immunofluorescent assay, Scher et al examined the association of AR-V7 protein in the nuclei of CTCs and clinical outcomes from 161 men with mCRPC who were undergoing a change in their treatment (additional AATT for 128 men and taxanes for 63 men). In their cross-sectional study, the choice of therapy was at the discretion of the physician without knowledge of AR-V7 status. AR-V7—positive CTCs were rarely observed in those with first-line mCRPC (3%) and were present in 18% and 31% of patients treated with second- and third-line or subsequent therapies, respectively (P < .001). These findings were consistent with the tissue-based study of AR-V7 expression by Sharp et al.

None of the 47 patients who had a > 50% decline in PSA levels during therapy had detectable AR-V7—positive CTCs. In contrast,
16 of the 81 patients (20%) without a > 50% decline in PSA levels had AR-V7-positive CTCs. In addition, the AR-V7-positive patients had significantly shorter PFS and OS compared with the AR-V7-negative patients during AATT. In a multivariable analysis adjusting for key clinical covariates, AR-V7-positive patients treated with taxanes had a lower HR for death compared with AR-V7-positive patients treated with ATTT (HR, 0.24; \( P = .035 \)).

Finally, nuclear localization of the AR-V7 protein was an independent prognostic marker for OS (HR, 2.38; 95% confidence interval [CI], 1.02-5.53; \( P = .045 \)) in patients treated with taxanes.

Three major conclusions emerged from their study. First, the prevalence of AR-V7 positivity significantly increased with the number of lines of therapy received. Second, AR-V7 positivity was predictive of a lack of clinical benefit from AATT. Third, the results were a clinical validation of AR-V7 positivity in the CTCs of patients with mCRPC as a predictor of resistance to salvage AR-directed therapies but was not a predictor of resistance to taxanes.

The same group performed a follow-up analysis of their original study to determine whether the detection of intranuclear AR-V7 protein was critical to the predictive value of this same immunofluorescent assay of CTCs. In the new analysis, Scher et al. compared AR-V7 positivity and the clinical outcomes, using 2
different definitions of AR-V7 positivity (their original definition, which required the detection of AR-V7 protein in the nucleus, and an alternative “nuclear agnostic” definition, which merely required detection of AR-V7 somewhere in the cell, either cytoplasmic or nuclear). The analysis showed that, although none of the 16 patients with nuclear-specific AR-V7 protein had experienced a PSA response to AR-directed therapy, 6 of the 32 patients with AR-V7 positivity (19%) and only cytoplasmic AR-V7 expression had experienced a PSA response. Furthermore, although both definitions of AR-V7 positivity were prognostic for survival, nuclear-specific AR-V7 expression was more strongly associated with survival than was “nuclear agnostic” expression (HR, 10.4 vs. 4.3, respectively). Finally, with additional death events since the original report, Scher et al53 were able to confirm the statistically significant interaction between nuclear AR-V7 positivity and treatment in the multivariable analysis that was observed in the first study. AR-V7—positive patients continued to show a significantly lower risk of death with taxanes compared with AR-directed therapies (HR, 0.24; \( P = .019 \)). This association was not statistically significant when using the “nuclear agnostic” definition of AR-V7 positivity (HR, 0.73; \( P = .055 \)). Thus, the ability of an AR-V7 assay to determine the nuclear localization of AR-V7 has improved its utility.

The predictive value of AR-V7 positivity was again confirmed in a multicenter study using the same automated immunofluorescent assay of CTCs in 142 patients with mCRPC who were undergoing a change in therapy (72 patients to additional AR-directed therapy and 70 patients to taxanes). The investigators also compared the survival times of AR-V7—positive and AR-V7—negative patients by drug class. Just as in the previous study, the choice of therapy was at the discretion of the physician, without knowledge of AR-V7 status. The patients who started taxanes generally had worse clinical features (ie, higher PSA levels, higher lactate dehydrogenase levels, and more previous lines of therapy) compared with patients given AATT and had an overall greater risk of death. To estimate the median survival times of the patients with positive and negative AR-V7 status by drug class, these clinical factors were consolidated into a risk score for cohort comparison. In the “higher risk” group, as defined by the study-specific criteria, the AR-V7—positive patients had significantly better OS when treated with taxanes than with AR-directed therapy (14 vs. 6 months, respectively; \( P = .03 \)). The investigators concluded that AR-V7 testing can identify a subset of patients with mCRPC, who will live longer with taxanes than with AATT.

**Clinical Utility and Health Economics of AR-V7 Testing in mCRPC**

A key clinical question for any new biomarker is how it influences treatment decision-making by physicians. Markowski et al44 conducted a questionnaire-based physician survey of 38 American and Canadian providers who had ordered AR-V7 testing for 150 patients to determine how often the treatment decision was altered by the AR-V7 result. The surveys were completed for 142 patients (95%), and treatment decisions had been modified in 53% of the patients overall. The change rate was especially striking for the AR-V7—positive patients (86%). Thus, the test result appeared to have a major effect on treatment decisions in this setting.

Formal studies examining the cost-effectiveness of AR-V7 testing in advanced prostate cancer have been limited to date. Markowski et al45 provided a preliminary economic analysis before AR-V7 testing was commercially available and predicted that testing would provide a cost savings. Additional contemporaneous evidence (eg, drug and test prices, rates of AR-V7 positivity, characteristics of patients tested and across multiple clinical settings) would be valuable to estimate more precisely the value of AR-V7 testing on clinical outcomes and economics.

**Potential Future Applications for AR-V7 Testing**

Studies of AR-V7 status as a potential prognostic marker for CRPC in patients treated with taxanes are still ongoing. Inferior OS was observed in AR-V7—positive patients compared with AR-V7—negative patients treated with taxanes in the original study by Scher et al.50 In the TAX3TERGY study [early switch from first-line docetaxel/prednisone to cabazitaxel/prednisone and the opposite sequence, exploring molecular markers in men with mCRPC] of 54 patients treated with docetaxel or cabazitaxel, 67% had AR-V7—positive CTCs using a novel digital droplet PCR assay.51 AR-V7—positive patients had lower PSA response rates (78% vs. 58%; \( P = .23 \)) and shorter median PFS (8 vs. 12 months; HR, 0.38; \( P = .01 \)) compared with the AR-V7—negative patients. However, the clinical utility of this difference was questionable because both AR-V7—positive and —negative patients appeared to derive some clinical benefit from taxane treatment.

A recent clinical trial of ipilimumab and nivolumab in 15 AR-V7—positive patients suggested poor clinical efficacy in this subset of patients, with only 2 patients (13%) experiencing a PSA response.56 Additional studies are required to determine whether AR-V7—positive patients have a high mutational burden and respond differentially to immunotherapy and/or PARP inhibitors.

Finally, whether it is clinically useful to monitor AR-V7 status by repeat testing of CTCs is unclear. However, data have revealed that some patients will become AR-V7—negative again when restaged after treatment with taxanes, raising the possibility that the splice variant clone burden can be significantly decreased with this approach and that patients might become resensitized to AATT.57

**Commercially Available CLIA-Grade Assays for AR-V7 Expression in Peripheral Blood**

Currently available assays for use in CTCs can be divided into 2 categories: assays that detect intranuclear AR-V7 protein (eg, Genomic Health/Epic Sciences CTC Oncotype DX AR-V7 Nucleus Detect Test); and PCR-based assays that detect AR-V7 mRNA (eg, Qiagen AdnaTest ProstateCancerPanel AR-V7 test). The biomarkers used in clinical practice and treatment decisions should be scrutinized for analytic and clinical validation and should be shown to have clinical utility.58

The recent PROPHECY trial (prospective circulating prostate cancer predictors in higher risk mCRPC study) is the only head-to-head prospective comparison of the 2 commercial-grade AR-V7 assays.52 In that multicenter study of 118 patients with progressive mCRPC and high-risk features, baseline AR-V7 status was assessed in CTCs using both assays before starting either abiraterone or enzalutamide. The investigators were unaware of the AR-V7 results when determining the treatment selection. The objectives of the
study were to assess the association of AR-V7 status, as determined by each assay, with the radiographic PFS, OS, and PSA response during AATT. With both tests, AR-V7 positivity was predictive of decreased radiographic PFS and a poor prognosis. With the AdnaTest, AR-V7-positive patients had shortened radiographic PFS compared with AR-V7-negative patients (3.1 vs. 6.9 months, respectively; HR, 2.4; 95% CI, 1.5-3.7 months; Figure 3A). Similarly, with the AR-V7 Nucleus Detect test, the AR-V7-positive patients had shortened radiographic PFS (3.1 vs. 6.1 months, respectively; HR, 2.5; 95% CI, 1.3-4.7 months; Figure 3C). The AR-V7-positive patients also had poorer survival compared with the AR-V7-negative patients (median OS, 10.8 vs. 27.2 months [AdnaTest] and 8.4 versus 25.5 months [Epic test]; Figure 3B and D).52 On univariable and multivariable analyses, both assays were predictive of a reduced likelihood of a >50% decline in PSA levels (odds ratio, 0.31 for the AdnaTest; and not estimable because of 0 events for the AR-V7-positive group for the AR-V7 Nucleus Detect test).52 The investigators concluded that AR-V7-positive patients, determined using either assay, had a very low likelihood of benefiting from AATT. Their prospective, multicenter, blinded study of 118 patients with mCRPC validated AR-V7 detection as predictive of a lack of benefit from AATT and supports the clinical utility of AR-V7 testing before treatment selection, especially in high-risk patients with mCRPC.

In the study by Armstrong et al,57 the inequivalently rate for both tests was low: 1% for the AdnaTest and 9% for the Epic test. Both tests require fresh blood samples and cannot be used with stored blood specimens. The cost for both tests is ~$3000, and the test turnaround times are ≤1 week.

Discussion

The mechanisms regulating the generation of AR splice variants in normal or malignant prostate cells are not fully understood, and the emergence of AR-V7 splice variants in prostate tumors has been proposed to be an indirect consequence of AR gene amplification and increased transcription.59 In the study by Sharp et al,59 AR-V7 expression was correlated with AR full-length expression and AR gene copy number; however, discordant cases of AR-V7 and AR full-length expression were found, suggesting that the emergence of AR-V7 expression after ADT was not simply a consequence of AR gene amplification.

The mechanism of action for the AR-V7 splice variant as a mediator of resistance to AATT has been primarily ascribed to the lack of a ligand-binding domain in its encoded protein, thus enabling constitutive activation of AR target genes in the complete absence of androgen. This model has been supported by in vitro studies showing that the AR-V7 protein can form heterodimers with full-length AR, activate canonical AR target genes such as PSA, and stimulate cell growth in a castration-resistant setting.60,61 Furthermore, pharmacologic downregulation of AR-V7 protein by the antihelminthic drug niclosamide inhibited prostate cancer cell growth in vitro and in vivo and restored sensitivity to enzalutamide in enzalutamide-resistant tumors.62 These latter observations have led to a clinical study currently underway to test the combination of niclosamide and enzalutamide in patients with CRPC.63

Conclusions

Given the increasing number of treatment options and the limited survival of patients with mCRPC, biomarkers that can guide clinical decision-making will have clear clinical utility. The expression of the AR-V7 splice variant in prostate cancer cells appears to be the first well-established (negative) predictive factor to guide patient treatment for men with mCRPC. The detection of AR-V7 in the CTCs of patients with mCRPC has been clinically validated as a predictor of resistance to AR-directed therapies in sequential lines of treatment. Multiple reported studies have shown that AR-V7-positive patients will have improved outcomes if treated with taxanes instead of AATT. AR-V7 testing represents an important actionable advance toward personalizing the treatment selection for men with high-risk mCRPC.

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