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REVIEW ARTICLE

# Androgen receptor variant-driven prostate cancer II: advances in clinical investigation

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## Abstract

**Background** Approximately 10–30% of men with mCRPC will test positive for AR-V7 using one of two analytically and clinically validated circulating tumor cell (CTC)-based assays. These men have poor outcomes with approved AR-targeting therapies but may retain sensitivity to chemotherapy. Here, we discuss the clinical implications of testing and strategies that may benefit AR splice variant (AR-V)-positive men and discuss whether such variants are passengers or drivers of aggressive clinical behavior.

**Methods** We conducted a systemic review of the literature, covering updates since our 2016 review on androgen receptor variants in mCRPC, outcomes, and existing and novel approaches to therapy. We provide an expert opinion about management strategies for AR-V7-positive men and key unanswered research questions.

**Results** AR-V7-positive men, defined by Epic nuclear protein detection or the modified AdnaTest mRNA detection in CTCs, identify a subset of men with mCRPC that have a low probability of response to AR-targeting therapy with short progression-free and overall survival in multivariable analyses. AR-variants do not exist in isolation, but rather in the context of a complex, heterogeneous, and evolving mCRPC genome and phenotype as well as patient-specific clinical heterogeneity, and multiple mechanisms of resistance likely exist in patients regardless of AR-V7 detection. Efforts to develop broader resistance assays are needed, and effective treatment strategies beyond taxanes are needed to address the causal driver role of AR-variants and to benefit patients with AR-V-expressing prostate cancer.

**Conclusions** CTC AR-V7 detection using the AdnaTest mRNA or Epic nuclear protein assays represents the first analytically and prospective clinically validated liquid biopsy assays that may inform treatment decisions in men with mCRPC, particularly after failure of first-line AR-therapy. The importance of AR-variants is likely to increase with the earlier use of AR-targeting strategies in other settings, and novel interventions for these men are needed.

## Introduction

The hallmark of castration resistant prostate cancer (CRPC) development is the resumption of active AR signaling in the setting of castration. Many AR-dependent mechanisms for CRPC have been previously hypothesized including, but not limited to, AR amplification, AR gene point mutations, increased DHT production, adrenal and autocrine androgen production, and constitutively activated AR splice variants (AR-Vs) [1, 2]. The most common and clinically relevant AR-V is AR-V7. AR-V7 lacks the ligand-binding domain and has been predicted to be a major mechanism of resistance to androgen receptor signaling inhibitors (ARSI) such as next-generation androgen receptor (AR) antagonists (enzalutamide, apalutamide, and darolutamide) and CYP17 inhibitors (abiraterone).

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AR-V7 was first described in 2008 [3], and is a result of a variant-specific cryptic exon 3 within intron 3 resulting in a truncated AR-V LBD after 16 variant-specific amino acids [4]. Multiple AR-variants can coexist within patients and may arise either from AR genomic structural rearrangements (GSRs), high AR transcriptional rates, or alterations in alternative splicing either broadly or more specifically for AR splicing (e.g., intron retention). Notably, since 2016 AR-GSRs have been described as giving rise to additional AR-Vs, which have yet to be functionally characterized and remain of uncertain clinical significance [5–8]. Currently, only AR-V7 has a clear protein product and clinically and analytically validated detection method in patients. The functional characterization of AR-variants beyond AR-V7 and the valid detection of additional variants in patients remains an area of ongoing investigation.

This review is an update to a previously published review from 2016 on the topic of AR-Vs in prostate cancer [9]. Here, we will discuss the updates in the clinical relevance of AR-V7 testing in men with mCRPC.

## Detection of AR-V7

Part II of this review will provide an in-depth discussion of the current analytically and clinically validated methods of detection of AR-V7 in patients. Two major validated methods have emerged, the first of which is by detecting AR-V7 mRNA in EpCAM-captured circulating tumor cells (CTC). This method was first reported in 2014 [10] and utilizes an RT-PCR based detection of AR-FL/AR-V7 mRNA. This method has been developed at Johns Hopkins University and licensed to Qiagen, and has undergone further analytic and clinical validation in the past 3 years [11–13]. The second validated method is the Epic Sciences/Genomic Health AR-V7 CTC Liquid Biopsy test, which assesses nuclear AR-V7 protein expression by immunofluorescence [14–16]. CTC IHC based AR-V7 detection was facilitated by the development of AR-V7 antibodies and has been implemented for clinical use by Epic Sciences. This testing platform has been characterized in clinical cohorts and relies on the detection of the nuclear localized protein product. These two methods were most recently investigated in the blinded, multicenter PROPHECY trial. The Epic Sciences CTC AR-V7 nuclear assay is currently commercially available and Medicare reimbursed. The AdnaTest CTC mRNA assay is being marketed by Qiagen to individual diagnostic laboratories, for internal research or clinical use within a given hospital or healthcare system.

## AR-V7 and response to systemic therapy

Table 1 and Figs. 1 and 2 summarize key outcomes from the major prospective AR-V7 mCRPC cohort studies stratified by method of AR-V7 detection, AR-V7 status, and type of systemic treatment. Outcomes are listed in the same figure/table for convenience, but not intended for direct cross-trial comparison.

## ARSI response and CTC AR-V7 mRNA detection

A pilot study in 2014 of patients with mCRPC and detectable CTCs starting on enzalutamide ($n = 31$) and abiraterone ($n = 31$) showed that 39% and 19%, respectively, of patients with detectable CTCs were positive for AR-V7 (AR-V7+) by mRNA detection [10]. The study found no PSA responses (confirmed ≥50% decline) among patients who were AR-V7+. Among men who were negative for AR-V7 (AR-V7−), confirmed PSA response rate was 10/19, 53% (95% CI 26–76%) among those receiving enzalutamide and 17/25, 68% (95% CI 46–85) among those receiving abiraterone. However, CTCs were not enumerated in this study, and it was unclear if the poor prognosis association of AR-V7 was related to high CTC burden rather than AR-V7 itself.

This method of CTC-based AR-V7 mRNA detection (AdnaTest) was further investigated as a prognostic biomarker in an expanded single-center prospective mCRPC cohort ($n = 202$) of men treated with first-line and second-line abiraterone or enzalutamide [11]. In this study, men were divided into three subgroups depending on detection of CTCs (yes, no) and AR-V7 (yes, no) using the modified AdnaTest mRNA assay: CTC−, CTC+/AR-V7−, and CTC+/AR-V7+. Of note, CTCs were not determined by the FDA-approved Cellsearch assay, but rather based on the detection of PSA/PSMA and AR transcripts in EpCAM-captured CTCs. Overall, 53 of the 202 men (26.2%) were CTC−, 113 of the 202 men (56.0%) were CTC+/AR-V7−, and 36 of the 202 men (17.8%) were CTC+/AR-V7+. The study revealed a relatively high CTC− rate (1st ARSI vs 2nd ARSI: 29% vs 21.8%) and relatively low CTC+/AR-V7+ rate (1st ARSI vs 2nd ARSI: 12.1% vs 26.9%). The study demonstrated that CTC− patients have the best treatment outcomes to AR-directed therapies with OS, PFS, and confirmed $PSA_{50}$ responses being 28.7 months, 13.9 months, and 75.5%, respectively. CTC+/AR-V7− patients showed OS, PFS, and confirmed $PSA_{50}$ of 29.5 months, 7.7 months, and 52.2%, respectively, while CTC+/ AR-V7+ showed the worst OS, PFS, and confirmed $PSA_{50}$ at 11.2 months, 3.1 months, and 13.9%, respectively. CTC+/AR-V7+

**Table 1** Selected AR-V7 outcomes trials: note that outcomes are listed in the same table for convenience and reference, not for direct cross-trial comparison.

| Study | *n* | Population | AR-V7+ rate (%) | PFS (mo) AR-V7+ (95% CI) | PFS (mo) AR-V7− (95% CI) | AR-V7+ vs −PFS HR (95% CI)[†] | AR-V7+ vs −PFS HR (95% CI)[‡] | PFS (mo) CTC− (95% CI) | $PSA_{50}$* AR-V7+ | $PSA_{50}$* AR-V7− | $PSA_{50}$* CTC− | OS (mo) AR-V7+ (95% CI) | OS (mo) AR-V7− (95% CI) | AR-V7+ vs −OS HR (95% CI)[†] | AR-V7+ vs −OS HR (95% CI)[‡] | Method of detection |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Antonarakis et al. [10] | 31 | mCRPC starting enzalutamide | 39% | 2.1 (2.0–NR) | 6.1 (4.7–NR) | 8.5 (2.8–25.5) | 3.0 (0.9–9.6)[d] | NA | 0% | 53% | NA | 5.5 | Not reached | 6.9 (1.7–28.1) | NA | AdnaTest CTC mRNA |
| | 31 | mCRPC starting abiraterone | 19% | 2.3 (1.4–NR) | 6.3 (6.3–NR) | 16.5 (3.3–82.9) | 7.6 (1.0–57.6)d | NA | 0% | 68% | NA | 10.6 | Not reached | 12.7 (1.3–125.3) | NA | AdnaTest CTC mRNA |
| Antonarakis et al. [18] | 37 | mCRPC starting taxane | 46% | 5.1 | 6.9 | 2.8 (1.2–6.9) | 2.7 (0.8–8.8)[c] | NA | 41% | 65% | NA | 9.2 | 14.2 | 2.5 (0.8– 8.1) | 0.7 (0.1–3.8)[c] | AdnaTest CTC mRNA |
| Antonarakis et al. [11] | 202 | mCRPC starting ARSI | 17.8% | 3.1 (2.3–3.7) | 7.7 (6.2–10.1) | NA | 2.5 (1.6–4.0)[b] | 13.9 (11.0–NR) | 13.9% | 52.2% | 75.5% | 11.2 (8.3–17.1) | 29.5 (18.4–NR) | NA | 3.0 (1.7–5.3)[b] | AdnaTest CTC mRNA |
| | 124 | mCRPC starting 1st line ARSI | 12.2% | 4.1 (3.0–NR) | 10.1 (10.1–NR) | NA | 1.6 (0.7–3.6)[b] | 21.6 (13.9–NR) | 26.7% | 65.8% | 86.1% | 21.5 (10.4–NR) | 30.7 (29.5–NR) | NA | 4.0 (1.5–10.6)[b] | AdnaTest CTC mRNA |
| | 78 | mCRPC starting 2nd line ARSI | 26.9% | 2.8 (2.1–3.4) | 5.3 (4.1–7.7) | NA | 3.14 (1.6–6.0)[b] | 6.2 (5.4–NR) | 4.8% | 27.5% | 52.9% | 8.5 (4.9–15.6) | 13.0 (10.0–22.6) | NA | 2.3 (1.2–4.5)[b] | AdnaTest CTC mRNA |
| Scher et al. [14] | 128 | mCRPC starting ARSI | 12.5% | 2.3 | 14.5 | 3.7 (1.4 –9.5) | NA | NA | 0% | 42.0% | NA | 4.6 | Not reached | 11.45 (5.67–23.82) | NA | Epic CTC nuclear AR-V7 expression |
| | 63 | mCRPC starting Taxane | 28.6% | 5.3 | 6.6 | 1.38 (0.63–3.0) | NA | NA | 33.3% | 37.7% | NA | 8.9 | 19.8 | 3.74 (1.95–7.20) | NA | Epic CTC nuclear AR-V7 expression |
| Scher et al. [15] | 142 | mCRPC starting ARSI | 20.0% | NA | NA | NA | NA | NA | NA | NA | NA | 7.3 (5.6 high risk) | 12.8 (9.7 high risk) | NA | NA | Epic CTC nuclear AR-V7 expression |
| | 70 | mCRPC starting Taxane | 30.6% | NA | NA | NA | NA | NA | NA | NA | NA | 14.3 (14.3 high risk) | 19.8 (16.9 high risk) | NA | NA | Epic CTC nuclear AR-V7 expression |
| PROPHECY Armstrong et al. [22] | 118 | High-risk mCRPC starting ARSI | 24% | 3.1 | 6.9 | 2.4 (1.5–3.7) | 1.9 (1.1–3.3)[a] | 7.5 | 11% | 26% | NA | 10.8 | 27.2 | 3.9 (2.2–6.9) | 4.2 (2.1–8.5)[a] | AdnaTest CTC mRNA |
| | 118 | High-risk mCRPC starting ARSI | 9% | 3.1 | 6.1 | 2.5 (1.3–4.7) | 2.4 (1.1–5.1)[a] | 5.6 | 0% | 28% | NA | 8.4 | 25.5 | 3.4 (1.6–7.0) | 3.5 (1.6–8.1)[a] | Epic CTC nuclear AR-V7 expression |
| Sharp et al. [23] | 162 | Unselected mCRPC | 33% | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | 1.26 (0.7–2.2) | AdnaTest CTC mRNA |

*$PSA_{50}$ = Confirmed PSA decline of ≥50%.

[†]Univariate HR for CTC+/AR-V7+ vs CTC+/AR-V7−.

[‡]Multivariable Cox regression model HR for CTC+/AR-V7+ vs CTC+/AR-V7−, see Fig. 2 for variables.

*NA* not applicable or reported.

[a]Model controls for baseline CTC enumeration (CELLSEARCH) and Halabi prognostic risk score.

[b]Model controls for PSA, Gleason sum, number of prior hormone therapies, prior abiraterone or enzalutamide use, prior taxane use, the presence of visceral metastases, and Eastern Cooperative Oncology Group score.

[c]Model controls for AR-FL expression levels, and prior use of abiraterone and/or enzalutamide.

[d]Model controls for expression of full-length androgen receptor mRNA and prior abiraterone and/or enzalutamide use.

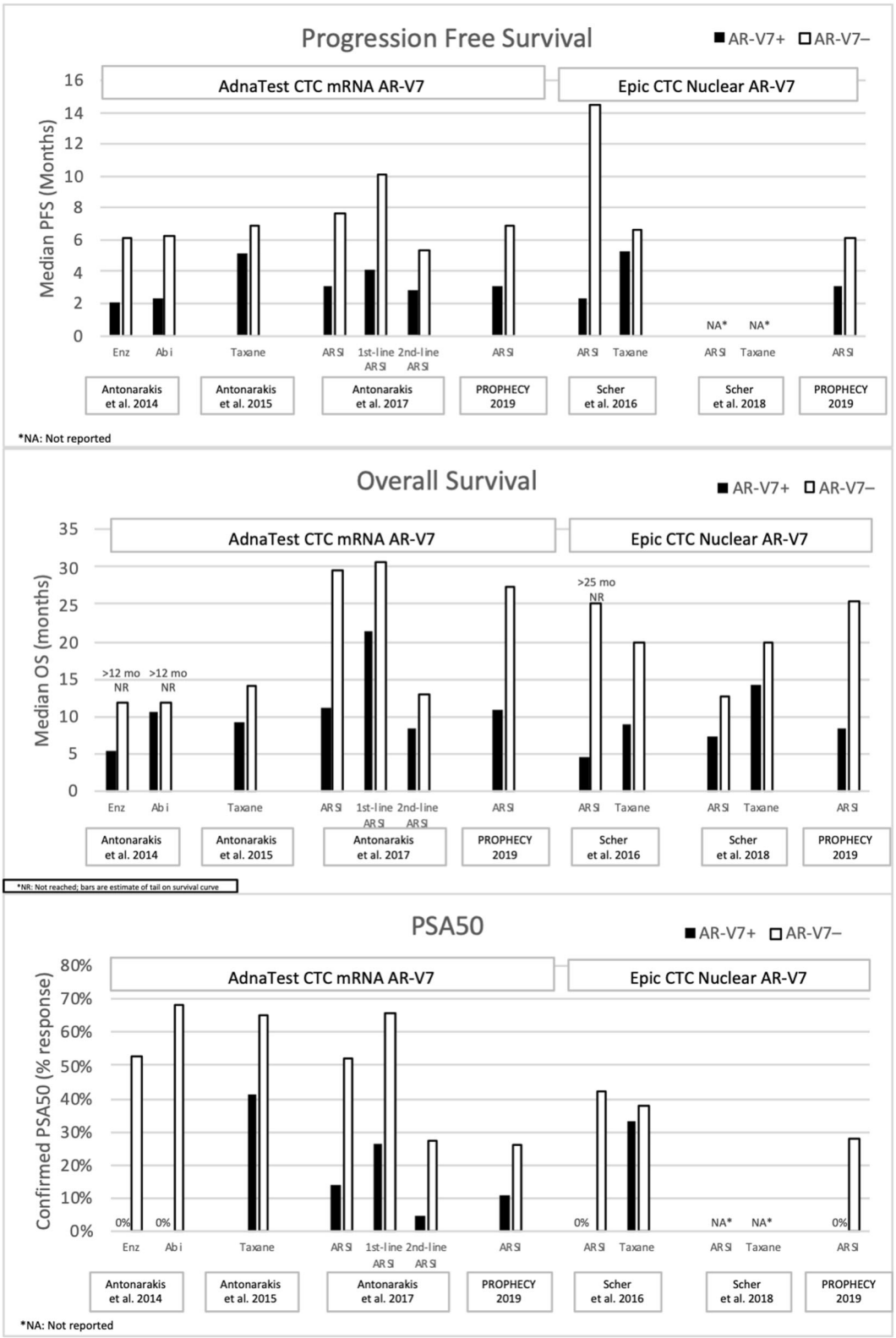
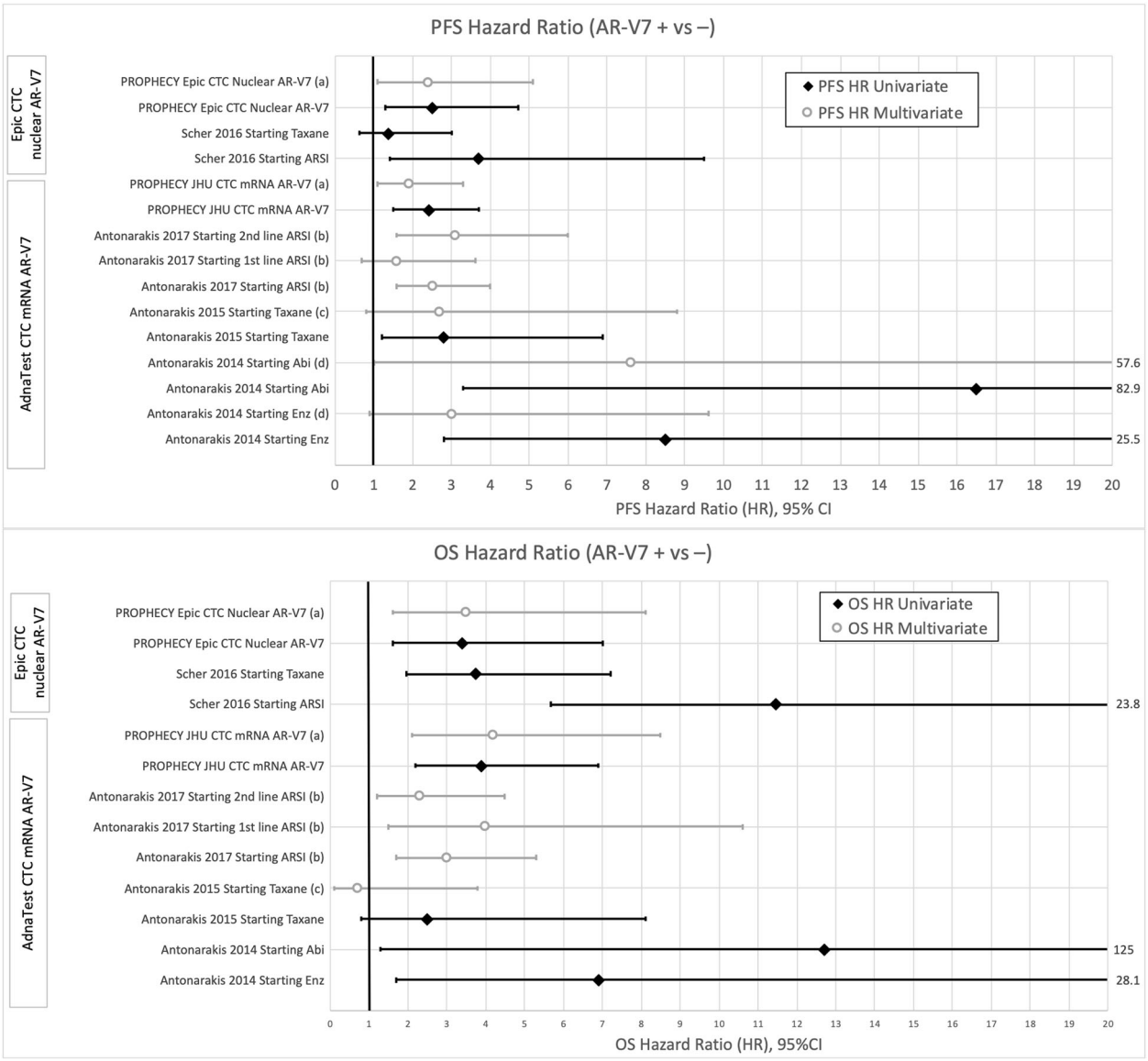



**Fig. 1 Radiographic or clinical progression-free survival (PFS), overall survival (OS), and $PSA_{50}$ from AR-V7 cohorts. a** The median progression-free survival (PFS), and (**b**) the median overall survival (OS) of AR-V7-positive and AR-V7-negative patients from select publications. **c** The confirmed 50% PSA decline ($PSA_{50}$) proportion among AR-V7-positive and AR-V7 patients from select publications. $PSA_{50}$ defined as 50% or greater PSA decline from baseline with additional confirmatory value >2 weeks later.




**(a)** Model controls for baseline CTC enumeration (CELLSEARCH) and Halabi prognostic risk score
**(b)** Model controls for PSA, Gleason sum, number of prior hormone therapies, prior abiraterone or enzalutamide use, prior taxane use, presence of visceral metastases, and Eastern Cooperative Oncology Group score
**(c)** Model controls for AR-FL expression levels, and prior use of abiraterone and/or enzalutamide
**(d)** Model controls for expression of full-length androgen receptor mRNA and prior abiraterone and/or enzalutamide use

**Fig. 2 Progression-free survival (PFS) and overall survival (OS) hazard ratios from AR-V7 cohorts. a** The PFS hazard ratios comparing AR-V7-positive versus AR-V7-negative patients from publications analyzing the AdnaTest CTC mRNA AR-V7 assay and the Epic CTC nuclear AR-V7 assay when treated with various systemic therapies. **b** The OS hazard ratios comparing AR-V7-positive versus negative patients from publications analyzing the AdnaTest CTC mRNA AR-V7 assay and the Epic CTC nuclear AR-V7 assay when treated with various systemic therapies. Solid black diamonds and lines show univariate HRs, while white circle/grey line shows multivariable models (variables used in each model are shown below the figure).

patients were also more likely to have a Gleason score ≥8, metastatic disease at diagnosis, higher pretreatment PSA level, higher alkaline phosphatase levels, more prior lines of therapy, the presence of pain, and ECOG performance status ≥1. This expanded study confirmed the finding that CTC+/AR-V7+ patients have inferior clinical outcomes compared with CTC+/AR-V7− and CTC− patients when treated with enzalutamide or abiraterone.

## Taxane response and CTC AR-V7 mRNA detection

Recent preclinical data have suggested that nuclear translocation of AR-V7 is microtubule-independent and suggested that taxanes may be less effective in AR-V7 dominant prostate cancer [17]. The AdnaTest CTC AR-V7 mRNA assay was prospectively investigated with respect to

taxane treatment response in 37 CTC-positive patients with mCRPC [18]. The primary outcome ($PSA_{50}$) was numerically, but not statistically, better in AR-V7− patients (65%, 13/20) than in those who were AR-V7+ (41%, 7/17). A secondary endpoint, clinical and/or radiographic PFS, was significantly different by AR-V7 status as median PFS was 5.1 months in AR-V7+ men versus 6.9 months in AR-V7− men (HR 2.8; 95% CI 1.2–6.9) in univariate analysis. However, this effect did not persist when controlling for AR-FL expression and prior ARSI use. Importantly, a cross-trial comparison showed that median PFS was longer in AR-V7+taxane-treated compared with ARSI-treated patients (HR 0.26, 95% CI 0.11–0.59) which remained superior when adjusted for AR-FL level and prior ARSI therapy (HR 0.21, 95% CI 0.07–0.59). These data seem to support the overall worse prognosis of AR-V7+ patients, but a higher response rate and longer PFS time with taxane chemotherapy as compared with ARSIs. The totality of the data, although not derived from prospective randomized trials, suggest that taxane therapies might be preferred in AR-V7+ men while both AR-directed therapies and taxanes are both reasonable options for AR-V7− men.

A final study that evaluated the prognostic impact of CTC-based AR-V detection on taxane outcomes was the TAXYNERGY trial, a study investigating an early taxane switch from docetaxel to cabazitaxel, or vice versa in 63 men with mCRPC who did not achieve a PSA response after 12 weeks of initial treatment [19]. CTC AR-V7 and ARv567es were detected by mRNA digital droplet RT-PCR (ddPCR) using a novel CTC chip-based assay and were evaluated for association with outcomes in a subset of 54 patients evaluable for CTC analyses. This correlative follow-up study of the TAXYNERGY trial showed that 36/54 (67%) patients were AR-V7+ [20]. PFS on taxane therapy was greater in the AR-V7− men compared with AR-V7+ men (12.0 vs 8.5 months, respectively; HR 0.38, $p = 0.01$); 58.3% of AR-V7+ patients achieved $PSA_{50}$ response during the study while 77.8% of AR-V7− achieved $PSA_{50}$ during the study. The initial report of the TAXYNERGY study demonstrated that a taxane-induced decrease in CTC percent AR nuclear localization (%ARNL) between days 1 and 8 of cycle 1 significantly correlated with $PSA_{50}$ response, which is hypothesized to be related to taxane effects on microtubule-mediated AR translocation, suggesting that %ARNL could be an early pharmacodynamics predictor of taxane response. AR-V7− evaluable patients demonstrated a %ARNL decrease of 21.5%, while AR-V7+ patients exhibited an increase of 0.4%. The authors suggest that given the differences in AR nuclear localization and the worse outcomes of AR-V7+ versus AR-V7− men with taxanes, this may support AR-V7 playing a role in taxane resistance as well. However, this was a small study that did not utilize a clinically validated AR-variant assay and relied on a surrogate endpoint of AR nuclear localization. Further, it did not have a control arm of hormonally treated patients in order to determine comparative efficacy by AR-V7 biomarker status. In addition, given that $PSA_{50}$ responses and reasonable PFS times were observed in both AR-V7-positive and -negative men, these data do not currently support AR-V7 or AR nuclear localization assays for clinical use as a negative predictor of taxane efficacy.

## ARSI versus taxane response and CTC Nuclear AR-V7 protein detection

The Epic EpCAM-independent automated staining system for detection of nuclear localized AR-V7 protein was first applied to 161 men with mCRPC by Scher et al. [14]. In this study, 191 blood samples were collected from 161 men starting on either an ARSI or taxane chemotherapy. The rate of AR-V7+ with first, second, and third or greater lines of therapy were 3% (2/67), 18% (9/50), and 31% (23/74), respectively. The presence of AR-V7+ CTCs were associated with worse PSA response rate, shorter rPFS, and lower OS in patients treated with ARSI (Table 1). Conversely, AR-V7+ patients treated with taxane therapy did not have significantly different rPFS (5.3 vs 6.6 months, $p = 0.46$) or time on therapy (3.0 vs 3.7 months, $p = 0.23$). AR-V7+ patients had a longer median survival with taxanes compared with ARSIs (8.9 vs 4.6 months), despite taxanes being administered later in the disease course. When controlling for various clinical prognostic factors using a multivariable Cox proportional hazards model, AR-V7 status was the most significant predictor of mortality. AR-V7+ patients treated with a taxane had better survival than those treated with an ARSI (HR 0.24, 95% CI 0.10–0.57; $p = 0.035$), while AR-V7− patients did not. However, in this study, Cellsearch CTC enumeration was not included in the adjusted analysis, and ARSI and taxane patients were not prospectively assigned, thus supporting a prognostic rather than truly predictive role of nuclear AR-V7 protein detection for ARSI versus taxane therapy.

Scher et al. subsequently published a multicenter retrospective blinded validation cohort of the Epic CTC nuclear AR-V7 protein assay investigating 142 patients starting on second-line therapy or later mCRPC with 70 samples prior to an ARSI and 72 prior to taxane therapy [15]. To enrich their population for AR-V7+ patients, the authors stratified patients into low and high-risk groups based on a risk score calculated with standard clinical prognostic variables such as LDH, hemoglobin, visceral metastases, and serum PSA. When using Cox proportion hazards regression, high-risk AR-V7− men had a longer median OS when treated with an ARSI versus a taxane (16.9 vs 9.7 months; HR 2.38; 95% CI,

1.12–5.06). The same analysis showed that AR-V7+ men had a shorter median OS when treated with an ARSI versus a taxane (5.6 vs 14.3 months; HR 0.35; 95% CI, 0.14–0.88). AR-V7 detection, however, did not discriminate outcomes in low-risk men with mCRPC, who generally lack evaluable CTCs. The authors concluded that high-risk patients being treated in the second-line setting and are AR-V7+ are more likely to benefit from a taxane than an ARSI agent.

The Epic CTC nuclear localized AR-V7 protein assay was also compared with a nuclear-agnostic assay by Scher et al. [21]. They reported that the nuclear-specific assay detected AR-V7 in 3%, 18%, and 31% prior to patients receiving first, second, and third line or above therapy, respectively, while the nuclear-agnostic method detected AR-V7 in 16%, 26%, and 43%, respectively. AR-V7 positivity by either assay pre-ARSI had a worse OS compared with AR-V7-negative samples. However, the nuclear-agnostic AR-V7 assay failed to show a treatment-specific interaction in multivariable analysis while the nuclear-specific assay did. The authors concluded that the nuclear-specific AR-V7 assay is a superior assay to predict differential responses while cytoplasmic-only AR-V7 detection lacked specificity for ARSI outcome prediction.

## Multicenter prospective validation of AR-V7: the PROPHECY trial

The PROPHECY study was a multicenter, blinded, prospective study designed to evaluate the prognostic values of these two analytically validated AR-V7 assays using pre-treatment AR-V7 status in CTCs in high-risk mCRPC patients treated with abiraterone or enzalutamide [22]. High risk was defined by having two or more of the following: anemia, elevated alkaline phosphatase, elevated serum LDH, prior ARSI, the presence of visceral metastasis, pain requiring opiates, Cellsearch CTC counts of >5 cells per 7.5 mL, a PSA-doubling time of <3 months, or radiographic progression at study entry. High-risk men were included given their unmet medical need, the enrichment of CTC+ patients with informative liquid biopsy results and given that these men could be reasonably treated with either an AR-targeted therapy or a taxane chemotherapy. Men were treated with either abiraterone or enzalutamide according to physician choice and followed through progression and subsequent therapies, including taxane chemotherapy, until death.

The primary endpoint of PROPHECY was the association of AR-V7 detection (by each method) with radiographic or clinical PFS on abiraterone or enzalutamide. The modified AdnaTest CTC AR-V7 mRNA assay and the Epic Sciences CTC nuclear AR-V7 protein assay were each evaluated for their prognostic significance for predicting clinical outcomes with ARSI therapy. Notably, each assay was performed at a central lab (Johns Hopkins University and Epic Sciences, respectively), and the central labs were blinded to clinical outcomes (as were clinical sites that were blinded to assay results). AR-V7 detection differed according to the assay at baseline, as trial participants were 10% and 24% AR-V7+ by the Epic and the AdnaTest assays, respectively, with 82% of samples concordant between the two tests. Most discordant results were AdnaTest AR-V7+ but Epic AR-V7− due to lack of sufficient Epic CTCs or cytoplasmic-only AR-V7 detection. AR-V7 positivity was more likely in men with higher LDH, CTCs, and alkaline phosphatase, as well as those with liver metastasis. AR-V7+ status by either detection method was independently associated with worse radiographic or clinical PFS and OS following ARSI treatment. The median PFS for AdnaTest AR-V7-positive versus -negative patients was 3.1 vs 6.9 months (HR, 2.4; 95% CI, 1.5–3.7) and the median PFS for Epic AR-V7-positive versus -negative men was 3.1 vs 6.1 months (HR, 2.5; 95% CI, 1.3–4.7). The AdnaTest AR-V7 assay showed a median OS for AR-V7-positive versus -negative cases of 10.8 vs 27.2 months (HR 3.9, 95% CI 2.2–6.9). The Epic AR-V7 assay showed a median OS for AR-V7-positive versus -negative cases of 8.4 vs 25.5 months (HR 3.4, 95% CI 1.6–7.0). In multivariable analysis, the HRs for PFS and OS for AR-V7 positivity by the AdnaTest assay were 1.9 (95% CI 1.1–3.3) and 4.2 (95% CI, 2.1–8.5), respectively, while HRs for PFS and OS for AR-V7 positivity by the Epic assay were 2.4 (95% CI, 1.1–5.1) and 3.5 (95% CI, 1.6–8.1), respectively.

No Epic AR-V7+ patients had a confirmed PSA or objective radiographic response to an ARSI. However, 11% of AdnaTest AR-V7+ patients had a confirmed PSA response and 6% had an objective response to an ARSI (albeit for a short duration), suggesting that the AR-V7 assays slightly differ in their sensitivity and specificity. Overall, both AR-V7 biomarkers met their primary endpoint of being independently associated with poor PFS and OS during abiraterone/enzalutamide therapy and confirm that AR-V7-positive patients with either assay have a low probability of clinical benefits with these ARSIs. Importantly, this was the first study to adjust for CTC enumeration (using the CellSearch method) and showed that AR-V7 status was independently prognostic for ARSI outcomes even after accounting for CTC burden. Future analyses of responses in these same men to subsequent taxane chemotherapy are planned.

Finally, CTC measurements were taken at disease progression and AR-V7 positivity increased to 20% of men by the Epic test and 34% by the AdnaTest, up from 10% to 24%, respectively, prior to therapy. We concluded that men with high-risk mCRPC who are AR-V7 positive by either assay have little clinical benefit from an ARSI. Important

differences between the assays highlighted by this investigation include the Epic assay having a relatively lower rate of sensitivity and detection but greater specificity, with no false-positive results based on PSA50 or radiographic responses, while the JHU assay had greater sensitivity and prevalence of detection, but resulted in 6–11% of AR-V7 men with PSA or radiographic responses and thus lower specificity.

## Tissue nuclear AR-V7 protein detection

The correlation between the AdnaTest CTC AR-V7 mRNA assay in 181 mCRPC patients and tissue-based IHC nuclear AR-V7 protein assay in 58 of these patients with a contemporaneous metastatic tissue biopsy was evaluated by Sharp et al. [23]. False negative CTC results were observed in 13/21 men who had AR-V7-positive metastatic tissue, typically related to low CTC number or low AR-V7 RT-PCR signal, and CTC AR-V7 detection was more common in patients with tumors than had high nuclear AR-V7 and AR genomic amplification. This study also found that CTC+/AR-V7+ men had a significantly higher nuclear AR-V7 protein expression by tissue IHC when compared with CTC+/AR-V7− men (median HS 100 vs 15, $p = 0.004$). However, 2/28 (7%) of CTC+/AR-V7+ patients were negative for nuclear AR-V7 by tissue IHC and 13/21 (62%) of CTC+/AR-V7− patients were positive for nuclear AR-V7 by tissue IHC. 10/16 (63%) CTC− patients had detectable AR-V7 on matched biopsy samples. This highlights that CTC mRNA and IHC AR-V7 detection are correlated but significant discordance between the two methods of detection remain that relate to CTC burden and AR mRNA levels. CTC AR-V7 status did correlate with survival in univariate analysis; however, by multivariate analysis controlling for differences between CTC− and CTC+ patients, there was a weaker association with inferior OS in CTC+/AR-V7+ patients compared with CTC+/AR-V7− patients (HR 1.26; 95% CI 0.73–2.17). However, this was a heterogeneous collection of patients who received a range of therapies, not just ARSIs, and confidence intervals are wide. This suggests that CTC AR-V7 may identify patients with higher CTC burden and thus a worse prognosis but cannot address the causal driver role of AR-V7 in these poor outcomes.

In addition, the same group published AR-V7 nuclear protein expression levels by IHC in 358 primary prostate samples and 293 metastatic biopsies [24]. The study found that <1% of ADT-naive prostatectomy samples express AR-V7 compared with 75% of biopsies in CRPC cases, suggesting that AR-V7 is specific to CRPC. Also reported was that AR-V7 expression was not equivalent across biopsy sites with higher AR-V7 expression in lymph node biopsies compared with bone, liver, prostate, and "other" sites. In addition, AR-V7 expression by IHC was lower in 40 biopsies prior to ARSI (*H* score: 40, IQR: 1–107.5) compared with higher levels after treatment with ARSI (*H* score: 90, IQR: 20–150), as expected. Finally, AR-V7 detection in mCRPC tissue was associated with worse responses and inferior OS in the context of ARSI agents.

## Response to ARSI observed in CTC AR-V7+ patients

Despite the strong evidence that AR-V7 positivity can convey resistance to an ARSI, there is evidence that a small group may still respond albeit transiently. For instance, Bernemann et al. identified four out of 21 AR-V7+ mCRPC patients by CTC analysis who responded by PSA criteria to either abiraterone or enzalutamide, with PFS ranging from 26 to 188 days [25]. This study used the AdnaTest platform for CTC collection and mRNA extraction similar to prior studies; however, it used different AR-V7 PCR primers and PCR conditions for detection, limiting its interpretation. Steinestel et al. also observed 1 out of 15 AR-V7+ mCRPC patients who responded to abiraterone treatment after failing ADT and docetaxel [26]. Collectively, these studies suggest a subset of men positive for AR-V7 may respond briefly to abiraterone/enzalutamide, dependent, at least in part, on the detection method as evident in the PROPHECY study. However, these responses appear to be short lived with shorter PFS times, and their clinical relevance is unclear.

In the ARMOR3-SV trial (a randomized, open-label, multicenter phase 3 study of galeterone vs enzalutamide), men with treatment-naive mCRPC were screened for CTC-specific AR-V7 by the Qiagen modified AdnaTest qRT-PCR method, and only AR-V7+ men were randomized 1:1 to receive galeterone or enzalutamide [27]. The trial was discontinued early due to its unlikeness to meet the primary endpoint of radiographic PFS. A total of 38 men had been randomized equally in the two arms (19 men in galeterone group, 19 in enzalutamide group). At the time of the study end, median time on therapy was 2.0 vs 2.8 months in the galeterone and enzalutamide arms, respectively, and median time to PSA progression was 3.9 vs 3.8 months. Unconfirmed $PSA_{50}$ response rates were 2/16 (13%) and 8/19 (42%). The fact that $PSA_{50}$ rates in the ARMOR-3V study are unconfirmed and thus short lived likely explain why results differ from studies such as PROPHECY, which reported only confirmed $PSA_{50}$ rates. Many of these unconfirmed PSA declines are transient and thus of unclear clinical significance. The results suggested that treatment-naive mCRPC patients positive for AR-V7 may have short-

**Table 2** AR-V7: passenger or driver?

| Passenger | Driver |
|---|---|
| • We have yet to develop AR inhibitors that are effective in AR-V7 (+) patients<br>• Model systems demonstrate heterogeneity of AR-V7 dependence versus full-length AR dependence [67, 68]<br>• Most patients with resistance to abi/enza lack CTCs with detectable AR-V7 [22]<br>• Many other AR-variants (AR-GSRs) likely exist and many additional drivers are likely present in the same patients and cells [69]<br>• AR-V7 detection is associated with phenotypic heterogeneity suggesting that driver may be broader defects in splicing or AR transcription [22]<br>• AR-V7's association with poor prognosis cannot prove mechanism and oncogene addiction | • Ligand-independent growth is mechanistically plausible and has been preclinically validated in some models [68, 70]<br>• Some models do exhibit AR-V7-dependent growth and resistance to AR inhibitors [71]<br>• Some patients do have nuclear AR-V7 dominant clones in CTCs and biopsies which increases over time with therapy [14, 24]<br>• AR-V7 appears to be the dominant AR-variant with a protein product and is strongly associated with inhibitor outcomes independent of disease burden [7, 10, 11, 14, 15, 22, 23] |

term PSA declines with first-line ARSI, but that both galeterone and enzalutamide resulted in very short PFS in these AR-V7+ patients.

## AR-V7 as passenger or driver

Based on the above studies, AR-V7 is now well established as a prognostic (and potentially predictive) biomarker; however, its role as mediator of resistance to therapy remains unclear. Table 2 summarizes the key issues that argue for or against AR-V7 acting as a pathogenic driver of resistance to hormonal therapy versus simply being associated with more aggressive disease. Testing the passenger versus driver etiology of AR-variants in these contexts ultimately will require novel therapies that block AR function proximal to the missing ligand-binding domains in AR-variant-driven mCRPC.

## AR-V7 as a therapeutic target

Recent efforts have focused on the development of therapies other than docetaxel or cabazitaxel that may target AR and/or AR-V7 independent of the ligand-binding domain and systemic therapies that retain efficacy independently of the AR-signaling axis altogether. Given the concerns over the rapid pace of disease and progression of men who test AR-V7 positive, new therapeutic strategies are clearly needed. Table 3 presents selected compounds that may have activity in the face of AR-variants which are in early clinical development.

## Galeterone

See above for discussion of the early-terminated ARMOR3-SV trial. Galeterone is no longer in clinical development

## Niclosamide

Niclosamide, an FDA-approved antihelminthic drug, was identified as a potent AR-V7 inhibitor in prostate cancer cells [28]. In a Phase I trial, safety was tested on niclosamide plus standard-dose enzalutamide in five mCRPC patients with previous abiraterone treatment. The Data Safety Monitoring Board for the trial recommended that the trial terminated prematurely (NCT02532114), due to lack of efficacy, as well as the difficulty in reaching a minimal effective concentration possibly due to poor absorption [29].

## AR NTD inhibitors, EPI derivatives

Derived from endocrine disruptor bisphenol A, several EPI compounds including EPI-101 and EPI-506 (a prodrug of EPI-002) were found to interact with the transcriptional activation domains TAU1 and TAU5 in AR N-terminal domain (NTD) to exhibit antiandrogen effects [30]. These compounds may also exert their functions via an androgen-independent manner as EPI-001 could selectively activate peroxisome proliferator-activated receptor-gamma (PPAR-γ) in prostate cancer cells [31]. Among these compounds, only EPI-506 was advanced to a Phase 1/2 study. The trial was terminated due to lack of efficacy and high burden of oral pill taken daily (NCT02606123). More recently, EPI-7386 has been found to have increased stability and activity in vitro and clinical studies are in development [32].

## Bromodomain/BET inhibitors

The bromodomain and extraterminal (BET) domain-containing protein 4 (BRD4) was originally identified as an epigenetic adapter. BRD4 interacts with the AR NTD

**Table 3** Selected clinical trials investigating AR-V7 targeting therapies.

| Agent | Trial phase | Description | Outcomes | NCT number, and/or reference |
|---|---|---|---|---|
| Niclosamide + enzalutamide | 1 | Single-arm trial of niclosamide plus enzalutamide in mCRPC | Safety | NCT03123978 [29] |
| Niclosamide + abiraterone | 2 | Single-arm trial of niclosamide plus abiraterone in CRPC (M0 or M1) | $PSA_{50}$ | NCT02807805 |
| ZEN-3694 | 1 | Open-label trial of ZEN-3694 for mCRPC | Safety | NCT02705469 |
| ZEN-3694 | 1b/2a | Open-label trial of ZEN-3694 with enzalutamide in mCRPC | Safety, PSA response | NCT02711956 |
| GS-5829 | 1b/2 | Open-label trial of GS-5829 for mCRPC alone (phase 1) and with enzalutamide (phase 2) | Safety (phase 1), PFS (phase 2) | NCT02607228 |
| GSK525762 | 1 | Open-label trial of GSK525762 in solid tumors including CRPC | Safety, response rate | NCT01587703 |
| Cabazitaxel | 2 | Single-arm open-label trial of cabazitaxel in AR-V7 + men with mCRPC previously treated with docetaxel | PSA response | NCT03050866 |
| Ipilimumab + Nivolumab (STARVE-PC) | 2 | Single-arm trial of ipilimumab plus nivolumab in AR-V7-positive men with mCRPC | PSA response, safety | NCT02601014 [65] |
| Radium-223 (EXCAAPE) | 2 | Single-arm open-label trial of Radium-223 in men with mCRPC with asymptomatic progression on abiraterone or enzalutamide | rPFS and rPFS by AR-V7 status | NCT03002220 |
| Docetaxel and enzalutamide | 2 | Single-arm open-label trial of sequential docetaxel and enzalutamide to correlate AR-V7 status with PSA response to enzalutamide post docetaxel | PSA decline | NCT03700099 |
| Bipolar androgen therapy alone, or combined with nivolumab | 2 | Single-arm open-label multicenter trial of intramuscular supraphysiologic testosterone combined with nivolumab | PSA decline | NCT02090114, NCT03554317 [66] |
| ARV-110 | 1 | Open-label trial of AR degrader AR-110 in men with mCRPC who have progressed on >2 prior therapies | Safety | NCT03888612 |
| Onvansertib | 2 | Open-label trial of onvansertib in combination with abiraterone/prednisone in men with mCRPC progressing on abiraterone. | Lack of PSA progression | NCT03414034 [43] |

to facilitate transcriptional activity [33]. Several BET inhibitors (BETi) (e.g., GSK525762, GS-5829, OTX015, JQ1, ABBV-075, ZEN-3694, PFI-1) are under development for CRPC treatment [34–38]. BETi could disrupt BRD4-AR interaction and prevent DNA binding of AR-FL or AR-variants. BETi also decreased AR-V7 expression by regulating splicing factors required for its generation [36, 39] ZEN-3694 is a BETi that has entered clinical trials for men with mCRPC as a single-agent phase I study in mCRPC (NCT02705469) and a phase 1b/2a safety and tolerability study in combination with enzalutamide (NCT02711956).

## PLK1 inhibition: onvansertib

A novel target that might be relevant in AR-V-expressing mCRPC is polo-like kinase 1 (PLK1), a cell-cycle control and proliferation enzyme. Interestingly, previous studies have shown an association with AR-V7 expression in human CRPC and overexpression of cell-cycle transcripts including the AR-driven cell-cycle gene UBE2C [40]. Onvansertib (formerly NMS-1286937) is an orally bioavailable next-generation PLK1 inhibitor that has completed phase 1 clinical testing [41], and has been shown preclinically to inhibit the growth of AR-V7+ CRPC cell lines and xenograft models while also reducing AR-V7 protein expression in CRPC models [42]. An ongoing phase 2 study (NCT03414034) is now evaluating onvansertib when added to abiraterone in men with mCRPC who have developed PSA progression on abiraterone alone (i.e., a salvage strategy). Interestingly, an interim analysis from this trial preliminarily suggests that the clinical activity of onvansertib when added to abiraterone (at least in terms of PSA responses) is more evident in AR-V7+ compared with AR-V7− patients [43]. Ongoing analyses from this study will further define whether this agent may work better in patients with AR-V7+ disease (i.e., correlated with greater cellular

proliferation) or whether the initial results may have been artefactual.

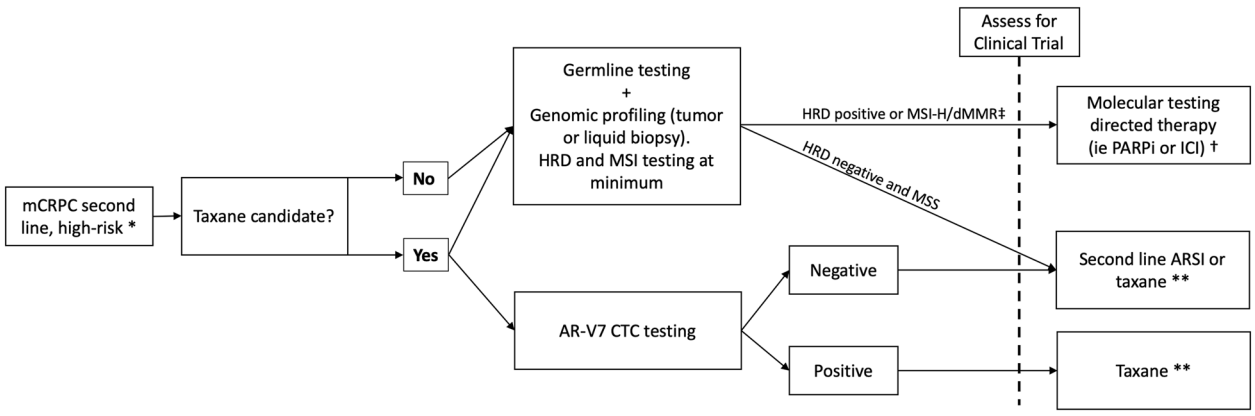
## Interpreting AR-V7 status in the context of other mechanisms of resistance

AR-V7-positive mCRPC represents a critical part of disease resistance to novel AR inhibitors; however, AR-V7 likely mediates only a minority of such resistance. There are a number of other key molecular alterations in mCRPC including but are not limited to AR-independent mechanisms such as neuroendocrine evolution, lineage plasticity, RB1 loss, TP53 loss, and BRCA1/2 loss as well as other AR-related mechanisms such as AR gain, AR-GSRs, and other AR-variants such as AR-V567 [6, 7, 20, 44–47]. Due to this cross-resistance between ARSI's due to these multiple mechanisms, second-line ARSI therapy commonly fails to be effective, even in AR-V7-negative men. A recent randomized study, the CARD trial, demonstrated that cabazitaxel improved PFS and OS as compared with second-line ARSI in men with mCRPC who failed a prior ARSI and docetaxel, suggesting that further taxane therapy should be offered to eligible patients [48]. In addition, many men will have overlapping resistance mechanisms as recent studies have demonstrated detection of AR-V7 and AR itself in NEPC tissues, raising the possibility that AR and AR-V7 may be operating through novel mechanisms to promote lineage plasticity [49, 50]. Moving forward AR-V7 will need to be interpreted in the context of the growing knowledge of molecular derangements driving disease biology and treatment response in mCRPC.

## AR-V7 in clinical practice

The author's recommendations for integrating AR-V7 testing and decision-making into standard clinical practice are shown in Fig. 3. Given the low rates of AR-V7 detection and the occasional responses seen in the first-line mCRPC setting, we primarily recommend AR-V7 testing in the second-line post-ARSI setting and particularly in "high-risk" men (using PROPHECY prognostic criteria). In this population, AR-V7 positivity can inform the patient and clinician of a likely more aggressive disease course and improved outcomes with taxane chemotherapy as opposed to another ARSI immediately after the first. These recommendations are based on the very low response rate and short PFS with ARSI treatment in PROPHECY and the superior OS seen with taxane versus ARSI in publications by Scher et al. This scenario may become increasingly common with the earlier use of potent ARSIs in the mHSPC and nonmetastatic CRPC settings [51–58].

The Epic AR-V7 nuclear assay is actively being incorporated into clinical practice, though it should not be ordered indiscriminately in mCRPC given the additional costs of testing as well as the waiting time required for results. When considering ordering AR-V7, clinicians should only order the assay when the results of testing will change their management (i.e., chemotherapy contraindicated or will chemotherapy be recommended even if




* High risk as determined by > 1 high risk clinical feature from PROPHECY trial including anemia with a hemoglobin <12.0 g/dl, elevated alkaline phosphatase above the institution upper limit of normal, elevated serum LDH above the institutional upper limit of normal, prior therapy with enzalutamide or abiraterone, presence of visceral metastasis on imaging, presence of clinically significant pain requiring opiate analgesics, Cellsearch CTC counts of ≥5 cells per 7.5 mL, a PSA doubling time of <3 months, or radiographic progression at entry.
† Poly ADP-ribose polymerase inhibitor (PARPi), immune checkpoint inhibitor (ICI)
‡ Homologous repair deficiency (HRD), Microsatellite instability high (MSI-H), Mismatch repair deficient (dMMR), Microsatellite stable (MSS)
** Consider Radium-223 if symptomatic, bone-predominant mCRPC, independent of AR-V7 status

**Fig. 3** Clinical algorithm.

AR-V7 testing is negative). As mentioned above, selecting a high-risk patient population with a high enough likelihood of testing positive is also essential. AR-V7 results must also be interpreted in the context of the many other known resistance mechanisms to hormonal therapy. A negative AR-V7 result still is associated with a suboptimal response rate to ARSI as shown in the PROPHECY study.

In addition, testing for germline or tumor mutations in homologous recombination genes and microsatellite instability and/or mismatch repair deficiency should be performed in parallel for all men with mCRPC [59]. Comprehensive genomic panels either using tumor sample testing or liquid biopsy approaches should be considered, as they may open doors to clinical trials (i.e., ATR inhibitors in ATM-mutated patients) or suggest a response to an off-label agent (i.e., anti-PD-1 immunotherapy for CDK12 or LRP1B mutations or TMB high) [60–64]. Independent of somatic genetic testing, comprehensive germline testing, and genetic counseling should also be considered in all mCRPC patients, especially those with a strong family history of other malignancy, not just prostate cancer. Lastly, we would argue that all AR-V7+ patients should be offered clinical trial options, whether AR-V7 specifically targeting or not, due to the poor prognosis of AR-V7+ mCRPC and the limited therapeutic options.

## Conclusions

Men with mCRPC harbor a complex, heterogeneous, and evolving cancer genome and transcriptome, and germline and somatic molecular characterization of patients in this setting is increasingly being recommended and utilized for treatment decision-making. AR-V7 testing may provide one additional critical aspect of disease resistance linked to poor outcomes to ARSI and can inform treatment decisions particularly in the second-line post-ARSI treatment setting.

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### Compliance with ethical standards

**Conflict of interest** AJA has served as a paid consultant for Astrazeneca, Merck, Dendreon, Janssen, Clovis, Bayer, and Medivation/Astellas; is on the speaker's bureau for Bayer and Dendreon; and receives research funding to his institution from Janssen, Medivation/Astellas, Sanofi-aventis, Active Biotech, Bayer, Dendreon, Merck, Astrazeneca, Genentech/Roche, BMS, Constellation, Novartis, and Pfizer. ESA has served as a paid consultant/advisor for Janssen, Pfizer, Sanofi, Dendreon, Essa, Merck, Bristol-Myers Squibb, AstraZeneca, Clovis, Eli Lilly and Amgen; has received research funding to his institution from Janssen, Johnson & Johnson, Sanofi, Dendreon, Genentech, Novartis, Tokai, Merck, Bristol-Myers Squibb, AstraZeneca and Constellation; and is a co-inventor of an AR-V7 biomarker technology that has been licensed to Qiagen. JL has served as a paid consultant/advisor for Sun Pharma, Janssen, Tolero, and Sanofi; has received research funding to his institution from Orion, Mirati, Astellas, Sanofi, Constellation, Calibr, Pandomedx, and Gilead; and is a co-inventor of a technology that has been licensed to Tokai, Qiagen, and A&G. CL is a co-inventor of a technology that has been licensed to Tokai and Qiagen.



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