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Brief Correspondence

Expression of Androgen Receptor Splice Variant 7 or 9 in Whole Blood Does Not Predict Response to Androgen-Axis–targeting Agents in Metastatic Castration-resistant Prostate Cancer

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Abstract

In 2014, a landmark study was published demonstrating that the expression of androgen receptor splice variant (AR-V) 7 was a negative predictive biomarker for response to abiraterone acetate and enzalutamide in metastatic castration-resistant prostate cancer (mCRPC) patients. However, these results were not supported by the recently reported ARMOR3-SV phase III clinical trial, which employed an identical circulating tumour cell assay to assess AR-V7 expression. Therefore, the predictive utility of AR-V7 expression in mCRPC remains uncertain, as does any potential association between other AR-Vs and treatment response. To further investigate, we designed a highly sensitive and specific whole blood assay for detecting AR-V7 and AR-V9. We then examined for a correlation between baseline AR-V7/V9 status and treatment outcome in 37 mCRPC patients commencing abiraterone or enzalutamide. Of the patients, 24% (9/37) were AR-V–positive. Notably, prostate-specific antigen (PSA) response rates did not significantly differ between AR-V–positive (6/9) and AR-V–negative (18/28) patients (66% vs 64%, $p = 0.9$). Likewise, median PSA progression-free survival was not significantly different between AR-V–positive and AR-V–negative patients (9.2 mo vs not reached; $p = 0.9$). These data, which support the findings of the pivotal ARMOR3-SV clinical trial, suggest that baseline AR-V expression does not predict outcomes in mCRPC patients receiving abiraterone or enzalutamide.

Patient summary: Detection of androgen receptor splice variants (AR-Vs) in circulating tumour cells of advanced prostate cancer patients has been linked to resistance to abiraterone and enzalutamide. We designed a blood test to detect AR-Vs that can be performed more routinely than tests involving circulating tumour cells and found that patients with AR-Vs still benefit from these effective treatments.

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Androgen receptor splice variants (AR-Vs) are truncated isoforms of the androgen receptor (AR). Two of the most abundantly expressed AR-Vs in metastatic castration-resistant prostate cancer (mCRPC) are AR-V7 and AR-V9. Both remain constitutively active despite lacking the ligand-binding domain in comparison with full-length AR, making them potentially impervious to AR-axis–targeted therapies. Several studies have concluded that AR-V7 expression in circulating tumour cells (CTCs) is a negative predictive biomarker for response to the next-generation hormonal agents abiraterone acetate and enzalutamide [1–3]. In contrast, two recent studies, including the randomised phase III ARMOR3-SV clinical trial (which reported a striking 42% response rate to enzalutamide in patients with AR-V7–positive CTCs) [4,5], have cast doubt about whether AR-V7 is indeed a negative predictive biomarker in mCRPC. AR-V9 has also been previously linked to abiraterone resistance [6], warranting a broader investigation incorporating both AR variants. Of note, no prior study has evaluated the predictive utility of both circulating AR-V7 and AR-V9 transcripts in mCRPC patients.

Despite advances in the field, blood-based biomarker detection with CTCs remains limited in its broader clinical applications for mCRPC patients. CTCs are more abundant in patients with a high tumour burden [7], which may present a source of selection bias in biomarker investigations. Furthermore, sample processing for CTCs is time sensitive and can require highly specialised equipment, pitfalls that make clinical translation challenging. Accordingly, more recent studies have sought to utilise whole blood assays to detect AR-V7 expression, bypassing the need for CTC enrichment [8,9].

We have developed an assay for detecting AR-V7 and AR-V9 that requires only 2.5 ml of whole blood collected in PAXgene RNA tubes. Immediate sample processing is not required, and tubes can be stored frozen for up to 7 yr without loss of RNA integrity. RNA is isolated and subject to reverse transcription using a primer specific for the AR-V7 or AR-V9 transcript (Supplementary material). Quantitative polymerase chain reaction (qPCR) is then performed using Taqman chemistry, and a probe/primer is set for specifically targeted unique sequences within the AR-V7 or AR-V9 transcript (Supplementary Fig. 1). Importantly, the combination of gene-specific reverse transcription and Taqman probe qPCR detection allows for both high specificity and sensitivity. By serially diluting RNA from VCaP prostate cancer cells (which are known to express AR-V7), we established that the lower limit of detection of our assay for AR-V7 is 0.1% (Supplementary material). The assay also exhibits 100% specificity with neither AR-V7 nor AR-V9 detected in any of the 13 healthy male controls (data not shown).

We next applied our assay to a prospectively collected cohort of 37 mCRPC patients, commencing abiraterone or enzalutamide, across three institutions. Blood samples were taken immediately before the commencement of therapy. Patient characteristics are listed in Supplementary Table 1. Clinical outcome data were collected, including prostate-specific antigen (PSA) response rates (defined as PSA decrease $\geq$50%, confirmed $\geq$3 wk later) and PSA progression-free survival (PSA-PFS; PCWG3 criteria). Median follow-up time of the cohort was 7.3 mo (interquartile range 4.7–10.6 mo). Patients positive for either AR-V7 or AR-V9 were defined as AR-V positive, while patients negative for both AR-V7 and AR-V9 were defined as AR-V negative.

In total, nine out of 37 patients (24%) were AR-V positive, with seven being AR-V7 positive and two being AR-V9 positive (no patients were positive for both AR-Vs). The overall PSA response rate was 65% (24/37). Importantly, we observed similar PSA response rates in the AR-V7–positive (4/7) patients to those in the AR-V7–negative (20/30) patients (57% vs 66%, $p = 0.6$, chi-square test). When both AR-V7 and AR-V9 were analysed together, PSA response rates to abiraterone or enzalutamide remained similar across AR-V–positive (6/9) patients compared with AR-V–negative (18/28) patients (66% vs 64%; $p = 0.9$, chi-square test; Fig. 1). We also evaluated the probability of AR-V–positive and AR-V–negative patients attaining a PSA response by 12 wk after starting therapy. We found no significant difference in the proportion of AR-V–positive (5/

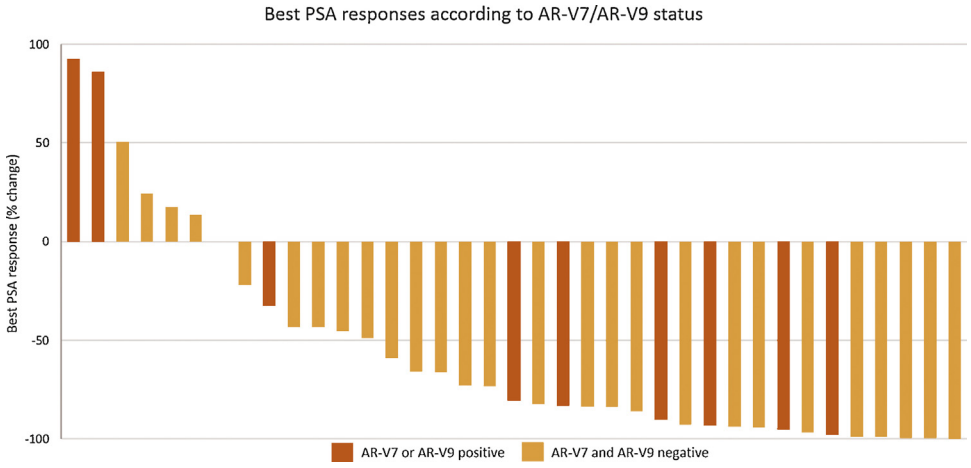

Fig. 1 – Waterfall plot of best prostate-specific antigen (PSA) responses for 37 patients treated with abiraterone or enzalutamide, according to AR-V7 and AR-V9 status. AR-V = androgen receptor splice variant.