



Circulating tumor cell-based or tissue biopsy-based AR-V7 detection: which provides the greatest clinical utility?

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Metastatic castration-resistant prostate cancer (mCRPC) is a condition characterized by cancer progression in the setting of low serum testosterone levels (1,2), and represents the lethal phase of the disease. Biologically, the development of castration-resistance occurs through a multitude of primarily androgen receptor (AR)-dependent mechanisms such as AR amplification, AR mutation, AR splice variant expression, and increase in synthesis of intratumoral and adrenal androgens through CYP17 and other pathways (3,4).

A number of next-generation hormonal therapies (such as enzalutamide, an AR antagonist; and abiraterone, a CYP17 lyase inhibitor) have demonstrated improvement in survival outcomes in patients with mCRPC (5,6), providing significant gains for patients with this condition. However, it is well recognized that a proportion of mCRPC patients have *de novo* resistance to these next generation hormonal therapies, and acquired resistance will eventually develop in all others. This combination of therapeutic advancements, yet with a high burden of mortality, has made mCRPC an area of active investigation for biomarker research (7).

AR splice variant 7 (AR-V7), originally identified in the CWR22Rv1 and VCaP human prostate cancer cell lines, lacks the ligand-binding domain of the full-length AR (AR-FL) and is constitutively active (8,9). AR-V7 has been proposed as an independent mechanism of *de novo* and acquired resistance to next-generation hormonal therapy (10).

A number of clinical studies have demonstrated the associations between presence of AR-V7 in tumor cells and resistance to novel antiandrogen therapies as well as shorter progression-free and overall survival when AR-V7 is expressed (11-14).

Several assays including the AdnaTest AR-V7 assay (Qiagen) and the Oncotype DX AR-V7 Nucleus Detect assay (Genomic Health) have been developed for assessment of AR-V7 status in circulating tumor cells (CTCs). The AdnaTest EpCAM-based assay uses peripheral blood to identify and enrich CTCs that express prostate-specific membrane antigen (PSMA) and/or prostate specific antigen (PSA) transcripts. These CTCs are subjected to quantitative real-time reverse-transcription polymerase chain reaction analysis with primers for AR-FL and AR-V7, and the AR-V7 status is presented as binary (present ≥ 1 transcript copies/mL or absent < 1 copy/mL) or continuous (transcript copies/mL) outcomes (15). In contrast, the Oncotype DX AR-V7 Nucleus Detect assay is an EpCAM-independent CTC detection assay that uses immunofluorescence to detect AR-V7 protein (not mRNA), and a positive call requires presence of nuclear-specific (not just cytoplasmic) AR-V7 protein localization (16). This assay employs high-throughput imaging of DAPI expression and CD45/cytokeratin immunofluorescence on all circulating nucleated cells to identify cancer cells for

downstream analyses (16).

In a recent issue of *European Urology*, Drs. Sharp and colleagues published a comprehensive investigation of the AdnaTest mRNA AR-V7 platform across two institutions, with collection of concurrent tumor biopsies in a subset of patients (15). In this study, AR-V7 status was assessed from CTCs using the AdnaTest assay on 181 patients with mCRPC initiating treatment at the Royal Marsden Hospital (RMH) in England. Intra-laboratory (at RMH) and inter-laboratory (RMH and Johns Hopkins) assay validations were performed. Correlations between AdnaTest mRNA sensitivity and CellSearch CTC count (another EpCAM-based detection method), as well as the metastatic biopsy AR-V7 expression [by immunohistochemistry (IHC)] were evaluated in a subset of patients.

Baseline characteristics of the study cohort were representative of the typical mCRPC population. One hundred fifty-two of 162 (94%) patients evaluable for survival had at least one AR-targeting therapy, and 120 of 162 (74%) had at least one taxane chemotherapy. Of 277 peripheral blood samples collected, CTC AR-V7 positivity using the AdnaTest was noted in 96 (35%) samples. Another 86 (31%) samples had detectable CTCs but negative AR-V7, and the remaining 95 (34%) samples had no detectable CTCs by the AdnaTest. The CTC AR-V7 positive group had more advanced disease and a higher disease burden, as characterized by a worse Eastern Cooperative Oncology Group Performance Status (ECOG PS; $P=0.03$), higher number of prior taxane therapies received (53.1% with 2 taxanes; $P<0.001$), lower hemoglobin levels (median 10.7 g/dL; $P=0.009$), higher alkaline phosphatase levels (median 180 U/L; $P=0.0006$), higher lactate dehydrogenase levels (median 230 U/L; $P=0.001$), and higher PSA levels (median 244.5 microgram/L; $P=0.0002$) compared with the other two groups comprised of CTC⁺/AR-V7⁻ and CTC⁻ cases.

These results are generally consistent with earlier studies evaluating the AdnaTest mRNA-based AR-V7 assay (10,17). In the largest prior study of 202 men starting either abiraterone or enzalutamide for mCRPC, 53 (26.2%) were CTC⁻, 113 (56.0%) were CTC⁺/AR-V7⁻, and 36 (17.8%) were CTC⁺/AR-V7⁺. CTC⁺/AR-V7⁺ patients were more likely to have Gleason scores ≥ 8 , metastatic disease at diagnosis, higher PSA and alkaline phosphatase levels, more prior novel antiandrogen and taxane therapies, presence of pain or ECOG PS ≥ 1 (17).

In the current study, CTC⁺/AR-V7⁺ patients had a median overall survival of 12.5 months (95% CI: 9.8–14.6), which is comparable to similar recent results using the

AdnaTest and Oncotype DX AR-V7 assays (11,17,18). In univariate analysis, CTC⁺/AR-V7⁺ patients had a higher risk of mortality (HR 2.62; 95% CI: 1.68–4.07), and CTC⁻ patients had a 41% lower risk of mortality (HR 0.59; 95% CI: 0.37–0.93), compared with CTC⁺/AR-V7⁻ patients ($P<0.0001$). However, this effect diminished significantly in multivariate analysis after adjustment for baseline characteristics and CellSearch CTC counts, with CTC⁺/AR-V7⁺ patients having a statistically non-significant higher risk of mortality (HR 1.26; 95% CI: 0.73–2.17; $P=0.4$), and CTC⁻ patients having a lower risk of mortality (HR 0.59; 95% CI: 0.35–1.00; $P=0.05$), compared with CTC⁺/AR-V7⁻ patients.

The loss of statistical significance in overall survival after adjustment for CellSearch CTC count is provocative, but contrary to the findings of other large studies, including a prospective trial of 118 patients starting a novel antiandrogen therapy for mCRPC (18). In that trial, called PROPHECY, AR-V7 positivity remained significantly associated with inferior PFS (adjusted HR 1.9; 95% CI: 1.1 to 3.3) and OS (adjusted HR 4.2; 95% CI: 2.1 to 8.5) on a multivariable analysis adjusted for baseline variables including the CellSearch CTC count. This discrepancy can be explained by differences in the enrolled patient populations, a small sample size in each group, or variables used in the multivariate model in the current study. Therefore, the question of whether AR-V7 detection is simply a surrogate of greater CTC burden or more aggressive disease characteristics cannot be answered reliably at this time.

Dr. Sharp's study is also the first to find a positive correlation ($P=0.004$) between nuclear AR-V7 protein expression in tumor tissue and the detection of CTC-derived AR-V7 mRNA in an IHC-cohort of 58 patients. To this end, CTC⁺/AR-V7⁺ samples had higher biopsy-derived AR-V7 protein expression than CTC⁺/AR-V7⁻ samples [median H-score (IQR): 100 [63 to 148] vs. 15 (0 to 113)]. They also showed that false positive results (CTC AR-V7 mRNA positive but tissue IHC negative; 2 of 28 patients; 7%) and false negative results (CTC AR-V7 mRNA negative but tissue IHC positive; 13 of 21 patients; 62%) were common. Additionally, 10 of 16 AdnaTest CTC⁻ patients (63%) had detectable tissue AR-V7 expression. These results support the argument that tissue-based AR-V7 protein expression should not be used in lieu of the CTC-based assay. These results are not surprising given the amount of intra-patient clonal heterogeneity observed in advanced prostate cancer patients. It also supports the notion that CTC-based AR-V7 detection, rather than

tissue-based AR-V7 detection, is most clinically useful with respect to prognostication and making treatment-selection considerations (19).

A limitation of the current study is the lack of data on PSA/objective response rates and progression-free survival analysis. This makes an in-depth comparison with other published literature infeasible. Specifically, an analysis of the correlation between AR-V7 mRNA levels and biochemical or radiographic responses in this large prospective study could shed further light on the ongoing debate regarding the prognostic utility of AR-V7 in mCRPC (20).

In closing, we would like to congratulate Dr. Sharp and colleagues for this comprehensive investigation of tumoral AR-V7 status in men with mCRPC. Their study adds to the growing body of evidence that AR-V7 can reliably be detected using blood-based assays, that the prevalence of AR-V7 is associated with a higher tumor burden and increases with treatment exposure, and that AR-V7 positivity in CTCs is associated with inferior clinical outcomes. Whether detection of CTC-derived AR-V7 may serve as a predictive marker remains an open question which can only be answered reliably from prospective randomized trials comparing at least two different therapeutic modalities (e.g., AR-targeted therapy *vs.* taxane chemotherapy), representing a great challenge for the future.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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