Editorial Commentary

Considerations for AR-V7 testing in clinical routine practice

Christof Bernemann, Laura-Maria Krabbe, Andres Jan Schrader

Department of Urology, University of Muenster Medical Center, Muenster, Germany

Correspondence to: Prof. Dr. Andres Schrader. Department of Urology, Muenster University Medical School, Albert-Schweitzer-Campus 1, D-48149 Muenster, Germany. Email: andresjan.schrader@ukmuenster.de.

Provenance: This is an invited article commissioned by the Section Editor Dr. Xiao Li, MD (Department of Urology, Jiangsu Cancer Hospital & Jiangsu Institute of Cancer Research & Nanjing Medical University Affiliated Cancer Hospital, Nanjing, China).

Comment on: Sharp A, Welti JC, Lambros MBK, *et al*. Clinical Utility of Circulating Tumour Cell Androgen Receptor Splice Variant-7 Status in Metastatic Castration-resistant Prostate Cancer. Eur Urol 2019;76:676-85.

Submitted Nov 12, 2019. Accepted for publication Dec 20, 2019.

doi: 10.21037/atm.2019.12.136

View this article at: http://dx.doi.org/10.21037/atm.2019.12.136

Although a number of patients suffering from prostate cancer respond initially to androgen deprivation therapy (ADT), the majority of patients will progress to metastatic castration resistant prostate cancer (mCRPC) over time (1). Within this stage of disease, different therapeutic options are available, e.g., novel hormonal therapies (NHT) like abiraterone and enzalutamide, taxane based chemotherapy as well as novel, more experimental treatments (e.g., PARP inhibition, PSMA targeted radionuclide therapy, etc.) (2). Nonetheless, virtually all tumors develop resistance mechanisms to the respective therapies. Thus, preemptive knowledge of likelihood of response to a certain type of treatment is of high clinical interest.

Clinical biomarkers might serve as a tool in both prognosticating a disease course as well as predicting response to treatments. One of the most promising biomarkers in mCRPC is AR-V7, the most abundant one of androgen receptor splice variants. AR splice variants occur as either conditionally active or constitutively active. The latter ones are able to translocate into the nucleus without co-factors. Once nuclear localized, AR splice variants act as transcription factors regulating AR target gene expression (3).

Expression of AR-V7 seems to correlate with poor prognosis and decreased response to NHT, whereas response to taxane-based chemotherapeutics seems to not differ significantly between AR-V7 positive and AR-V7 negative patients. Thus, the role of AR-V7 as a predictive biomarker for patient stratification and choice of treatment is currently being discussed intensively (4).

Several studies reported on detection systems for either AR-V7 mRNA or nuclear localized AR-V7 protein in tissue biopsies or circulating tumor cells (CTCs) (5-10). However, analyses of CTC AR-V7 expression along with CTC enumeration approaches has not been performed extensively. Given the technical challenges in interpretation of results obtained by analysis of rare events, especially detection of AR-V7 mRNA in low numbers of CTCs, contradicting results among detection systems as well as differences between CTC analysis and matched tumor tissue samples might weaken the use of CTC AR-V7 as potential novel biomarker in prostate cancer.

The study by Sharp and colleagues reports on the analytical and clinical validity of the AdnaTest CTC AR-V7 mRNA detection with respect to intralaboratory and interlaboratory differences, correlation to CTC counts as a surrogate for advanced stage of disease, and concordance between CTC detection and matched tissue samples (11).

First, the authors determined the correlation between CTC AR-V7 expression and clinical characteristics, e.g., Hb, ALP and LDH levels, demonstrating more advanced disease in AR-V7 positive patients.

The AdnaTest AR-V7 system is supposed to dichotomously define patients to be either AR-V7 positive or AR-V7 negative, irrespective of quantitative factors, e.g., AR-V7 copy numbers. Thus, the authors performed AdnaTest AR-V7 analysis along with AR-V7 copy number calculation and detected concordance in intralaboratory and interlaboratory analyses in AR-V7 high copy number samples. However, the concordance rates dropped when analyzing samples containing AR-V7 low copy numbers. Particularly, these results demonstrate wide variability of detection of in AR-V7 low copy number samples,

which might influence of predictive accuracy when binary outcomes are used for patient stratification.

Given that AR-V7 expression correlates with characteristics of an advanced stage of disease the authors further combined AdnaTest AR-V7 detection with contemporaneous CellSearch CTC counts. When correlating AdnaTest results for CTC-, CTC+/AR-V7- and CTC+/AR-V7+ samples with CellSearch CTC counts the authors detected significantly lower CTC counts in AdnaTest CTC negative samples. In addition, a number of patients displayed presence of CTC in the CellSearch approach without detection using the AdnaTest platform. Furthermore, CTC counts in CTC+/AR-V7- samples were significantly lower compared to CTC+/AR-V7+ samples. These results demonstrate both limitations of the AdnaTest system which is not detecting as many CTCs compared to the CellSearch system as well as a strong correlation between AR-V7 status and high CTC counts which might serve as a surrogate for a more advanced stage of disease.

By comparing CTC AR-V7 along with matched tissue biopsies the authors detected high levels of tissue expression in CTC AR-V7 positive patients. However, even in AdnaTest CTC+/AR-V7- as well as AdnaTest CTC- patients, a number of tissue samples displayed nuclear expression of AR-V7 protein, suggesting false negative detection of the CTC AR-V7 system. Contrary, two AdnaTest CTC+/AR-V7+ patients did not show AR-V7 expression in matched tissue samples. Conclusively, the authors detected correlation between CTC AR-V7 positivity and nuclear tissue expression although a number of false positive or false negative samples were identified.

Finally, the authors performed survival analysis based on baseline characteristics correlating with advanced disease as well as CTC metrics in both univariable and multivariable analyses. Using univariable analysis they detected correlation between CTC AR-V7 status, CTC count, higher ECOG PS, more taxane based therapies, lower hemoglobin, higher alkaline phosphatase, lower albumin, higher LDH and higher PSA. Using multivariable analyses there remained a statistically significant association between CTC AR-V7 status, CellSearch CTC count, ECOG PS and high ALP. Interestingly, differences in OS by CTC AR-V7 status appeared to be related to worse survival of CTC+/AR-V7+ patients compared to CTC- patients. However, no significant difference was detected between CTC+/AR-V7- and CTC+/AR-V7+ patients. Taken together, this data demonstrates a more advanced stage of disease in AR-V7 positive patients, which is accompanied by worse prognosis.

The results of this study clearly demonstrate the necessity of proper biomarker validation before use in clinical applications. Since its first report on predictive property for patient stratification in 2014, AR-V7 has been analyzed using a plethora of distinct detection technologies, i.e., mRNA expression and nuclear protein localization (5,12-14).

Initially, CTC AR-V7 mRNA expression has been found to entirely correlate with non-response of mCRPC patients to NHT, i.e., abiraterone and enzalutamide (5).

During the process of research, however, several studies reported a benefit of patients to these agents, irrespective of AR-V7 expression (8,14-17). The response rates differ among studies with lower response rates in second or later lines of treatment. In the first line enzalutamide cohort of the discontinued ARMOR3-SV trial, 42% (8 out of 19 patients) demonstrated PSA response irrespective of AR-V7 positive CTCs (18). Nonetheless, several reports still describe the property of CTC AR-V7 expression as being a predictive biomarker for treatment selection, although currently mostly discussed in second or later lines of treatment (16,17).

The majority of studies suggesting a predictive role for AR-V7 in treatment selection proclaim an alternative treatment for AR-V7 positive patients. Nevertheless, an AR-V7 related alternative treatment strategy is still missing. The most often proposed treatment would be taxane based chemotherapy. However, given the limited benefit of this particular therapy in all mCRPC patients in comparison with some degree of benefit to NHT in AR-V7 positive patients, the true clinical benefit of taxane based chemotherapy is still under discussion. Also, response rates to NHT are far from perfect even with AR-V7 negativity, therefore other underlying mechanisms of resistance need to be evaluated (19).

A way to circumvent the challenging aspect of predicting response to treatment by AR-V7 mRNA detection might be to detect the functional AR-V7 protein localized inside the nucleus. Biologically, a cell which contains the constitutively active AR splice variant within the nucleus would be resistant to anti-AR targeted therapies, given that AR-V7 will perform as a transcription factor even in the presence of these agents in order to stimulate expression of AR target genes. The detection of AR-V7 nuclear localized protein in CTCs has been performed in a set of publications, elegantly demonstrating the necessity of AR-V7 being localized within the nucleus; whereas cytoplasmatic AR-V7 protein positive patients still showed benefit from NHT. These

patients are likely to be AR-V7 mRNA positive, thereby explaining the phenomenon that AR-V7 mRNA positive patients might still benefit from these agents.

In a recent study by Armstrong *et al.*, the authors describe initial results of the PROPHECY trial, a prospective, blinded trial on AR-V7 response to NHT (17). The authors compared the validity of two AR-V7 detection systems i.e., mRNA and protein-based assays. Although there was a high concordance rate between these assays, this high rate was mostly based on detection of AR-V7 negative samples, whereas concordance among AR-V7 positive samples was diminished. This is presumably based on the distinct nature of analytes (mRNA *vs.* fully functional, nuclear localized protein). Nonetheless, even in this study, AR-V7 mRNA positive patients showed response to NHT. Further, the number of AR-V7 protein positive samples was lower compared to AR-V7 mRNA samples.

Therefore, the question still remains which assay to use to obtain the most predictive information in the highest number of samples analyzed. Likewise, one has to keep in mind, that all studies performed so far still demonstrated a high number of patients negative for AR-V7 (either mRNA or protein), which despite this fact do not respond to NHT, making the use of AR-V7 as a sole biomarker largely ineffective (20).

It is tempting to speculate that a combination of AR variants might offer more insights into patient resistance mechanisms. However, the true nature of such combinations has yet to be determined. Recently, another AR splice variant—AR-V567es—has been linked to clinical applications (21). Yet, in a comparative analysis it has been shown that different AR-V567es detection systems demonstrate high variability, representing a reason for discrepant results. Additionally, it reveals the risk of implying non-valid biomarkers into clinical considerations (20).

For sure, AR-V7 could serve as a tool to prognosticate the volume and therefore stage of disease to both patients and physicians, thereby aiding clinical decision making. However, the assay is not broadly available, expensive and therefore the question remains on how much information is added by performance of this test. Whether or not AR-V7 might one day reach the stage of being a routinely used prognostic or predictive clinical biomarker, is currently doubtful.

Acknowledgments

None.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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.

References

1. Rathkopf D, Scher HI. Androgen receptor antagonists in castration-resistant prostate cancer. Cancer J 2013;19:43-9.
2. Sumanasuriya S, De Bono J. Treatment of Advanced Prostate Cancer-A Review of Current Therapies and Future Promise. Cold Spring Harb Perspect Med 2018;8. doi: 10.1101/cshperspect.a030635.
3. Ware KE, Garcia-Blanco MA, Armstrong AJ, et al. Biologic and clinical significance of androgen receptor variants in castration resistant prostate cancer. Endocr Relat Cancer 2014;21:T87-T103.
4. Bastos DA, Antonarakis ES. AR-V7 and treatment selection in advanced prostate cancer: are we there yet?. Precis Cancer Med 2018. doi: 10.21037/pcm.2018.09.01.
5. Antonarakis ES, Lu C, Wang H, et al. AR-V7 and resistance to enzalutamide and abiraterone in prostate cancer. N Engl J Med 2014;371:1028-38.
6. Scher HI, Graf RP, Schreiber NA, et al. Nuclear-specific AR-V7 Protein Localization is Necessary to Guide Treatment Selection in Metastatic Castration-resistant Prostate Cancer. Eur Urol 2017;71:874-82.
7. Scher HI, Lu D, Schreiber NA, et al. Association of AR-V7 on Circulating Tumor Cells as a Treatment-Specific Biomarker With Outcomes and Survival in Castration-Resistant Prostate Cancer. JAMA Oncol 2016;2:1441-9.
8. Steinestel J, Luedeke M, Arndt A, et al. Detecting predictive androgen receptor modifications in circulating prostate cancer cells. Oncotarget 2015;10:4213-23.
9. Robinson D, Van Allen EM, Wu YM, et al. Integrative clinical genomics of advanced prostate cancer. Cell 2015;161:1215-28.
10. Miyamoto DT, Zheng Y, Wittner BS, et al. RNA-Seq of single prostate CTCs implicates noncanonical Wnt signaling in antiandrogen resistance. Science 2015;349:1351-6.
11. Sharp A, Welti JC, Lambros MBK, et al. Clinical Utility of Circulating Tumour Cell Androgen Receptor Splice

Variant-7 Status in Metastatic Castration-resistant Prostate Cancer. Eur Urol 2019;76:676-85.
12. Scher HI, Graf RP, Schreiber NA, et al. Assessment of the Validity of Nuclear-Localized Androgen Receptor Splice Variant 7 in Circulating Tumor Cells as a Predictive Biomarker for Castration-Resistant Prostate Cancer. JAMA Oncol 2018;4:1179-86.
13. Onstenk W, Sieuwerts AM, Kraan J, et al. Efficacy of Cabazitaxel in Castration-resistant Prostate Cancer Is Independent of the Presence of AR-V7 in Circulating Tumor Cells. Eur Urol 2015;68:939-45.
14. Bernemann C, Steinestel J, Humberg V, et al. Performance comparison of two androgen receptor splice variant 7 (AR-V7) detection methods. BJU Int 2018;122:219-26.
15. Bernemann C, Schnoeller TJ, Luedeke M, et al. Expression of AR-V7 in Circulating Tumour Cells Does Not Preclude Response to Next Generation Androgen Deprivation Therapy in Patients with Castration Resistant Prostate Cancer. Eur Urol 2017;71:1-3.
16. Antonarakis ES, Lu C, Luber B, et al. Clinical Significance of Androgen Receptor Splice Variant-7 mRNA Detection in Circulating Tumor Cells of Men With Metastatic Castration-Resistant Prostate Cancer Treated With First- and Second-Line Abiraterone and Enzalutamide. J Clin Oncol 2017;35:2149-56.
17. Armstrong AJ, Halabi S, Luo J, et al. Prospective Multicenter Validation of Androgen Receptor Splice Variant 7 and Hormone Therapy Resistance in High-Risk Castration-Resistant Prostate Cancer: The PROPHECY Study. J Clin Oncol 2019;37:1120-9.
18. Taplin ME, Antonarakis ES, Ferrante KJ, et al. Clinical factors associated with AR-V7 detection in ARMOR3-SV, a randomized trial of galeterone (Gal) vs enzalutamide (Enz) in men with AR-V7+ metastatic castration-resistant prostate cancer (mCRPC). J Clin Oncol 2017;35:5005.
19. Steinestel J, Bernemann C, Schrader AJ, Lennerz JK. Re: Emmanuel S. Antonarakis, Changxue Lu, Brandon Luber, et al. Clinical Significance of Androgen Receptor Splice Variant-7 mRNA Detection in Circulating Tumor Cells of Men with Metastatic Castration-resistant Prostate Cancer Treated with First- and Second-line Abiraterone and Enzalutamide. J Clin Oncol 2017;35:2149-56: AR-V7 Testing: What's in it for the Patient?. Eur Urol 2017;72:e168-9.
20. Bernemann C, Humberg V, Thielen B, et al. Comparative Analysis of AR Variant AR-V567es mRNA Detection Systems Reveals Eminent Variability and Questions the Role as a Clinical Biomarker in Prostate Cancer. Clin Cancer Res 2019;25:3856-64.
21. Tagawa ST, Antonarakis ES, Gjyrezi A, et al. Expression of AR-V7 and ARv567es in Circulating Tumor Cells Correlates with Outcomes to Taxane Therapy in Men with Metastatic Prostate Cancer Treated in TAXYNERGY. Clin Cancer Res 2019;25:1880-8.

Cite this article as: Bernemann C, Krabbe LM, Schrader AJ. Considerations for AR-V7 testing in clinical routine practice. Ann Transl Med 2019;7(Suppl 8):S378. doi: 10.21037/atm.2019.12.136

