PROSTATE CANCER IN FOCUS

Current Developments in the Management of Prostate Cancer

Section Editor: Andrew J. Armstrong, MD

The Role of AR-V7 Testing in the Management of Metastatic CRPC

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H&O What is the rationale behind detection of the androgen receptor splice variant 7 (AR-V7) protein in men with prostate cancer?

AA Prostate cancer is a hormonally driven malignancy. Ever since Huggins and Hodges published their seminal work in *Cancer Research* in 1941, eventually receiving a Nobel Prize, we have understood that androgens are a major driver of prostate cancer. We see great responses to androgen deprivation therapy (ADT) in men with prostate cancer, and further improvements in survival with more potent inhibition of androgen receptor (AR) signaling through the use of oral agents such as abiraterone acetate, apalutamide (Erleada, Janssen), darolutamide (Nubeqa, Bayer), and enzalutamide (Xtandi, Astellas), particularly when they are given earlier in hormone-sensitive settings or nonmetastatic castration-resistant settings.

Although resistance to ADT alone eventually develops, the biology of these tumors suggests that they are not truly hormone-refractory. Instead, the tumors become resistant to the castrating effects of testosterone-lowering therapies. Two of the major resistance mechanisms to hormonal therapy are amplification of the *AR* gene and induction of enzymes that fuel androgen synthesis. The emergence of resistance mechanisms allows prostate cancer to evade ADT, but despite these mechanisms, treatment responses and benefit are still possible with our novel potent AR therapies.

Most AR blockers bind to the ligand-binding domain, which is located at the C terminal of the AR protein. Even if that area is deleted in the AR, however, it is still present at the N terminal and can constitutively activate the AR program, driving the production of prostate-specific antigen (PSA), growth and division of cancer cells, and tumor metastasis. Multiple AR variants exist that can promote resistance to AR inhibitors, but AR-V7 is the most common one. Structural rearrangements in the *AR* gene or alternative splicing of the *AR* gene gives rise to these variants. We know that AR-V7 is strongly associated with poor survival, even after adjustment for clinical features and disease burden.

AR-V7 also has a protein product, and assays have been developed to detect it specifically. Assays to detect AR-V7 in circulating tumor cells (CTCs) with liquid biopsy have been studied over the past 10 years in an effort to identify men with metastatic castration-resistant prostate cancer (mCRPC) that will not respond to abiraterone or enzalutamide. The rationale for AR-V7 detection is to identify those men who may benefit more from subsequent AR targeting, as well as men who may benefit more from alternative approaches.

Therapeutic alternatives should be considered for a patient who is predicted not to respond to AR therapy on the basis of AR-V7 detection in CTCs; these include docetaxel, cabazitaxel (Jevtana, Sanofi-Aventis), a poly(ADP-ribose) polymerase (PARP) inhibitor such as olaparib (Lynparza, AstraZeneca) or rucaparib (Rubraca, Clovis Oncology), radium Ra dichloride 223 (Xofigo, Bayer), and eventually lutetium Lu 177 prostate-specific membrane antigen-617 (^{177}Lu-PSMA-617). A patient whose CTCs are AR-V7–negative has an additional second-line option: switching to abiraterone if the first agent used was enzalutamide, or switching to enzalutamide if the first agent used was abiraterone. However, additional

cross-resistance mechanisms beyond AR-V7 are also important, and thus a negative test result does not guarantee benefit from a second AR inhibitor. Switching from one AR inhibitor to another usually does not provide durable benefits, although sometimes it does, so it can be worth trying in some patients who—after informed counseling—are seeking additional options.

H&O What has research shown regarding AR-V7 assays?

AA Two assays have been rigorously studied with both analytic and clinical validation: the modified AdnaTest CTC messenger RNA (mRNA) assay, from Johns Hopkins University, and the nuclear-specific Oncotype DX AR-V7 Nucleus Detect test, from Epic Sciences. The Hopkins test detects mRNA derived from CTCs, whereas the Epic test detects AR-V7 in the nuclei of CTCs. The Hopkins assay is commercialized at Johns Hopkins but is not widely available, whereas the Epic Sciences test is widely available in the United States and is now reimbursed by Medicare.

Each assay first underwent clinical validation at a single institution; researchers led by Drs Emmanuel Antonarakis and Jun Luo at Johns Hopkins studied the modified AdnaTest, and researchers led by Dr Howard Scher at Memorial Sloan Kettering studied the nuclear assay. Initially, these were relatively small, exploratory studies suggesting that the presence of the AR-V7 protein in CTCs could identify patients who might live longer with chemotherapy than with AR inhibition. Both subsequently led to larger, confirmatory studies, which also showed a lack of response to AR therapies in AR-V7–positive men but no predictive value for response to taxane chemotherapy, suggesting that either of these assays might have clinical utility.

In the PROPHECY study, we validated the use of both of these assays in 118 men with high-risk mCRPC who were starting abiraterone or enzalutamide treatment. This was really the first prospective, blinded multicenter study to evaluate both assays, in which laboratory investigators were blinded to outcomes and clinical investigators were blinded to test results. We found that after adjustment for the number of CTCs and other clinical prognostic factors, the detection of AR-V7 was independently associated with worse outcomes—a lower chance of response by PSA testing or imaging, shorter progression-free survival (PFS), and shorter overall survival (OS)—when patients received abiraterone or enzalutamide. AR-V7 was not associated with worse outcomes of subsequent taxane chemotherapy, however, suggesting that AR-V7 is a driver of selective hormone therapy resistance. The 2 tests were very similar in their prognostic and predictive abilities, with an observed percentage agreement between them of 82%.

There are some small differences between the tests. The Hopkins assay can detect a bit more AR-V7 in CTCs than the Epic Sciences assay can, which makes it more sensitive but also produces more false-positive results, meaning that a patient in whom AR-V7 is detected could still have a response to treatment. Approximately 11% of patients who were AR-V7–positive on the Hopkins assay actually did have a response to abiraterone or enzalutamide; however, these responses were short-lived. The Epic Sciences assay was a little less sensitive in that the detection rate was lower, but it was very specific, with no chance of a response by PSA or imaging criteria if a patient was AR-V7–positive. This strong specificity makes the Epic Sciences assay an especially valuable test, as potentially beneficial therapy might be withheld after a false-positive result.

> AR-V7 is one of many AR alterations that may mediate resistance.

H&O Is AR-V7 a passenger or a driver of resistance to hormonal therapy?

AA This is the question I am asked most frequently when I present our research at meetings, and one that the field is still struggling to answer. In some preclinical models, AR-V7 is the major driver of resistance, which means that enzalutamide sensitivity might be restored by knocking down AR-V7. In other preclinical models, wild-type *AR* is the major driver of resistance. In some models, blocking either AR or AR variants improves disease control, thus illustrating the heterogeneity of both full-length AR and AR-variant dependency. We do not have drugs at this time that selectively target AR-V7 without affecting full-length AR, so this question is impossible to answer right now. Most of the drugs that are in development for this use block the signaling of AR and AR variants downstream, selectively degrade wild-type *AR*, or block cofactors that engage both AR and AR variants. Pure AR-variant degraders would permit us to test this passenger-vs-driver conundrum. What we can say, however, is that the associations are strong in clinical studies, but that many CTCs lack AR-V7 in patients even when AR-V7 is detected,

illustrating that optimal therapies will likely need to be directed not just against AR-V7 alone but also against a range of aggressive biological factors that give rise to AR signaling and AR-variant production and activity.

H&O Is AR-V7 detected earlier in hormone-sensitive disease?

AA AR-V7 and other AR structural rearrangements, including *AR* amplifications and *AR* point mutations, are almost never found in localized prostate cancer or in hormone-sensitive prostate cancer except through very sensitive RNA sequencing assays of tissue. CTC AR-V7, *AR* amplification, and *AR* mutations or *GSR* mutations are likewise not commonly detected in men with metastatic hormone-sensitive prostate cancer or in patients with newly diagnosed disease, indicating that they are adaptive forms of hormone resistance that occur in response to selection pressure from treatment. Prostate cancer cells are quite hardy and can adapt to survive a low testosterone level and AR blockade. Adaptive techniques include making more copies of themselves, increasing the transcriptional rate, and undergoing alternative splicing. We tend to see more and more mutations and deletions after abiraterone and enzalutamide, and with each line of therapy.

H&O How widely is AR-V7 testing used in mCRPC?

AA Medicare does reimburse for the AR-V7 assay from Epic Sciences. I would say that some centers in the United States are using it, but a good deal of heterogeneity is seen that is based on geographic location and type of practice—community or academic. The test is not yet available outside the United States. I would add that the usefulness of AR-V7 testing is probably decreasing a bit as more drugs become available that do not depend on AR-V7. For example, the test for poly(ADP-ribose) polymerase (PARP) inhibitors is the identification of a homologous repair deficiency, such as mutated *BRCA1* or *BRCA2*, through germline or somatic tumor assays. We expect eventually to use prostate-specific membrane antigen positron emission tomography (PSMA-PET) to evaluate who is a candidate for ^{177}Lu-PSMA-617 treatment. Radiopharmaceuticals such as ^{177}Lu-PSMA-617 and radium-223 are not dependent on AR-V7 testing, and AR-V7 testing is not needed if a decision has already been made to start docetaxel or cabazitaxel.

H&O How often do you use AR-V7 testing?

AA To facilitate precision medicine and optimal care for men with mCRPC, I generally do comprehensive genetic testing, both tumor testing and germline testing. I include AR-V7 testing when I'm on the fence about using a second AR inhibitor vs a taxane chemotherapy, particularly if the patient has minimal symptoms and is a candidate for either approach. If the patient has already received 2 AR inhibitors, or if we have already determined that the next step is chemotherapy, we do not need AR-V7 testing because the results will not change management.

H&O Are strategies being developed to reduce AR-V7 expression?

AA Drugs are being developed to reduce AR-V7 expression in several different ways. One approach would be to degrade AR-V7 directly, through the use of an agent that binds to AR-V7 specifically or through proteolysis targeting chimera (PROTAC) technology. Such drugs are not yet in the clinic, but degraders of full-length AR are in phase 1 testing now. These degraders may indirectly affect AR-V7 signaling by interfering with heterodimerization between AR and AR-V7, reducing the activity of both. A second approach would be to block cofactors that work with both AR and AR-V7, through the use of agents targeting these cofactors, such as SRC, FOXA1, HOXB13, and CBP/p300. This is another active area of drug development. A third approach would be to block targets that are downstream of the AR.

H&O What are the mechanisms of resistance to AR therapy in men who are AR-V7–negative?

AA This is an important question because AR-V7 explains resistance to second-line AR therapy in only about one-quarter of patients in the mCRPC setting. This means that approximately 75% of resistance is mediated by other factors, ranging from AR itself, *AR* amplifications and mutations, other AR variants besides AR-V7, AR enhancer amplification, and AR structural rearrangement. AR-V7 is one of many AR alterations that may mediate resistance and would have to be considered as part of comprehensive AR profiling. My guess is that AR mediates 30% to 50% of the resistance we see in this setting.

An emerging area of concern is the development of non-AR mechanisms of resistance to AR inhibition. For example, the loss of tumor suppressor genes such as *TP53*, *PTEN*, and *RB1* is associated with neuroendocrine transformation and lineage plasticity, in which tumors convert to a small-cell histology and do not respond to hormonal therapy. This type of non–AR-mediated cross-resistance accounts for approximately 20% of resistance. Other mediators of resistance include homologous

repair deficiencies; dysregulation of WNT, cell cycle, and epigenetic or metabolic programs; and mutated *FOXA1*. Finally, immune evasion can be a significant feature of mCRPC mediated through many of these alterations, and approaches targeting the key pathways are in trials now.

H&O What do you expect the status of liquid biopsy for prostate cancer to be in 5 to 10 years?

AA I think that in 5 to 10 years, we will have a comprehensive liquid biopsy assay that will include plasma DNA to detect the genotype, along with CTC biomarkers that capture phenotypic assays missed by DNA-only tests. Much more information can be gleaned from liquid biopsy than just the circulating tumor DNA (ctDNA) sequence. For example, liquid biopsy can reveal ctDNA methylation patterns, serum androgens, germline alterations, the presence of clonal hematopoiesis of indeterminate potential (CHIP), and structural rearrangements. More can be made of tumor biopsies and CTC assays of phenotype, including functional/growth assays, drug resistance assays, immune phenotyping, and protein expression, such as PSMA or other tumor antigens. The more targeted agents that we have available to capitalize on these findings, the more useful liquid biopsy will be.

Disclosure

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Suggested Readings

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