Androgen receptor splice variant V7 (AR-V7) in circulating tumor cells: a coming of age for AR splice variants?

Prostate cancer continues to be the most common non-cutaneous malignancy afflicting men in developed countries [1]. Although surgery or radiotherapy for the primary disease appears curative at the time of initial treatment, upward of 25% of men will have metastatic or residual disease (ACS Facts and Figures 2015). Since the advent of prostate-specific antigen assays, the recurrence of the cancer has become evident even before the presence of tumor can be observed by methods such as isotopic bone scan or computer-assisted tomography. Of further significance, the overwhelming majority of the recurrent tumors are driven by the androgen receptor [2]. When the recurrent tumor becomes symptomatic, e.g. bone pain or symptoms from metastases to soft tissues, castration either surgically or medically with estrogens or a gonadotropin releasing hormone agonists and antagonists has been demonstrated to ameliorate symptoms and prolong survival [3, 4]. The beneficial effect of castration was initially demonstrated by Huggin’s studies over 70 years ago [5]. However, the tumor invariably recurs [6]. At this juncture, historically, the patient was considered to be androgen depleted and signaling through the AR stopped; however, more recent studies clearly demonstrate that the AR drives tumor progression [2].

The mechanisms for the continued AR activation appear to be varied but include increased AR expression that allows activation by intracrine or paracrine androgen synthesis or expression of constitutively active AR-splice variants [7]. When the tumor begins to progress and the patient is symptomatic, the treatment tree becomes crucial. Dr Antonarakis et al. have previously shown that detection of AR-V7 mRNA in RNA derived from circulating tumor cells (CTCs) is a powerful determinant of who will fail to respond to further AR inhibition at this juncture [8] (Figure 1). No patient among the 31 AR-V7-positive patients in their initial study responded to enzalutamide or abiraterone. In contrast, 53% of those patients who were AR-V7 negative responded to those therapies. In their follow-up study, the important questions that Nakazawa et al. attempt to answer in the present paper appear to be the durability of the AdnaTest AR-V7 assay once it is positive and characteristics of the patients who convert from positive to negative and vice versa [9].

Although the patient numbers in this current study are too small for statistical confirmation (14 patients), this series of case studies permits some important observations. First, men who present as AR-V7 positive and remain on their second line of AR-directed therapy remained AR-V7 positive. Second, two subjects converted from AR-V7 negative to AR-V7 positive while on standard androgen deprivation therapy. These data suggest that any androgen deprivation therapy can result in expression of AR-V7 mRNA in CTCs, which correlates with resistance to second-line AR-directed therapy. Third, it appears that once conversion occurs and the patient remains on abiraterone or enzalutamide they will remain AR-V7 positive and it may be inferred that they will remain resistant to therapy. Finally, the most important observation from this series of patients is that those patients who had been AR-V7 positive but reverted to AR-V7-negative status had stopped their second-line AR-directed therapy and were on taxane therapies, implying that withdrawal of AR-directed therapies resulted in downregulation of AR-V7. This finding raises several points for further investigation. First, what were the patients’ responses to docetaxel in comparison to men treated with docetaxel who already were AR-V7 negative? The AR is potentially a target for taxanes as they affect cytoplasmic-to-nuclear AR-trafficking. In a paper by Thandi-Mule, the presence of AR-V7 impaired response to taxanes in vitro [10]. For the patients in this study, looking at PSA response as an indicator of response to therapy (as was done in this paper) suggests that there was no difference, with half in each group showing PSA declining by >50%. This issue could be studied in more depth with the original study’s patient population since 50% of those patients who failed second-line AR-directed therapy were AR-V7 negative [8]. Second, the patients who reverted to AR-V7 negative had all stopped abiraterone or enzalutamide therapy before starting taxane therapies, suggesting that AR-directed therapy could be driving AR-V7 positivity, not taxanes inhibiting AR-V7 synthesis. Third, if the AR-V7 status remains negative after stopping the taxanes does this indicate that the patients will again respond to AR-directed therapy or would they quickly revert back to AR-V7 positivity and be resistant again? None of the patients in this current study who reverted to AR-V7-negative status were put on AR-directed therapies following taxane therapy. Although there is a study showing the benefit of abiraterone after taxanes [11], no studies have shown the benefit of abiraterone or enzalutamide in an abiraterone/enzalutamide—docetaxel—abiraterone/enzalutamide sequence. Certainly, there are reports of patients regaining responses to abiraterone after taxane therapy but no assessment of AR-V7 status was available for those patients [11]. Further, a recent paper from Antonarakis’ group using testosterone therapy in advanced disease suggests that the loss of AR-V7 may be an indication that re-sensitization to AR-directed therapy may occur [12]. Of note, in Antonarakis et al.’s original paper, although the CTCs tested positive for AR-V7, the in situ detection of AR-V7 mRNA in biopsies demonstrated heterogeneous expression of AR-V7 [8]. If the pressure of AR-directed therapies is loosened, then the AR-V7-negative cells could
repopulate the metastasis. If those cells express AR-FL, which appears to be the case with the current patient population, then those cells would respond to a second round of abiraterone or enzalutamide. The issue with this hypothesis is that patients could remain unresponsive to enzalutamide or abiraterone for reasons other than AR-V7 expression, e.g. intra-tumoral androgen synthesis or activating mutations of the AR. Additionally, Nakazawa et al point out that, in some cases, ‘reversion’ may actually have been due the absence of CTCs (because of effectiveness of therapy), not necessarily downregulation of AR-V7. The AdnaTest does not detect CTCs but rather isolates RNA from presumed CTCs based on a selection of cells that bind to a proprietary panel of antibodies.

What is novel about the current paper by Nakazawa et al. is that it demonstrates for the first time the dynamic changes in expression of AR-V7 during the course of cancer progression and therapies [9]. Although this paper deals with small patient numbers that do not permit statistical evaluation-based conclusions, it does point out that the use of ‘liquid biopsies’ of prostate cancer patients may be of value in determination of treatment. Some of the potential treatment forks that may be determined by this test are shown in Figure 1 above. This paper also demonstrates that patients may revert to an AR-V7-negative status when they are transferred to taxane therapies. However, whether the test has become negative because the tumor has stopped generating AR-V7, the tumor has become AR negative, or because there are no longer CTCs around is not clear but the distinctions between these possibilities are important and could affect response to future therapies.

As with any biomarker, appropriately sized and rigorously evaluated prospective studies are needed before a test should be put into clinical practice. At best, use of the test as currently available outside of confirmative biomarker studies should be limited to clinical trials (e.g. http://finance.yahoo.com/news/tokai-pharmaceuticals-announces-initiation-phase-110000361.html). Most importantly, as Nakazawa et al. point out, no clinical conclusions should be drawn from these data until larger studies have been carried out across multiple institutions [9]. Of further importance is the distinction between a biomarker and mechanism of action. For instance, PSA is a biomarker that is used for progression of prostate cancer but there is no evidence that it is a mechanism driving prostate cancer progression. Although AR-splice variants have been shown to be resistant mechanisms in preclinical models, their role in the patient with castration-resistant prostate cancer has yet to be confirmed. If AR-V7 is both a biomarker and a mechanism of resistance, it will be imperative to follow its expression patterns in patients through the course of their therapies.

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references


