AR-V7 biomarker testing for primary prostate cancer: The ongoing challenge of analytical validation and clinical qualification

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Development of therapies targeting the androgen receptor (AR) signaling axis have improved the outcome for patients with metastatic castration-resistant prostate cancer (CRPC) and treatment-naïve metastatic castration-sensitive PC (CSPC) [1]. However, primary and acquired resistance to therapies targeting the AR signaling axis, which include both LHRH analogues and second generation inhibitors such as abiraterone and enzalutamide, is inevitable, and the development of predictive biomarkers that identify those patients that benefit from these therapies remains critically important; such tests could impact survival, quality of life and reduce the high costs associated with these therapies.

Qualification of molecular biomarkers for standard clinical use remains a challenge. AR splice variant-7 (AR-V7) is one such promising biomarker having been associated with resistance to currently available AR-targeted therapies. Retrospective studies assessing AR-V7 mRNA and protein levels from tissue biopsies, circulating tumor cells (CTCs), and whole blood have demonstrated that expression of AR-V7 is associated with resistance to AR-targeted therapies [2–6]. Additionally, a prospective study suggests CTC AR-V7 mRNA and protein expression are associated with worse progression-free survival and poorer overall survival in patients with CRPC receiving abiraterone and/or enzalutamide [7].

Although AR-V7 has been well studied in patients with advanced CRPC, its role as a predictive biomarker earlier in the disease course, specifically in primary prostate cancer,

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remains less well studied [8–10]. We read with interest the recent study by Kaczorowski and colleagues that utilized two AR-V7 antibodies (AG10008 and RM7) to determine the incidence and clinical impact of AR-V7 protein expression in primary disease [11]. The authors directly compared the two antibodies; however, common challenges of antibody (and molecular biomarker) validation should be considered when interpreting these data. We wish to highlight concerns regarding the test patient cohort, antibody specificity, and orthogonal validation.

First, the authors report a relatively common incidence (~20%) of nuclear AR-V7 positivity using both AG10008 and RM7, compared to our own study of primary prostate cancer, where nuclear AR-V7 positivity was low (<1%) [2, 11]. Unlike our study, where patients were completely treatment-naïve, Kaczorowski and colleagues indicated that 43 patients had received neoadjuvant hormonal therapy (with a median duration of 35 days). This may explain the striking difference between studies, and they should clarify if these were the tumors expressing AR-V7. Indeed, we have recently shown that a subset of patients undergoing intense neoadjuvant ADT express high levels of nuclear AR-V7 protein by RM7 and RNA *in situ* hybridization (ISH) with probes specific for AR-V7 [12]. Furthermore, 75% of patients progressing on primary ADT (with or without bicalutamide) before starting standard systemic therapy for CRPC had detectable nuclear AR-V7 protein expression by RM7 [2]. Therefore, it is expected that tumors exposed to neoadjuvant hormonal therapy would express substantial nuclear AR-V7 protein and it would be important to know if these tumors are those expressing nuclear AR-V7 in this current study [11]. Furthermore, this could influence the clinical outcomes described, as patients treated with neoadjuvant hormones may have had intrinsically adverse clinical features that would reduce the interval until biochemical recurrence, as opposed to truly treatment-naïve patients.

Second, the authors also report that AG10008 is specific for AR-V7 protein by demonstrating that AG10008 recognizes eGFP-tagged AR-V7, but not eGFP or full-length AR by Western blot analysis with AG10008 reactivity being lost in both Western blot and immunohistochemistry (IHC) with an AR-V7 blocking peptide. Prior work from the same group demonstrates differences in AR-V7 expression between mRNA and protein expression in a small patient cohort [8]. It is important to note, that although the authors demonstrate similar analyses and outcomes with RM7, RM7 has been rigorously validated by multiple laboratories [2, 12, 13]. These studies have shown RM7 to be specific for AR-V7 protein by Western blot and IHC (using multiple cell lines with varying full-length AR and AR-V7 expression; both endogenously and through genomic manipulation), by immunoprecipitation, and by demonstrating nuclear AR-V7 protein to be associated with AR-V7 mRNA expression (by RNA ISH and RNA sequencing) in primary prostate cancer and metastatic CRPC [2, 12, 13]. Although the head-to-head comparison of AG10008 and RM7 is admirable, it would be of interest to determine the performance of AG10008 after further extensive analytical validation, similar to what was performed for RM7. Indeed, we have observed through rigorous testing that another AR-V7 antibody used clinically, clone EP343 from Epitomics, has shown off-target protein binding by such analyses [14].

Finally, a more detailed description of the reported heterogeneity amongst AG10008 derived nuclear AR-V7 protein expression and AR-V7 mRNA expression is of interest [8, 11].

Although this may be due to the small number of cases studied; the primary technique utilized for assessing mRNA levels was RT-PCR using primers detecting either AR-V7 or full-length AR [15]. This technique, although rapid, is not without limitations, as the sequences of AR-V7 primers can also detect other AR splice variants. Consequently, these discrepancies, and the absence of orthogonal methods (such as RNA ISH or RNA sequencing), the primary evidence for AG10008 sensitivity and specificity seems to be focused on the IHC analysis of primary prostate cancer [8, 11]. Furthermore, abundant cytoplasmic staining is consistently seen with AG10008, and it would be of interest to know how common this is observed since AR-V7 is a largely nuclear protein. Cytoplasmic staining is rarely seen with RM7 under validated conditions (less than 6% of 144 mCRPC cases); this raises the question whether either antibody was used at too high a concentration leading to false positive signals represented by strong cytoplasmic staining for AR-V7 [2, 8, 11]. As previously reported, even tumors known not to express a protein will stain positive at high antibody concentrations [16]. This has been a challenge with other AR-V7 antibodies with the requirement of a nuclear-specific AR-V7 score with cytoplasmic staining being disregarded [17, 18].

Taken together, studies that compare different antibodies for biomarker identification, such as the one presented by Kaczorowski and colleagues, are important for the development of assays to inform clinical practice [11]. However, many of the differences in nuclear AR-V7 protein detection between AG10008 and RM7 described within this and other studies are due, at least in part, to the differences in antibody validation, assay development, and patient cohorts [2, 8, 11]. Finally, as with CRPC, if nuclear AR-V7 protein expression is identified in localized or metastatic CSPC using analytically validated assays, its clinical significance may only be realized when specific therapies targeting AR-V7 are developed or when AR-V7 levels can be used as a predictive biomarker.

Declaration of competing interest

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