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Association of AR-V7 and Prostate-Specific Antigen RNA Levels in Blood with Efficacy of Abiraterone Acetate and Enzalutamide Treatment in Men with Prostate Cancer

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Abstract

Purpose: We evaluated the association of PSA and androgen receptor splice variant-7 (AR-V7) transcript levels in patients' blood with time to treatment failure (TTF) and overall survival (OS) with abiraterone acetate and/or enzalutamide treatment in castration-resistant prostate cancer (CRPC) patients.

Experimental Design: RNA levels of AR-V7 and PSA in peripheral blood collected before treatment were quantified using droplet digital-PCR in retrospective cohorts treated with abiraterone acetate ($N = 81$) or enzalutamide ($N = 51$) for CRPC. Multivariable Cox regression adjusted for known prognostic factors was used for analyses.

Results: PSA transcripts were detected in 57% of abiraterone acetate–treated patients and in 63% of enzalutamide-treated patients. PSA-positive patients had a shorter TTF than PSA-negative patients [adjusted HR = 2.27 (95% confidence interval (CI) 1.26–4.10) and 2.60 (95% CI, 1.19–5.69); $P = 0.006$ and 0.017 in abiraterone acetate and enzalutamide cohorts, respectively]. Patients with a higher-AR-V7 transcript level had a shorter TTF with abiraterone acetate and enzalutamide in univariate analysis (median 8.0 months vs. 15.6 months, $P = 0.046$ in abiraterone acetate-cohort and 3.6 months vs. 5.6 months; $P = 0.050$ in enzalutamide cohort). In multivariable models, the association with TTF remained significant in the enzalutamide cohort (adjusted HR = 2.02; 95% CI, 1.01–4.05; $P = 0.048$), but statistically insignificant in the abiraterone acetate cohort. In both cohorts, we observed potential prognostic value of both PSA and AR-V7 RNA expression on OS; patients with detectable PSA transcripts and high AR-V7 predicted the poorest OS.

Conclusions: PSA and AR-V7 transcripts in blood potentially serve as biomarkers predicting TTF and OS with abiraterone acetate or enzalutamide treatment. If validated prospectively, their detection could be facilitated without isolation of circulating tumor cells. *Clin Cancer Res; 23(3); 726–34. ©2016 AACR.*

Introduction

Androgen deprivation therapy (ADT) is the most effective and widely used treatment for advanced hormone-sensitive prostate cancer (HSPC) patients (1, 2). Most HSPCs initially respond to ADT but eventually progress to castration-resistant prostate cancer (CRPC), usually leading to death (3–5). The androgen receptor (AR) remains a principal target in CRPC, which can be sustained by intratumoral androgens from circulating adrenal androgens or from *de novo* synthesis (6–10). New therapies targeting the AR signaling axis have emerged for CRPC (11, 12). The FDA recently approved several drugs for the treatment of metastatic CRPC and two of these drugs target the AR or the androgen synthesis pathway. Abiraterone acetate is an inhibitor of CYP17A1 that blocks androgen production (13, 14). Abiraterone acetate can also be converted to a more active Δ4-abiraterone *in vivo*, which blocks multiple steroidogenic enzymes and antagonizes AR (15). Enzalutamide is an AR antagonist that binds to the ligand-binding domain of the AR, competing with testosterone and dihydrotestosterone and thereby blocking the translocation of AR to nucleus, inhibiting AR function (16, 17). Even though studies have shown that these drugs prolong overall survival (OS), a significant proportion of patients does not respond to these drugs or develops resistance shortly after the treatment (18–23).

The AR splice variant 7 (AR-V7), which lacks the ligand-binding domain but retains functional transcriptional element binding domains, mediates intracellular AR signaling in a ligand-independent manner (24–26). Accumulating evidence has suggested the association of the presence of AR-V7 with CRPC development and resistance to treatment with abiraterone acetate and enzalutamide (27–35). In a small study, Antonarakis and colleagues (36) reported that detection of AR-V7 mRNA in circulating tumor cells (CTC) from CRPC

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Translational Relevance

Our study indicated that the quantity of PSA and AR-V7 transcripts detected in the blood is inversely associated with time to treatment failure (TTF) and overall survival (OS) in castration-resistant prostate cancer patients who were treated with abiraterone acetate and/or enzalutamide treatment. This result suggests that PSA and AR-V7 transcripts in patients' blood may potentially serve as biomarkers predicting TTF and OS for treatments targeting at androgen receptor axis in castration-resistant prostate cancer patients. The combination of PSA and AR-V7 signals likely provides a more accurate assessment. If validated prospectively, the direct detection of AR-V7 and PSA transcripts in the blood could be a simple and powerful prognostic tool without the need for the isolation of circulating tumor cells.

patients was associated with resistance to abiraterone acetate and enzalutamide. Later, Antonarakis and colleagues and other groups showed that detection of AR-V7 in CTCs from men with metastatic CRPC did not appear to be associated with primary resistance to taxanes (37, 38). However, a more recent study by Scher and colleagues demonstrated that the CTC nuclear expression of AR-V7 protein in men with mCRPC is associated with superior survival on taxane therapy over AR-directed therapy in a clinical practice setting (39). Nevertheless, these results suggested that AR-V7 might serve as a biomarker for treatments targeting the AR axis in CRPC.

We previously demonstrated the prognostic value of detection of PSA transcripts in the blood of men with CRPC (40–42). This study was intended to evaluate the potential application of whole blood AR-V7 and PSA transcript levels as prognostic markers for mCRPC patients treated with abiraterone acetate and enzalutamide. We performed quantitative analysis of AR-V7 and PSA transcripts in blood to determine their association with time to treatment failure (TTF) and OS. Because expression of AR-V7 and PSA are thought to be prostate tumor cell–specific, signals detected in the peripheral blood mononuclear cell fraction would presumably represent those from CTCs. Thus, we evaluated the expression levels of AR-V7 and PSA in the peripheral blood mononuclear cell fraction using droplet-digital PCR (ddPCR; refs. 43, 44), from samples derived pre-abiraterone and/or pre-enzalutamide treatment, in retrospective cohorts of men.

Materials and Methods

Patients

Patients were retrospectively identified from Dana-Farber Cancer Institute Prostate Clinical Research Information System (CRIS) database (45). Eligible patients were those who had CRPC treated with abiraterone acetate and/or enzalutamide between April 2010 and May 2015 and had the peripheral blood mononuclear cell fraction–derived RNA available within 1 year prior to treatment initiation. All patients were consented to an Institutional Review Board-approved protocol that permits collection of clinical and specimen data. Baseline patient characteristics and treatment history were extracted from the database and medical record review.

Sample collection and RNA extraction

The peripheral blood mononuclear cell fraction were isolated by density separation over Ficoll-Hypaque (GE Healthcare) from 8 mL of whole blood collected in Vacutainer cell preparation tubes (BD Biosciences) with EDTA within 2 hours after blood drawn (46). Samples generated from patients were then blind-coded. Total RNA from the peripheral blood mononuclear cell fraction was extracted with TRIzol reagent (Life Technologies) according to the manufacturer's protocol. The resulting RNA was precipitated in 70% ethanol and 0.3 mol/L sodium acetate, and stored at −80°C until use. RNA was further purified by phenol/chloroform extraction and resuspended in nuclease-free water prior to experiments. Quality of purified RNA samples was verified on 2% agarose E-gels (Life Technologies) and no visible signs of degradation were shown; RNA concentrations were determined using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies).

cDNA synthesis

Reverse transcription (RT) was conducted using iScript advanced cDNA Synthesis Kit (Bio-Rad) with random hexamer primers as per manufacturer's instruction. Briefly, approximately 2.5 μg of total RNA sample was subjected to RT-PCR in the 15-μL reaction system; and incubated at 42°C for 30 minutes and 85°C for 5 minutes. cDNA was stored at −20°C until use for ddPCR.

Droplet-digital PCR

ddPCR (QX200; Bio-Rad) was used in this study. Droplet generation, PCR reactions, and detection were carried out according to the manufacturer's instruction (43, 47). Briefly, the reactions were performed in 20-μL reaction volume that consisted of 10 μL of 2× ddPCR Supermix for probes (No dUTP; Bio-Rad), 1 μL of gene-specific primers (900 nmol/L) and probes (250 nmol/L) and 2 μL of the cDNA sample. Each reaction mix was converted to droplets with the QX200 droplet generator (Bio-Rad). Droplet-partitioned samples were then transferred to a 96-well plate, sealed, and cycled in a C1000 Touch thermal cycler (Bio-Rad) under the following cycling protocol: 95°C for 10 minutes, followed by 40 cycles of 94°C for 30 seconds, 60°C for 60 seconds, and a 10-minute incubation at 98°C. The cycled plates were then read on a Bio-Rad QX200 droplet reader. At least two negative control wells with no cDNA template were included in every run. The data analysis was performed with QuantaSoft droplet reader software v1.7.4 (Bio-Rad). The target mRNA concentrations were calculated using Poisson statistics (44) and the background was corrected on the basis of the data of the no template control. Absolute transcript levels were initially presented as copies per microliter and converted to copies per microgram RNA based on the input amount of RNA.

Primers and probes for the full-length AR (AR-FL), AR-V7, and PSA are provided in Supplementary Data.

Statistical analysis

The cutoff for high AR-V7 was set at the upper tertile of the expression value normalized to the amount of input-RNA because similar results (TTF or OS) were observed between the lowest and middle tertile so they were combined. Samples with detectable PSA transcripts were considered PSA positive. Patient disease characteristics and prior treatments were

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evaluated by descriptive statistics; Fisher exact and Wilcoxon rank-sum tests were used to compare the expression status of AR-V7 (high versus low) or PSA (positive or negative) for categorical and continuous characteristics.

TTF was defined as time from treatment initiation until the date of drug discontinuation for any reason, censored at the date of last follow-up for patients who were still on therapy. OS was defined as time from treatment initiation to death from any cause, censored at the date of last follow-up for patients who were still alive. The distributions of TTF and OS were estimated using the Kaplan–Meier method, with 95% confidence intervals (CI); their associations with AR-V7 and PSA expression status were evaluated using the log-rank test or the Wald χ^2 test in multivariable Cox regression model adjusted for known prognostic factors. The multivariable model was constructed by including variables with $P < 0.10$ in univariate analysis; no formal model selection was used. For patients treated with abiraterone acetate, we included baseline PSA (dichotomized at the median), albumin < lower limit of normal (yes versus no), ECOG performance status (0 versus >0), prior use of docetaxel (yes versus no) as covariates. Similar covariates were used for those treated with enzalutamide except "prior use of abiraterone acetate (yes versus no)" was used as a covariate instead of albumin. Statistical analyses were performed with SAS version 9.4 (SAS Institute) and all statistical tests were two-sided.

Results

Detection of AR-V7 and PSA in the peripheral blood mononuclear cell fraction by ddPCR

We first conducted a series of experiments intended to optimize conditions for the detection of AR-V7 and PSA by ddPCR. The absolute quantification and linearity detection of the transcripts were validated by using a series of diluted plasmid DNAs; cross-reaction between AR-V7 and AR-FL were not detected (Supplementary Fig. S1). To document its sensitivity, AR-V7 transcripts were determined in DU145 (an AR-negative and PSA-negative prostate cancer cell line) cells spiked with different numbers of 22RV1 cells, a prostate-cancer cell line known to express both AR-FL and AR-V7. As shown in Supplementary Fig. S2, AR-V7 transcripts were detectable in samples with one 22RV1 cell to 10^4 DU145 cells. We measured the level of AR-FL in the peripheral blood mononuclear cell fraction and found expectedly high levels of expression of AR-FL, potentially masking the detection of tumor-derived AR-FL. After the validation of linearity of detection, we applied the assay to patient samples. As examples, droplet plots and histogram of six representative samples and the negative control are shown in Supplementary Fig. S3.

In total, we assayed 171 pretreatment samples: 102 were abiraterone acetate–treated and 69 were enzalutamide-treated patients. In both cohorts, samples were removed if (i) they were from the same patient ($N = 11$ and 12), (ii) they were ineligible

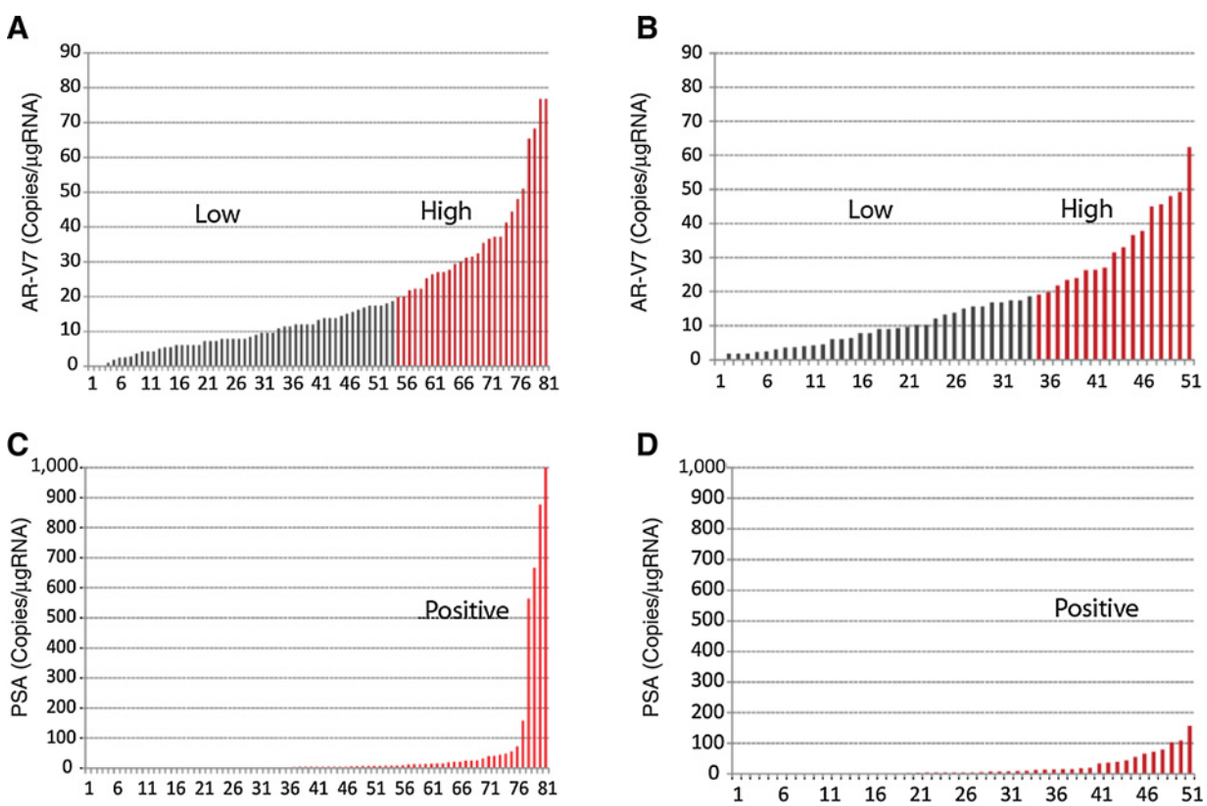

Figure 1.

The distribution of AR-V7 and PSA transcript levels in the peripheral blood mononuclear cell fraction of samples. AR-V7 transcripts in abiraterone acetate (AA; **A**) and enzalutamide cohort (**B**); PSA transcripts in AA (**C**) and enzalutamide cohort (**D**). *x*-axis represents the number of samples; y-axis represents the normalized transcription numbers.

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Table 1. Patient and disease characteristics

	Abiraterone acetate cohort ($N = 81$) *N* (%)	Enzalutamide cohort ($N = 51$) *N* (%)
At diagnosis of cancer		
Gleason		
6 or less	7 (8.6)	8 (15.7)
7	27 (33.3)	16 (31.4)
8–10	40 (49.4)	21 (41.2)
Unknown	7 (8.6)	6 (11.8)
At treatment initiation		
ECOG performance status		
0	62 (76.5)	28 (54.9)
1	9 (11.1)	16 (31.4)
≥2	5 (6.2)	5 (9.8)
Unknown	5 (6.2)	2 (3.9)
Presence of metastasis	79 (97.5)	50 (98.0)
Low albumin (<LLN)[a]	5 (6.8)	7 (14.3)
Prior use of docetaxel[b]	25 (30.9)	23 (45.1)
Prior use of abiraterone acetate[b]	—	35 (68.6)
Prior use of enzalutamide[b]	4 (4.9)	—
Age, years (median)	68.3 (IQR: 62–74; range: 46–89)	69.0 (IQR: 63–74; range: 50–88)
PSA, ng/mL (median)	16.4 (IQR: 4.8–52.1; range: 0.1–972.1)	45.5 (IQR: 7.4–126.6; range: 0.3–1148.4)
Months from sample collection to treatment start		
≤3	33 (40.7)	19 (37.3)
3–6	20 (24.7)	13 (25.5)
>6	28 (34.6)	19 (37.3)

Abbreviation: IQR, interquartile range.
[a]Evaluable $N = 74$ and 49 for abiraterone acetate and enzalutamide cohort, respectively.
[b]Treatment for metastasis or as a secondary hormone for castration-resistant prostate cancer.

(i.e., sample was collected post therapy initiation; abiraterone acetate or enzalutamide was used in the adjuvant setting; patients had been treated with prior abiraterone acetate before sample collection in the abiraterone acetate cohort; or outcome data was not assessable) after we systemically reviewed clinical data ($N = 7$ and 5), or (iii) the housekeeping TBP gene expression was extremely low ($N = 3$ and 1) in the abiraterone acetate and enzalutamide cohort, respectively. The final number was 81 and 51 in the abiraterone acetate and enzalutamide cohort, respectively. The number of transcripts was normalized to the amount of input RNA prior to the analysis.

The overall distributions of AR-V7 and PSA transcripts detected in the abiraterone acetate and the enzalutamide cohorts are shown in Figure 1. PSA transcripts were detected in 46 (57%) of abiraterone acetate–treated patients and in 32 (63%) of enzalutamide-treated patients. AR-V7 transcripts were detected in greater than 95% of patients in both cohorts. The distribution of AR-V7 expression level was similar in the abiraterone acetate- and enzalutamide-treated patients [median and interquartile range: 13.2 (7.2, 26.4) and 13.8 (6.0, 24.0) copies/μgRNA, respectively). In the abiraterone acetate cohort, among 27 patients with high AR-V7 expression (defined as the top tertile, i.e., >19 copies/μgRNA), 21 (78%) patients demonstrated PSA transcripts and 6 (22%) patients did not. Similarly, in the enzalutamide cohort, the majority of patients with high AR-V7 patients were also positive for PSA transcripts (77%).

Patient characteristics and outcomes

Patient and disease characteristics were presented in Table 1. Eighty-one were abiraterone acetate–treated patients and 51 were enzalutamide-treated patients. Twenty-five (30.9%) of patients in the abiraterone acetate cohort received prior docetaxel. In the enzalutamide cohort, 23 (45.1%) patients received prior docetaxel and 35 (68.8%) also received prior abiraterone acetate for CRPC or HSPC. Median follow-up was 29.7 (range: 3.6^{+}, 47.5) and 23.9 (range: 0.9^{+}, 48.3) months in the abiraterone acetate and enzalutamide cohorts, respectively. In the abiraterone acetate cohort, median TTF and OS were 10.3 (95% CI, 8.0–19.9) and 34.4 (95% CI, 25.5–38.7) months, respectively. In the enzalutamide cohort, median TTF and OS were only 3.7 (95% CI, 2.8–6.0) months and 21.4 (15.1–35.3) months, respectively. These differences in outcomes were likely due to baseline characteristics including high prior treatment with abiraterone acetate in the enzalutamide treated cohort.

Association of PSA and AR-V7 expression with patient characteristics

Median serum PSA value was higher in patients with detectable PSA transcripts (62.7 ng/mL vs. 8.5 ng/mL, $P = 0.051$) in the enzalutamide cohort; but this trend was not statistically significant in the abiraterone acetate cohort (median 18.2 vs. 16.4, $P = 0.357$). Serum PSA value was not associated with patient AR-V7 expression status in both cohorts ($P = 0.502$ and 0.811, respectively). In addition, PSA and AR-V7 transcript status in the peripheral blood mononuclear cell fraction was not associated with patients' other baseline characteristics, such as ECOG performance status and albumin ($P > 0.30$, data not shown). However, we observed that patients who had started chemotherapy at time of sample collection more likely had high AR-V7 or positive PSA transcripts than those who had not received chemotherapy in the abiraterone acetate cohort. Similar trend was also observed in the enzalutamide cohort (Supplementary Table S1).

Association of PSA and AR-V7 transcript level in the peripheral blood mononuclear cell fraction with TTF

PSA transcript positive patients had a shorter TTF than PSA-negative patients; the adjusted HR was 2.27 (95% CI, 1.26–4.10)

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Table 2. TTF and OS from treatment initiation (abiraterone acetate cohort)

	TTF			OS		
	***N/N* events**	**Median, months**	**Adjusted HR[a] (95% CI)**	***N/N* events**	**Median, months**	**Adjusted HR[a] (95% CI)**
All patients ($N = 81$)	81/58	10.3 (8.0–19.9)	—	81/35	34.4 (25.5–38.7)	—
By AR-V7 expression						
Low	54/36	15.6 (8.1–21.3)	1.00 (reference)	54/19	35.6 (27.3–43.2)	1.00 (reference)
High (top 33th percentile)	27/22	8.0 (4.2–12.1)	1.31 (0.74–2.32)	27/16	27.2 (13.0–34.4)	1.73 (0.83–3.60)
P		0.046	0.353		0.060	0.145
By PSA expression						
Negative	35/20	21.1 (14.8–27.6)	1.00 (reference)	35/10	43.2 (29.8–NR)	1.00 (reference)
Positive	46/38	7.7 (5.5–9.1)	2.27 (1.26–4.10)	46/25	27.3 (20.0–34.4)	1.84 (0.81–4.19)
P		0.002	0.006		0.019	0.146
By PSA and ARV7 status						
PSA negative	35[a]/20	21.1 (14.8–27.6)	1.00 (reference)	35/10	43.2 (29.8–NR)	1.00 (reference)
PSA positive and low ARV7	25/18	8.1 (6.5–9.2)	2.04 (1.03–4.02)	25/10	34.4 (21.4–NR)	1.30 (0.50–3.37)
PSA positive and high ARV7	21/20	5.6 (3.2–8.6)	2.58 (1.29–5.15)	21/15	21.3 (10.8–33.6)	2.82 (1.11–7.18)
P		0.0001	0.018		0.004	0.071

Abbreviation: NR, no response.

[a]Adjusted for variables with $P < 0.10$ in univariate analysis, including baseline PSA (dichotomized at the median), low albumin (yes vs. no), ECOG PS (0 vs. >0), prior use of docetaxel (yes vs. no).

in the abiraterone acetate cohort and 2.60 (95% CI, 1.19–5.69) in the enzalutamide cohort (adjusted $P = 0.006$ and 0.017, respectively). Patients with high AR-V7 expression tended to have a shorter TTF: median 8.0 months versus 15.6 months in the abiraterone acetate cohort (log-rank $P = 0.046$) and median 3.6 months versus 5.6 months in the enzalutamide cohort (log-rank $P = 0.050$). In multivariable analysis when adjusted for the above-mentioned covariates, AR-V7 remained significant in the enzalutamide cohort [adjusted HR = 2.02 (95% CI, 1.01–4.05), $P = 0.048$], but not in the abiraterone acetate cohort [adjusted HR = 1.31 (95% CI, 0.74–2.32), $P = 0.353$], as shown in Tables 2 and 3 and Figs. 2A, C, and E and 3A, C, and E. For both cohorts, the effect of PSA and AR-V7 transcripts on TTF appeared additive. Those with both PSA positive and high AR-V7 expression had the shortest TTF compared with those with no PSA transcript detected or those who were PSA transcript positive but low AR-V7 expression [median 21.1, 8.1, and 5.6 months, adjusted HR = 1.00, 2.04 (95% CI, 1.03–4.02) and 2.58 (95% CI, 1.29–5.15), respectively in the abiraterone acetate cohort; median 13.4, 3.0, and 2.8 months, adjusted HR = 1.00, 2.02 (95% CI, 0.85–4.82) and 3.70 (95% CI, 1.52–8.96) in the enzalutamide cohort; Tables 2 and 3].

Association of PSA and AR-V7 expression with OS

In both cohorts, we observed that patients with high AR-V7 expression had a shorter OS (median OS: 35.6 months versus 27.2 months in the abiraterone acetate cohort and 29.1 months versus 13.8 months in the enzalutamide cohort). Similarly, median OS was shorter among men with detectable PSA transcripts than among those with negative PSA transcripts (Tables 2 and 3 and Figs. 2 and 3B, C, and F). However, these observed associations became insignificant in the multivariable models adjusted for other baseline factors, most likely due to the limited number of deaths (i.e., 35 deaths in the abiraterone acetate cohort and 25 deaths in the enzalutamide cohort) in these analyses (HR ranging 1.6–2.1, $P > 0.05$). In addition, we observed an additive effect of PSA transcript detection and AR-V7 expression in the prediction for OS (Figs. 2F and 3F). When compared with those with negative PSA or those with PSA positive but low AR-V7 expression, those with both positive PSA transcripts and high AR-V7 transcripts had the worst OS [median 43.2, 34.4, and 21.3 months, adjusted HR = 1.00, 1.30 (95% CI, 0.50–3.37) and 2.82 (95% CI, 1.11–7.18), respectively in the abiraterone acetate cohort; median 29.4, 20.4 and 12.5 months, adjusted HR = 1.00, 1.08 (95% CI, 0.37–3.14)

Table 3. TTF and OS from treatment initiation (enzalutamide cohort)

	TTF			OS		
	***N/N* events**	**Median, months**	**Adjusted HR[a] (95% CI)**	***N/N* events**	**Median, months**	**Adjusted HR[a] (95% CI)**
All patients ($N = 51$)	51/43	3.7 (2.8–6.0)		51/25	21.4 (15.1–35.3)	
By AR-V7 expression						
Low	34/28	5.6 (2.9–13.4)	1.00 (reference)	34/16	29.1 (16.6–39.8)	1.00 (reference)
High	17/15	3.6 (0.9–5.1)	2.02 (1.01–4.05)	17/9	13.8 (7.4–35.3)	2.08 (0.83–5.24)
P		0.050	0.048		0.242	0.119
By PSA expression						
Negative	19/13	13.4 (3.6–21.5)	1.00 (reference)	19/6	29.4 (17.6–NR)	1.00 (reference)
Positive	32/30	2.9 (2.0–4.5)	2.60 (1.19–5.69)	32/19	16.6 (7.9–35.3)	1.61 (0.63–4.17)
P		0.003	0.017		0.053	0.322
By PSA and ARV7 status						
PSA negative	19/13	13.4 (3.6–21.5)	1.00 (reference)	19/6	29.4 (17.6–NR)	1.00 (reference)
PSA positive and low ARV7	19/17	3.0 (2.0–5.6)	2.02 (0.85–4.82)	19/10	20.4 (7.8–39.8)	1.08 (0.37– 3.14)
PSA positive and high ARV7	13/13	2.8 (0.9–4.9)	3.70 (1.52–8.96)	13/9	12.5 (4.6–35.3)	3.08 (1.02–9.30)
P		0.003	0.015		0.045	0.074

[a]Adjusted for variables with $P < 0.10$ in univariate analysis, including baseline PSA (dichotomized at the median), ECOG PS (0 vs. >0), prior use of docetaxel (yes vs. no), prior use of abiraterone acetate (yes vs. no).

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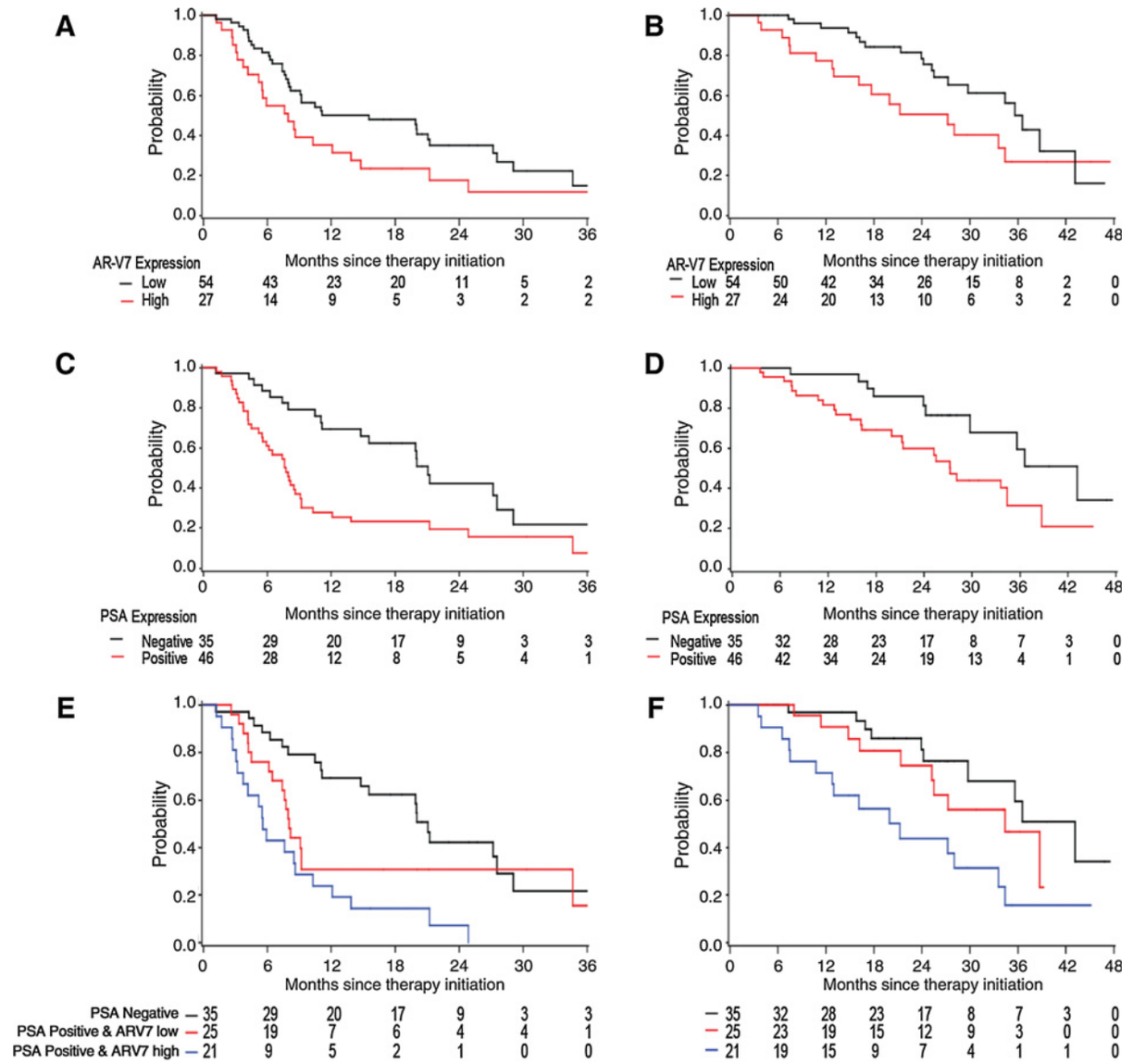

Figure 2.

Kaplan–Meier plots in abiraterone acetate cohort. Plots of TTF according to AR-V7 expression (**A**), PSA expression (**C**), combination of PSA and AR-V7 expression (**E**). Plots of OS according to AR-V7 expression (**B**), PSA expression (**D**), and combination of PSA and AR-V7 expression (**F**) in abiraterone acetate cohort.

and 3.08 (95% CI, 1.02–9.30) in the enzalutamide cohort, as shown in Tables 2 and 3).

Subgroup and sensitivity analysis

All samples for this study were collected within 1 year prior to treatment initiation. We suspected that the time of the blood draw may potentially impact the level of AR-V7 and PSA expression and confound the observed association. Thus, we conducted a subgroup analysis by restricting the samples to those with blood draw within 6 months prior to treatment initiation. The results suggested that there was no significant difference in the groups (HRs ranging 1.3–2.5) but there was limited statistical power for the subgroup analysis (Supplementary Table S2).

In addition, we noticed that a few patients (six in the abiraterone acetate cohort and four in the enzalutamide cohort) who had no PSA transcripts detected had high AR-V7 transcripts, though the majority (77%) of patients with high AR-V7 was also positive for PSA transcripts. It is possible that for these cases, AR-V7 might be mainly derived from non-prostate cells. If we consider that these patients may have been misclassified, by placing them in the low AR-V7 group, we found that the association of AR-V7 with TTF and OS became stronger [for TTF: adjusted HR = 1.93 (95% CI, 1.04–3.55) and 2.52 (95% CI, 1.2–5.15); for OS: adjusted HR = 2.47 (95% CI, 1.12–5.41) and 2.95 (95% CI, 1.17–7.5), in the abiraterone acetate and enzalutamide cohort, respectively].

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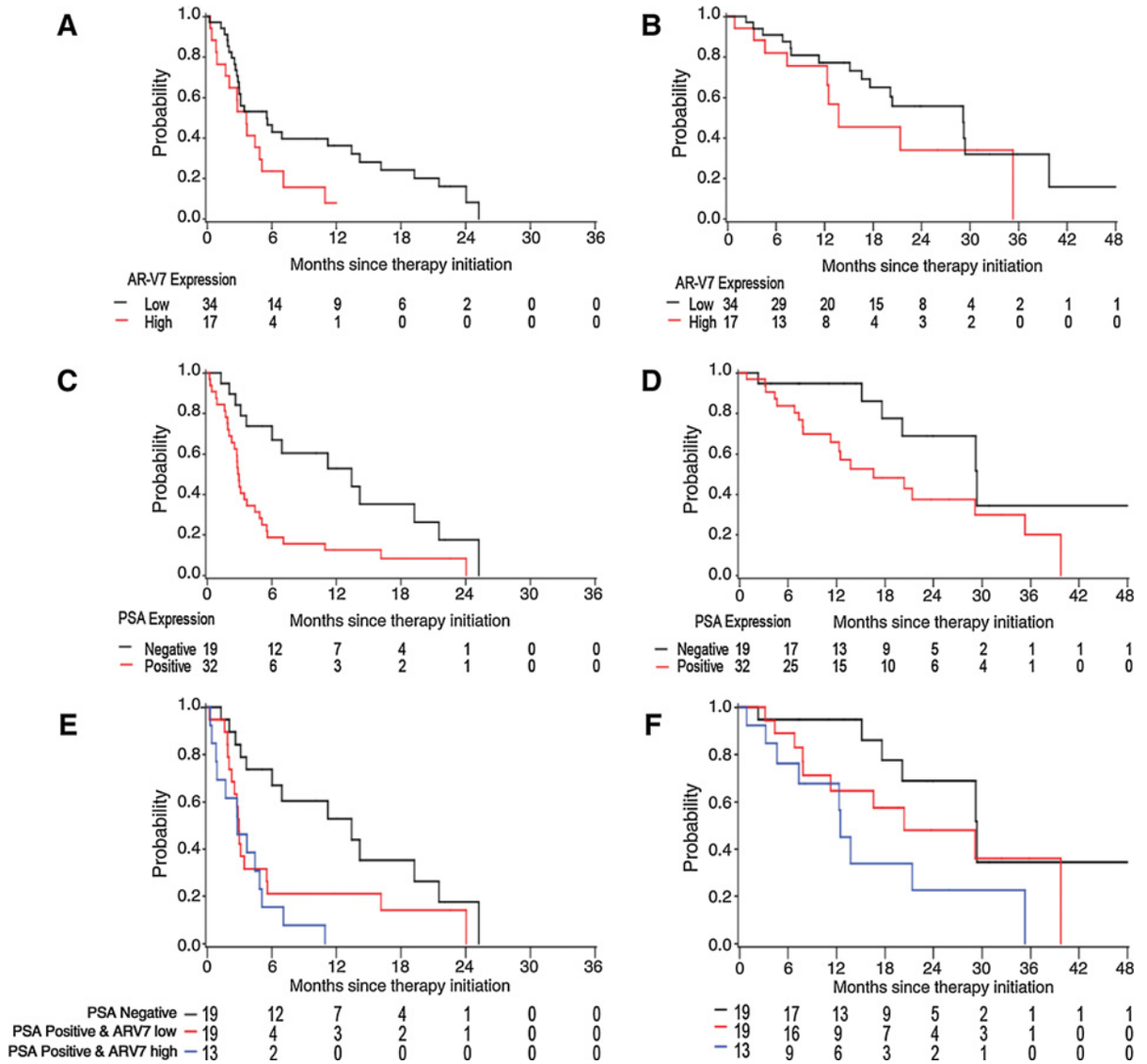

Figure 3.

Kaplan–Meier plots in enzalutamide cohort. Plots of TTF according to AR-V7 expression (**A**), PSA expression (**C**), and combination of PSA and AR-V7 expression (**E**). Plots of OS according to AR-V7 expression (**B**), PSA expression (**D**), and combination of PSA and AR-V7 expression (**F**) in enzalutamide cohort.

Discussion

We had previously shown that PSA detected by RT-PCR was predictive of OS (40–42). Under the assumption that tumor-specific transcripts in blood are most likely derived from CTCs, we directly analyzed the tumor specific RNAs, AR-V7, and PSA, in the peripheral blood mononuclear cell fraction, thus bypassing the isolation of CTCs. Our data showed that the detection of PSA transcripts in the peripheral blood mononuclear cell fraction predicted TTF in CRPC patients who were treated with abiraterone acetate or enzalutamide, with adjustment of serum PSA, albumin, ECOG performance status, prior use of docetaxel, and/or prior use of abiraterone acetate. The association of AR-V7 expression with the TTF was statistically significant in univariable analyses but insignificant in multivariable analysis although trends remained. Both the transcript level of PSA and AR-V7 in the peripheral blood mononuclear cell fraction were associated with shorter OS (HR ranging from 1.6 to 2.1) and the prognostic effect was additive. These results demonstrate the potential application of PSA and AR-V7 transcripts in the peripheral blood mononuclear cell fraction as biomarkers for predicting OS and potentially TTF on abiraterone acetate or enzalutamide treatment.

Among 132 samples analyzed, we found that almost all of the samples had detectable AR-V7, whereas only approximately 60% of samples had detectable PSA. There was some degree of correlation as most patients with high AR-V7 also had positive PSA expression. We noticed that a few patients had no detectable PSA transcripts but high AR-V7 transcripts. It is possible that some CTCs express AR-V7 but not PSA. Another possibility is that in some cases, the AR-V7 may originate from non-

prostate cells as noted in the report by Takeuchi and colleagues (48). Our re-grouping study supported the possibility that in some cases, AR-V7 in the peripheral blood mononuclear cell fraction may not be derived from CTCs and this event may potentially alter the assessment of AR-V7 alone when using this methodology.

For this study, we defined high AR-V7 expression as the upper tertile of expression level (i.e., $\geq$19 copies/μgRNA). The analysis was initially conducted with three tertile groups, and subsequently condensed into two groups as we observed similar results (TTF and OS) among those with low and intermediate AR-V7 expression (data not shown). In addition, classifying 33% of patients to the high expression group was consistent with the reported detection rate (ranging 20%–40%) in circulating tumor cells from patients treated with abiraterone acetate or enzalutamide (37, 38).

The limitation of this study includes its retrospective nature and a wide range of time window for the sample collection. However, we observed a consistent trend when the analysis was restricted to samples collected within 6 months prior to therapy initiation. In addition, due to limited sample size and absent data issue, we could not include some important covariates, such as LDH, hemoglobin, AP, which have been shown to be of prognostic value for patients treated on abiraterone acetate. Finally, we could not analyze treatment response based on PSA and/or radiographic criteria due to incomplete data issue. Also, as no data were available from untreated patients as the control group, it was not possible to investigate the interaction between treatment and these biomarkers to assess its potential predictive value, in addition to its prognostic role.

It is interesting to note that Reig and colleagues recently has reported that the detection of TMPRSS2–ERG fusion transcripts in the peripheral blood mononuclear cell fraction predicted a lower PSA progression-free survival to docetaxel or cabazitaxel treatments (49). Because the TMPRSS2–ERG fusion is driven by AR and unique to prostate cancer cells, we would consider the detection of TMPRSS2–ERG fusion transcript in our future validation studies. This additional control may help eliminate issues relating to signals derived from non-tumor cells. Currently, we are performing validation analyses in cohorts obtained from other institutions.

In conclusion, this study suggests that PSA and AR-V7 transcripts detected in the peripheral blood mononuclear cell fraction may potentially serve as biomarkers predicting TTF and OS with abiraterone acetate or enzalutamide treatment. The combination of PSA and AR-V7 signals may provide a more accurate assessment. If validated prospectively, the direct detection of AR-V7 and PSA transcripts in the peripheral blood mononuclear cell fraction could be a simple and powerful prognostic tool without the need for CTC isolation.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): G.-S. Mary Lee
Study supervision: G.-S. Mary Lee, P.W. Kantoff

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Association of AR-V7 and Prostate-Specific Antigen RNA Levels in Blood with Efficacy of Abiraterone Acetate and Enzalutamide Treatment in Men with Prostate Cancer

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