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

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ABSTRACT

Purpose

We reported previously that the detection of androgen receptor splice variant-7 (AR-V7) mRNA in circulating tumor cells (CTCs) correlated with poor outcomes from the use of abiraterone and enzalutamide in patients with castration-resistant prostate cancer (CRPC). Here, we expanded our cohort size to better characterize the prognostic significance of AR-V7 in this setting.

Methods

We prospectively enrolled 202 patients with CRPC starting abiraterone or enzalutamide and investigated the prognostic value of CTC detection (+ v -) and AR-V7 detection (+ v -) using a CTC-based AR-V7 mRNA assay. We examined $\geq 50\%$ prostate-specific antigen (PSA) responses, PSA progression-free survival, clinical and radiologic progression-free survival, and overall survival. We constructed multivariable models adjusting for PSA, Gleason sum, number of prior hormone therapies, prior abiraterone or enzalutamide use, prior taxane use, presence of visceral metastases, and Eastern Cooperative Oncology Group score. We also separately examined the first-line and second-line novel hormonal therapy (NHT) settings.

Results

Median follow-up times were 15.0, 21.7, and 14.6 months for CTC-, CTC+/AR-V7-, and CTC+/AR-V7+ patients, respectively. CTC+/AR-V7+ patients were more likely to have Gleason scores ≥ 8 ($P = .05$), metastatic disease at diagnosis ($P = .01$), higher PSA ($P < .01$), prior abiraterone or enzalutamide use ($P = .03$), prior taxane use ($P = .02$), and Eastern Cooperative Oncology Group ≥ 1 ($P = .01$). Outcomes for the overall cohort (and separately for the first-line and second-line NHT cohorts) were best for CTC- patients, intermediate for CTC+/AR-V7- patients, and worse for CTC+/AR-V7+ patients. These correlations remained significant in multivariable models.

Conclusion

This expanded analysis further characterizes the importance of CTC-based AR-V7 mRNA detection in predicting outcomes in patients with CRPC receiving first- and second-line NHT and, to the best of our knowledge, is the first to suggest that this assay be interpreted using three separate prognostic categories: CTC-, CTC+/AR-V7-, and CTC+/AR-V7+.

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INTRODUCTION

Treatment-specific biomarkers (eg, markers that help select or exclude a particular therapy) for metastatic castration-resistant prostate cancer (CRPC) are currently lacking. It has emerged recently that detection of androgen receptor splice variant-7 (AR-V7) in circulating tumor cells (CTCs) may represent one such treatment-

selection marker in men with metastatic CRPC.¹ AR-V7 is an abnormally spliced mRNA isoform of the androgen receptor, producing a protein product lacking the C-terminal ligand-binding domain but retaining the transcriptionally active N-terminal domain. Despite its inability to bind ligand (eg, dihydrotestosterone), AR-V7 remains constitutively active in a ligand-independent manner and is capable of driving CRPC growth.¹

ASSOCIATED CONTENT

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Data Supplement
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Accumulating evidence suggests that CTC-based detection of AR-V7 may be associated with a lack of benefit of novel hormonal therapies (NHT) including abiraterone² and enzalutamide.³ An initial pilot study conducted by our group (n = 62) suggested that AR-V7 mRNA detection was associated with resistance to abiraterone and enzalutamide.⁴ This was confirmed subsequently by additional studies showing a similar lack of benefit of NHT in patients with detectable AR-V7 mRNA or protein.^{5,6} In addition, it has been suggested that CTC-based AR-V7 detection is compatible with sensitivity to taxane chemotherapies such as docetaxel and cabazitaxel.^{7,8} Furthermore, the relative benefit of taxane chemotherapy over NHT may be greater in AR-V7+ patients than in AR-V7- patients where chemotherapy and NHT seem to have comparable efficacy.^{6,7}

These prior studies suffered from two significant limitations. First, because of small sample sizes, these studies were generally not able to explore the prognostic value of AR-V7 separately in the first-line and second-line NHT settings (and typically included a mix of patients, some of whom had received prior NHT). Indeed, the usefulness of the AR-V7 biomarker could be different in the first-line and second-line settings. Second, none of the above-mentioned studies included data on patients without detectable CTCs. This is problematic because some patients with CRPC may not harbor CTCs and therefore cannot be evaluated for AR-V7. The purpose of our analysis was to expand on our study examining AR-V7 in men receiving abiraterone or enzalutamide (currently, n = 202). By doing so, we aimed to better understand the clinical significance of AR-V7 in both the first-line and second-line NHT settings and to explore the prognostic value of CTC- results compared with CTC+/AR-V7- and CTC+/AR-V7+ results.

METHODS

Patients

We prospectively enrolled men with metastatic CRPC who were beginning treatment with enzalutamide or abiraterone. Patients had to have histologically confirmed prostate adenocarcinoma, progressive disease despite castration levels of serum testosterone (< 50 ng/dL), and radiographic metastases on computed tomography (CT) or technetium-99 bone scans. Patients had to have three or more rising serum prostate-specific antigen (PSA) values taken $\geq$ 2 weeks apart, consistent with Prostate Cancer Working Group guidelines.⁹ Patients were excluded if they planned to receive additional concurrent anticancer therapies. Prior taxane chemotherapy was permitted, as was previous treatment with the alternative NHT (ie, prior abiraterone in enzalutamide-treated patients, and vice versa). This study was approved by the Johns Hopkins University institutional review board, and patients provided written informed consent.

Study Design

This was a prospective study evaluating the ability of baseline CTC status (+ v -) and AR-V7 status (+ v -) to predict clinical benefit from NHT. Patients were asked to provide peripheral blood CTC samples at baseline (before beginning NHT) and at the time of progression. Enzalutamide was administered at 160 mg once daily, and abiraterone was administered at 1,000 mg once daily (with prednisone 5 mg twice a day). Follow-up was prospectively defined: patients had PSA measurements every 1 to 2 months, as well as CT (chest, abdomen, and pelvis) and technetium-99 bone scans every 2 to 4 months. Therapy with enzalutamide

or abiraterone was continued until PSA progression, clinical or radiographic progression, or unmanageable drug-related toxicity.

CTC Assay and AR-V7 Detection

CTC analyses were conducted using a modified AdnaTest platform (QIAGEN, Hannover, Germany), as described previously.⁴ Capture of CTCs was performed using the EpCAM-based ProstateCancerSelect kit, and mRNA expression analyses were performed using the ProstateCancerDetect kit with multiplexed reverse-transcription polymerase chain reaction primers to establish the presence or absence of CTCs. Custom primers were designed to detect full-length androgen receptor (AR-FL) mRNA and AR-V7 mRNA, as described previously.⁴ The relative abundance of AR-V7 was determined by calculating the ratio of AR-V7 transcript to AR-FL transcript.

In our previous reports,^{4,7} we presented data only on CTC+ patients, whereas CTC- patients were excluded from analysis (because their AR-V7 status could not be determined). Here, we aimed to consider our biomarker readout in three separate categories: CTC- (AR-V7 agnostic), CTC+/AR-V7-, and CTC+/AR-V7+. In this way, all enrolled patients, even those without detectable CTCs, would contribute data to our study.

Outcome Measures

The primary end point was clinical and radiographic progression-free survival (PFS); progression was defined as symptomatic progression (worsening disease-related symptoms or new cancer-related complications) or radiologic progression (on CT scan: $\geq$ 20% enlargement in sum diameter of target lesions [Response Evaluation Criteria in Solid Tumors⁹]; on bone scan: two or more new bone lesions not caused by flare), or death, whichever occurred first.¹⁰ Secondary end points included PSA response rate, PSA progression-free survival (PSA-PFS), and overall survival (OS). PSA response was defined as the proportion of patients with a $\geq$ 50% PSA decline from baseline at any time point after therapy (and maintained for $\geq$ 3 weeks); best PSA response (maximal percentage decrease from baseline) was also determined. PSA progression was defined as a $\geq$ 25% increase in PSA from nadir (and by $\geq$ 2 ng/mL), requiring confirmation $\geq$ 3 weeks later (Prostate Cancer Working Group criteria).¹⁰ OS was defined as the interval from enrolment to death from any cause.

Statistical Analyses

Clinical outcomes were analyzed separately in the three biomarker groups: CTC-, CTC+/AR-V7-, and CTC+/AR-V7+. PSA response rates were compared using Fisher's exact test. Time-to-event outcomes (PFS, PSA-PFS, and OS) were evaluated using Kaplan-Meier analysis, and survival-time differences were compared using the log-rank test. Univariable and multivariable logistic regressions (for PSA response) and Cox regressions (for time-to-event end points) were used to assess the independent effect of biomarker status on clinical outcomes. Covariates included in the multivariable models were baseline PSA, number of prior hormonal therapies, presence of visceral metastases, Gleason sum, Eastern Cooperative Oncology Group score, prior taxane chemotherapy, and prior abiraterone or enzalutamide. These variables were strongly associated with clinical outcomes in our prior AR-V7 studies.^{4,7} Because of fewer events in the multivariable models for OS, only three covariates were included in these models (baseline PSA, prior chemotherapy, and prior abiraterone or enzalutamide).

Statistical analyses were performed for the cohort as a whole (n = 202; primary analysis) and also separately for the first-line NHT (n = 124) and second-line NHT (n = 78) cohorts. Sample size was determined on the basis of the primary comparison of PFS between CTC+/AR-V7- and CTC+/AR-V7+ patients in the overall cohort. Assuming a 30% prevalence of AR-V7 detection among CTC+ men, 148 CTC+ patients provided 90% power to detect a hazard ratio of 2.0 when median PFS in CTC+/AR-V7- men was 8.0 months, using a two-sided log-rank test at a significance level of .05. This calculation assumed 36 months of accrual time and a 2% dropout rate. Fifty-three of the enrolled patients were CTC- when the

study reached the required sample size of 148 men with CTC+, and these patients were included in the analysis as a separate group. The first-line NHT cohort included patients beginning abiraterone or enzalutamide who had not previously received the alternative agent. The second-line NHT cohort included men beginning abiraterone or enzalutamide who had previously received the alternative drug. All statistical tests were two sided, and *P* values were not corrected for multiple comparisons. Statistical analyses were performed using *R* (version 2.15.1).

RESULTS

Patients

Between December 2012 and November 2015, 202 men (95 starting abiraterone, 107 starting enzalutamide) were prospectively enrolled. As of February 2016, median follow-up times for the at-risk population, calculated using the reverse Kaplan-Meier method, were 15.0, 21.7, and 14.6 months for CTC-, CTC+/AR-V7-, and CTC+/AR-V7+ patients, respectively. Baseline patient characteristics are summarized in Table 1. Overall, 53 of the 202 men (26.2%) were CTC-, 113 of the 202 men (56.0%) were CTC+/AR-V7-, and 36 of the 202 men (17.8%) were CTC+/AR-V7+. CTC+/AR-V7+ patients were more likely to have Gleason scores ≥ 8 ($P = .05$), metastatic disease at diagnosis ($P = .01$), higher PSA levels ($P < .01$), higher alkaline phosphatase levels ($P < .01$), prior abiraterone or enzalutamide use ($P = .03$), prior taxane use ($P = .02$), presence of pain ($P < .01$), and Eastern Cooperative Oncology Group status ≥ 1 ($P = .01$).

One hundred twenty-four patients had not received abiraterone or enzalutamide previously (first-line NHT cohort). Of these, 36 (29.0%) were CTC-, 73 (58.9%) were CTC+/AR-V7-, and 15 (12.1%) were CTC+/AR-V7+. Seventy-eight men had received abiraterone or enzalutamide previously (second-line NHT cohort). Of these, 17 (21.8%) were CTC-, 40 (51.3%) were CTC+/AR-V7-, and 21 (26.9%) were CTC+/AR-V7+.

The prevalence of CTC- patients was lower and the prevalence of CTC+/AR-V7+ patients was higher in the second-line compared with the first-line NHT cohorts.

PSA Responses

Overall ($n = 202$), PSA response rates to enzalutamide or abiraterone were 75.5% (40 of 53) in CTC- patients, 52.2% (59 of 113) in CTC+/AR-V7- patients, and 13.9% (5 of 36) in CTC+/AR-V7+ patients ($P < .001$; Fig 1A). In multivariable logistic regression analysis (Data Supplement), biomarker status remained an independent predictor of PSA response. To understand the clinical characteristics that may permit PSA response to abiraterone or enzalutamide despite detection of AR-V7, we compared baseline characteristics among all CTC+/AR-V7+ patients stratified by whether they achieved a PSA response (Data Supplement). PSA responders had more favorable clinical characteristics (less prior abiraterone or enzalutamide use, less prior docetaxel use, less visceral metastases, less bone pain, lower PSAs, lower AR-FL levels, and lower AR-V7/AR-FL ratios) than did PSA nonresponders. We also discovered that two of these PSA responders received concurrent palliative radiotherapy to an osseous metastatic site, which may have influenced PSA trends.

Among first-line NHT patients ($n = 124$), PSA response rates in CTC- patients, CTC+/AR-V7- patients, and CTC+/AR-V7+ patients were 86.1% (31 of 36), 65.8% (48 of 73), and 26.7% (four of 15), respectively ($P < .001$; Fig 2A). In multivariable logistic regression analysis (Data Supplement), biomarker status remained an independent predictor of PSA response. Among second-line NHT patients ($n = 78$), PSA response rates in CTC- men, CTC+/AR-V7- men, and CTC+/AR-V7+ men were 52.9% (nine of 17), 27.5% (11 of 40), and 4.8% (one of 21), respectively ($P = .003$; Fig 3A). In multivariable logistic regression analysis (Data Supplement), biomarker status generally remained an independent predictor of PSA response.

Table 1. Baseline Characteristics of Patients With CRPC Starting Treatment With Abiraterone or Enzalutamide ($N = 202$)

Baseline Characteristic	CTC- ($n = 53$ [26.2%])	CTC+/AR-V7- ($n = 113$ [56.0%])	CTC+/AR-V7+ ($n = 36$ [17.8%])	<i>P</i> *
Median age, years	70	71	70	.888
Nonwhite ethnicity, %	9	14	17	.575
Years since diagnosis, mean	7.9	7.3	5.6	.098
Gleason sum ≥ 8 at diagnosis, %	68	60	83	.052
Type of primary local therapy, %				.011
Surgery only	12	24	25	
Radiation only	31	30	19	
Both	35	20	8	
None	23	26	47	
M1 disease at diagnosis, %	16	22	47	
No. prior hormonal therapies, median	2	2	3	.158
Time to castration resistance, median no. months	23.0	20.5	14.0	.148
Prior use of abiraterone or enzalutamide, %	32	35	58	.025
Prior use of docetaxel, %	19	25	44	.022
Presence of bone metastases, %	76	83	100	.010
Presence of visceral metastases, %	23	27	34	.559
ECOG performance status ≥ 1 , %	41	24	53	.006
Presence of pain, %	32	40	72	.002
Baseline PSA, ng/mL, median	13.7	31.4	92.0	< .001
Baseline alkaline phosphatase, U/L, median	80	96	120	< .001

Abbreviations: AR-V7, androgen receptor splice variant-7; CRPC, castration-resistant prostate cancer; CTC, circulating tumor cell; ECOG, Eastern Cooperative Oncology Group; PSA, prostate-specific antigen.

**P* values are based on Fisher's exact test and the Mann-Whitney *U* test for categorical and continuous variables, respectively.

Fig 1. Clinical outcomes in the overall cohort of men starting treatment with abiraterone or enzalutamide (N = 202), according to CTC status and AR-V7 status. (A) Waterfall plots depicting best PSA responses according to CTC status and AR-V7 status, expressed in three categories: CTC-, CTC+/AR-V7-, and CTC+/AR-V7+. The dotted line illustrates the threshold for defining a PSA response ($\geq 50\%$ PSA reduction from baseline). PSA response rates in CTC- patients, CTC+/AR-V7- patients, and CTC+/AR-V7+ patients were 75.5% (40 of 53), 52.2% (59 of 113), and 13.9% (5 of 36), respectively ($P < .001$). All three groups were significantly different from each other (CTC- v CTC+/AR-V7-, $P = .005$; CTC- v CTC+/AR-V7+, $P < .001$; CTC+/AR-V7- v CTC+/AR-V7+, $P < .001$). (B) Kaplan-Meier curves indicating PSA progression-free survival according to CTC status and AR-V7 status. Median PSA progression-free survival in CTC- patients, CTC+/AR-V7- patients, and CTC+/AR-V7+ patients was 11.3 months (95% CI, 8.7 to 13.8), 6.2 months (95% CI, 5.8 to 7.3), and 2.1 months (95% CI, 1.9 to 3.1), respectively ($P < .001$). (C) Kaplan-Meier curves indicating clinical and radiographic progression-free survival according to CTC status and AR-V7 status. Median progression-free survival in CTC- patients, CTC+/AR-V7- patients, and CTC+/AR-V7+ patients was 13.9 months (95% CI, 11.0 to not reached), 7.7 months (95% CI, 6.2 to 10.1), and 3.1 months (95% CI, 2.3 to 3.7), respectively ($P < .001$). (D) Kaplan-Meier curves indicating overall survival according to CTC status and AR-V7 status. Median overall survival in CTC- patients, CTC+/AR-V7- patients, and CTC+/AR-V7+ patients was 28.7 months (95% CI, 28.4 to not reached), 29.5 months (95% CI, 18.4 to not reached), and 11.2 months (95% CI, 8.3 to 17.1), respectively ($P < .001$). AR-V7, androgen receptor splice variant-7; CTC, circulating tumor cell; PSA, prostate-specific antigen.

PSA-PFS

In the overall cohort, median PSA-PFS to enzalutamide or abiraterone was 11.3 months (95% CI, 8.7 to 13.8) in CTC- patients, 6.2 months (95% CI, 5.8 to 7.3) in CTC+/AR-V7- patients, and 2.1 months (95% CI, 1.9 to 3.1) in CTC+/AR-V7+ patients ($P < .001$; Fig 1B). In multivariable Cox regression analysis (Data Supplement), biomarker status remained independently prognostic for PSA-PFS.

In the first-line NHT cohort, median PSA-PFS in CTC- patients, CTC+/AR-V7- patients, and CTC+/AR-V7+ patients was 12.7 months (95% CI, 11.7 to 23.9), 7.3 months (95% CI, 6.2 to 12.0), and 2.9 months (95% CI, 2.0 to not reached), respectively ($P < .001$; Fig 2B). In multivariable Cox regression analysis (Data Supplement), biomarker status remained independently prognostic for PSA-PFS. In the second-line NHT cohort, median PSA-PFS in CTC- patients, CTC+/AR-V7- patients, and CTC+/AR-V7+ patients

was 6.4 months (95% CI, 5.1 to not reached), 4.4 months (95% CI, 3.2 to 6.0), and 1.1 months (95% CI, 1.0 to 3.1), respectively ($P < .001$; Fig 3B). In multivariable Cox regression analysis (Data Supplement), biomarker status remained independently prognostic for PSA-PFS.

PFS

In the overall cohort, median PFS was 13.9 months (95% CI, 11.0 to not reached) in CTC- patients, 7.7 months (95% CI, 6.2 to 10.1) in CTC+/AR-V7- patients, and 3.1 months (95% CI, 2.3 to 3.7) in CTC+/AR-V7+ patients ($P < .001$; Fig 1C). In multivariable Cox regression analysis (Data Supplement), biomarker status remained independently prognostic for PFS.

In the first-line NHT cohort, median PFS in CTC- patients, CTC+/AR-V7- patients, and CTC+/AR-V7+ patients was 21.6 months (95% CI, 13.9 to not reached), 10.1 months

Fig 2. Clinical outcomes for men starting abiraterone or enzalutamide in the first-line novel hormonal therapy setting (N = 124), according to CTC status and AR-V7 status. (A) Waterfall plots depicting best PSA responses according to CTC status and AR-V7 status, expressed in three categories: CTC-, CTC+/AR-V7-, and CTC+/AR-V7+. The dotted line illustrates the threshold for defining a PSA response ($\geq 50\%$ PSA reduction from baseline). PSA response rates in CTC- patients, CTC+/AR-V7- patients, and CTC+/AR-V7+ patients were 86.1% (31 of 36), 65.8% (48 of 73), and 26.7% (4 of 15), respectively ($P < .001$). All three groups were significantly different from each other (CTC- v CTC+/AR-V7-, $P = .03$; CTC- v CTC+/AR-V7+, $P < .001$; CTC+/AR-V7- v CTC+/AR-V7+, $P = .009$). (B) Kaplan-Meier curves indicating PSA progression-free survival according to CTC status and AR-V7 status. Median PSA progression-free survival in CTC- patients, CTC+/AR-V7- patients, and CTC+/AR-V7+ patients was 12.7 months (95% CI, 11.7 to 23.9), 7.3 months (95% CI, 6.2 to 12.0), and 2.9 months (95% CI, 2.0 to not reached), respectively ($P < .001$). (C) Kaplan-Meier curves indicating clinical and radiographic progression-free survival according to CTC status and AR-V7 status. Median progression-free survival in CTC- patients, CTC+/AR-V7- patients, and CTC+/AR-V7+ patients was 21.6 months (95% CI, 13.9 to not reached), 10.1 months (95% CI, 7.9 to 14.9), and 4.1 months (95% CI, 3.0 to not reached), respectively ($P < .001$). (D) Kaplan-Meier curves indicating overall survival according to CTC status and AR-V7 status. Median overall survival in CTC- patients, CTC+/AR-V7- patients, and CTC+/AR-V7+ patients was 29.7 months (95% CI, 28.7 to not reached), 30.7 months (95% CI, 29.5 to not reached), and 21.5 months (95% CI, 10.4 to not reached), respectively ($P = .003$). AR-V7, androgen receptor splice variant-7; CTC, circulating tumor cell; PSA, prostate-specific antigen.

(95% CI, 7.9 to 14.9), and 4.1 months (95% CI, 3.0 to not reached), respectively ($P < .001$; Fig 2C). In multivariable Cox regression analysis (Data Supplement), biomarker status generally remained independently prognostic for PFS. In the second-line NHT cohort, median PFS in CTC- patients, CTC+/AR-V7- patients, and CTC+/AR-V7+ patients was 6.2 months (95% CI, 5.4 to not reached), 5.3 months (95% CI, 4.1 to 7.7), and 2.8 months (95% CI, 2.1 to 3.4), respectively ($P < .001$; Fig 3C). In multivariable Cox regression analysis (Data Supplement), biomarker status generally remained independently prognostic for PFS.

OS

In the overall cohort, median OS was 28.7 months (95% CI, 28.4 to not reached) in CTC- patients, 29.5 months (95% CI, 18.4 to not reached) in CTC+/AR-V7- patients, and 11.2 months

(95% CI, 8.3 to 17.1) in CTC+/AR-V7+ patients ($P < .001$; Fig 1D). In multivariable Cox regression analysis (Data Supplement), biomarker status generally remained independently prognostic for OS.

In the first-line NHT cohort, median OS in CTC- patients, CTC+/AR-V7- patients, and CTC+/AR-V7+ patients was 29.7 months (95% CI, 28.7 to not reached), 30.7 months (95% CI, 29.5 to not reached), and 21.5 months (95% CI, 10.4 to not reached), respectively ($P = .003$; Fig 2D). In multivariable Cox regression analysis (Data Supplement), biomarker status generally remained independently prognostic for OS. In the second-line NHT cohort, median OS in CTC- patients, CTC+/AR-V7- patients, and CTC+/AR-V7+ patients was 18.8 months (95% CI, 12.5 to not reached), 13.0 months (95% CI, 10.0 to 22.6), and 8.5 months (95% CI, 4.9 to 15.6), respectively ($P < .001$; Fig 3D). In multivariable Cox regression analysis (Data Supplement), biomarker status generally remained independently prognostic for OS.

Fig 3. Clinical outcomes for men starting abiraterone or enzalutamide in the second-line novel hormonal therapy setting (N = 78), according to CTC status and AR-V7 status. (A) Waterfall plots depicting best PSA responses according to CTC status and AR-V7 status, expressed in three categories: CTC-, CTC+/AR-V7-, and CTC+/AR-V7+. The dotted line illustrates the threshold for defining a PSA response ($\geq 50\%$ PSA reduction from baseline). PSA response rates in CTC- patients, CTC+/AR-V7- patients, and CTC+/AR-V7+ patients were 52.9% (nine of 17), 27.5% (11 of 40), and 4.8% (one of 21), respectively ($P = .003$). All three groups were significantly different from each other (CTC- vs CTC+/AR-V7-, $P = .078$; CTC- vs CTC+/AR-V7+, $P = .002$; CTC+/AR-V7- vs CTC+/AR-V7+, $P = .044$). (B) Kaplan-Meier curves indicating PSA progression-free survival according to CTC status and AR-V7 status. Median PSA progression-free survival in CTC- patients, CTC+/AR-V7- patients, and CTC+/AR-V7+ patients was 6.4 months (95% CI, 5.1 to not reached), 4.4 months (95% CI, 3.2 to 6.0), and 1.1 months (95% CI, 1.0 to 3.1), respectively ($P < .001$). (C) Kaplan-Meier curves indicating clinical and radiographic progression-free survival according to CTC status and AR-V7 status. Median progression-free survival in CTC- patients, CTC+/AR-V7- patients, and CTC+/AR-V7+ patients was 6.2 months (95% CI 5.4–not reached), 5.3 months (95% CI 4.1–7.7), and 2.8 months (95% CI 2.1–3.4), respectively ($P < .001$). (D) Kaplan-Meier curves indicating overall survival according to CTC status and AR-V7 status. Median overall survival in CTC- patients, CTC+/AR-V7- patients, and CTC+/AR-V7+ patients was 18.8 months (95% CI 12.5–not reached), 13.0 months (95% CI 10.0–22.6), and 8.5 months (95% CI 4.9–15.6), respectively ($P < .001$).

Conversions

Fifty-nine patients had evaluable paired blood samples from baseline and progression. Of the 14 patients with baseline CTC- samples, six (43%) remained CTC-, and the remainder converted to CTC+/AR-V7- ($n = 6$, 43%) or CTC+/AR-V7+ ($n = 2$, 14%). Of the 35 men with baseline CTC+/AR-V7- samples, 20 (57%) remained CTC+/AR-V7-, and the remainder converted to CTC- ($n = 5$, 14%) or CTC+/AR-V7+ ($n = 10$, 29%). Of the 10 men with baseline CTC+/AR-V7+ samples, nine (90%) remained CTC+/AR-V7+, and one patient (10%) converted to CTC+/AR-V7- (none of these patients converted to CTC-).

DISCUSSION

We report, to the best of our knowledge, the largest prospective study to date examining the prognostic significance of CTC-based AR-V7 testing in patients with CRPC receiving NHT with

abiraterone or enzalutamide. The current results confirm our previous pilot data that CTC+/AR-V7+ patients have inferior clinical outcomes compared with CTC+/AR-V7- individuals, with respect to PSA responses, PSA-PFS, PFS, and OS. Furthermore, we report, we believe for the first time, the prognostic value of CTC- results using the AdnaTest platform. As expected, CTC- patients demonstrated clinical outcomes that were superior even to CTC+/AR-V7- individuals. To this end, CTC- patients seem to have the best outcomes with NHT, CTC+/AR-V7- patients have intermediate outcomes, and CTC+/AR-V7+ patients have the worst outcomes. Furthermore, because of the increased sample size of this study, we were able to evaluate the clinical significance of biomarker status separately in patients receiving first-line NHT and second-line NHT. To this end, biomarker status remained prognostic for all clinical outcomes in both the first-line and the second-line NHT settings, although there was no statistical difference in survival between CTC- and CTC+/AR-V7- subgroups.

The fact that a small proportion (13.9%) of CTC+/AR-V7+ patients achieved PSA responses with NHT was an important observation of our study (Data Supplement). Interestingly, responding patients generally had lower AR-FL transcript levels (median 21 v 36 copies) and lower AR-V7/AR-FL ratios (median, 8.8% v 21.2%), perhaps suggesting that a higher abundance of AR-FL and higher AR-V7 ratios may be associated with worse prognosis. It is also possible that some CTCs in a given blood sample expressed detectable levels of AR-V7, whereas others did not, reflecting individual tumor cell heterogeneity as demonstrated recently by RNA sequencing.¹¹ Moreover, our assay, which detects AR-V7 mRNA, does not document the presence or nuclear localization of AR-V7 protein,⁶ and it is possible that untranslated mRNA was detected, which would not be expected to be pathogenic in the absence of nuclear-localized protein. Finally, it should be highlighted that despite the possibility of a PSA response in some CTC+/AR-V7+ patients, PSA responses in the CTC+/AR-V7- and CTC- populations were much higher (52.2% and 75.5%, respectively). Nevertheless, this observation highlights the notion that not all CTC+/AR-V7+ patients may have an absolute primary resistance to abiraterone and enzalutamide. Rare PSA responses in CTC+/AR-V7+ patients have also been observed in other series,^{5,12} although PSA reductions alone do not constitute a clinical benefit.

The current prostate cancer clinical states model¹³ recommends considering metastatic CRPC as a series of states defined by the number of prior systemic therapies received. Accordingly, it was important to examine our biomarker outcomes separately in the first-line NHT and second-line NHT settings, representing two distinct contexts of use. Interestingly, but not surprisingly, the prevalence of CTC- patients was lower and the prevalence of CTC+/AR-V7+ patients was higher in the second-line compared with the first-line NHT setting. Similarly, in another study using a CTC-based assay relying on immunofluorescence staining of nuclear AR-V7 protein,⁶ AR-V7 prevalence in that analysis also increased with subsequent lines of CRPC therapy. Importantly, our current data show that our biomarker assay retains its prognostic value in both the first-line and the second-line NHT settings, with clinical outcomes remaining distinct for each of the three biomarker categories in each setting. Our data also underscore the value of considering CTC- patients as a distinct category from CTC+/AR-V7- patients. Other CTC-based AR-V7 platforms should also evaluate the prognostic implications of CTC- results.

This study has some limitations, the most significant of which was that there was some variability in the timing of PSA assessments and imaging assessments (which may have influenced our PSA-PFS and PFS estimates). In addition, this study as designed

allowed us to draw conclusions only on the prognostic usefulness of our biomarker in the context of androgen-directed therapy, because all patients exclusively received NHT and we did not include chemotherapy-treated patients. Therefore, the predictive usefulness of this biomarker and the interaction between biomarker status and treatment type could not be evaluated and will form the basis of future work. Finally, this study was not powered to assess OS, and we have not yet observed enough death events to make conclusive statements about biomarker status and survival. In addition, because of the exploratory nature of this study, *P* values were not corrected for multiple comparisons.

In conclusion, this expanded analysis confirms the negative prognostic impact of CTC-based AR-V7 detection in patients with CRPC undergoing therapy with abiraterone and enzalutamide and suggests that this biomarker panel may be useful in the prediction of response to AR-targeted treatment applied in the first- and second-line NHT settings. Furthermore, we believe our study is the first to suggest that the modified-AdnaTest CTC-based AR-V7 mRNA assay should be interpreted using three separate prognostic categories: CTC-, CTC+/AR-V7-, and CTC+/AR-V7+. Prospective studies ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT02269982) identifier NCT02269982) are currently underway to validate these findings.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at jco.org.

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REFERENCES

- Antonarakis ES, Armstrong AJ, Dehm SM, et al: Androgen receptor variant-driven prostate cancer: Clinical implications and therapeutic targeting. *Prostate Cancer Prostatic Dis* 19:231-241, 2016
- de Bono JS, Logothetis CJ, Molina A, et al: Abiraterone and increased survival in metastatic prostate cancer. *N Engl J Med* 364:1995-2005, 2011
- Scher HI, Fizazi K, Saad F, et al: Increased survival with enzalutamide in prostate cancer after chemotherapy. *N Engl J Med* 367:1187-1197, 2012
- Antonarakis ES, Lu C, Wang H, et al: AR-V7 and resistance to enzalutamide and abiraterone in prostate cancer. *N Engl J Med* 371:1028-1038, 2014
- Steinestel J, Luedeke M, Arndt A, et al: Detecting predictive androgen receptor modifications in circulating prostate cancer cells. *Oncotarget* 10.18632/oncotarget.3925 [epub ahead of print on April 23, 2015]
- 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* 2: 1441-1449, 2016
- Antonarakis ES, Lu C, Lubber B, et al: Androgen receptor splice variant 7 and efficacy of taxane chemotherapy in patients with metastatic castration-resistant prostate cancer. *JAMA Oncol* 1:582-591, 2015
- 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* 68:939-945, 2015

9. Eisenhauer EA, Therasse P, Bogaerts J, et al: New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1). *Eur J Cancer* 45:228-247, 2009

10. Scher HI, Halabi S, Tannock I, et al: Design and end points of clinical trials for patients with progressive prostate cancer and castrate levels of

testosterone: Recommendations of the Prostate Cancer Clinical Trials Working Group. *J Clin Oncol* 26:1148-1159, 2008

11. Miyamoto DT, Zheng Y, Wittner BS, et al: RNA-Seq of single prostate CTCs implicates non-canonical Wnt signaling in antiandrogen resistance. *Science* 349:1351-1356, 2015

12. 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* 71:1-3, 2017

13. Scher HI, Morris MJ, Stadler WM, et al: Trial design and objectives for castration-resistant prostate cancer: Updated recommendations from the Prostate Cancer Clinical Trials Working Group 3. *J Clin Oncol* 34:1402-1418, 2016

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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

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