

Award Number: W81XWH-12-1-0605

TITLE: Molecular Indicators of Castration-Resistant Prostate Cancer

PRINCIPAL INVESTIGATOR: Jun Luo, Ph.D.

CONTRACTING ORGANIZATION: Johns Hopkins University
Baltimore, MD 21218-2680

REPORT DATE: December 2015

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.					
1. REPORT DATE December 2015		2. REPORT TYPE Final		3. DATES COVERED 30 Sep 2012 - 29 Sep 2015	
4. TITLE AND SUBTITLE Molecular Indicators of Castration-Resistant Prostate Cancer				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER W81XWH-12-1-0605	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Jun Luo, Ph.D. E-Mail: jluo1@jhmi.edu				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Johns Hopkins University Baltimore, MD 21218-2680				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT Metastatic prostate cancers are commonly treated by agents designed to suppress androgen receptor (AR) signaling mediated by the full-length AR (AR-FL). Why some patients progress rapidly after treatment while others benefit with prolonged remission is an unsolved question. We propose approaches to develop molecular indicators of response and resistance that will enable prediction (before therapy) or early detection (during therapy) of therapeutic benefit. We will test the hypothesis that AR splice variants (AR-Vs) are molecular indicators of castration-resistant prostate cancer (CRPC). During the fundgin period, we achieved major milestones by completing RNA sequencing of 55 clinical specimens and yielding data supporting the clinical importance of AR-V7, by establishing an association of AR-V7 with resistance to two current FDA-approved AR-targeting therapies, and by demonstrating the feasibility of serial AR-V7 testing in men undergoing standard-of-care treatments for metastatic CRPC. We conclude that detection of AR-V7 predicts treatment outcome in men with metastatic castration-resistant prostate cancer initiating AR-targeting therapies.					
15. SUBJECT TERMS					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT UU	18. NUMBER OF PAGES 71	19a. NAME OF RESPONSIBLE PERSON USAMRMC
a. REPORT U	b. ABSTRACT U	c. THIS PAGE U			19b. TELEPHONE NUMBER (include area code)

Table of Contents

	<u>Page</u>
Introduction.....	1
Body.....	1
Key Research Accomplishments.....	5
Reportable Outcomes.....	5
Conclusion.....	7
References.....	7
Appendices.....	8
Supporting Data.....	n/a

Introduction

Hormone therapies block androgen production and/or androgen receptor (AR) function leading to a period of clinical regression varying from months to more than 6 years among patients treated for metastatic prostate cancer. Because restored AR signaling is the key determinant of castration-resistant prostate cancer (CRPC), aberrant variants of the androgen receptor may mediate and indicate therapeutic resistance. Our recent efforts (1-3) have established that truncated androgen receptor splice variants (AR-Vs) are resistant to castration therapies that target the canonical full-length AR (AR-FL). We are now equipped with the knowledge that AR-Vs are both structurally and functionally distinguishable from the full-length AR (AR-FL), which remains the key target in prostate cancer drug design. We view these new discoveries and knowledge as opportunities that have yet to be capitalized to address the unmet need of developing indicators of castration resistance. In our previous studies, we have shown elevated expression of the AR-Vs following suppression of AR-FL (3). The primary purpose of the project is to discover and develop molecular indicators of CRPC by targeting the AR-Vs. We will test the hypothesis that AR-Vs are molecular indicators of CRPC. The scope of the proposed research is: 1) to perform RNA-Seq targeting transcripts originated from the human AR gene locus for *ab initio* cataloging of AR-Vs that define CRPC; and 2) to establish the proof-of-principle that AR-Vs detected prior to the initiation of hormone therapies may be used to predict CRPC progression.

Body

Findings resulting from Task 1: To employ a novel targeted RNA-Seq method for *ab initio* cataloging of androgen receptor variants that define CRPC (Months 1-24).

Summary: By generating raw sequencing data using RNA-seq from 48 clinical specimens, we have achieved a critical project milestones associated with Task 1 as outlined in SOW during the first year of the project period. This accomplishment was reported in our 2013 annual progress report. Expanded studies involving data analysis and validation of some of the findings were conducted during year 2 and year 3 of the projected according to the timeline outlined in SOW. In our first annual report submitted in 2013, we presented initial findings from three such specimens and concluded that AR-V7, one of the most important androgen receptor splice variant (4), is abundantly expressed in CRPC and detected by RNA-seq. In our second annual report submitted in 2014, we further presented published findings from the analysis of the AR-V7-driven expression signature in four metastatic castration-resistant prostate cancer specimens (5), concluded that AR-V7 drives the expression of a unique set of genes, and further confirmed that AR-V7 is the most abundantly expressed androgen receptor splice variant (4, 6). During year 3 of the project period, we have compiled generated RNA-Seq data from a total of 55 clinical specimens, including 31 metastatic CRPC (mCRPC) and 24 hormone naïve radical prostatectomy specimens (RRP). We have compiled the expression data related to AR-V7 in all these

clinical specimens we have sequenced so far, and we now include aforementioned data in Appendix of this Final Report.

Supporting data: Supporting data Figures and Tables can be found in the Appendix of this Final Report.

Year 1: Quantitative RT-PCR results suggest that the expression level of each individual AR-V is often a small fraction of the total level for AR-FL. For example, AR-V7 represents only about 1% to 5% of the total AR in the vast majority of CRPC specimens examined (1, 7). Because of the lower abundance of AR-V than AR-FL, the clinical significance and functional role of AR-V has been debated (4, 7, 8). However, we posit that 1) accurate quantification of AR-V transcripts would be compromised by unknown splice junctions as well as various technical constraints of the conventional RT-PCR methodology and 2) even at a small fraction of the AR-FL, AR-V expression in treatment-resistant specimens may reach levels equivalent to those of AR-FL in untreated tumors because AR-FL is often massively overexpressed in CRPC (9), thus conferring functional significance when AR-FL is suppressed. To address accurate quantification, we performed RNA-seq in three independent CRPC specimens using the Illumina Hi-Seq 2000 platform (Figure 1). Comparing to qRT-PCR data from the same specimens, we found that RT-PCR *underestimated* the AR-V7 abundance by an average of 5 fold, and that in one CRPC specimen the ratio of AR-V7 to AR-FL reached 41%, using the number of AR-FL- and AR-V7-specific exon-spanning sequence reads as proxies for relative transcript abundance (Figure 1). Taken together, these data suggest that AR-V7 is a high-abundance transcript in a subset of clinical CRPC, and may be readily detected in CRPC cells. Other known AR-Vs are either orders of magnitude lower in abundance than AR-V7, or functionally inactive (2). Another constitutively active AR-V, ARV567ES, is frequently expressed in CRPC (10). Interestingly, splice junctions specific for ARV567ES are not detected by the three RNA samples sequenced so far (Figure 1). These findings suggest that although other variants may contribute to CRPC, AR-V7 is the best candidate for designing detection assays to be used in clinical specimens.

Year 2: Supporting data can be found in the Appendix of this progress report (5). Briefly, we published findings from genome-wide comparisons of two AR-V7–negative and two AR-V7–positive metastatic tumor samples by means of gene-set enrichment analysis of RNA sequencing data (Figs. S9 and S10 in the Supplementary Appendix of the attached paper (5)), or by means of targeted analysis of a set of genes regulated by the canonical androgen receptor (Table S5 in the Supplementary Appendix of the attached paper (5)) revealed alterations consistent with a shift toward AR-V7–driven transcription in AR-V7–positive samples.

Year 3: We have quantified the AR-V7 as well as the full-length AR (AR-FL) levels in a total of 55 clinical specimens, including 31 metastatic CRPC (mCRPC) and 24 hormone naïve radical prostatectomy (RRP) tumor specimens. Results are summarized in

Tables 2 and 3. In average, AR-V7 levels in mCRPC specimens were found to be over 30-fold higher than those in hormone naïve prostate cancer specimens, and AR-FL levels in mCRPC specimens were found to be ~3.7 fold higher than those in hormone naïve prostate cancer specimens (Table I and II). The ratio of AR-V7/AR-FL was calculated for each sample. In average, the AR-V7/AR-FL ratio in mCRPC specimens were found to be ~5.4 fold higher than those in hormone naïve prostate cancer specimens (Tables I and II). Importantly, we found that the median normalized AR-V7 expression level (~6.9) in mCRPC is nearly equivalent to the median normalized AR-FL expression level in RRP specimens (Tables I and II), further corroborating the clinical relevance of AR-V7 with respect to its mRNA abundance. These findings also suggest the importance and clinical relevance of AR-V7/AR-FL ratio measurement. However, these early discovery-phase findings are mainly hypothesis- generating. To evaluate the clinical utility of AR-V7/AR-FL ratio measurement, a more in-depth investigation will be necessary but would be beyond the tasks outlined in SOW. Therefore studies relevant to the evaluation of AR-V7/AR-FL ratios in prospectively collected specimens will be pursued on other funded research activities.

Findings resulting from Task 2: To establish the proof-of-principle that AR-Vs detected prior to the initiation of hormone therapies may be used to predict CRPC progression (Months 1-36).

Summary: We have focused on the predictive value of AR-V7 in men with metastatic prostate cancer initiating therapies with the FDA-approved second-generation agents targeting the androgen and androgen receptor axis. These newer agents, abiraterone and enzalutamide, have transformed the treatment landscape for prostate cancer, and provided an ideal treatment context to test our hypothesis. In our last annual progress report submitted in 2014, we presented a summary of our recent study published in the New England Journal of Medicine (Appendix of this Final Report) (5). During year 3 of the project period, we published two additional research studies relevant to this task (Appendix of this Final Report) (11, 12). First, we hypothesized that AR-V7–positive patients would retain sensitivity to taxane chemotherapy, and that AR-V7 status would have a differential impact on taxane-treated men compared to enzalutamide/abiraterone-treated men (11). Our study findings (11) suggest that detection of AR-V7 in men with metastatic CRPC is not associated with primary resistance to taxane chemotherapy. In AR-V7–positive men, taxanes appear to be more efficacious than enzalutamide/abiraterone. These findings, in conjunctions with findings reported in our 2014 annual progress report (5), suggest that AR-V7 detection may serve as a treatment-selection biomarker in CRPC. In addition, we published our findings after conducting serial measurements of AR-V7 and evaluated patterns of longitudinal AR-V7 dynamics over the course of multiple sequential therapies (12), supporting the feasibility of serial blood-based AR-V7 testing in routine clinical practice.

In summary, we conclude that pre-treatment detection of androgen receptor splice variant-7 (AR-V7) in men with castration-resistant prostate cancer (CRPC) is associated with resistance to abiraterone and enzalutamide, but not to taxane chemotherapies, and serial measurements of AR-V7 is feasible for evaluation of patterns of longitudinal AR-V7 dynamics over the course of multiple sequential therapies.

We now include aforementioned data in Appendix of this Final Report.

Supporting data: Supporting data Figures and Tables can be found in the Appendix of this Final Report containing copies of our published studies (5, 11, 12).

Years 1 and 2: Briefly, 39% of enzalutamide-treated patients (12 of 31 patients) and 19% of abiraterone-treated patients (6 of 31 patients) had detectable AR-V7 mRNA in baseline CTC samples. Among the samples with detectable AR-V7, the median ratio of AR-V7 to full-length androgen receptor mRNA was 21.0% (range, 1.8 to 208.0) (Fig. 1 of the attached Paper #1 in Appendix). In the enzalutamide cohort, the PSA response rate among AR-V7–positive patients was 0% (95% CI, 0 to 26; 0 of 12 men), and the rate among AR-V7–negative patients was 53% (95% CI, 29 to 76; 10 of 19 men; $P = 0.004$) (Figure 2A of the attached Paper #1 in Appendix). In the abiraterone cohort, the PSA response rate among AR-V7–positive patients was 0% (95% CI, 0 to 46; 0 of 6 men), and the rate among AR-V7–negative patients was 68% (95% CI, 46 to 85; 17 of 25 men; $P = 0.004$) (Figure 2B of the attached Paper #1 in Appendix). Overall inferior treatment outcome in AR-V7 positive men was also demonstrated in Figure 3 of the attached Paper #1 in Appendix, in which clinical progression-free survival and overall survival rates were evaluated and compared between AR-V7 positive and AR-V7 negative patients. During the project period, 6 patients (4 receiving enzalutamide and 2 receiving abiraterone) who were negative for AR-V7 at baseline subsequently converted to AR-V7–positive status during the course of treatment, while all evaluable patients with detectable AR-V7 at baseline who had at least one follow-up sample available remained AR-V7–positive during treatment. Clinical outcomes for all patients according to AR-V7 conversion status are summarized in Table S4 in the attached Paper #1 in Appendix. In addition, changes in levels of AR-V7 expression during the course of treatment are summarized in Figure S5 in the attached Paper #1 in Appendix. Finally, we also showed high concordance of AR-V7 status in CTC and matched biopsy specimens from the same patient (Fig. 4 of the attached Paper #1 in Appendix).

Year 3: To test the hypothesis that AR-V7–positive patients would retain sensitivity to taxane chemotherapy, and that AR-V7 status would have a differential impact on taxane-treated men compared to enzalutamide/abiraterone-treated men, thirty-seven taxane-treated patients were enrolled, of which 17/37 (45.9%) had detectable AR-V7 in CTCs (Table I in the attached Paper #2 in Appendix). PSA responses were achieved in both AR-V7–positive and AR-V7–negative men (41% vs 65%, $P=0.19$) (Figure 1A in the attached Paper #2 in Appendix). Similarly, median PSA-PFS and PFS were comparable in AR-V7–positive and

AR-V7–negative patients (Figure 1B and 1C in the attached Paper #2 in Appendix). A significant interaction was observed between AR-V7 status and treatment type ($P<0.001$) (Figure 2 in the attached Paper #2 in Appendix). Clinical outcomes were superior with taxanes than with enzalutamide/abiraterone in AR-V7–positive men (Figure 3A and 3B in the attached Paper #2 in Appendix), while outcomes did not differ by treatment type in AR-V7–negative men (Figure 3D and 3E in the attached Paper #2 in Appendix). In AR-V7–positive patients, PSA responses were higher in taxane-treated *versus* enzalutamide/abiraterone-treated men (41% vs 0%, $P<0.001$), and median PSA-PFS and PFS were significantly longer in taxane-treated men (HR 0.19 for PSA-PFS, $P=0.001$; HR 0.21 for PFS, $P=0.003$) (Figure 3A and 3B in the attached Paper #2 in Appendix).

In a subsequent study (12), we also conducted serial measurements of AR-V7 and evaluated patterns of longitudinal AR-V7 dynamics over the course of multiple sequential therapies (see attached Paper #3 in Appendix). By selecting men who provided ≥ 4 samples with at least one AR-V7–positive samples and received at least two consecutive therapies, we identified 14 patients who received a total of 37 therapies and contributed 70 samples for AR-V7 analysis during a median follow-up period of 11 months (Table I in attached Paper #2 in Appendix). Three patients remained AR-V7–positive during the entire course of therapy (Figure 1 in attached Paper #2 in Appendix). The remainder underwent transitions in AR-V7 status: there were 8 instances of ‘conversions’ from AR-V7–negative to positive status (during treatment with first-line ADT, abiraterone, enzalutamide, and docetaxel), and 6 instances of ‘reversions’ from AR-V7–positive to negative status (during treatment with docetaxel and cabazitaxel) (Table II and Figure 2 in attached Paper #2 in Appendix). These observations suggest that while ‘conversions’ to AR-V7–positive status were observed with both AR-directed therapies and taxane chemotherapies, ‘reversions’ to AR-V7–negative status only occurred during taxane therapies.

Key Research Accomplishments

1. Completed RNA-seq data generation and analysis of AR-V7 expression levels in clinical specimens demonstrating clinical importance of AR-V7 (n=55).
2. Published a high-impact study demonstrating an association between AR-V7 and treatment outcome in men (n=62) with metastatic prostate cancer initiating treatments with abiraterone and enzalutamide.
3. Published a study demonstrating retained sensitivity to chemotherapy in AR-V7–positive patients with metastatic prostate cancer (n=37).
4. Published a study demonstrating the feasibility of serial AR-V7 testing in patients receiving multiple therapies (n=14).

Reportable Outcomes

Publications:

1. Antonarakis ES, Lu C, Lubner B, Wang H, Chen Y, Nakazawa M, Nadal R, Paller CJ, Denmeade SR, Carducci MA, Eisenberger MA, Luo J. Androgen Receptor Splice Variant 7 and Efficacy of Taxane Chemotherapy in Patients With Metastatic Castration-Resistant Prostate Cancer. *JAMA oncology*. 2015;1(5):582-91. Epub 2015/07/17. doi: 10.1001/jamaoncol.2015.1341. PubMed PMID: 26181238; PubMed Central PMCID: PMC4537351.
2. Antonarakis ES, Lu C, Wang H, Lubner B, Nakazawa M, Roeser JC, Chen Y, Mohammad TA, Chen Y, Fedor HL, Lotan TL, Zheng Q, De Marzo AM, Isaacs JT, Isaacs WB, Nadal R, Paller CJ, Denmeade SR, Carducci MA, Eisenberger MA, Luo J. AR-V7 and resistance to enzalutamide and abiraterone in prostate cancer. *The New England journal of medicine*. 2014;371(11):1028-38. Epub 2014/09/04. doi: 10.1056/NEJMoa1315815. PubMed PMID: 25184630.
3. Nakazawa M, Lu C, Chen Y, Paller CJ, Carducci MA, Eisenberger MA, Luo J, Antonarakis ES. Serial blood-based analysis of AR-V7 in men with advanced prostate cancer. *Annals of oncology : official journal of the European Society for Medical Oncology / ESMO*. 2015;26(9):1859-65. Epub 2015/06/29. doi: 10.1093/annonc/mdv282. PubMed PMID: 26117829; PubMed Central PMCID: PMC4551160.

Presentations:

1. Antonarakis E, Lu C, Wang H, Lubner B, Nakazawa M. Androgen receptor splice variant, AR-V7, and resistance to enzalutamide and abiraterone in men with metastatic castration-resistant prostate cancer (mCRPC). *J Clin Oncol*. 2014;32(5 Suppl).
2. Antonarakis ES, Lu C, Wang H, Lubner B, Nakazawa M, Roeser JC, Chen Y, Fedor HL, Lotan TL, De Marzo AM. Androgen receptor splice variant-7 predicts resistance to enzalutamide in patients with castration-resistant prostate cancer. *Cancer research*. 2014;74(19 Supplement):2910-.

Grant Applications:

Title: Noninvasive Detection of AR-FL/AR-V7 as a Predictive Biomarker for Therapeutic Resistance in Men with Metastatic Castration-Resistant Prostate Cancer

Mechanism: 2015 DOD Biomarker Development Award (Luo - PI)

Performance Period: 9/30/2015 - 9/29/2018

Status: Funded

Title: The aberrant androgen receptor underlies abiraterone/enzalutamide resistance

Mechanism: NIH, R01CA185297 (Luo/Antonarakis - PI)

Performance Period: 05/01/2015 - 4/30/2019

Status: Funded

Conclusion

We conclude that AR-V7 drives the expression of a unique set of genes, and is the most abundantly expressed androgen receptor splice variant. We further conclude that detection of AR-V7 in CTCs from patients with CRPC is associated with resistance to AR-targeting therapies but not to chemotherapies, and it is feasible to conduct serial testing of AR-V7, supporting expanded validation studies aimed at evaluating the clinical utility of this blood-based treatment selection marker.

References

1. Hu R, Dunn TA, Wei S, Isharwal S, Veltri RW, Humphreys E, Han M, Partin AW, Vessella RL, Isaacs WB, Bova GS, Luo J. Ligand-independent androgen receptor variants derived from splicing of cryptic exons signify hormone-refractory prostate cancer. *Cancer Res.* 2009;69(1):16-22. Epub 2009/01/02. doi: 10.1158/0008-5472.CAN-08-2764. PubMed PMID: 19117982; PubMed Central PMCID: PMC2614301.
2. Hu R, Isaacs WB, Luo J. A snapshot of the expression signature of androgen receptor splicing variants and their distinctive transcriptional activities. *Prostate.* 2011;71(15):1656-67. Epub 2011/03/30. doi: 10.1002/pros.21382. PubMed PMID: 21446008; PubMed Central PMCID: PMC3360954.
3. Hu R, Lu C, Mostaghel EA, Yegnasubramanian S, Gurel M, Tannahill C, Edwards J, Isaacs WB, Nelson PS, Bluemn E, Plymate SR, Luo J. Distinct transcriptional programs mediated by the ligand-dependent full-length androgen receptor and its splice variants in castration-resistant prostate cancer. *Cancer Res.* 2012;72(14):3457-62. Epub 2012/06/20. doi: 10.1158/0008-5472.CAN-11-3892. PubMed PMID: 22710436; PubMed Central PMCID: PMC3415705.
4. Plymate SR, Luo J. The Expression Signature of Androgen Receptor Splice Variants and Their Distinctive Transcriptional Activities in Castration-Resistant Prostate Cancer. *Androgen-Responsive Genes in Prostate Cancer*: Springer; 2013. p. 201-13.
5. Antonarakis ES, Lu C, Wang H, Luber B, Nakazawa M, Roeser JC, Chen Y, Mohammad TA, Chen Y, Fedor HL, Lotan TL, Zheng Q, De Marzo AM, Isaacs JT, Isaacs WB, Nadal R, Paller CJ, Denmeade SR, Carducci MA, Eisenberger MA, Luo J. AR-V7 and resistance to enzalutamide and abiraterone in prostate cancer. *The New England journal of medicine.* 2014;371(11):1028-38. Epub 2014/09/04. doi: 10.1056/NEJMoa1315815. PubMed PMID: 25184630.
6. Nakazawa M, Antonarakis ES, Luo J. Androgen receptor splice variants in the era of enzalutamide and abiraterone. *Hormones & cancer.* 2014;5(5):265-73. Epub 2014/07/23. doi: 10.1007/s12672-014-0190-1. PubMed PMID: 25048254; PubMed Central PMCID: PMC4167475.
7. Watson PA, Chen YF, Balbas MD, Wongvipat J, Socci ND, Viale A, Kim K, Sawyers CL. Constitutively active androgen receptor splice variants expressed in

- castration-resistant prostate cancer require full-length androgen receptor. *Proc Natl Acad Sci U S A*. 2010;107(39):16759-65. Epub 2010/09/09. doi: 10.1073/pnas.1012443107. PubMed PMID: 20823238; PubMed Central PMCID: PMC2947883.
8. Sadar MD. Small molecule inhibitors targeting the "achilles' heel" of androgen receptor activity. *Cancer Res*. 2011;71(4):1208-13. Epub 2011/02/03. doi: 10.1158/0008-5472.CAN_10-3398. PubMed PMID: 21285252; PubMed Central PMCID: PMC3132148.
 9. Isaacs JT, D'Antonio JM, Chen S, Antony L, Dalrymple SP, Ndikuyeze GH, Luo J, Denmeade SR. Adaptive auto-regulation of androgen receptor provides a paradigm shifting rationale for bipolar androgen therapy (BAT) for castrate resistant human prostate cancer. *Prostate*. 2012;72(14):1491-505. Epub 2012/03/08. doi: 10.1002/pros.22504. PubMed PMID: 22396319; PubMed Central PMCID: PMC3374010.
 10. Sun S, Sprenger CC, Vessella RL, Haugk K, Soriano K, Mostaghel EA, Page ST, Coleman IM, Nguyen HM, Sun H, Nelson PS, Plymate SR. Castration resistance in human prostate cancer is conferred by a frequently occurring androgen receptor splice variant. *J Clin Invest*. 2010;120(8):2715-30. Epub 2010/07/21. doi: 10.1172/JCI41824. PubMed PMID: 20644256; PubMed Central PMCID: PMC2912187.
 11. Antonarakis ES, Lu C, Luber B, Wang H, Chen Y, Nakazawa M, Nadal R, Paller CJ, Denmeade SR, Carducci MA, Eisenberger MA, Luo J. Androgen Receptor Splice Variant 7 and Efficacy of Taxane Chemotherapy in Patients With Metastatic Castration-Resistant Prostate Cancer. *JAMA oncology*. 2015;1(5):582-91. Epub 2015/07/17. doi: 10.1001/jamaoncol.2015.1341. PubMed PMID: 26181238; PubMed Central PMCID: PMC4537351.
 12. Nakazawa M, Lu C, Chen Y, Paller CJ, Carducci MA, Eisenberger MA, Luo J, Antonarakis ES. Serial blood-based analysis of AR-V7 in men with advanced prostate cancer. *Annals of oncology : official journal of the European Society for Medical Oncology / ESMO*. 2015;26(9):1859-65. Epub 2015/06/29. doi: 10.1093/annonc/mdv282. PubMed PMID: 26117829; PubMed Central PMCID: PMC4551160.

Appendix

Figure 1 and legend

Figure 1. RNA-seq results illustrating the splice junction tracks for the androgen receptor locus in 3 CRPC specimens. The number of reads specific to each splice junction were compared with data derived from conventional RT-PCR. For example, the number of reads spanning AR-V7 splice junction (second blue arrow) is 41 in CRPC #2 and reads spanning AR-FL splice junction is 111, while the V7/FL ratio in this sample by QRT-PCR is 8%, suggesting QRT-PCR underestimates the true ratio of AR-V7/AR-FL by 5 fold. Blue

arrows indicate splice junctions. The number of reads were extracted from 3 specimens (CRPC #1, #2, and #3).

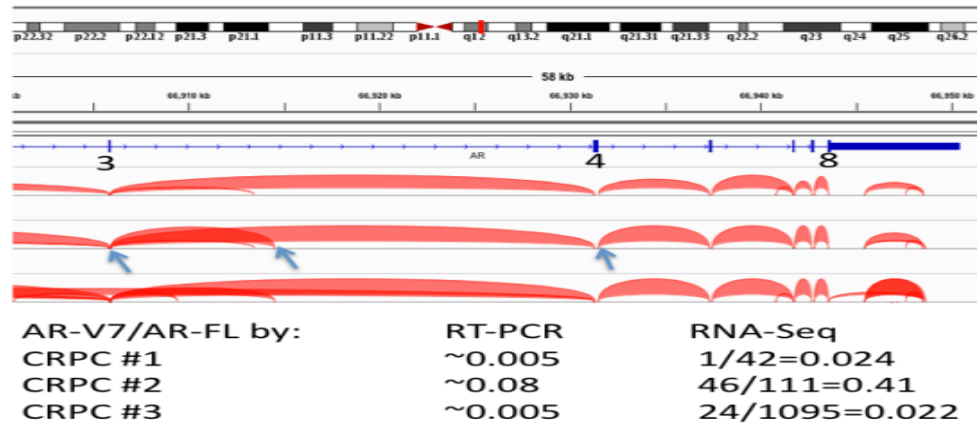


Table I. Quantification of AR-FL and AR-V7 in Metastatic CRPC Tumor Specimens (n=31).

mCRPC Tumor ID	Total Reads	AR-V7 Reads	AR-FL Reads	Ratio (AR-V7/AR-FL)	AR-V7 Normalized	AR-FL Normalized
1	59,976,657	0	51	0.000	0.000	8.503
2	51,750,612	23	150	0.153	4.444	28.985
3	62,168,931	17	212	0.080	2.734	34.101
4	49,167,795	0	338	0.000	0.000	68.744
5	31,260,609	3	60	0.050	0.960	19.193
6	66,706,808	39	267	0.146	5.846	40.026
7	65,345,349	0	4	0.000	0.000	0.612
8	51,197,114	0	8	0.000	0.000	1.563
9	44,453,512	2	17	0.118	0.450	3.824
10	75,733,517	20	721	0.028	2.641	95.202
11	53,724,016	1	303	0.003	0.186	56.399
12	66,280,566	24	115	0.209	3.621	17.350
13	87,040,451	60	334	0.180	6.893	38.373
14	97,152,778	143	144	0.993	14.719	14.822
15	81,645,037	226	65	3.477	27.681	7.961
16	61,401,226	75	58	1.293	12.215	9.446
17	64,680,598	54	797	0.068	8.349	123.221
18	70,974,620	77	721	0.107	10.849	101.586
19	63,656,191	48	31	1.548	7.541	4.870
20	63,671,295	86	66	1.303	13.507	10.366
21	86,636,264	185	147	1.259	21.354	16.967
22	67,713,362	151	108	1.398	22.300	15.950
23	74,217,413	146	565	0.258	19.672	76.128
24	75,542,483	145	703	0.206	19.194	93.060
25	89,349,212	19	155	0.123	2.126	17.348
26	102,295,110	69	700	0.099	6.745	68.429
27	87,498,826	22	511	0.043	2.514	58.401
28	71,428,402	50	473	0.106	7.000	66.220
29	79,111,716	55	522	0.105	6.952	65.983
30	163,028,609	583	1636	0.356	35.761	100.350
31	60,588,456	468	378	1.238	77.242	62.388

Table II. Quantification of AR-FL and AR-V7 in Hormone Naïve RRP Tumor Specimens (n=24).

RRP Tumor ID	Total Reads	AR-V7 Reads	AR-FL Reads	Ratio (AR-V7/AR-FL)	AR-V7 Normalized	AR-FL Normalized
32	59189762	0.000	85.000	0.000	0.000	14.361
33	69603348	1.000	82.000	0.012	0.144	11.781
34	58529089	0.000	43.000	0.000	0.000	7.347
35	84057060	0.000	78.000	0.000	0.000	9.279
36	53893237	4.000	41.000	0.098	0.742	7.608
37	74271569	1.000	77.000	0.013	0.135	10.367
38	65314974	1.000	88.000	0.011	0.153	13.473
39	60337249	4.000	75.000	0.053	0.663	12.430
40	67361736	0.000	53.000	0.000	0.000	7.868
41	75082339	19.000	127.000	0.150	2.531	16.915
42	54700266	3.000	28.000	0.107	0.548	5.119
43	73720860	3.000	118.000	0.025	0.407	16.006
44	62861912	2.000	48.000	0.042	0.318	7.636
45	69704085	4.000	47.000	0.085	0.574	6.743
46	71714729	3.000	62.000	0.048	0.418	8.645
47	72392511	4.000	65.000	0.062	0.553	8.979
48	80869159	4.000	73.000	0.055	0.495	9.027
49	89227403	1.000	90.000	0.011	0.112	10.087
50	64235251	0.000	56.000	0.000	0.000	8.718
51	51357702	2.000	44.000	0.045	0.389	8.567
52	58115454	0.000	67.000	0.000	0.000	11.529
53	70046729	2.000	80.000	0.025	0.286	11.421
54	60889329	1.000	49.000	0.020	0.164	8.047
55	63093482	1.000	83.000	0.012	0.158	13.155
Median Values	66338355	1.5	70	0.023	0.225	9.153

Published paper #1: New England Journal of Medicine (41 pages in total including supplemental data).

Published paper #2: JAMA Oncology (10 pages).

Published paper #3: Annals of Oncology (7 pages).

ORIGINAL ARTICLE

AR-V7 and Resistance to Enzalutamide and Abiraterone in Prostate Cancer

Emmanuel S. Antonarakis, M.D., Changxue Lu, Ph.D., Hao Wang, Ph.D., Brandon Luber, Sc.M., Mary Nakazawa, M.H.S., Jeffrey C. Roeser, B.S., Yan Chen, Ph.D., Tabrez A. Mohammad, Ph.D., Yidong Chen, Ph.D., Helen L. Fedor, B.S., Tamara L. Lotan, M.D., Qizhi Zheng, M.D., Angelo M. De Marzo, M.D., Ph.D., John T. Isaacs, Ph.D., William B. Isaacs, Ph.D., Rosa Nadal, M.D., Channing J. Paller, M.D., Samuel R. Denmeade, M.D., Michael A. Carducci, M.D., Mario A. Eisenberger, M.D., and Jun Luo, Ph.D.

ABSTRACT

BACKGROUND

The androgen-receptor isoform encoded by splice variant 7 lacks the ligand-binding domain, which is the target of enzalutamide and abiraterone, but remains constitutively active as a transcription factor. We hypothesized that detection of androgen-receptor splice variant 7 messenger RNA (AR-V7) in circulating tumor cells from men with advanced prostate cancer would be associated with resistance to enzalutamide and abiraterone.

METHODS

We used a quantitative reverse-transcriptase–polymerase-chain-reaction assay to evaluate AR-V7 in circulating tumor cells from prospectively enrolled patients with metastatic castration-resistant prostate cancer who were initiating treatment with either enzalutamide or abiraterone. We examined associations between AR-V7 status (positive vs. negative) and prostate-specific antigen (PSA) response rates (the primary end point), freedom from PSA progression (PSA progression–free survival), clinical or radiographic progression–free survival, and overall survival.

RESULTS

A total of 31 enzalutamide-treated patients and 31 abiraterone-treated patients were enrolled, of whom 39% and 19%, respectively, had detectable AR-V7 in circulating tumor cells. Among men receiving enzalutamide, AR-V7–positive patients had lower PSA response rates than AR-V7–negative patients (0% vs. 53%, $P=0.004$) and shorter PSA progression–free survival (median, 1.4 months vs. 6.0 months; $P<0.001$), clinical or radiographic progression–free survival (median, 2.1 months vs. 6.1 months; $P<0.001$), and overall survival (median, 5.5 months vs. not reached; $P=0.002$). Similarly, among men receiving abiraterone, AR-V7–positive patients had lower PSA response rates than AR-V7–negative patients (0% vs. 68%, $P=0.004$) and shorter PSA progression–free survival (median, 1.3 months vs. not reached; $P<0.001$), clinical or radiographic progression–free survival (median, 2.3 months vs. not reached; $P<0.001$), and overall survival (median, 10.6 months vs. not reached, $P=0.006$). The association between AR-V7 detection and therapeutic resistance was maintained after adjustment for expression of full-length androgen receptor messenger RNA.

CONCLUSIONS

Detection of AR-V7 in circulating tumor cells from patients with castration-resistant prostate cancer may be associated with resistance to enzalutamide and abiraterone. These findings require large-scale prospective validation. (Funded by the Prostate Cancer Foundation and others.)

From the Departments of Oncology (E.S.A., H.W., B.L., J.T.I., R.N., C.J.P., S.R.D., M.A.C., M.A.E.), Pathology (H.L.F., T.L.L., Q.Z., A.M.D.M.), and Urology (C.L., M.N., J.C.R., Yan Chen, W.B.I., J.L.), Johns Hopkins University School of Medicine, Baltimore; and Greehey Children's Cancer Research Institute (T.A.M., Yidong Chen) and the Department of Epidemiology and Biostatistics (Yidong Chen), University of Texas Health Science Center at San Antonio, San Antonio. Address reprint requests to Dr. Antonarakis at the Prostate Cancer Research Program, Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, 1650 Orleans St., CRB1–1M45, Baltimore, MD 21287, or at eantonar@jhmi.edu; or to Dr. Luo at the James Buchanan Brady Urological Institute and the Department of Urology, Johns Hopkins University School of Medicine, 600 N. Wolfe St., Baltimore, MD 21287, or at jlul@jhmi.edu.

This article was published on September 3, 2014, at NEJM.org.

N Engl J Med 2014;371:1028–38.

DOI: 10.1056/NEJMoa1315815

Copyright © 2014 Massachusetts Medical Society.

IT IS NOW ACCEPTED THAT CASTRATION-resistant prostate cancer is not androgen-independent and continues to rely on androgen signaling.¹ Owing to this new understanding, several drugs have recently emerged for the treatment of castration-resistant prostate cancer; these agents either suppress the synthesis of extragonadal androgens or target the androgen receptor directly.² Enzalutamide is an inhibitor of androgen-receptor signaling that exerts its activity by binding avidly to the ligand-binding domain of the androgen receptor, competing with and displacing the natural ligands of this receptor (testosterone and dihydrotestosterone) while also inhibiting translocation of the androgen receptor into the nucleus and impairing transcriptional activation of androgen-responsive target genes.^{3,4} Abiraterone is an inhibitor of cytochrome P450 17A1 (CYP17A1) that impairs androgen-receptor signaling by depleting adrenal and intratumoral androgens.^{5,6} After studies showed improved survival with these drugs,⁷⁻⁹ both agents were approved by the Food and Drug Administration for the treatment of metastatic castration-resistant prostate cancer.

Although enzalutamide and abiraterone represent breakthroughs in the treatment of metastatic castration-resistant prostate cancer, approximately 20 to 40% of patients have no response to these agents with respect to prostate-specific antigen (PSA) levels (i.e., they have primary resistance).^{4,7-9} Among patients who initially have a response to enzalutamide or abiraterone, virtually all eventually acquire secondary resistance. One plausible explanation for the resistance to both agents may involve the presence of androgen-receptor splice variants.^{10,11} These alternatively spliced variants encode a truncated androgen-receptor protein that lacks the C-terminal ligand-binding domain but retains the transactivating N-terminal domain.^{12,13} Although the resultant truncated proteins are unable to bind ligand, they are constitutively active as transcription factors and capable of promoting activation of target genes.

Because enzalutamide exerts its antitumor activity through its interaction with the ligand-binding domain of the androgen receptor, it would be expected that the presence of the protein encoded by the androgen-receptor variant (which lacks the ligand-binding domain) may be associated with enzalutamide resistance. Furthermore, this pro-

tein is ligand-independent and yet constitutively active, and its activity would not be expected to be inhibited by ligand-depleting agents such as abiraterone. Although these hypotheses are supported by preclinical studies,^{10,11,14,15} the clinical significance of androgen-receptor variants in patients receiving enzalutamide or abiraterone is unknown.

To investigate the clinical relevance of androgen-receptor variants in castration-resistant prostate cancer, we prospectively evaluated androgen-receptor splice variant 7 messenger RNA (AR-V7) in circulating tumor cells from patients receiving enzalutamide or abiraterone. Although multiple androgen-receptor variants have been discovered, we focused on AR-V7 because it is the only known androgen-receptor variant encoding a functional protein product that is detectable in clinical specimens.^{13,16} We hypothesized that detection of AR-V7 in circulating tumor cells may be associated with resistance to enzalutamide and abiraterone in patients with castration-resistant prostate cancer.

METHODS

PATIENTS

We prospectively enrolled men with metastatic castration-resistant prostate cancer who were beginning standard-of-care treatment with enzalutamide or abiraterone. Patients were required to have histologically confirmed prostate adenocarcinoma, progressive disease despite “castration levels” of serum testosterone (<50 ng per deciliter [1.73 nmol per liter]) with continued androgen-deprivation therapy, and documented metastases, as confirmed on computed tomography (CT) or bone scanning with technetium-99m-labeled methylene diphosphonate. Patients had to have three or more rising serum PSA values obtained 2 or more weeks apart, with the last value being 2.0 ng per milliliter or higher — criteria for PSA progression that are consistent with Prostate Cancer Clinical Trials Working Group 2 (PCWG2) guidelines.¹⁷ Patients were excluded if they planned to receive additional concurrent anticancer therapies. Prior chemotherapy was permitted, as was previous treatment with the alternative agent directed at the androgen receptor (i.e., prior abiraterone use in enzalutamide-treated patients and vice versa). All enrolled patients provided written informed consent.

STUDY DESIGN AND ASSESSMENTS

This was a prospective study evaluating the ability of baseline (pretreatment) AR-V7 status (positive vs. negative) in circulating tumor cells to predict a response or resistance to agents directed at the androgen receptor. The study was approved by the institutional review board at Johns Hopkins University. All the authors vouch for the completeness and integrity of the data and for the fidelity of the study to the clinical protocol (available with the full text of this article at NEJM.org). Peripheral-blood samples, for analysis of circulating tumor cells, were obtained from eligible patients at three prespecified time points: baseline, the time of a clinical or biochemical response (if a response occurred), and the time of clinical or radiographic progression. In addition, patients were encouraged to undergo core-needle biopsies of metastatic tumors at baseline and at the time of progression. Enzalutamide was given at a dose of 160 mg daily; abiraterone was given at a dose of 1000 mg daily, with prednisone at a dose of 5 mg twice daily.

The times of follow-up assessments were prospectively defined: PSA measurements were obtained every 1 to 2 months, and CT of the chest, abdomen, and pelvis and technetium-99m bone scanning were performed every 2 to 4 months. Therapy with enzalutamide or abiraterone was continued until PSA progression, clinical or radiographic progression, or the occurrence of unmanageable drug-related toxic effects.

All the clinical investigators were unaware of the AR-V7 status of the participants. All the laboratory investigators were unaware of clinical information when determining AR-V7 status. The study statisticians were the first to unblind the data, after at least 30 patients had been enrolled per cohort.

ANALYSIS OF CIRCULATING TUMOR CELLS AND TUMOR TISSUE

Descriptions of the methods used for the capture of circulating tumor cells and of messenger RNA (mRNA) analysis for full-length androgen receptor and AR-V7 are provided in the Supplementary Appendix, available at NEJM.org. Quantitative reverse-transcriptase–polymerase-chain-reaction (RT-PCR) assays were used for mRNA detection.

The analysis of AR-V7 in metastatic tumor tissue is also described in the Supplementary Appendix. RNA in situ hybridization assays were used.

CLINICAL OUTCOMES

The primary end point was the proportion of patients with a PSA response ($\geq 50\%$ decline in PSA level from baseline, maintained for ≥ 4 weeks) at any time after the initiation of therapy; the end point was assessed separately for enzalutamide-treated patients and abiraterone-treated patients. The best PSA response (maximal percentage decrease in PSA level from baseline) for each patient was also determined.

Secondary end points were freedom from PSA progression (PSA progression-free survival), freedom from clinical or radiographic progression (clinical or radiographic progression-free survival), and overall survival. PSA progression was defined as an increase in the PSA level of 25% or more above the nadir (and by ≥ 2 ng per milliliter), with confirmation 4 or more weeks later (PCWG2 criteria).¹⁷ Clinical or radiographic progression was defined as symptomatic progression (worsening disease-related symptoms or new cancer-related complications), radiographic progression ($\geq 20\%$ increase in the sum of the diameters of soft-tissue target lesions on CT scanning [according to the Response Evaluation Criteria in Solid Tumors¹⁸] or ≥ 2 new bone lesions on bone scanning), or death, whichever occurred first.¹⁷ Overall survival was defined as the time to death from any cause.

STATISTICAL ANALYSIS

Statistical analyses were performed separately in the enzalutamide and abiraterone cohorts. The sample size was determined on the basis of the primary end point of PSA response. We assumed that AR-V7 would be detectable from baseline samples of circulating tumor cells in 50% of enzalutamide-treated patients and 50% of abiraterone-treated patients. In both cohorts, we hypothesized that PSA response rates would be 10% or less in AR-V7–positive patients and 60% or more in AR-V7–negative patients.^{7,8} With this assumption, we calculated that a sample of 30 patients per cohort would give the study 85% power to detect a difference of 50 percentage points in PSA response rates (i.e., a rate of 10% in AR-V7–positive men and 60% in AR-V7–negative men), with the use of a two-sided test at an alpha level of 0.10.

In each cohort, clinical outcomes were compared between AR-V7–positive patients and AR-

V7–negative patients. PSA response rates were compared with the use of Fisher's exact test. Time-to-event outcomes (i.e., PSA progression-free survival, clinical or radiographic progression-free survival, and overall survival) were evaluated with the use of Kaplan–Meier methods, and survival-time differences were compared with the use of the log-rank test. Univariate and multivariable Cox regressions were used to assess the effect of AR-V7 status on the prediction of time-to-event outcomes. Owing to the small sample size and the limited number of events, each multivariable model included only three variables (AR-V7 status, the level of expression of full-length androgen receptor, and prior use of the alternative therapy directed at the androgen receptor), to prevent overfitting.

We also performed propensity-score-weighted multivariable Cox analyses for PSA progression-free survival and clinical or radiographic progression-free survival, in which the propensity score (the probability of being AR-V7–positive) was calculated from logistic regression with the use of variables including the Gleason score, the baseline PSA level, the number of prior hormonal treatments, the presence or absence of visceral metastases, the Eastern Cooperative Oncology Group (ECOG) score, prior use of abiraterone or enzalutamide, and the level of full-length androgen receptor. All tests were two-sided, and P values of 0.05 or less were considered to indicate statistical significance. Statistical analyses were performed with the use of R software, version 2.15.1.

RESULTS

AR-V7 DETECTION IN CIRCULATING TUMOR CELLS

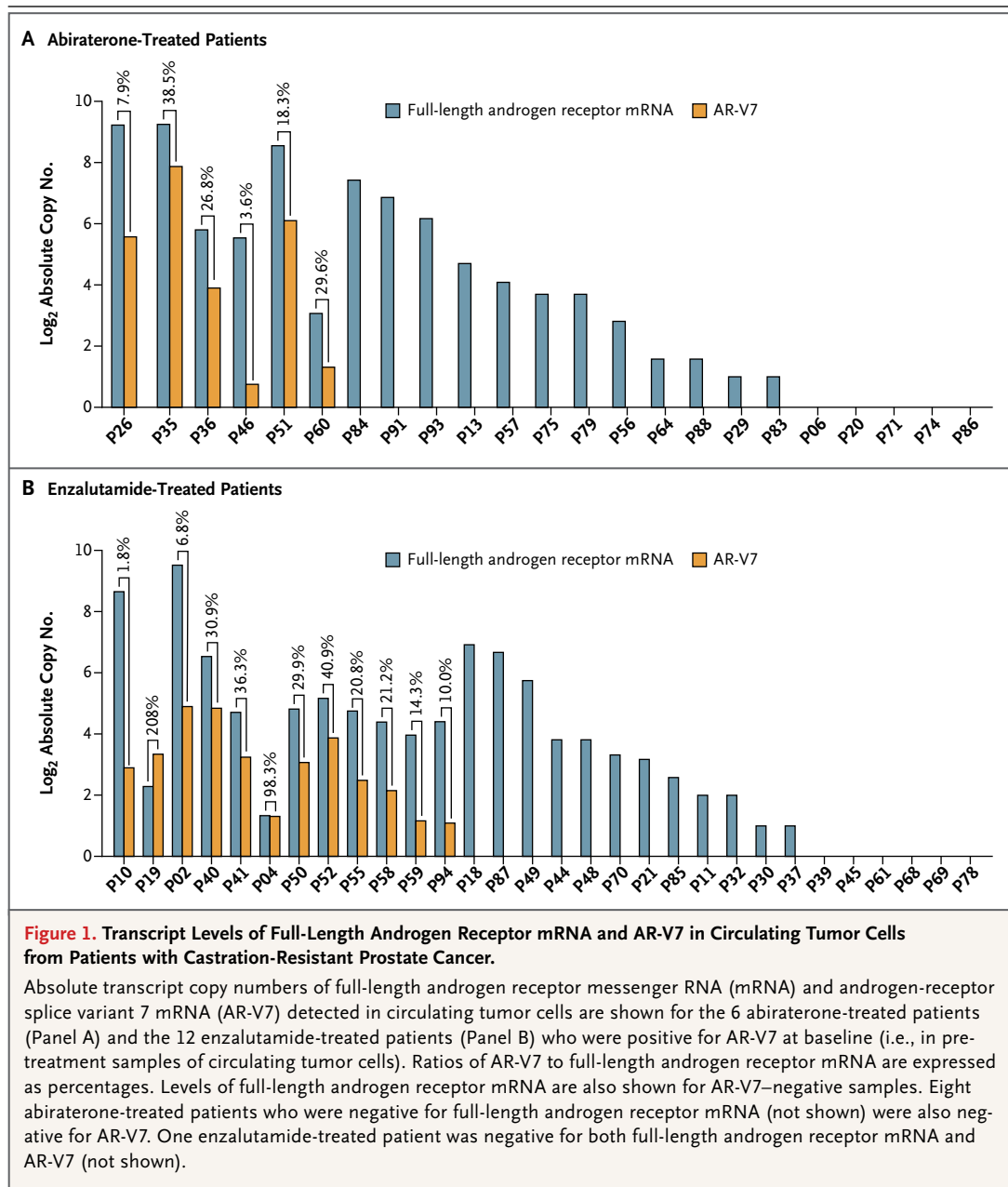
We first demonstrated our ability to detect AR-V7 transcript in cells by looking for AR-V7 in normal human blood spiked with VCaP cells (Fig. S1A in the Supplementary Appendix), a prostate-cancer cell line known to express both full-length androgen receptor and AR-V7.¹³ We then assayed the patient samples; examples of positive and negative detection of AR-V7 in blood samples from two patients are shown in Figure S1B in the Supplementary Appendix. After the validity of the assay was established (not shown), AR-V7 positivity was defined as detection of the AR-V7 transcript by means of a quantitative RT-PCR assay at 36 or fewer PCR cycles, corresponding to detection

of one or more copies of AR-V7 complementary DNA as determined by the relationship between cycle number and serial dilutions of prequantified AR-V7 (Fig. S2 in the Supplementary Appendix).

PATIENT CHARACTERISTICS

From December 2012 through September 2013, we prospectively enrolled 62 patients with detectable circulating tumor cells, of whom 31 received enzalutamide and 31 received abiraterone (Table S1 in the Supplementary Appendix). A total of 35 enzalutamide-treated men were screened to identify 31 with detectable circulating tumor cells (89% yield); 36 abiraterone-treated men were screened to identify 31 with detectable circulating tumor cells (86% yield). The 9 men with no detectable circulating tumor cells were excluded from further analysis. The median follow-up time was 5.4 months (range, 1.4 to 9.9) among enzalutamide-treated patients and 4.6 months (range, 0.9 to 8.2) among abiraterone-treated patients. A total of 39% of enzalutamide-treated patients (12 of 31 patients) and 19% of abiraterone-treated patients (6 of 31 patients) had detectable AR-V7 mRNA in baseline samples of circulating tumor cells. Among the 18 men with detectable AR-V7 from the entire study cohort, the median ratio of AR-V7 to full-length androgen receptor mRNA was 21.0% (range, 1.8 to 208.0) (Fig. 1); detection of AR-V7 was associated with increased expression of full-length androgen receptor mRNA ($P < 0.001$).

In the enzalutamide cohort, AR-V7–positive patients had higher levels of full-length androgen receptor mRNA and PSA than did AR-V7–negative patients and were more likely than AR-V7–negative patients to have an ECOG performance-status score of 1 or 2 (scores range from 0 to 5, with 0 indicating no symptoms and higher scores indicating increasing disability), visceral metastases, and six or more bone metastases and to have had prior docetaxel treatment and prior abiraterone treatment (Table S1A in the Supplementary Appendix). A total of 55% of the patients who had previously received abiraterone (11 of 20 patients) had detectable AR-V7, as compared with 9% of the patients who had not previously received the drug (1 of 11 patients). Table S2A in the Supplementary Appendix shows clinical outcomes separately for patients who had previously received abiraterone and those who had not previously received the drug.



In the abiraterone cohort, AR-V7-positive patients had higher levels of full-length androgen receptor mRNA, PSA, and alkaline phosphatase, and a higher number of prior hormonal therapies than did AR-V7-negative patients and were more likely than AR-V7-negative patients to have an ECOG performance-status score of 1 or 2 and prior enzalutamide treatment (Table S1B in the Supplementary Appendix). A total of 50% of patients who had previously received enzalutamide (2 of 4 patients) had detectable AR-V7, as

compared with 15% of patients who had not previously received the drug (4 of 27 patients). Table S2B in the Supplementary Appendix shows clinical outcomes separately for patients who had previously received enzalutamide and those who had not previously received the drug.

PRIMARY END POINT

The overall proportion of patients who had a PSA response while receiving enzalutamide was 32% (95% confidence interval [CI], 17 to 51; 10 of 31

men). In the enzalutamide cohort, the PSA response rate among AR-V7–positive patients was 0% (95% CI, 0 to 26; 0 of 12 men), and the rate among AR-V7–negative patients was 53% (95% CI, 29 to 76; 10 of 19 men; $P=0.004$). The best PSA responses are shown in Figure 2A. In linear regression modeling, AR-V7 status remained predictive of PSA response after adjustment for the expression of full-length androgen receptor mRNA ($P<0.001$).

The overall proportion of patients who had a PSA response while receiving abiraterone was 55% (95% CI, 36 to 73; 17 of 31 men). In the abiraterone cohort, the PSA response rate among AR-V7–positive patients was 0% (95% CI, 0 to 46; 0 of 6 men), and the rate among AR-V7–negative patients was 68% (95% CI, 46 to 85; 17 of 25 men; $P=0.004$). The best PSA responses are shown in Figure 2B. In linear regression modeling, AR-V7 status remained predictive of PSA response after adjustment for the expression of full-length androgen receptor mRNA ($P=0.02$).

SECONDARY END POINTS

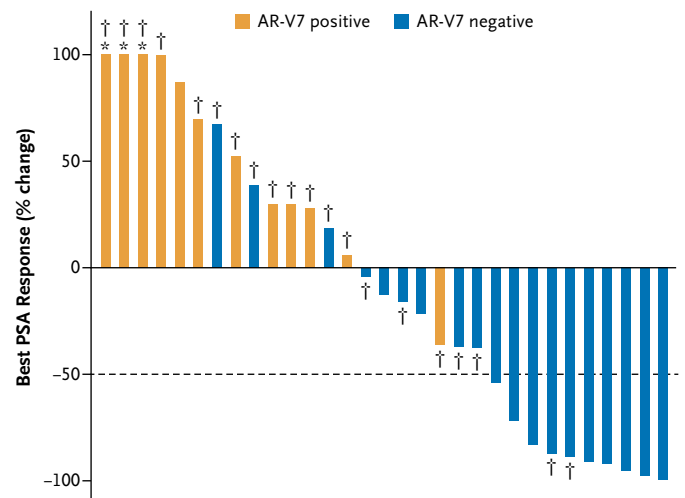
PSA Progression–free Survival

Among enzalutamide-treated patients, PSA progression–free survival was shorter among men with detectable AR-V7 at baseline than among those with undetectable AR-V7 ($P<0.001$ in a univariate analysis) (Fig. 3A). In a multivariable Cox model adjusted for the expression of full-length androgen receptor mRNA and prior abiraterone use, the detection of AR-V7 remained independently predictive of shorter PSA progression–free survival (hazard ratio for PSA progression, 3.1; 95% CI, 1.0 to 9.2; $P=0.046$); the level of full-length androgen receptor mRNA was also predictive of shorter PSA progression–free survival (hazard ratio, 1.4; 95% CI, 1.0 to 1.9), but previous abiraterone use was not (hazard ratio, 2.5; 95% CI, 0.4 to 14.5). Results of the propensity-score-weighted multivariable model are shown in Table S3A in the Supplementary Appendix.

Among abiraterone-treated patients, PSA progression–free survival was shorter among men with detectable AR-V7 at baseline than among those with undetectable AR-V7 ($P<0.001$ in a univariate analysis) (Fig. 3B). In a multivariable Cox model adjusted for the expression of full-length androgen receptor mRNA and prior enzalutamide use, the detection of AR-V7 was the only independent predictor of shorter PSA progression–free survival (hazard ratio for PSA

progression, 15.7; 95% CI, 2.1 to 117.5; $P=0.007$); neither the level of full-length androgen receptor mRNA (hazard ratio, 1.0; 95% CI, 0.8 to 1.2) nor previous enzalutamide use (hazard ratio, 0.9; 95% CI, 0.1 to 5.2) was predictive. Results of the propensity-score-weighted multivariable model

A Enzalutamide-Treated Patients



B Abiraterone-Treated Patients

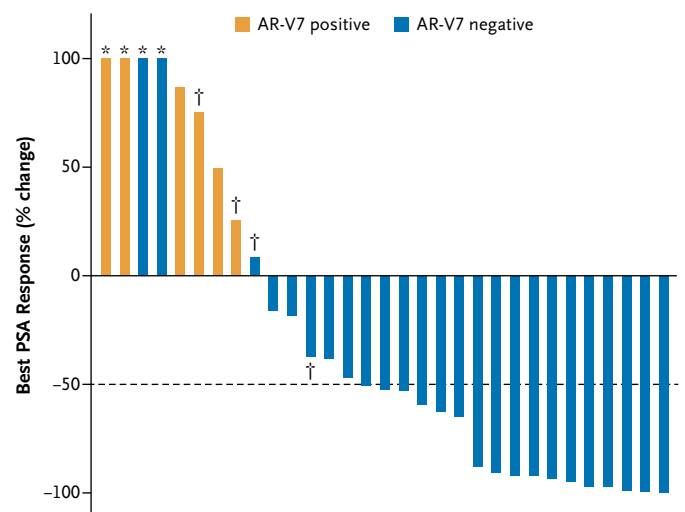


Figure 2. Waterfall Plots of Best Prostate-Specific Antigen (PSA) Responses According to AR-V7 Status.

Panel A shows the 31 enzalutamide-treated patients, and Panel B the 31 abiraterone-treated patients. The dotted line shows the threshold for defining a PSA response ($\geq 50\%$ reduction in PSA level from baseline). Asterisks indicate an increase of more than 100% in best PSA response. Daggers indicate patients in the enzalutamide cohort who had previously received abiraterone and patients in the abiraterone cohort who had previously received enzalutamide.

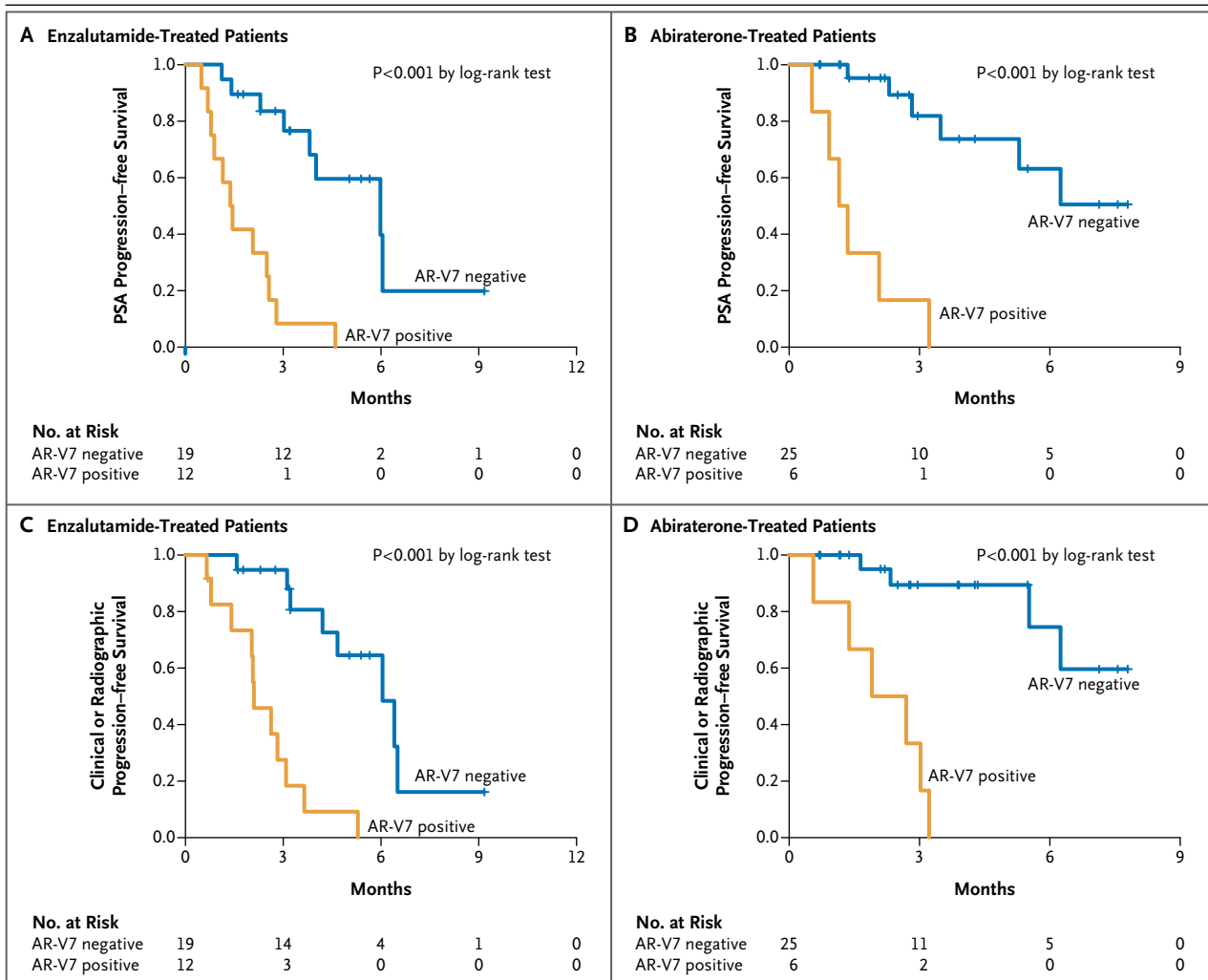


Figure 3. Kaplan-Meier Analysis of PSA Progression-free Survival and Clinical or Radiographic Progression-free Survival According to AR-V7 Status.

The median PSA progression-free survival in enzalutamide-treated patients (Panel A) was 1.4 months (95% CI, 0.9 to not reached) in AR-V7-positive patients and 6.0 months (95% CI, 3.8 to not reached) in AR-V7-negative patients (hazard ratio for PSA progression with AR-V7 positivity, 7.4; 95% CI, 2.7 to 20.6; $P < 0.001$ by the log-rank test). The median PSA progression-free survival in abiraterone-treated patients (Panel B) was 1.3 months (95% CI, 0.9 to not reached) in AR-V7-positive patients and more than 5.3 months (95% CI, 5.3 to not reached) in AR-V7-negative patients (hazard ratio for PSA progression with AR-V7 positivity, 16.1; 95% CI, 3.9 to 66.0; $P < 0.001$ by the log-rank test). The median clinical or radiographic progression-free survival in enzalutamide-treated patients (Panel C) was 2.1 months (95% CI, 2.0 to not reached) in AR-V7-positive patients and 6.1 months (95% CI, 4.7 to not reached) in AR-V7-negative patients (hazard ratio for clinical or radiographic progression with AR-V7 positivity, 8.5; 95% CI, 2.8 to 25.5; $P < 0.001$ by the log-rank test). The median clinical or radiographic progression-free survival in abiraterone-treated patients (Panel D) was 2.3 months (95% CI, 1.4 to not reached) in AR-V7-positive patients and more than 6.3 months (95% CI, 6.3 to not reached) in AR-V7-negative patients (hazard ratio for clinical or radiographic progression with AR-V7 positivity, 16.5; 95% CI, 3.3 to 82.9; $P < 0.001$ by the log-rank test).

are shown in Table S3C in the Supplementary Appendix.

Clinical or Radiographic Progression-free Survival
Among enzalutamide-treated patients, clinical or radiographic progression-free survival was short-

er among men with detectable AR-V7 at baseline than among those with undetectable AR-V7 ($P < 0.001$ in a univariate analysis) (Fig. 3C). In a multivariable Cox model adjusted for the expression of full-length androgen receptor mRNA and prior abiraterone use, the detection of AR-V7 re-

mained marginally predictive of shorter clinical or radiographic progression-free survival (hazard ratio for clinical or radiographic progression, 3.0; 95% CI, 0.9 to 9.6; $P=0.06$); the level of full-length androgen receptor mRNA was also predictive (hazard ratio, 1.7; 95% CI, 1.1 to 2.6), but previous abiraterone use was not (hazard ratio, 2.6; 95% CI, 0.2 to 27.6). Table S3B in the Supplementary Appendix shows the results of the propensity-score-weighted multivariable model.

Among abiraterone-treated patients, clinical or radiographic progression-free survival was shorter among men with detectable AR-V7 at baseline than among those with undetectable AR-V7 ($P<0.001$ in a univariate analysis) (Fig. 3D). In a multivariable Cox model adjusted for the expression of full-length androgen receptor mRNA and prior enzalutamide use, the detection of AR-V7 was the only factor that was independently predictive of shorter clinical or radiographic progression-free survival (hazard ratio for clinical or radiographic progression, 7.6; 95% CI, 1.0 to 57.6; $P=0.05$); the level of full-length androgen receptor mRNA (hazard ratio, 1.1; 95% CI, 0.9 to 1.5) and previous enzalutamide use (hazard ratio, 1.9; 95% CI, 0.4 to 10.0) were not predictive. Table S3D in the Supplementary Appendix shows the results of the propensity-score-weighted multivariable model.

Overall Survival

A preliminary survival analysis was conducted at 32% maturity in the enzalutamide-treated cohort (i.e., after 32% of the patients [10 patients] had died) (median follow-up, 8.4 months) and at 16% maturity in the abiraterone-treated cohort (i.e., after 16% of the patients [5 patients] had died) (median follow-up, 9.3 months). Overall survival was shorter in men with detectable AR-V7 at baseline than among those with undetectable AR-V7 both in the enzalutamide cohort (median, 5.5 months vs. not reached; hazard ratio for death, 6.9; 95% CI, 1.7 to 28.1; $P=0.002$ by the log-rank test) (Fig. S3A in the Supplementary Appendix) and in the abiraterone cohort (median, 10.6 months vs. not reached; hazard ratio for death, 12.7; 95% CI, 1.3 to 125.3; $P=0.006$ by the log-rank test) (Fig. S3B in the Supplementary Appendix). Owing to the small number of events in each cohort, multivariable models were not constructed.

COMBINED ANALYSIS

As an exploratory analysis, we evaluated PSA responses, PSA progression-free survival, clinical or radiographic progression-free survival, and overall survival in the combined patient population of all 62 participants. The effect of AR-V7 status on these outcomes remained significant (Fig. S4 in the Supplementary Appendix).

CONVERSION FROM AR-V7-NEGATIVE TO AR-V7-POSITIVE STATUS

Of 42 men with undetectable AR-V7 at baseline who had at least one follow-up sample available, 6 patients (4 receiving enzalutamide and 2 receiving abiraterone) subsequently converted to AR-V7-positive status during the course of treatment. All 16 patients with detectable AR-V7 at baseline who had at least one follow-up sample available remained AR-V7-positive during treatment. Clinical outcomes for all patients according to AR-V7 conversion status are summarized in Table S4 in the Supplementary Appendix. Changes in levels of AR-V7 expression during the course of treatment are summarized in Figure S5 in the Supplementary Appendix.

TISSUE-BASED ANALYSES

Seven patients consented to additional tissue-based studies: five underwent biopsies of metastatic tumors, and two consented to allow research autopsies to be performed after their death. Three of the seven patients had detectable AR-V7 in circulating tumor cells; these three patients also had detectable AR-V7 in metastatic tumor tissue according to RNA in situ hybridization analysis (Fig. 4). In addition, AR-V7 and full-length androgen receptor were detected at the protein level with the use of Western blot analysis in these patients (Fig. S7 in the Supplementary Appendix). Conversely, none of the four patients with undetectable AR-V7 in circulating tumor cells had detectable AR-V7 in metastatic tissue on RNA in situ hybridization, a finding that suggests good concordance. Finally, sequencing of the AR transcript with the use of RNA sequencing in metastatic lesions from two AR-V7-positive patients (autopsy specimens) did not identify mutations in the androgen-receptor gene that could explain resistance but did confirm the presence of AR-V7 splice junctions in both patients (Fig. S9 in the Supplementary Appendix).

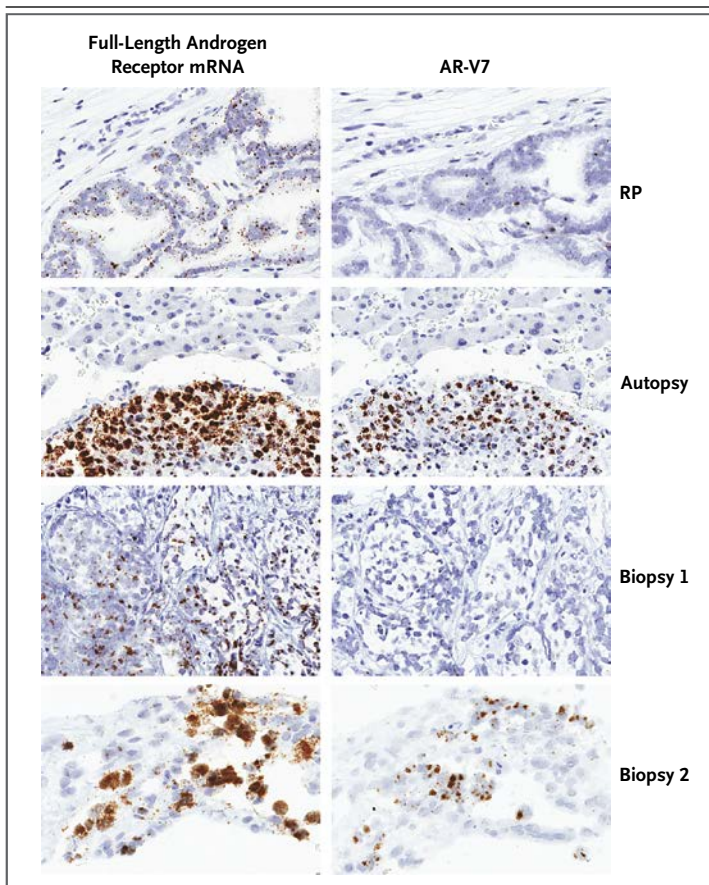


Figure 4. Detection of Full-Length Androgen Receptor mRNA and AR-V7 in Metastatic Prostate-Cancer Tissue.

In situ detection of full-length androgen receptor mRNA and AR-V7 in prostate-cancer tumor specimens was performed with the use of RNA in situ hybridization analysis. The tumor-tissue specimens shown are a radical-prostatectomy (RP) specimen that lacks AR-V7 expression from a patient (not enrolled in this study) who had not received hormonal treatment, an autopsy-derived specimen of a liver metastasis from a patient whose circulating tumor cells were shown to express AR-V7 (Autopsy), and core-needle biopsy specimens from a patient in whom AR-V7 was not detected (Biopsy 1) and a patient in whom AR-V7 was detected (Biopsy 2) in circulating tumor cells. All the tumor specimens show expression of full-length androgen receptor mRNA. The prostate-cancer cell lines that served as positive and negative controls for AR-V7 detection by means of RNA in situ hybridization are shown in Figure S6 in the Supplementary Appendix.

RELATIONSHIP BETWEEN FULL-LENGTH ANDROGEN RECEPTOR mRNA AND AR-V7

In all patients with detectable AR-V7, full-length androgen receptor mRNA was also expressed and at higher levels (with one exception) than the levels of AR-V7; increased expression of AR-V7 was generally (but not always) coupled with that of full-length androgen receptor mRNA (Fig. 1). Al-

though expression of PSA (an indicator of canonical androgen-receptor signaling) was generally suppressed in AR-V7–negative patients during treatment with enzalutamide or abiraterone, PSA expression did not decrease in post-treatment samples of circulating tumor cells from men with detectable AR-V7 at baseline (Fig. S8 in the Supplementary Appendix). This observation is consistent with continued androgen-receptor signaling despite potent inhibition of full-length androgen receptor when AR-V7 coexists with full-length androgen receptor and contrasts with previous findings from a cell-line model of prostate cancer.¹⁹

In addition, genomewide comparisons of two AR-V7–negative and two AR-V7–positive metastatic tumor samples by means of gene-set enrichment analysis of RNA sequencing data (Figs. S9 and S10 in the Supplementary Appendix) or by means of targeted analysis of a set of genes regulated by the canonical androgen receptor (Table S5 in the Supplementary Appendix) revealed alterations consistent with a shift toward AR-V7–driven transcription in AR-V7–positive samples. Finally, the addition of AR-V7 status to regression models that included only levels of full-length androgen receptor mRNA resulted in significant improvements in model fit across all clinical outcomes evaluated, confirming the added value of AR-V7 status in predicting outcomes with enzalutamide or abiraterone (Table S6 in the Supplementary Appendix).

DISCUSSION

Enzalutamide and abiraterone, two new therapies directed at the androgen receptor, represent important advances in the management of castration-resistant prostate cancer.^{4,7–9} However, a proportion of men do not benefit from these agents, and a clearer understanding of the mechanisms underlying resistance to these drugs would facilitate selection of alternative therapies (e.g., chemotherapies) for such patients. We found that AR-V7 can be detected reliably from circulating tumor cells and that detection of AR-V7 in tumor cells appears to be associated with resistance to both enzalutamide and abiraterone. This conceptually simple model is biologically plausible, because the protein encoded by AR-V7 lacks the ligand-binding domain of the androgen

receptor (the direct target of enzalutamide and the indirect target of abiraterone) while remaining constitutively active as a transcription factor in a ligand-independent manner.^{13,16}

In our study, no AR-V7–positive patient had any appreciable clinical benefit from enzalutamide or abiraterone therapy. Moreover, although AR-V7 detection was associated with increased expression of full-length androgen receptor mRNA, the prognostic effect of AR-V7 was maintained after adjustment for levels of full-length androgen receptor mRNA. Finally, although prior treatment with abiraterone or enzalutamide was associated with AR-V7 positivity, AR-V7 status remained prognostic after adjustment for this factor. Therefore, the current study shows a strong association between the presence of AR-V7 and resistance to enzalutamide and abiraterone. If this finding is substantiated by others, it is possible that AR-V7 could be used as a biomarker to predict resistance to enzalutamide and abiraterone and to facilitate treatment selection. However, our study does not prove a causal role for AR-V7 in mediating resistance to enzalutamide or abiraterone, and it remains possible that AR-V7 is a marker of more advanced disease or a higher disease burden.

Preclinical studies have shown that androgen-receptor variants are much more common in castration-resistant prostate cancer than in hormone-sensitive prostate cancer,¹³ that they represent one potential mechanism driving the emergence of the castration-resistant phenotype,¹⁰ and that they may be responsible for the progression of castration-resistant prostate cancer.¹⁴ Studies involving patients with castration-resistant prostate cancer have shown that androgen-receptor variants are often expressed in metastases^{20,21} and that high levels of these variants in metastatic tissue are associated with faster disease progression and shorter cancer-specific survival than are low or undetectable levels.^{13,16,20} However, all these studies have been retrospective in nature, and none have obtained serial specimens across time or investigated the clinical significance of androgen-receptor variants in patients receiving enzalutamide or abiraterone.

Several studies have shown that although the protein isoforms encoded by androgen-receptor splice variants are constitutively active, their function may be dependent on the activity of full-

length androgen receptor.¹⁹ Therefore, despite the fact that the protein isoforms encoded by androgen-receptor splice variants cannot be targeted directly by currently available drugs, it has been hypothesized that inhibition of full-length androgen receptor by enzalutamide or abiraterone could partially reverse resistance mediated by androgen-receptor variants. Our clinical data do not support this claim, because we did not observe any PSA responses in men harboring AR-V7 (all of whom also expressed full-length androgen receptor mRNA). An alternative treatment approach for AR-V7–positive patients would be to design agents targeting the N-terminal domain of the androgen receptor,^{22–24} which would theoretically inhibit both full-length androgen receptor and androgen-receptor isoforms that lack the ligand-binding domain; such inhibitors are in early stages of drug development.^{23,24}

There are likely to be multiple additional explanations for primary or acquired resistance to enzalutamide and abiraterone. For instance, overexpression of CYP17A1 (or other steroidogenic enzymes) leading to increased synthesis of intracrine or paracrine androgens has been shown to occur in patients receiving these agents.^{25–28} In addition, point mutations affecting the ligand-binding domain of the androgen receptor have been shown to confer agonistic activity to enzalutamide.^{29,30} Furthermore, expression of androgen-regulated genes may be driven by alternative steroid receptors, such as the glucocorticoid receptor.^{31,32} Finally, inhibition of the androgen receptor may lead to reciprocal up-regulation of other oncogenic pathways, such as the PI3K–AKT pathway.³³ It is unlikely that all cases of enzalutamide or abiraterone resistance will be explained by a single cause.

In conclusion, our data support an association between AR-V7 and resistance to both enzalutamide and abiraterone in patients with castration-resistant prostate cancer. These findings require large-scale prospective validation.

Supported by a Prostate Cancer Foundation (PCF) Young Investigator Award (to Dr. Antonarakis), a PCF Challenge Award, grants from the Department of Defense Prostate Cancer Research Program (W81XWH-10-2-0056 and W81XWH-10-2-0046, to the Prostate Cancer Biorepository Network [PCBN]; and W81XWH-12-1-0605, to Dr. Luo), a Johns Hopkins Prostate Cancer Specialized Program of Research Excellence grant (P50 CA058236), and a grant from the National Institutes of Health (P30 CA006973).

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

We thank the entire PCBN team at Johns Hopkins University School of Medicine, including Drs. Bruce Trock and Karen Sfianos, for providing valuable input and organizational support; Drs. Carla Ellis, Christine Iacobuzio-Donahue, and Barbara

Crain for assistance with the research autopsies; Dr. Nate Brennen and Ms. Jessica Hicks for technical assistance; and Ms. Medha Darshan and Ms. Guifang Yan for assistance in preparing the cryostat sections; and the patients and their families who participated in this study.

REFERENCES

1. Longo DL. New therapies for castration-resistant prostate cancer. *N Engl J Med* 2010;363:479-81.
2. Ryan CJ, Tindall DJ. Androgen receptor rediscovered: the new biology and targeting the androgen receptor therapeutically. *J Clin Oncol* 2011;29:3651-8.
3. Tran C, Ouk S, Clegg NJ, et al. Development of a second-generation antiandrogen for treatment of advanced prostate cancer. *Science* 2009;324:787-90.
4. Scher HI, Beer TM, Higano CS, et al. Antitumour activity of MDV3100 in castration-resistant prostate cancer: a phase 1-2 study. *Lancet* 2010;375:1437-46.
5. O'Donnell A, Judson I, Dowsett M, et al. Hormonal impact of the 17 α -hydroxylase/C(17,20)-lyase inhibitor abiraterone acetate (CB7630) in patients with prostate cancer. *Br J Cancer* 2004;90:2317-25.
6. Attard G, Reid AH, Yap TA, et al. Phase I clinical trial of a selective inhibitor of CYP17, abiraterone acetate, confirms that castration-resistant prostate cancer commonly remains hormone driven. *J Clin Oncol* 2008;26:4563-71.
7. Scher HI, Fizazi K, Saad F, et al. Increased survival with enzalutamide in prostate cancer after chemotherapy. *N Engl J Med* 2012;367:1187-97.
8. de Bono JS, Logothetis CJ, Molina A, et al. Abiraterone and increased survival in metastatic prostate cancer. *N Engl J Med* 2011;364:1995-2005.
9. Ryan CJ, Smith MR, de Bono JS, et al. Abiraterone in metastatic prostate cancer without previous chemotherapy. *N Engl J Med* 2013;368:138-48.
10. Nadiminty N, Tummala R, Liu C, et al. NF- κ B2/p52 induces resistance to enzalutamide in prostate cancer: role of androgen receptor and its variants. *Mol Cancer Ther* 2013;12:1629-37.
11. Mostaghel EA, Marck BT, Plymate SR, et al. Resistance to CYP17A1 inhibition with abiraterone in castration-resistant prostate cancer: induction of steroidogenesis and androgen receptor splice variants. *Clin Cancer Res* 2011;17:5913-25.
12. Dehm SM, Schmidt LJ, Heemers HV, Vessella RL, Tindall DJ. Splicing of a novel androgen receptor exon generates a constitutively active androgen receptor that mediates prostate cancer therapy resistance. *Cancer Res* 2008;68:5469-77.
13. Hu R, Dunn TA, Wei S, et al. Ligand-independent androgen receptor variants derived from splicing of cryptic exons signify hormone-refractory prostate cancer. *Cancer Res* 2009;69:16-22.
14. Hu R, Lu C, Mostaghel EA, et al. Distinct transcriptional programs mediated by the ligand-dependent full-length androgen receptor and its splice variants in castration-resistant prostate cancer. *Cancer Res* 2012;72:3457-62.
15. Li Y, Chan SC, Brand LJ, Hwang TH, Silverstein KA, Dehm SM. Androgen receptor splice variants mediate enzalutamide resistance in castration-resistant prostate cancer cell lines. *Cancer Res* 2013;73:483-9.
16. Guo Z, Yang X, Sun F, et al. A novel androgen receptor splice variant is up-regulated during prostate cancer progression and promotes androgen depletion-resistant growth. *Cancer Res* 2009;69:2305-13.
17. 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* 2008;26:1148-59.
18. Therasse P, Arbuck SG, Eisenhauer EA, et al. New guidelines to evaluate the response to treatment in solid tumors: European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. *J Natl Cancer Inst* 2000;92:205-16.
19. Watson PA, Chen YF, Balbas MD, et al. Constitutively active androgen receptor splice variants expressed in castration-resistant prostate cancer require full-length androgen receptor. *Proc Natl Acad Sci U S A* 2010;107:16759-65.
20. Hörnberg E, Ylitalo EB, Crnalic S, et al. Expression of androgen receptor splice variants in prostate cancer bone metastases is associated with castration-resistance and short survival. *PLoS One* 2011;6(4):e19059.
21. Zhang X, Morrissey C, Sun S, et al. Androgen receptor variants occur frequently in castration resistant prostate cancer metastases. *PLoS One* 2011;6(11):e27970.
22. Sadar MD. Small molecule inhibitors targeting the "Achilles' heel" of androgen receptor activity. *Cancer Res* 2011;71:1208-13.
23. Ravindranathan P, Lee T-K, Yang L, et al. Peptidomimetic targeting of critical androgen receptor-coregulator interactions in prostate cancer. *Nat Commun* 2013;4:1923.
24. Andersen RJ, Mawji NR, Wang J, et al. Regression of castrate-recurrent prostate cancer by a small-molecule inhibitor of the amino-terminus domain of the androgen receptor. *Cancer Cell* 2010;17:535-46.
25. Mitsiades N, Sung CC, Schultz N, et al. Distinct patterns of dysregulated expression of enzymes involved in androgen synthesis and metabolism in metastatic prostate cancer tumors. *Cancer Res* 2012;72:6142-52.
26. Efstathiou E, Titus M, Wen S, et al. Molecular characterization of enzalutamide-treated bone metastatic castration-resistant prostate cancer. *Eur Urol* 2014 May 29 (Epub ahead of print).
27. Efstathiou E, Titus M, Tsavachidou D, et al. Effects of abiraterone acetate on androgen signaling in castrate-resistant prostate cancer in bone. *J Clin Oncol* 2012;30:637-43.
28. Chang KH, Li R, Kuri B, et al. A gain-of-function mutation in DHT synthesis in castration-resistant prostate cancer. *Cell* 2013;154:1074-84.
29. Balbas MD, Evans MJ, Hosfield DJ, et al. Overcoming mutation-based resistance to antiandrogens with rational drug design. *eLife* 2013;2:e00499.
30. Joseph JD, Lu N, Qian J, et al. A clinically relevant androgen receptor mutation confers resistance to second-generation antiandrogens enzalutamide and ARN-509. *Cancer Discov* 2013;3:1020-9.
31. Sahu B, Laakso M, Pihlajamaa P, et al. FoxA1 specifies unique androgen and glucocorticoid receptor binding events in prostate cancer cells. *Cancer Res* 2013;73:1570-80.
32. Arora VK, Schenkein E, Murali R, et al. Glucocorticoid receptor confers resistance to antiandrogens by bypassing androgen receptor blockade. *Cell* 2013;155:1309-22.
33. Carver BS, Chapinski C, Wongvipat J, et al. Reciprocal feedback regulation of PI3K and androgen receptor signaling in PTEN-deficient prostate cancer. *Cancer Cell* 2011;19:575-86.

Copyright © 2014 Massachusetts Medical Society.

Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Antonarakis ES, Lu C, Wang H, et al. AR-V7 and resistance to enzalutamide and abiraterone in prostate cancer. *N Engl J Med* 2014;371:1028-38. DOI: 10.1056/NEJMoa1315815

Supplementary Appendix

Table of Contents

1. METHODS (pages 2–6)
2. FIGURES [Figures S1-S10] (pages 7–20)
 - Figure S1.** Detection of AR-V7 transcript in CTCs (page 7)
 - Figure S2.** Justification for threshold of detection of AR-V7 (page 8)
 - Figure S3.** Overall survival analysis stratified by AR-V7 status in enzalutamide-treated patients and abiraterone-treated patients (page 9)
 - Figure S4.** Combined analysis of patient outcome by AR-V7 status (page 11)
 - Figure S5.** Changes in AR-V7 and AR-FL transcript copy numbers detected in CTCs before and after treatment with enzalutamide and abiraterone (page 14)
 - Figure S6.** Detection of AR-FL and AR-V7 using RNA-ISH in cell lines with known expression of AR-FL and AR-V7 (page 16)
 - Figure S7.** Detection of AR-V7 at the protein level using Western blot analysis in patients with detectable AR-V7 transcript in CTCs (page 17)
 - Figure S8.** Changes in expression levels of the PSA transcript before and after therapy with enzalutamide or abiraterone in men with baseline detectable AR-V7 (page 18)
 - Figure S9.** RNA-Seq analysis of the AR transcript in two AR-V7–positive patients and two AR-V7–negative patients (page 19)
 - Figure S10.** Gene set enrichment analysis of metastatic tumors from AR-V7–positive and AR-V7–negative patients (page 20)
3. TABLES [Tables S1-S6] (pages 21–28)
 - Table S1A.** Baseline characteristics of the 31 patients treated with enzalutamide (page 21)
 - Table S1B.** Baseline characteristics of the 31 patients treated with abiraterone (page 22)
 - Table S2.** Clinical outcomes (PSA responses, PSA-PFS and PFS) reported separately according to prior exposure to abiraterone (in the enzalutamide cohort) and prior exposure to enzalutamide (in the abiraterone cohort) (page 23)
 - Table S3.** Propensity score weighted multivariable Cox models adjusted for Gleason score, baseline PSA, number of prior hormonal treatments, presence of visceral metastases, ECOG score, prior abiraterone/enzalutamide use, and AR-FL levels (page 24)
 - Table S4.** Clinical outcomes (PSA responses, PSA-PFS and PFS) for the entire patient population according to their AR-V7 ‘conversion’ rates (page 25)
 - Table S5.** Expression profiles of AR-regulated genes in AR-V7–positive and AR-V7–negative metastatic tumors (page 26)
 - Table S6.** R² values for regression models that include only AR-FL levels (continuous variable) compared to regression models that additionally include AR-V7 status, for each clinical outcome (PSA response rate, PSA-PFS and PFS) (page 28)
4. REFERENCES (page 29)

Methods

Analysis of circulating tumor cells

CTC analyses were conducted using the commercially-available Alere™ CTC AdnaTest platform (AdnaGen, Langenhagen, Germany). This assay does not enable CTC enumeration. Isolation and enrichment of CTCs was performed using the ProstateCancerSelect kit, and mRNA expression analyses were performed using the ProstateCancerDetect kit with multiplexed reverse-transcription polymerase-chain-reaction (qRT-PCR) primers to detect the presence of CTCs, and custom primers designed to detect the full-length-AR (AR-FL) and AR splice variant-7 (AR-V7).^{1,2} The relative AR-V7 transcript abundance was determined by calculating the ratio of AR-V7 to AR-FL.^{1,2} Additional details are provided below.

Blood samples were collected using standard BD Vacutainer® lavender top blood collection tubes (Becton Dickinson, Franklin Lakes, NJ) (Product #: 367862) by venipuncture, and carried to the lab on ice. Laboratory processing was carried out within 2 hours of collection, according to instructions provided by the Alere™ CTC AdnaTest (Alere Inc., San Diego, CA). The AdnaTest is a CE-marked, RNA-based CTC enrichment and detection test with two components/kits. Briefly, the ProstateCancerSelect (Product No. T-1-520) kit was used to enrich CTC from 5ml blood using magnetic particles coated with a combination of antibodies recognizing prostate cancer cells, while the ProstateCancerDetect (Product No. T-1-521) kit was used to make cDNA for detection of prostate cancer-associated RNA transcripts using multiplexed polymerase chain reaction (PCR). On the basis of detection of PCR signals for PSA, PSMA, or EGFR (very rarely detected) by the Agilent Bioanalyzer (Agilent Technologies, Palo Alto, CA), CTC calls were made for each sample tested. The test was adapted for detection and quantification of AR-FL and AR-V7 by quantitative real-time PCR using custom primers specific for AR-FL (forward: 5'-CAGCCTATTGCGAGAGAGCTG-3', reverse: 5'-GAAAGGATCTTGGGCACTTGC-3') and AR-V7 (forward: 5'-CCATCTTGTCGTCTTCGGAAATGTTA-3', reverse: 5'-TTTGAATGAGGCAAGTCAGCCTTTCT-3'). Briefly, PCR reactions were carried out under optimized conditions at 95°C x 10s, 58°C x 30s, and 72°C x 30s for 39 cycles followed by melting curve analysis.

Standard dilution curves from known quantities of AR-FL and AR-V7 was generated for calculating absolute transcript copy numbers for AR-FL and AR-V7. Laboratory data was generated for each patient enrolled in the study in a blinded fashion and recorded into the master data sheet on a weekly basis. To rule out false positive and false negative findings, a number of quality control measures were implemented each time the assay was performed, including negative and positive controls at multiple levels for both CTC detection and AR quantification. This laboratory-developed RNA-based assay modified from a commercially available circulating tumor cell (CTC) detection platform was thoroughly evaluated and internally validated with standard quality measures implemented. First, all samples collected from healthy volunteers (n=4) and CTC-negative CRPC patients (n=9) were negative for AR-FL and AR-V7, excluding the possibility of false-positive detection due to contaminating leukocytes. Second, the test consistently detected both AR-FL and AR-V7 in normal blood samples spiked with 5 AR-V7 positive cells (n=6). Third, overnight shipping of blood samples and storage of partially processed cell lysates (up to two weeks) did not compromise assay results (although our current study does not involving shipping). Fourth, all tests on clinical specimens were performed using a fraction (10%) of the cDNA yield from 5 mL of blood, and were always accompanied by two negative and two positive controls. Detailed technical protocols will be made available upon request.

RNA *in situ* hybridization

RNA in situ hybridization (RISH) was performed to detect the androgen receptor (AR) and AR-V7 using the ACD (Advanced cell Diagnostics, Hayward, CA) RNAscope 2.0 Brown kit. Briefly, formalin-fixed paraffin-embedded (FFPE) tissue or cell pellet blocks were sectioned and the slides baked for one hour at 60°C. The slide were subsequently de-paraffinized with xylene for 20 min at room temperature, and allowed to air dry following two rinses using 100% ethanol. Following a series of pretreatment steps, the cells were permeablized using protease to allow probe access to the RNA target. ACD target probes, a series of paired oligonucleotides forming a binding site for a preamplifier, were custom designed to detect RNA corresponding to exon 1 of the human AR (ACD 401211), or the cryptic AR exon 3 sequence^{1,3} that encode human AR-V7 (ACD 401221). Hybridization of the probes to the AR

RNA targets was performed by incubation in the oven for 2 hours at 40 °C. Following two washes, the slides were processed for standard signal amplification steps per manufacturer's instructions.

Western Blot

Whole cell protein extracts were prepared from cultured prostate cancer cells or cryosections prepared from clinical specimens by using RIPA buffer (radioimmunoprecipitation assay buffer) (Cell Signaling Technology, Danvers, MA) supplemented with 1X protease inhibitors (Roche, Indianapolis, IN) and 1X phosphatase inhibitors (Thermo Fisher Scientific, Rockford, IL). Standard blots were prepared following electrophoresis of forty µg protein per sample on a 10% SDS-PAGE precast gel (Bio-Rad Laboratories, Hercules, CA), and incubated overnight with anti-AR-V7⁴ (1 µg/mL), anti-AR (N20) (1:2000 dilution) (sc-816, Santa Cruz Biotechnology, Dallas, TX), anti-PSA (C-19) (1:500) (sc-7638, Santa Cruz Biotechnology), and anti-β-actin (1:5000 dilution) (Sigma, St Louis, MO). Following incubation with horseradish-peroxidase(HRP)-conjugated secondary antibodies, immunoreactive bands were visualized using the SuperSignal West Pico Chemiluminescent Substrate system (I-34080) (Thermo Fisher Scientific, Rockford, IL) on HyBlot CL film (E3022) (Denville Scientific, South Plainfield, NJ).

Prostate cancer cell lines

LNCaP cells (ATCC, Manassas, VA) were maintained in RPMI1640 medium (Invitrogen, Carlsbad, CA) with 10% fetal bovine serum (FBS, Sigma–Aldrich, St. Louis, MO). LNCaP95 is an AR-V7-positive androgen-independent cell line derived from the parental LNCaP cells as described previously⁴. LNCaP95 cells were maintained in phenol red-free RPMI 1640 medium supplemented with 10% charcoal stripped FBS (CSS). For analysis of androgen-induced changes in AR-V7, LNCaP95 cells were treated with R1881 (NEN, Waltham, MA) or ethanol vehicle control as described previously⁴. These cell lines were authenticated (DDC Medical, Fairfield, OH) using short tandem repeats DNA profiling and tested negative for mycoplasma.

Metastatic prostate tumor tissue specimens

To investigate concordance in AR-V7 status between CTCs and tumor tissue, qRT-PCR analysis for AR-V7 was performed on fresh metastatic tumor biopsies (or autopsy specimens) from a subset of patients who consented to this. In addition, RNA in situ hybridization (RNA-ISH) was performed according to the manufacturer's instructions using the RNAscope platform (Advanced Cell Diagnostics, Hayward, CA) to visualize AR-V7 mRNA in formalin-fixed paraffin-embedded metastatic tumor tissues, and to correlate this with AR-V7 detection in CTCs. Additional details are provided below.

Research autopsies were performed on two patients who died during the course of treatment with enzalutamide. Both patients were AR-V7 positive as determined by the CTC assay before and after treatment. Metastatic prostate tumors were dissected and flash frozen blocks prepared. Following histological analysis, cryosections enriched for tumor cells were prepared following manual trimming of the frozen blocks, using a standard procedure as described previously ⁵. High-quality total RNA in adequate quantity was extracted from two specimens (one from each patient) and labeled as AR-V7(+) Met1 and AR-V7(+) Met2, respectively. To identify relevant AR-V7-negative metastatic CRPC samples for comparison, we analyzed AR-FL and AR-V7 expression levels in a separate collection of CRPC specimens from men consented for autopsy (before the development of enzalutamide and abiraterone) as described previously ^{1,6,7}. Two specimens that were AR-V7 negative but with AR-FL levels similar to those detected in other mCRPC specimens were identified from this collection of specimens. These two samples were labeled AR-V7(-) Met1 and AR-V7(-) Met2. Both samples were processed histologically in a similar fashion to enrich prostate carcinoma cells.

RNA-Seq analysis

Four metastatic prostate tissue specimens, AR-V7(+) Met1, AR-V7(+) Met2, AR-V7(-) Met1, and AR-V7(-) Met 2, were subjected to RNA-Seq following the standard TruSeq Stranded Total RNA Sample Prep Kit and sequenced using the Illumina HiSeq 2000 platform (Illumina Inc, San Diego, CA). We generated an average of ~63 million reads per sample. Sequences were aligned to UCSC hg19 genome build using TopHat, and mutation and splice junctions visualized using Integrated Genome

Viewer (IGV) ⁸. Read counts (gene expression levels) were obtained using HTSeq ⁹, and normalized per kilo base-pair gene length and per million reads library size (RPKM). Fold expression changes (FC) between the two conditions (AR-V7(+)) and AR-V7(-)) were calculated. Genes were pre-ranked by logFC and subjected to Gene Set Enrichment Analysis (GSEA) ¹⁰. Both raw and processed RNA-Seq data were deposited in the Gene Expression Omnibus (accession number: GSE56701).

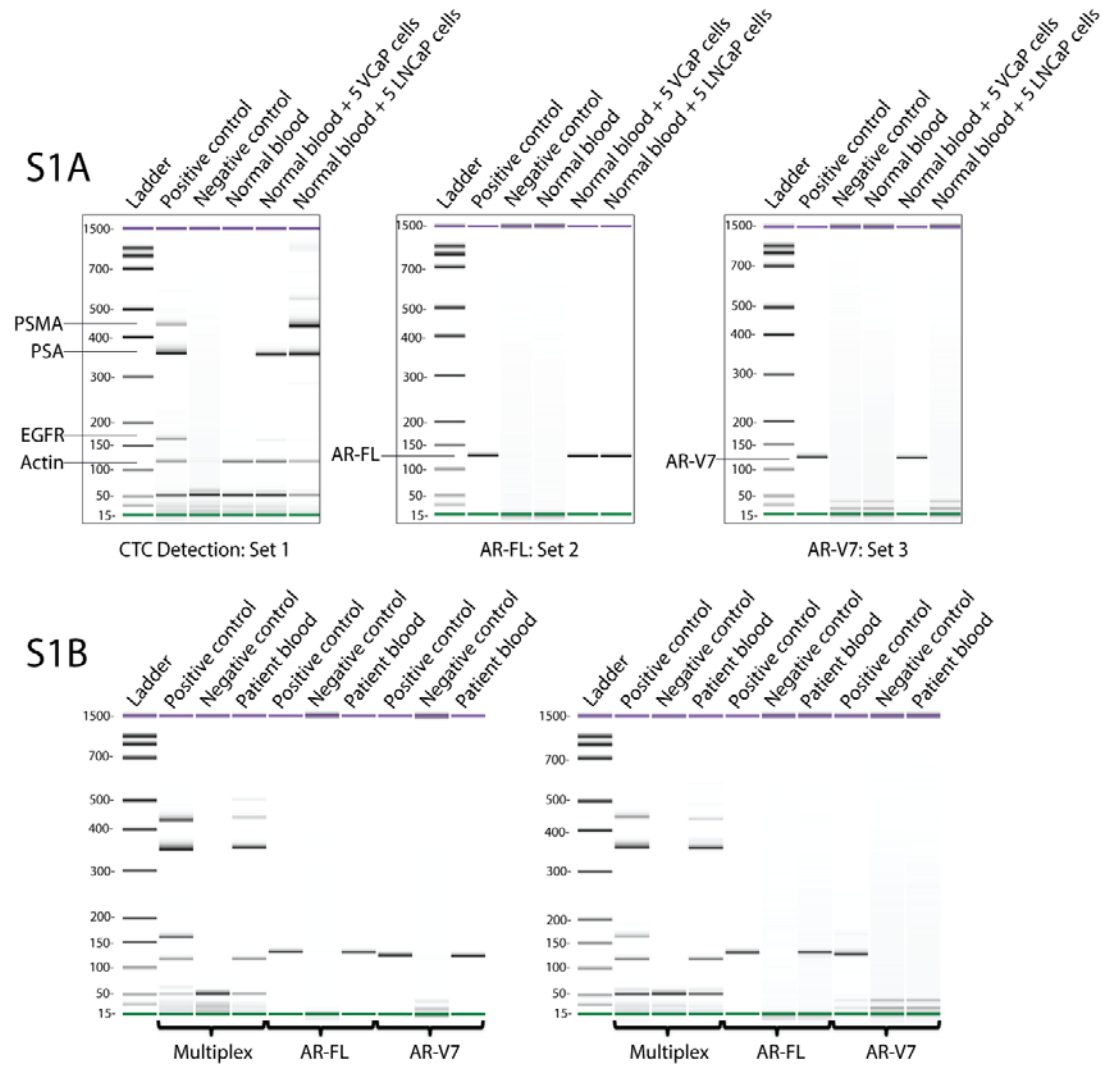
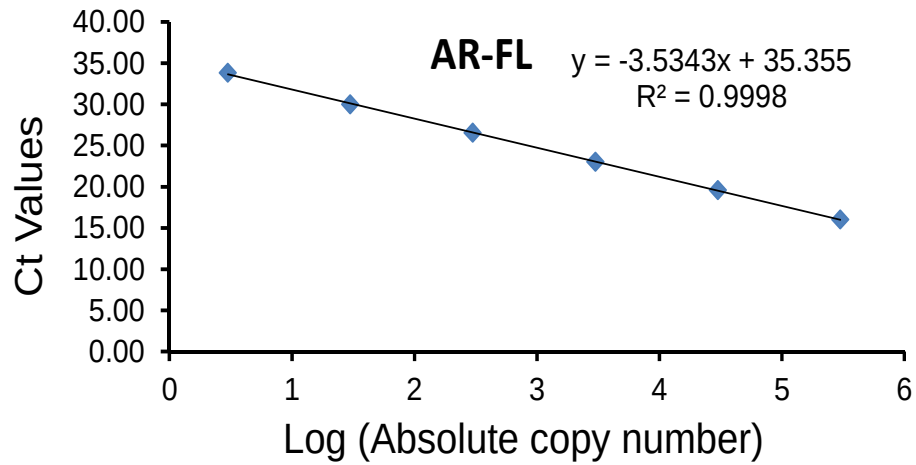


Figure S1. Detection of AR-V7 transcript in CTCs.

(A) Blood-based detection of full-length androgen receptor (AR-FL) and AR splice variant-7 (AR-V7) transcripts in tumor cells spiked into 5 mL of blood from normal human volunteers. Following CTC capture, lysis, and cDNA synthesis, three sets of independent PCR reactions were performed to examine the presence of CTC-specific mRNA transcripts by multiplex PCR (set 1), as well as transcripts for AR-FL (set 2) and AR-V7 (set 3). **(B)** Examples of positive and negative detection of AR-V7 in baseline (pre-treatment) blood samples from two enzalutamide-treated patients. The patient in the **left panel** is positive for both AR-FL and AR-V7, while the patient in the **right panel** is positive only for AR-FL but negative for AR-V7. Both patients were positive for CTCs, as determined by the multiplex PCR assay (based on the examination of PSMA, PSA, EGFR and Actin) per the manufacturer's instructions provided by AdnaGen.

S2 A



S2 B

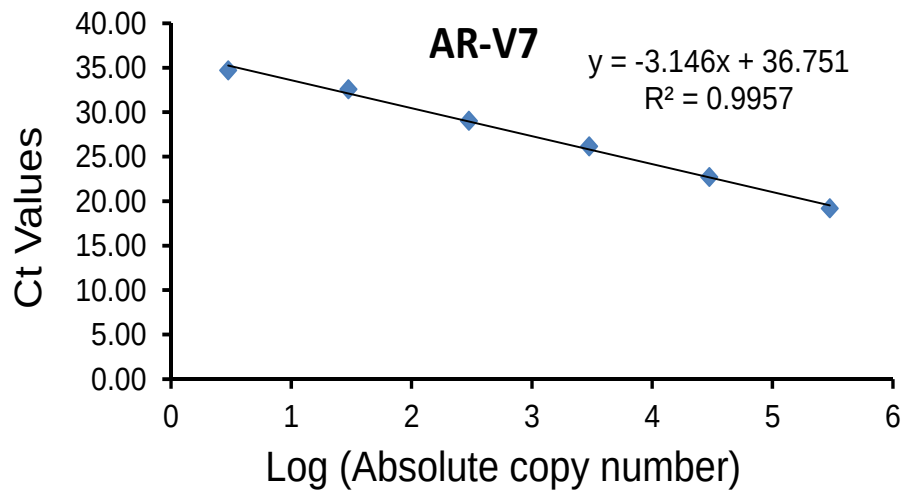
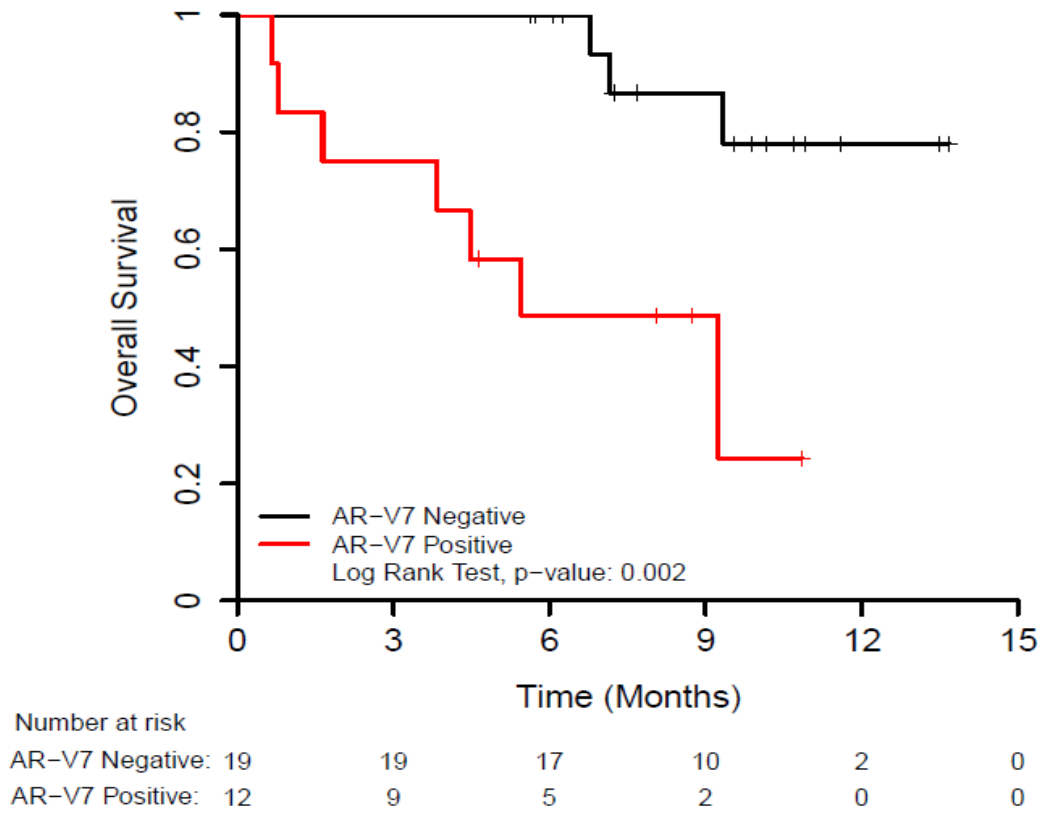


Figure S2. Justification for threshold of detection of AR-V7.

Standard dilution curves for AR-FL and AR-V7 are shown. Threshold cycle numbers (Y axis) in quantitative PCR reactions were determined for complementary DNA (cDNA) specific to AR-FL (**A**) and AR-V7 (**B**) at 6 dilutions containing the indicated number of copies of each transcript (X axis). Formulas were derived to quantify the absolute copy numbers on the basis of Ct values.

S3 A



S3 B

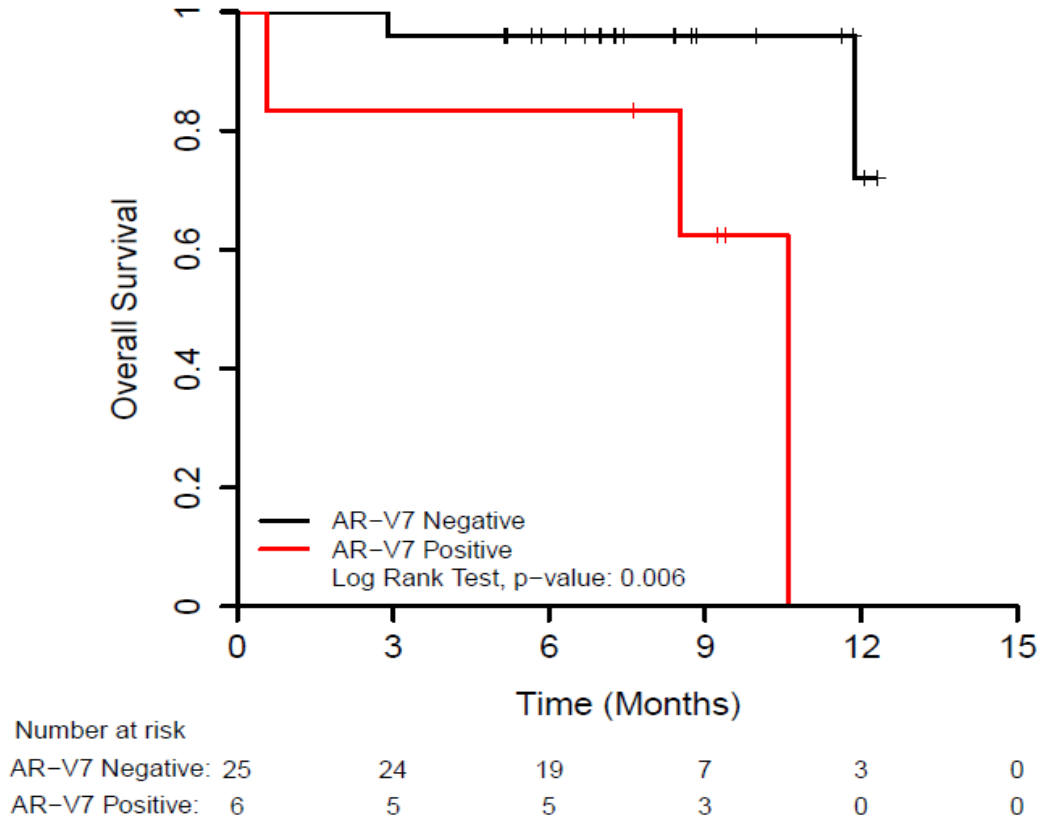
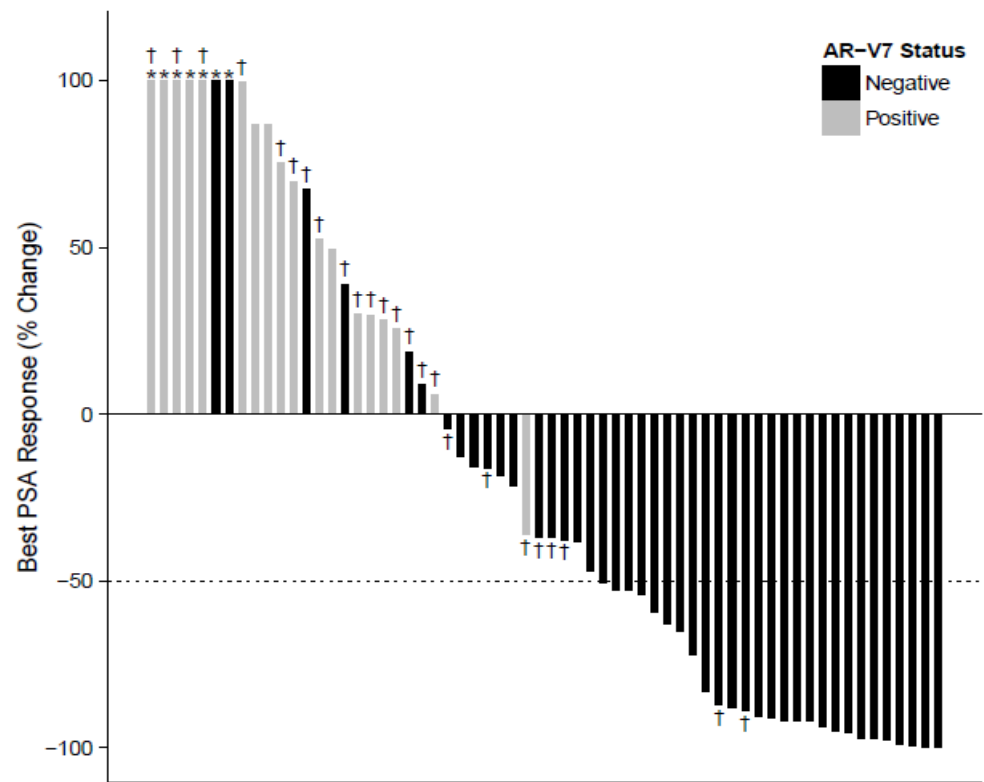


Figure S3. Overall survival (OS) analysis stratified by AR-V7 status in enzalutamide-treated patients and abiraterone-treated patients.

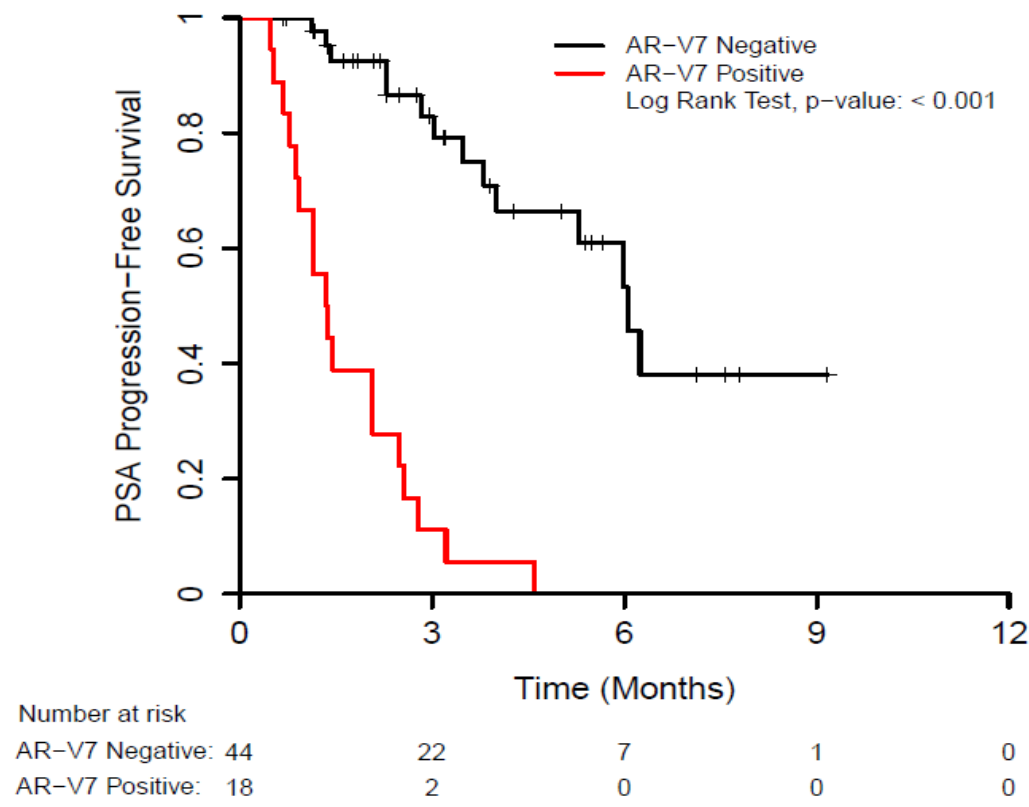
(A) Median OS in enzalutamide-treated patients was 5.5 months (95%CI, 3.9–not reached) and not reached (95%CI, not reached–not reached) in AR-V7–positive and AR-V7–negative patients, respectively (HR 6.9, 95%CI 1.7–28.1, log-rank $P=0.002$).

(B) Median OS in abiraterone-treated patients was 10.6 months (95%CI, 8.5–not reached) and >11.9 months (95%CI, 11.9–not reached) in AR-V7–positive and AR-V7–negative patients, respectively (HR 12.7, 95%CI 1.3–125.3, log-rank $P=0.006$).

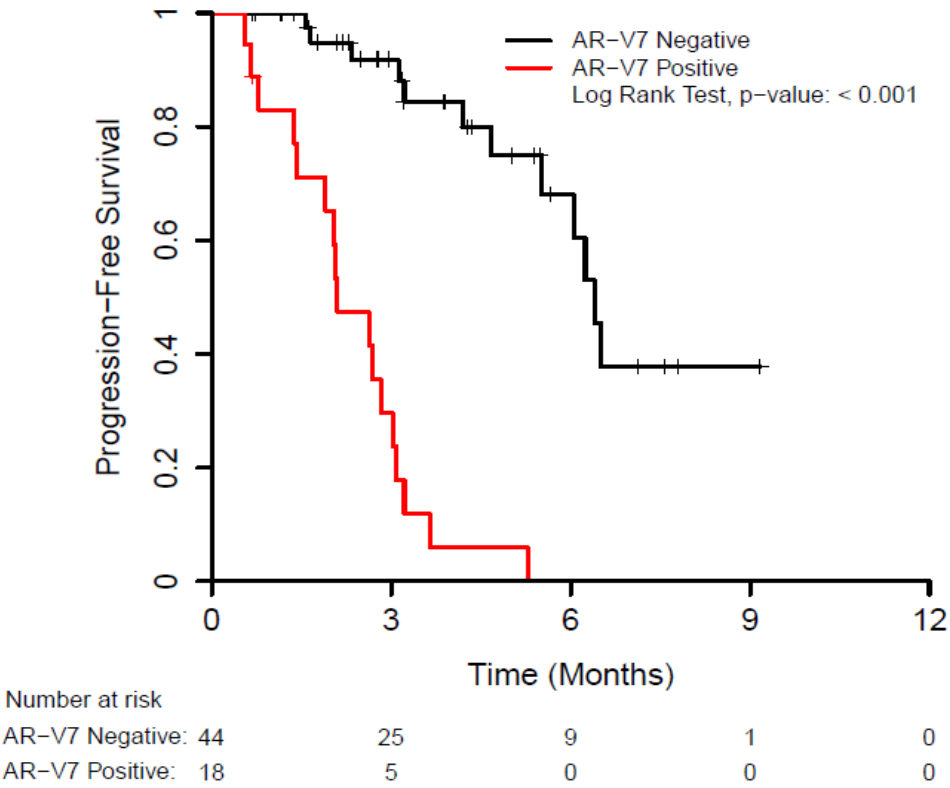
S4 A



S4 B



S4 C



S4 D

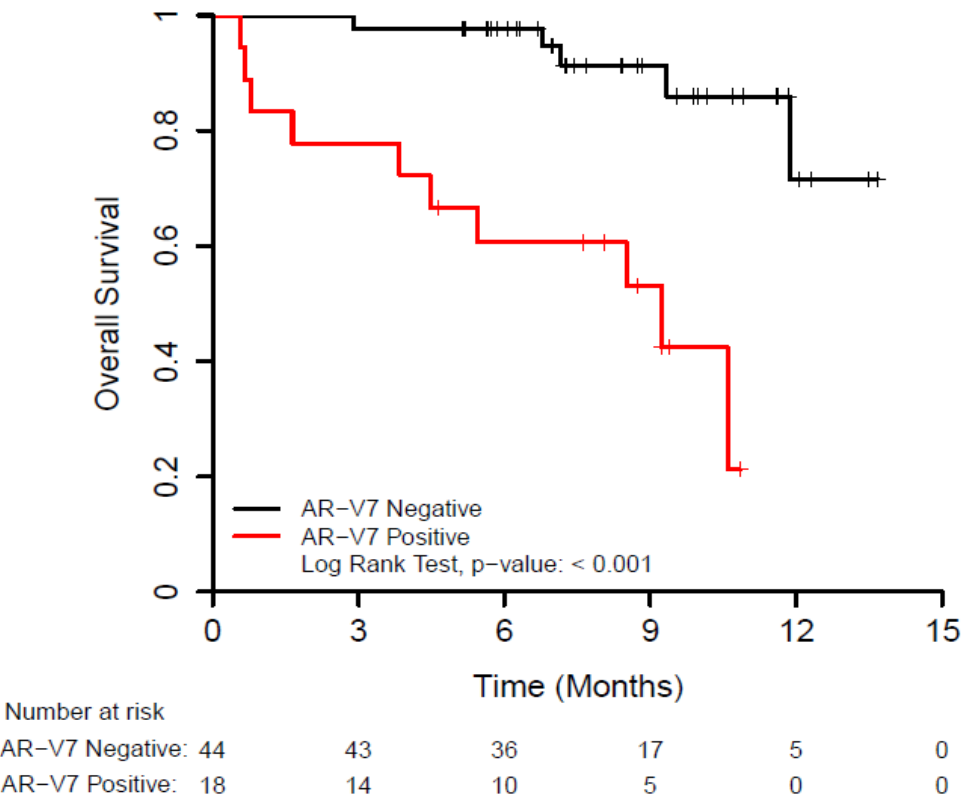


Figure S4. Combined analysis of patient outcome by AR-V7 status.

(A) Waterfall plot showing best PSA responses according to CTC AR-V7 status for all 62 patients.

The ‘asterisk’ marks (*) indicate clipped bars. The dotted line shows the threshold for defining a PSA response. Men who had previously received abiraterone and enzalutamide (in the enzalutamide and abiraterone cohorts, respectively) are denoted with ‘dagger’ marks (†). The overall proportion of patients who achieved a PSA response to either therapy was 44% (27/62 men; 95%CI, 31–57%). PSA response rates were 0% (0/18 men; 95%CI, 0–19%) in AR-V7–positive patients and 61% (27/44 men; 95%CI, 45–76%) in AR-V7–negative patients ($P<0.001$). Considered alternatively, among patients achieving a PSA response, 0% (0/27 men; 95%CI, 0–13%) were AR-V7–positive; while in patients not achieving a PSA response, 51% (18/35 men; 95%CI, 34–69%) were AR-V7–positive. In linear regression modeling, AR-V7 status remained predictive for PSA response after adjusting for AR-FL expression levels ($P<0.001$).

(B) Kaplan-Meier curves showing PSA-progression-free-survival [PSA-PFS] stratified by CTC AR-V7 status in all 62 patients.

Median PSA-PFS was 1.4 months (95%CI, 0.9–2.6) and 6.1 months (95%CI, 5.3–not reached) in AR-V7–positive and AR-V7–negative patients, respectively (HR 10.5, 95%CI 4.7–23.6, log-rank $P<0.001$). In multivariable Cox regression analysis stratified by treatment type, AR-V7 detection remained independently predictive of PSA-PFS (HR 8.2, 95%CI 2.7–24.9, $P<0.001$). Presence of visceral metastases and more prior hormonal therapies were also predictive of PSA-PFS; while AR-FL level, prior use of enzalutamide/abiraterone, and baseline PSA level were not predictive.

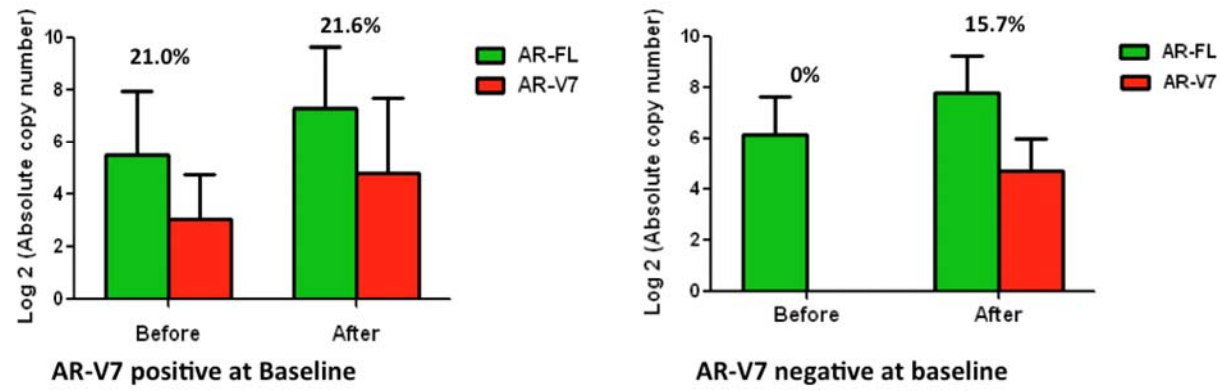
(C) Kaplan-Meier curves showing clinical/radiographic-progression-free-survival [PFS] stratified by CTC AR-V7 status in all 62 patients.

Median PFS was 2.1 months (95%CI, 1.9–3.1) and 6.4 months (95%CI, 6.1–not reached) in AR-V7–positive and AR-V7–negative patients, respectively (HR 12.7, 95%CI 5.1–31.9, log-rank $P<0.001$). In multivariable Cox regression analysis stratified by treatment type, AR-V7 detection remained independently predictive of PFS (HR 4.9, 95%CI 1.7–13.8, $P=0.003$). AR-FL levels, more prior hormonal therapies and prior use of enzalutamide/abiraterone were also predictive of PFS; while baseline PSA level, and presence of visceral metastases were not predictive.

(D) Kaplan-Meier curves showing overall survival [OS] stratified by CTC AR-V7 status in all 62 patients.

Median OS was 9.2 months (95%CI, 4.5–not reached) and >11.9 months (95%CI, 11.9–not reached) in AR-V7–positive and AR-V7–negative patients, respectively (HR 8.3, 95%CI 2.5–27.4, log-rank $P<0.001$). In multivariable Cox regression analysis stratified by treatment type, AR-V7 detection remained independently predictive of OS (HR 5.0, 95%CI 1.3–19.8, $P=0.021$). Prior use of enzalutamide/abiraterone was also predictive of OS, while AR-FL level was not predictive.

S5 A



S5 B

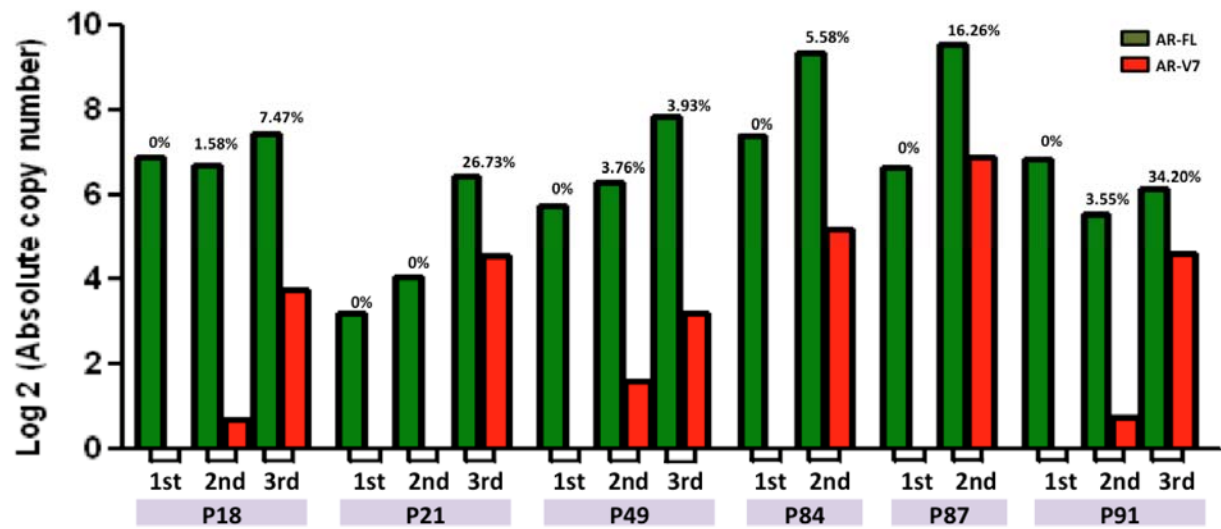


Figure S5. Changes in AR-V7 and AR-FL transcript copy numbers detected in CTCs before and after treatment with enzalutamide and abiraterone.

(A) Alterations in AR-FL and AR-V7 transcript copy numbers before and after enzalutamide/abiraterone treatment in patients with baseline detectable AR-V7 (n=16, with paired samples available) (*left panel*) and in patients who converted from initially undetectable to later detectable AR-V7 (n=6) (*right panel*). Higher copy numbers for both AR-FL and AR-V7 were detected in CTC samples collected after treatment (at the time of resistance to therapy) compared to baseline (pretreatment) samples. Note that the AR-V7/AR-FL ratio is similar between samples collected before and after treatment in patients with baseline detectable AR-V7 (~21%), and that an average AR-V7/AR-FL ratio of 15.7% was detected in patients who converted from initially undetectable to later detectable AR-V7. In patients who converted from initially undetectable to later detectable AR-V7, copy number values for AR-V7 were generated from the last follow-up CTC samples (see B, below).

(B) AR transcript copy numbers detected in the 6 patients whose AR-V7 status was negative at baseline but converted to positive during treatment. Absolute transcript copy numbers for AR-FL and AR-V7 are shown for each of the 6 patients before, during, and after treatment with enzalutamide or abiraterone (1st: before treatment; 2nd: during treatment; 3rd: at the time of progression). The percentage values represent the ratio of the absolute copy number of AR-V7 to AR-FL in CTCs. As shown, absolute AR-V7 levels (and AR-V7/AR-FL ratios) increased with time in all 6 cases. Patients P18, P21, P49 and P87 were treated with enzalutamide. Patients P84 and P91 were treated with abiraterone.

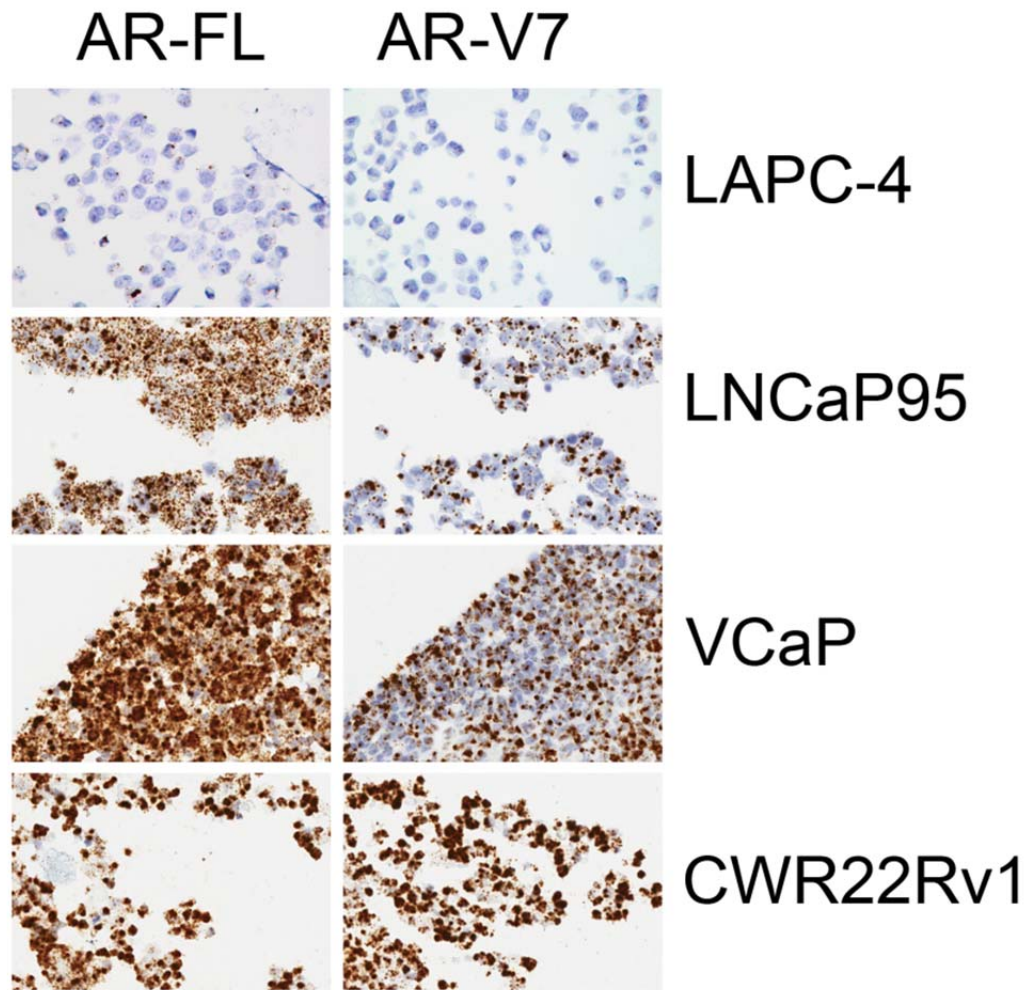


Figure S6. Detection of AR-FL and AR-V7 using RNA-ISH in cell lines with known expression of AR-FL and AR-V7.

Three of the prostate cancer cell lines shown (LNCaP95, VCaP and CWR22Rv1) express AR-FL as well as AR-V7, while the LAPC-4 line is positive only for AR-FL but negative for AR-V7, as visualized using RNA-ISH analysis. These prostate cancer cell lines served as positive and negative controls for AR-V7 detection by RNA-ISH in the patient-derived metastatic prostate cancer tissue samples shown in Fig. 4 of the main manuscript.

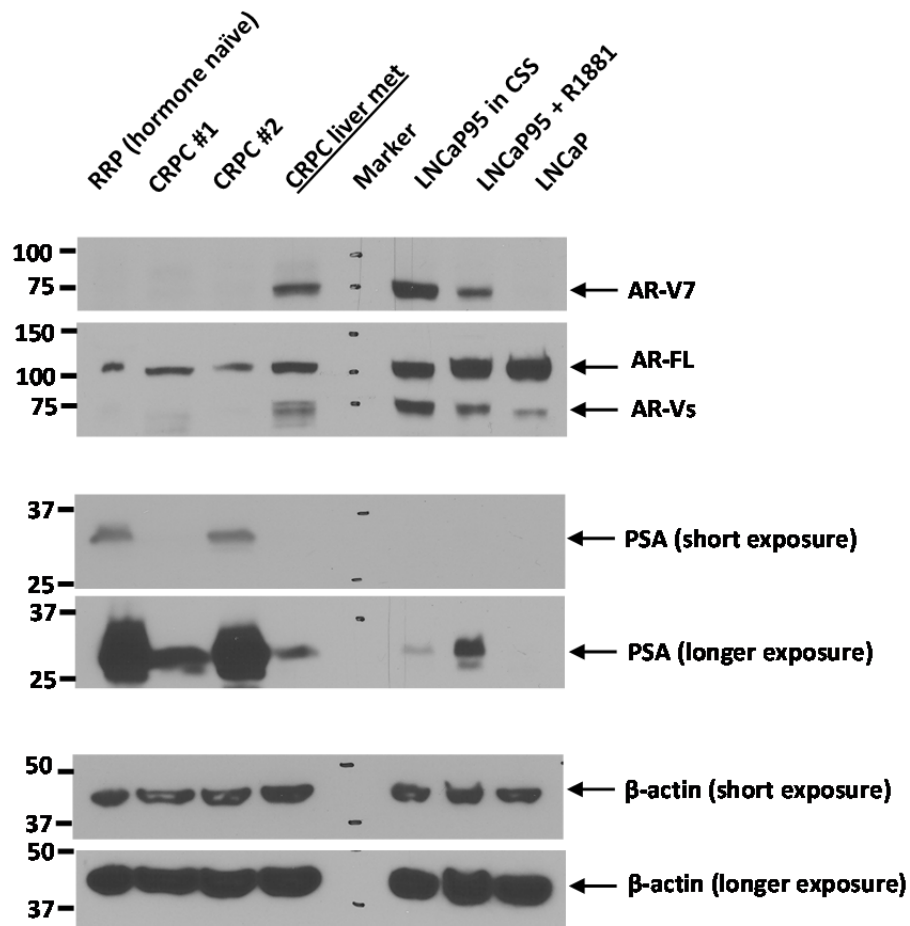


Figure S7. Detection of AR-V7 at the protein level using Western blot analysis in patients with detectable AR-V7 transcript in CTCs.

Detection of AR-V7 protein expression in a representative tissue sample, in this case from a liver metastasis from an AR-V7-positive patient (underlined label). Whole protein extractions were prepared from cryosections using RIPA buffer with protease inhibitors and phosphatase inhibitors. 40 µg of protein from each sample was separated on a 10% SDS-PAGE precast gel and blotted with anti-AR-V7, anti-AR (N20) (for detection of both AR-FL and AR-Vs), anti-PSA, and anti-β-actin antibodies. The LNCaP cell line served as the negative control for AR-V7; the LNCaP95 cell line (in the presence or absence of synthetic androgen, R1881) served as the positive control for AR-V7. Also shown for comparison are samples from a hormone-naïve radical prostatectomy specimen (RRP), and two metastatic tissue samples from AR-V7-negative patients (CRPC #1 and CRPC #2). Molecular weight marks are indicated to the left of the blots.

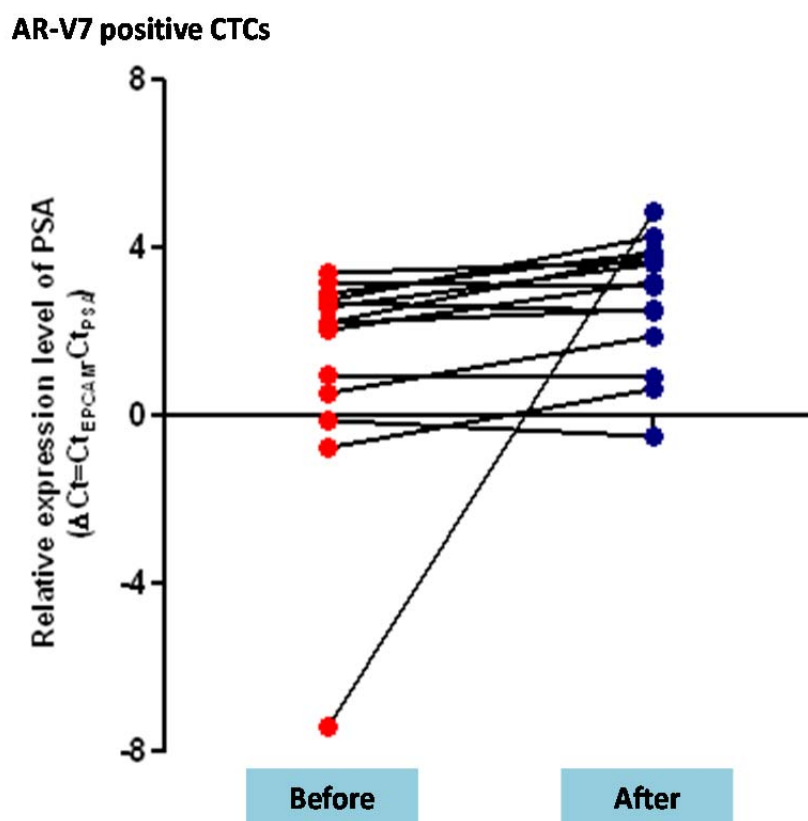


Figure S8. Changes in expression levels of the PSA transcript before and after therapy with enzalutamide or abiraterone in men with baseline detectable AR-V7.

PSA expression changes in CTC samples from AR-V7–positive patients before and after treatment with enzalutamide/abiraterone (n=14), as assessed by qRT-PCR. Results are shown as the difference in Ct value between EPCAM and PSA expression ($Ct_{EPCAM} - Ct_{PSA}$). As depicted, PSA expression was generally increased during treatment with enzalutamide/abiraterone in AR-V7–positive patients, suggesting that canonical AR signaling (as indicated by levels of PSA mRNA normalized to those of EPCAM) was not inhibited by enzalutamide/abiraterone in the presence of AR-V7.

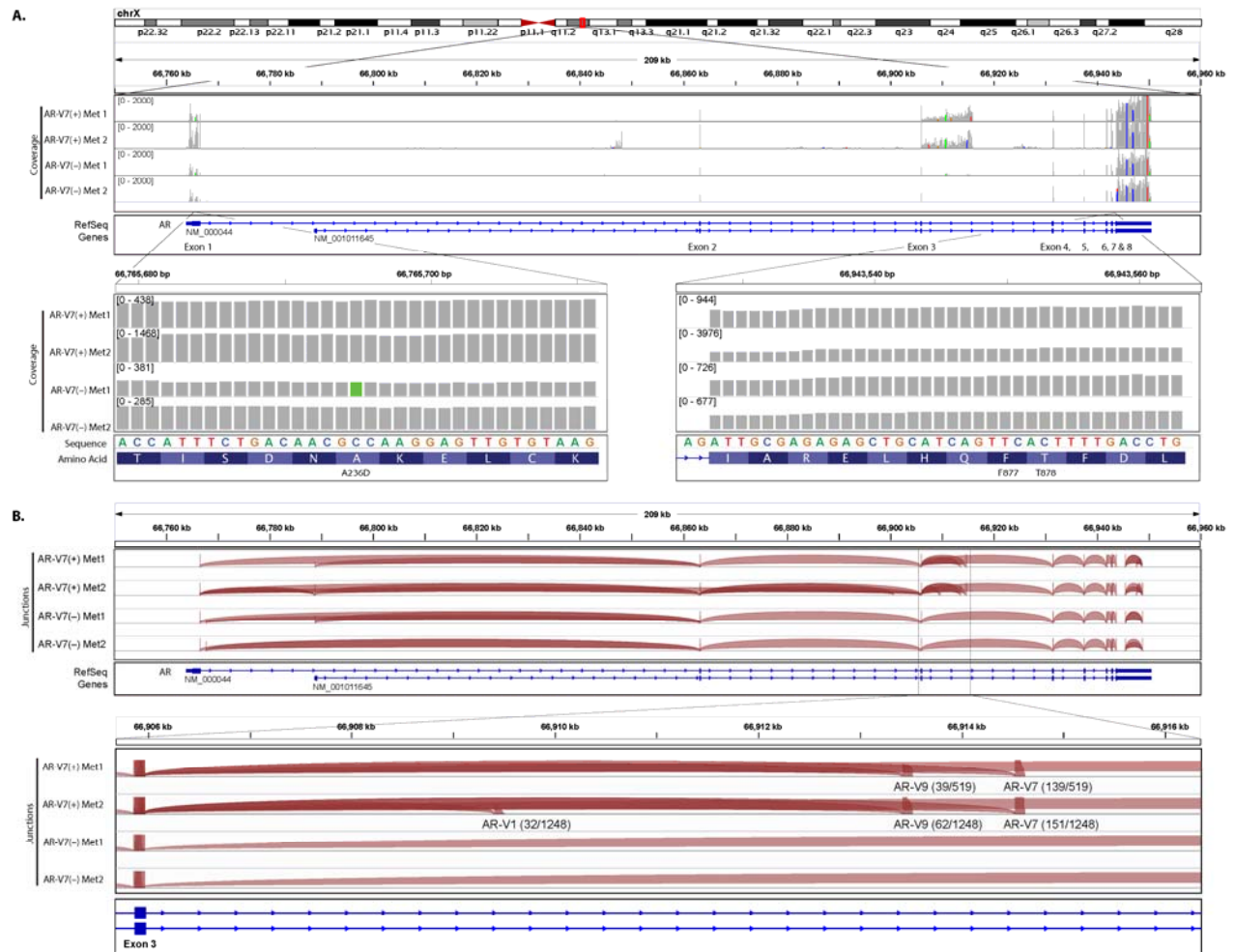


Figure S9. RNA-Seq analysis of the AR transcript in two AR-V7 (+) and two AR-V7 (-) patients.

(A) Read coverage along the AR gene, with the enlarged view showing a novel AR mutation (A236D) in exon 1 (of unknown significance) detected in an AR-V7-negative tumor, but a lack of known AR ligand-binding domain (LBD) mutations F876L and T877A previously implicated in castration resistance and enzalutamide resistance (note: due to Refseq sequence changes, the numbering of amino acid positions have increased by one). A236D is the only AR mutation detected in these four samples. (B) AR RNA splice junction tracks depicting sequence reads connecting canonical and cryptic AR exons (junctions supported by a read depth of at least 20 are shown in the figure). The enlarged region spanning exon 3 and intron 3 shows positively identified AR-V7 variants (along with AR-V1 and AR-V9) in the tissue samples from the two AR-V7-positive patients. Numbers in parentheses indicate the number of variant-specific reads over the number of AR-FL-specific reads. The AR-V7/AR-FL ratios were 26.8% and 12.1%, for AR-V7(+) Met1 and AR-V7(+) Met2, respectively.

Positive enrichment in prostate related gene sets

Gene Set Details	# of genes	ES	NES	Norm p-val	FDR q-val
1. RESPONSE_TO_WOUNDING	87	0.64	2.27	0	0
2. DEFENSE_RESPONSE	105	0.61	2.18	0	0
3. CELLULAR_DEFENSE_RESPONSE	16	0.86	2.18	0	0
4. RESPONSE_TO_EXTERNAL_STIMULUS	135	0.57	2.16	0	0
5. INFLAMMATORY_RESPONSE	60	0.63	2.12	0	0
6. M_PHASE_OF_MITOTIC_CELL_CYCLE	67	0.61	2.08	0	0
7. G_PROTEIN_COUPLED_RECEPTOR_PROTEIN_SIGNALING_PATHWAY	83	0.59	2.06	0	0.001
8. MITOSIS	64	0.6	2.02	0	0.002
9. M_PHASE	81	0.57	1.97	0	0.003
10. ORGAN_MORPHOGENESIS	79	0.56	1.96	0	0.003



Negative enrichment in prostate related gene sets

Gene Set Details	# of genes	ES	NES	Norm p-val	FDR q-val
1. GOLGI_VESICLE_TRANSPORT	47	-0.38	-1.49	0.033	1
2. CHROMATIN_ASSEMBLY_OR_DISASSEMBLY	22	-0.43	-1.33	0.115	1
3. GAMETE_GENERATION	44	-0.34	-1.27	0.114	1
4. MRNA_PROCESSING_GO_0006397	70	-0.28	-1.17	0.135	1
5. ER_TO_GOLGI_VESICLE_MEDIATED_TRANSPORT	18	-0.39	-1.16	0.223	1
6. NEGATIVE_REGULATION_OF_TRANSCRIPTION_DNA_DEPENDENT	94	-0.25	-1.12	0.184	1
7. MRNA_METABOLIC_PROCESS	81	-0.26	-1.11	0.194	1
8. NEGATIVE_REGULATION_OF_RNA_METABOLIC_PROCESS	95	-0.26	-1.1	0.259	1
9. SEXUAL_REPRODUCTION	50	-0.28	-1.1	0.288	1
10. PROTEIN_AUTOPROCESSING	22	-0.34	-1.1	0.303	1



Figure S10. Gene set enrichment analysis of metastatic tumors from AR-V7–positive and AR-V7–negative patients.

Top ranked ‘biological processes’ enriched in genes differentially expressed between AR-V7–positive and AR-V7–negative metastatic prostate cancer tissues are shown. Genes are pre-ranked based on fold expression changes. Consistent with the ‘AR-V7 up’ and ‘AR-FL up’ gene signatures reported in our previous study,⁴ cell cycle genes previously shown to be driven by AR-V7 (*i.e.*, ‘AR-V7 up’) are enriched for increased expression in AR-V7–positive samples, and genes involved in Golgi activities, previously linked to AR-FL activity (*i.e.*, ‘AR-FL up’), are downregulated in AR-V7-positive samples.

[ES: enrichment score. NES: normalized enrichment score].

Table S1 A. Baseline characteristics of the 31 patients treated with enzalutamide.

Baseline Characteristic	All Patients (N=31)	AR-V7 Negative (N=19)	AR-V7 Positive (N=12)	P-value*
Age (years) median (range)	70 (56–84)	72 (60–84)	69 (56–82)	0.223
Race, N (%) white non-white	26 (83.9%) 5 (16.1%)	16 (84.2%) 3 (15.8%)	10 (83.3%) 2 (16.7%)	0.999
Time since diagnosis (years) median (range)	5 (1–21)	5 (1–21)	7 (1–18)	0.760
Tumor stage at diagnosis, N (%) T1/T2 T3/T4	17 (54.8%) 14 (45.2%)	10 (52.6%) 9 (47.4%)	7 (58.3%) 5 (41.7%)	0.999
Gleason sum at diagnosis, N (%) ≤7 ≥8	12 (40.0%) 18 (60.0%)	9 (47.4%) 10 (52.6%)	3 (27.3%) 8 (72.7%)	0.442
Type of local treatment, N (%) surgery radiation none	13 (41.9%) 7 (22.6%) 11 (35.5%)	8 (42.1%) 6 (31.6%) 5 (26.3%)	5 (41.7%) 1 (8.3%) 6 (50.0%)	0.262
Number of prior hormonal therapies mean (range)	3.3 (2–5)	3.2 (2–5)	3.4 (3–5)	0.317
Prior use of abiraterone, N (%) yes no	20 (64.5%) 11 (35.5%)	9 (47.4%) 10 (52.6%)	11 (91.7%) 1 (8.3%)	0.020
Prior use of docetaxel, N (%) yes no	20 (64.5%) 11 (35.5%)	10 (52.6%) 9 (47.4%)	10 (83.3%) 2 (16.7%)	0.128
Presence of bone metastases, N (%) yes no	28 (90.3%) 3 (9.7%)	17 (89.5%) 2 (10.5%)	11 (91.7%) 1 (8.3%)	0.999
Number of bone metastases, N (%) ≤5 ≥6	20 (64.5%) 11 (35.5%)	15 (78.9%) 4 (21.1%)	5 (41.7%) 7 (58.3%)	0.056
Presence of visceral metastases, N (%) yes no	10 (32.3%) 21 (67.7%)	3 (15.8%) 16 (84.2%)	7 (58.3%) 5 (41.7%)	0.021
ECOG performance status score, N (%) 0 1 or 2	22 (71.0%) 9 (29.0%)	16 (84.2%) 3 (15.8%)	6 (50.0%) 6 (50.0%)	0.056
Baseline PSA (ng/mL) median (range)	44.3 (4.3–3204.2)	29.8 (4.3–452.0)	144.3 (14.5–3204.2)	0.047
Baseline alkaline phosphatase (U/L) median (range)	108 (58–872)	91 (58–872)	110 (82–744)	0.282
Baseline AR-FL level (copy number) median (range)	10 (0–734)	4 (0–121)	26 (3–734)	0.003

* P-value is based on Fisher's Exact test and Wilcoxon Mann-Whitney test for categorical and continuous variables, respectively.

Table S1 B. Baseline characteristics of the 31 patients treated with abiraterone.

Baseline Characteristic	All Patients (N=31)	AR-V7 Negative (N=25)	AR-V7 Positive (N=6)	P-value*
Age (years) median (range)	69 (48–79)	69 (48–79)	69 (58–79)	0.565
Race, N (%) white non-white	25 (80.6%) 6 (19.4%)	20 (80.0%) 5 (20.0%)	5 (83.3%) 1 (16.7%)	0.999
Time since diagnosis (years) median (range)	5 (1–21)	5 (1–13)	4 (1–21)	0.705
Tumor stage at diagnosis, N (%) T1/T2 T3/T4	12 (38.7%) 19 (61.3%)	10 (40.0%) 15 (60.0%)	2 (33.3%) 4 (66.7%)	0.999
Gleason sum at diagnosis, N (%) ≤7 ≥8	8 (26.7%) 22 (73.3%)	6 (24.0%) 19 (76.0%)	2 (40.0%) 3 (60.0%)	0.589
Type of local treatment, N (%) surgery radiation none	14 (45.2%) 10 (32.3%) 7 (22.6%)	10 (40.0%) 9 (36.0%) 6 (24.0%)	4 (66.6%) 1 (16.7%) 1 (16.7%)	0.520
Number of prior hormonal therapies mean (range)	2.5 (2–6)	2.2 (2–4)	3.7 (2–6)	0.020
Prior use of enzalutamide, N (%) yes no	4 (12.9%) 27 (87.1%)	2 (8.0%) 23 (92.0%)	2 (33.3%) 4 (66.7%)	0.159
Prior use of docetaxel, N (%) yes no	5 (16.1%) 26 (83.9%)	4 (16.0%) 21 (84.0%)	1 (16.7%) 5 (83.3%)	0.999
Presence of bone metastases, N (%) yes no	24 (77.4%) 7 (22.6%)	19 (76.0%) 6 (24.0%)	5 (83.3%) 1 (16.7%)	0.999
Number of bone metastases, N (%) ≤5 ≥6	17 (54.8%) 14 (45.2%)	15 (60.0%) 10 (40.4%)	2 (33.3%) 4 (66.7%)	0.370
Presence of visceral metastases, N (%) yes no	8 (25.8%) 23 (74.2%)	8 (32.0%) 17 (68.0%)	0 (0%) 6 (100%)	0.298
ECOG performance status score, N (%) 0 1 or 2	25 (80.6%) 6 (19.4%)	22 (88.0%) 3 (12.0%)	3 (50.0%) 3 (50.0%)	0.069
Baseline PSA (ng/mL) median (range)	37.8 (2.2–2045.0)	31.4 (2.2–262.2)	86.9 (19.4–2045.0)	0.084
Baseline alkaline phosphatase (U/L) median (range)	118 (59–1348)	109 (59–524)	263 (71–1348)	0.063
Baseline AR-FL level (copy number) median (range)	3 (0–609)	1 (0–173)	216 (8–609)	0.002

* P-value is based on Fisher's Exact test and Wilcoxon Mann-Whitney test for categorical and continuous variables, respectively.

Table S2. Clinical outcomes (PSA responses, PSA-PFS and PFS) reported separately according to prior exposure to abiraterone (in the enzalutamide cohort) and prior exposure to enzalutamide (in the abiraterone cohort).

A. Enzalutamide cohort: Clinical outcomes according to prior exposure (or not) to abiraterone

Outcome	No previous abiraterone (n=11)			Previous abiraterone (n=20)		
	AR-V7 [+] (n=1)	AR-V7 [-] (n=10)	<i>P</i> value	AR-V7 [+] (n=11)	AR-V7 [-] (n=9)	<i>P</i> value
PSA Response	0% (0/1)	80% (8/10)	0.273	0% (0/11)	22% (2/9)	0.189
PFA-PFS	HR (95%CI) not estimable		0.005	HR 3.34 (95%CI, 1.14–9.80)		0.021
PFS	HR (95%CI) not estimable		0.005	HR 2.93 (95%CI, 0.96–8.90)		0.048

B. Abiraterone cohort: Clinical outcomes according to prior exposure (or not) to enzalutamide

Outcome	No previous enzalutamide (n=27)			Previous enzalutamide (n=4)		
	AR-V7 [+] (n=4)	AR-V7 [-] (n=23)	<i>P</i> value	AR-V7 [+] (n=2)	AR-V7 [-] (n=2)	<i>P</i> value
PSA Response	0% (0/4)	74% (17/23)	0.012	0% (0/2)	0% (0/2)	N/A
PFA-PFS	HR 41.0 (95%CI, 4.5–376.8)		<0.001	HR (95%CI) not estimable		N/A
PFS	HR 28.2 (95%CI, 3.1–255.8)		<0.001	HR (95%CI) not estimable		N/A

C. Combined cohort: Clinical outcomes according to prior exposure to abiraterone/enzalutamide

Outcome	No prior abiraterone/enzalutamide (n=38)			Prior abiraterone/enzalutamide (n=24)		
	AR-V7 [+] (n=5)	AR-V7 [-] (n=33)	<i>P</i> value	AR-V7 [+] (n=13)	AR-V7 [-] (n=11)	<i>P</i> value
PSA Response	0% (0/5)	76% (25/33)	0.003	0% (0/13)	18% (2/11)	0.199
PFA-PFS	HR 55.9 (95%CI, 6.4–488.5)		<0.001	HR 2.91 (95%CI, 1.10–7.72)		0.023
PFS	HR 45.2 (95%CI, 5.1–398.1)		<0.001	HR 2.65 (95%CI, 0.97–7.25)		0.048

Table S3. Propensity score weighted multivariable Cox models adjusted for Gleason score, baseline PSA, number of prior hormonal treatments, presence of visceral metastases, ECOG score, prior abiraterone/enzalutamide use, and AR-FL levels.

A. Propensity score weighted multivariable Cox model for PSA-PFS in enzalutamide-treated men

Variable	Hazard Ratio (HR)	95% Confidence Interval (95% CI)	<i>P</i> value
AR-V7 Positive	3.40	(1.43 – 8.08)	0.006
AR-FL Level (log)	1.33	(1.03 – 1.72)	
Prior use of Abiraterone	2.66	(0.72 – 9.86)	

B. Propensity score weighted multivariable Cox model for PFS in enzalutamide-treated men

Variable	Hazard Ratio (HR)	95% Confidence Interval (95% CI)	<i>P</i> value
AR-V7 Positive	3.38	(1.35 – 8.46)	0.009
AR-FL Level (log)	1.64	(1.14 – 2.35)	
Prior use of Abiraterone	1.54	(0.31 – 7.79)	

C. Propensity score weighted multivariable Cox model for PSA-PFS in abiraterone-treated men

Variable	Hazard Ratio (HR)	95% Confidence Interval (95% CI)	<i>P</i> value
AR-V7 Positive	17.51	(3.53 – 87.03)	<0.001
AR-FL Level (log)	1.05	(0.87 – 1.25)	
Prior use of Enzalutamide	0.61	(0.17 – 2.19)	

D. Propensity score weighted multivariable Cox model for PFS in abiraterone-treated men

Variable	Hazard Ratio (HR)	95% Confidence Interval (95% CI)	<i>P</i> value
AR-V7 Positive	5.25	(1.09 – 25.21)	0.038
AR-FL Level (log)	1.36	(0.97 – 1.90)	
Prior use of Enzalutamide	1.72	(0.50 – 5.92)	

Table S4. Clinical outcomes (PSA responses, PSA-PFS and PFS) for the entire patient population according to their AR-V7 ‘conversion’ rates.

Footnote: Of the 44 patients who were AR-V7–negative at baseline, 42 had at least one follow-up sample collected; 36 of these men (86%) remained AR-V7–negative at follow-up (AR-V7[–] → AR-V7[–]), while 6 of these men (14%) converted to AR-V7–positive at follow-up (AR-V7[–] → AR-V7[+]). Of the 18 patients who were AR-V7–positive at baseline, 16 had at least one follow-up sample collected; all of these men remained AR-V7–positive at follow-up (AR-V7[+] → AR-V7[+]). The clinical outcomes of these patients are also shown.

These data should be interpreted with caution (and are hypothesis-generating only) because we have not taken into account the timing of the subsequent sample collection, and we have not performed time-dependent covariate analysis or landmark analysis to adjust for this. Therefore, the clinical outcomes in each group cannot be formally compared with each other, and are provided for descriptive purposes only.

Outcome	AR-V7[–] → AR-V7[–] (n=36)	AR-V7[–] → AR-V7[+] (n=6)	AR-V7[+] → AR-V7[+] (n=16)
PSA Response	68% (95%CI, 52 – 81%)	17% (95%CI, 4 – 58%)	0% (95%CI, 0 – 19%)
PFA-PFS	6.1 months (95%CI, 5.9 mo – NA)	3.0 months (95%CI, 2.3 mo – NA)	1.4 months (95%CI, 0.9 – 2.6 mo)
PFS	6.5 months (95%CI, 6.1 mo – NA)	3.2 months (95%CI, 3.1 mo – NA)	2.1 months (95%CI, 1.9 – 3.1 mo)

Table S5. Expression profiles of AR-regulated genes in AR-V7–positive and AR-V7–negative metastatic tumors.

Footnote: A total of 34 canonical AR regulated genes were identified by combined analysis of downloaded expression data reported in two published studies.^{1,11} The AR gene did not make the selection but was added for reference. For each of the 35 genes, the number of raw RNA-Seq reads and sequencing reads normalized by “Reads Per Kilo Gene Size Per Million of Total Reads” (RPKM= number of raw counts/(gene-length/1000)/(total-reads/1,000,000) in each of the four tumor samples, as well as the log fold expression change between AR-V7-positive and AR-V7-negative tumors calculated from RPKM-normalized data, are displayed. Annexed to the RNA-Seq data are previously published expression microarray data⁴ characterizing AR-V7– versus AR-FL–driven transcriptional programs (expression ratios normalized to a common reference sample were downloaded from GEO accession number GSE36549). Fold expression changes in AR-V7–positive samples when compared to AR-V7–negative tumors (log FC) are significantly correlated ($P<0.001$, with a correlation coefficient of 0.68) with fold expression changes induced by AR-V7 in the absence of AR-FL activation (log FC by ARV7), but not significantly correlated ($p>0.05$, with a correlation coefficient of 0.23) with fold expression changes induced by AR-FL activation (log FC by ARFL).

Gene	Size (bp)	Raw V7(–) Met 1	Raw V7(–) Met 2	Raw V7(+) Met 1	Raw V7(+) Met 2	RPKM V7(–) Met 1	RPKM V7(–) Met 2	RPKM V7(+) Met 1	RPKM V7(+) Met 2	log FC	CSS	R1881	CSS + ARV7	R1881 + ARV7	log FC by ARV7	log FC by ARFL
<i>AR</i>	4496	23044	20645	21722	62370	111.83	92.43	54.53	201.92	-0.22	0.10	-0.89	-0.01	-0.96	-0.11	-0.99
<i>C1orf116</i>	5502	3123	5063	3293	10168	12.38	18.52	6.75	26.90	-0.62	-0.90	1.05	-0.90	0.99	0.01	1.95
<i>CENPN</i>	5307	1337	11671	1799	907	5.50	44.27	3.83	2.49	-3.26	-2.67	0.52	-1.92	0.88	0.75	3.20
<i>CXCR4</i>	2005	654	695	10593	7739	7.12	6.98	59.63	56.18	2.65	-0.40	2.77	-0.26	3.51	0.13	3.17
<i>DBI</i>	1260	11624	10108	43384	10800	201.28	161.47	388.60	124.76	0.11	-0.50	3.07	0.33	3.19	0.82	3.57
<i>EAF2</i>	1020	66	212	3689	116	1.41	4.18	40.82	1.66	0.74	0.10	4.18	0.37	3.77	0.28	4.08
<i>EBP</i>	1157	784	3333	3322	2755	14.78	57.98	32.40	34.66	-0.20	-0.48	0.88	-0.07	0.75	0.41	1.35
<i>FASN</i>	8458	2272	5891	19983	46976	5.86	14.02	26.66	80.84	1.97	-0.50	0.61	-0.06	0.86	0.44	1.11
<i>FKBP5</i>	10628	3810	34978	55568	66987	7.82	66.24	59.01	91.74	1.32	-1.28	3.61	1.26	4.03	2.54	4.89
<i>FZD5</i>	6564	3247	6014	3756	2384	10.79	18.44	6.46	5.29	-1.98	-1.12	1.65	-0.93	1.80	0.19	2.77

HMGCR	4582	2395	3169	3526	2334	11.40	13.92	8.68	7.41	-1.25	0.06	1.54	0.07	1.27	0.01	1.49
HMGCS1	3510	2596	5207	12367	3282	16.14	29.86	39.76	13.61	-0.38	0.40	2.15	0.65	1.79	0.25	1.75
HPGD	3022	127	57	392	29067	0.92	0.38	1.46	140.00	3.64	-2.52	3.46	-0.05	4.05	2.47	5.98
INSIG1	3081	421	3684	3118	8029	2.98	24.07	11.42	37.93	0.90	-0.58	1.55	0.27	2.00	0.85	2.13
KLK2	2872	286904	327838	82985	16542	2179.51	2297.62	326.10	83.84	-3.76	-4.34	0.14	-3.86	0.37	0.48	4.48
KLK3	2214	213712	922556	121923	32693	2106.00	8387.23	621.51	214.93	-3.03	-3.75	-0.16	-2.95	0.37	0.80	3.59
KLK4	1347	2478	3956	813	4579	40.14	59.11	6.81	49.48	-1.81	-2.68	-0.19	-2.59	-0.20	0.08	2.49
LDLR	5283	149	1947	2714	5261	0.62	7.42	5.80	14.49	1.47	-0.82	1.70	-0.23	1.50	0.60	2.52
MAF	6878	425	778	4311	1659	1.35	2.28	7.07	3.51	0.59	-1.22	2.98	-1.54	2.81	-0.32	4.20
MICAL1	3644	685	88	2959	2630	4.10	0.49	9.16	10.51	2.00	0.32	3.28	0.28	3.14	-0.04	2.96
NCAPD3	5605	849	1024	2601	4880	3.30	3.68	5.24	12.67	0.63	-0.62	2.50	-0.22	2.55	0.41	3.12
NDRG1	3478	66845	19467	44162	124199	419.32	112.66	143.30	519.78	0.22	-1.02	2.99	0.11	3.35	1.14	4.01
NKX3-1	3281	35635	53042	44160	43298	236.96	325.40	151.90	192.08	-0.89	-2.62	1.17	-2.59	1.04	0.04	3.80
ORM1	839	3	2407	94992	21539	0.08	57.75	1277.81	373.67	7.90	-0.88	4.10	6.70	6.47	7.57	4.97
PMEPA1	5186	10061	16129	7660	5565	42.33	62.60	16.67	15.62	-2.12	-1.49	1.04	-0.81	1.22	0.67	2.52
PTPN21	6215	551	1684	565	2532	1.93	5.45	1.03	5.93	-1.28	1.08	3.51	0.84	3.47	-0.24	2.43
RAB3B	12844	2274	10492	8012	3620	3.86	16.44	7.04	4.10	-1.33	-1.39	1.25	-1.14	1.22	0.25	2.64
RHOU	4758	16379	28644	5876	7735	75.11	121.17	13.94	23.66	-2.77	0.28	3.58	0.34	3.71	0.06	3.30
SCAP	4255	609	869	1134	4593	3.12	4.11	3.01	15.71	0.34	-0.29	0.76	-0.11	0.99	0.18	1.05
SGK1	3965	105	24777	20302	1347	0.58	125.78	57.79	4.94	0.24	-0.10	3.35	-0.05	3.62	0.05	3.45
SLC45A3	3382	5108	7039	2578	15489	32.95	41.89	8.60	66.66	-1.03	-2.52	1.07	-2.29	1.42	0.23	3.59
TMPRSS2	3320	4457	54020	27831	19920	29.29	327.51	94.61	87.33	-0.53	-4.59	-0.38	-3.92	0.09	0.67	4.21
WIPI1	1924	209	435	6828	1472	2.37	4.55	40.05	11.14	2.15	-0.10	2.57	0.98	2.86	1.08	2.67
WWC1	6739	2465	1922	6536	5735	7.98	5.74	10.95	12.39	0.26	-0.75	0.16	-0.99	0.21	-0.23	0.91
ZNF350	2341	2985	3161	5862	1155	27.82	27.18	28.26	7.18	-1.54	0.27	1.27	0.18	1.35	-0.09	1.00

Table S6. R^2 values for regression models that include only AR-FL levels (continuous variable) compared to regression models that additionally include AR-V7 status, for each clinical outcome (PSA response rate, PSA-PFS and PFS).

Footnote: The R^2 values are based on the Sums-of-squares method of Mittleböck and Schemper (Computing measures of explained variation for logistic regression models. Computer Methods and Programs in Biomedicine 1999; 58:17-24) for PSA response rate, and on the method of Heller (A measure of explained risk in the proportional hazards model. Biostatistics 2012; 13:315-325) for PSA-PFS and PFS. P-values are based on the Likelihood ratio test.

Outcome	Regression Models – Enzalutamide Cohort			Regression Models – Abiraterone Cohort		
	AR-FL alone	AR-FL + AR-V7	P value	AR-FL alone	AR-FL + AR-V7	P value
PSA Response	$R^2 = 0.307$	$R^2 = 0.449$	0.019	$R^2 = 0.203$	$R^2 = 0.336$	0.021
PFA-PFS	$R^2 = 0.196$	$R^2 = 0.573$	<0.001	$R^2 = 0.651$	$R^2 = 0.793$	0.012
PFS	$R^2 = 0.341$	$R^2 = 0.649$	<0.001	$R^2 = 0.869$	$R^2 = 0.903$	0.083

Supplementary References

1. Hu R, Dunn TA, Wei S, et al. Ligand-independent androgen receptor variants derived from splicing of cryptic exons signify hormone-refractory prostate cancer. *Cancer Res* 2009;69:16-22.
2. Watson PA, Chen YF, Balbas MD, et al. Constitutively active androgen receptor splice variants expressed in castration-resistant prostate cancer require full-length androgen receptor. *Proceedings of the National Academy of Sciences* 2010.
3. Hu R, Isaacs WB, Luo J. A snapshot of the expression signature of androgen receptor splicing variants and their distinctive transcriptional activities. *The Prostate* 2011;71:1656-67.
4. Hu R, Lu C, Mostaghel EA, et al. Distinct transcriptional programs mediated by the ligand-dependent full-length androgen receptor and its splice variants in castration-resistant prostate cancer. *Cancer Res* 2012;72:3457-62.
5. Luo J, Duggan DJ, Chen Y, et al. Human prostate cancer and benign prostatic hyperplasia: molecular dissection by gene expression profiling. *Cancer Res* 2001;61:4683-8.
6. Aryee MJ, Liu W, Engelmann JC, et al. DNA methylation alterations exhibit intraindividual stability and interindividual heterogeneity in prostate cancer metastases. *Science translational medicine* 2013;5:169ra10.
7. Liu W, Laitinen S, Khan S, et al. Copy number analysis indicates monoclonal origin of lethal metastatic prostate cancer. *Nature medicine* 2009;15:559-65.
8. Robinson JT, Thorvaldsdottir H, Winckler W, et al. Integrative genomics viewer. *Nature biotechnology* 2011;29:24-6.
9. Anders S, Pyl PT, Huber W. HTSeq—A Python framework to work with high-throughput sequencing data. *bioRxiv* 2014.
10. Subramanian A, Tamayo P, Mootha VK, et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proceedings of the National Academy of Sciences of the United States of America* 2005;102:15545-50.
11. Norris JD, Chang CY, Wittmann BM, et al. The homeodomain protein HOXB13 regulates the cellular response to androgens. *Molecular cell* 2009;36:405-16.

Original Investigation

Androgen Receptor Splice Variant 7 and Efficacy of Taxane Chemotherapy in Patients With Metastatic Castration-Resistant Prostate Cancer

Emmanuel S. Antonarakis, MD; Changxue Lu, PhD; Brandon Lubner, ScM; Hao Wang, PhD; Yan Chen, PhD; Mary Nakazawa, MHS; Rosa Nadal, MD; Channing J. Paller, MD; Samuel R. Denmeade, MD; Michael A. Carducci, MD; Mario A. Eisenberger, MD; Jun Luo, PhD

IMPORTANCE We previously showed that detection of androgen receptor splice variant 7 (AR-V7) in circulating tumor cells (CTCs) from men with castration-resistant prostate cancer (CRPC) was associated with primary resistance to enzalutamide and abiraterone therapy, but the relevance of AR-V7 status in the context of chemotherapy is unknown.

OBJECTIVE To investigate whether AR-V7-positive patients would retain sensitivity to taxane chemotherapy and whether AR-V7 status would have a differential impact on taxane-treated men compared with enzalutamide- or abiraterone-treated men.

DESIGN, SETTING, AND PARTICIPANTS We examined CTCs for AR-V7 mRNA using a reverse-transcription polymerase chain reaction assay. From January 2013 to July 2014, we prospectively enrolled patients with metastatic CRPC initiating taxane chemotherapy (docetaxel or cabazitaxel) at a single academic institution (Johns Hopkins). Our prespecified statistical plan required a sample size of 36 taxane-treated men.

MAIN OUTCOMES AND MEASURES We evaluated associations between AR-V7 status and prostate-specific antigen (PSA) response rates, PSA progression-free survival (PSA PFS), and clinical and/or radiographic progression-free survival (PFS). After incorporating updated data from our prior study of 62 patients treated with enzalutamide or abiraterone, we also investigated the interaction between AR-V7 status (positive or negative) and treatment type (taxane vs enzalutamide or abiraterone).

RESULTS Of 37 taxane-treated patients enrolled, 17 (46%) had detectable AR-V7 in CTCs. Prostate-specific antigen responses were achieved in both AR-V7-positive and AR-V7-negative men (41% vs 65%; $P = .19$). Similarly, PSA PFS (hazard ratio [HR], 1.7, 95% CI, 0.6-5.0; $P = .32$) and PFS (HR, 2.7, 95% CI, 0.8-8.8; $P = .11$) were comparable in AR-V7-positive and AR-V7-negative patients. A significant interaction was observed between AR-V7 status and treatment type ($P < .001$). Clinical outcomes were superior with taxanes compared with enzalutamide or abiraterone therapy in AR-V7-positive men, whereas outcomes did not differ by treatment type in AR-V7-negative men. In AR-V7-positive patients, PSA responses were higher in taxane-treated vs enzalutamide- or abiraterone-treated men (41% vs 0%; $P < .001$), and PSA PFS and PFS were significantly longer in taxane-treated men (HR, 0.19 [95% CI, 0.07-0.52] for PSA PFS, $P = .001$; HR, 0.21 [95% CI, 0.07-0.59] for PFS, $P = .003$).

CONCLUSIONS AND RELEVANCE Detection of AR-V7 in CTCs from men with metastatic CRPC is not associated with primary resistance to taxane chemotherapy. In AR-V7-positive men, taxanes appear to be more efficacious than enzalutamide or abiraterone therapy, whereas in AR-V7-negative men, taxanes and enzalutamide or abiraterone may have comparable efficacy. Circulating tumor cell-based AR-V7 detection may serve as a treatment selection biomarker in CRPC.

JAMA Oncol. 2015;1(5):582-591. doi:10.1001/jamaoncol.2015.1341
Published online June 4, 2015.

← Editorial page 577

+ Supplemental content at
jamaoncol.org

Author Affiliations: Department of Oncology, Johns Hopkins University School of Medicine, Baltimore, Maryland (Antonarakis, Lubner, Wang, Nadal, Paller, Denmeade, Carducci, Eisenberger); Department of Urology, Johns Hopkins University School of Medicine, Baltimore, Maryland (Lu, Chen, Nakazawa, Luo).

Corresponding Authors: Emmanuel S. Antonarakis, MD, Prostate Cancer Research Program, Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, 1650 Orleans St, CRB1-1M45, Baltimore, MD 21287 (eanton1@jhmi.edu) and Jun Luo, PhD, Department of Urology, James Buchanan Brady Urological Institute, Johns Hopkins University School of Medicine, 600 N Wolfe St, Baltimore, MD 21287 (jluo1@jhmi.edu).

jamaoncol.org

There are currently 6 available therapies for the treatment of castration-resistant prostate cancer (CRPC), all of which have produced survival improvements.¹ These therapies fall into 4 classes: androgen receptor (AR)-directed therapies (abiraterone acetate,² enzalutamide³), taxane chemotherapies (docetaxel,⁴ cabazitaxel⁵), immunotherapies (sipuleucel-T⁶), and bone-targeting radiopharmaceuticals (radium-223).⁷ Of these, the most widely used are the AR-targeting therapies and the chemotherapies. However, mechanisms of response and resistance to these therapies remain poorly understood.^{8,9} Furthermore, predictive biomarkers aiding in treatment selection (ie, selecting for or against a particular therapy) are still lacking, although prognostic markers are abundant.¹⁰

We have recently shown that AR splice variants, in particular AR variant 7 (AR-V7), are strongly associated with primary resistance to abiraterone and enzalutamide therapy in men with CRPC.¹¹ Androgen receptor variants (AR-Vs) are alternatively spliced isoforms of the AR that encode a truncated AR protein lacking the C-terminal ligand-binding domain but retaining the transactivating N-terminal domain.¹²⁻¹⁴ Although these AR-Vs are unable to bind to the ligand (eg, dihydrotestosterone), they are constitutively active and capable of promoting transcription of target genes.¹⁴⁻¹⁶ To investigate the clinical relevance of AR-Vs in CRPC, we previously developed a circulating tumor cell (CTC)-based assay to interrogate AR-V7 in men undergoing therapy with abiraterone (an androgen synthesis inhibitor) or enzalutamide (an AR antagonist). We demonstrated that detection of AR-V7 in CTCs from such patients was associated with lack of a prostate-specific antigen (PSA) response and that AR-V7-positive patients had shorter progression-free survival (PFS) and overall survival (OS) than AR-V7-negative men.¹¹

Recent preclinical data have emerged suggesting that taxane chemotherapies may exert their antitumor activity in CRPC (at least partially) by impairing AR signaling along the microtubule network, thereby sequestering AR in the cytoplasm.¹⁷⁻²⁰ In addition, it has been shown that in patients with taxane-sensitive disease, treatment produces microtubule bundling resulting in exclusion of the AR from the nucleus. Conversely, AR often remains capable of trafficking into the nucleus despite therapy in patients with taxane-resistant disease.^{19,21} Furthermore, in certain xenograft mouse models it has been suggested that some AR splice variants may promote resistance to taxane chemotherapies while others may be compatible with taxane sensitivity.²² However, the clinical significance of AR-Vs in patients receiving taxanes is unknown.

The present study aimed to prospectively evaluate the predictive impact of AR-Vs in men with CRPC undergoing taxane chemotherapy. We hypothesized that men with detectable CTC-derived AR-V7 would retain sensitivity to taxanes and that AR-V7 status would have a differential effect on taxane-treated men vs enzalutamide- or abiraterone-treated men. Herein, we show that detection of AR-V7 is not associated with primary resistance to taxane chemotherapy and that taxanes may have superior efficacy compared with AR-targeting agents in AR-V7-positive patients.

At a Glance

- Androgen receptor splice variant 7 (AR-V7) is associated with resistance to enzalutamide and abiraterone, but its relevance in the context of taxane chemotherapy is unknown.
- Detection of AR-V7 in circulating tumor cells from men with metastatic castration-resistant prostate cancer is not associated with primary resistance to taxane chemotherapy; AR-V7-positive patients may retain sensitivity to taxanes.
- In AR-V7-positive men, taxanes may be more efficacious than AR-directed agents (enzalutamide and abiraterone).
- In AR-V7-negative men, taxanes appear to have comparable efficacy to AR-directed agents.
- Androgen receptor splice variant 7 may serve as a treatment selection marker in metastatic castration-resistant prostate cancer.

Methods

Patients

The study enrolled men with metastatic CRPC who were beginning standard-of-care treatment with docetaxel or cabazitaxel. Patients were required to have histologically confirmed prostate adenocarcinoma, progressive disease despite “castration levels” of serum testosterone (<50 ng/dL), and documented radiographic metastases on computed tomography (CT) or technetium-99 bone scans. Patients were required to have at least 3 increasing serum PSA values taken at least 2 weeks apart with the last value being at least 2.0 ng/mL, consistent with the Prostate Cancer Working Group (PCWG2) guidelines.²³ Patients were excluded if they planned to receive additional concurrent anticancer therapies (standard or investigational) during the course of taxane treatment. Prior treatment with abiraterone and/or enzalutamide was permitted, as was previous treatment with docetaxel among men starting cabazitaxel therapy (consistent with the labeled indication⁵). The 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 AR-V7 status to predict sensitivity or resistance to taxane agents. Patients who were about to begin docetaxel or cabazitaxel chemotherapy were enrolled and underwent peripheral blood CTC sampling at up to 3 time points: at baseline, at the time of a clinical and/or biochemical response (if a response occurred), and at the time of clinical and/or radiographic progression. Docetaxel was administered at a dose of 75 mg/m² intravenously every 3 weeks, and cabazitaxel was given at a dose of 25 mg/m² intravenously every 3 weeks (both with prednisone 5 mg twice daily).

Follow-up was prospectively defined: patients had PSA measurements every 1 to 2 months, as well as CT (chest/abdomen/pelvis) and technetium-99 bone scans every 2 to 4 months. Therapy with docetaxel or cabazitaxel was continued until PSA progression or clinical and/or radiographic pro-

gression, or until patients developed unmanageable drug-related toxic effects.

CTC-Based AR-V7 Detection

The CTC analyses were conducted using a modification of the commercially available AdnaTest platform (Qiagen), as previously described.¹¹ Isolation and enrichment of CTCs was performed using the 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 used to detect the full-length AR (AR-FL) mRNA and AR-V7 mRNA, as previously described.¹¹ The relative abundance of AR-V7 was determined by calculating the ratio of AR-V7 transcript to AR-FL transcript.

Outcome Measures

The primary end point was PSA response: the proportion of patients who achieved at least a 50% PSA level decline from baseline at any time point after therapy (and maintained it for ≥ 3 weeks). Secondary end points included PSA PFS and clinical and/or radiographic PFS (referred to hereafter as PFS). Overall survival was an exploratory end point. Prostate-specific antigen progression was defined as at least a 25% increase in PSA level from nadir (and by ≥ 2 ng/mL), requiring confirmation at least 3 weeks later (PCWG2 criteria).²³ Clinical and/or radiographic progression was defined as symptomatic progression (worsening disease-related symptoms or new cancer-related complications), radiologic progression (on CT scan, $\geq 20\%$ enlargement in sum diameter of soft-tissue target lesions [RECIST {Response Evaluation Criteria in Solid Tumors} criteria²⁴]; on bone scan, ≥ 2 new bone lesions), or death, whichever occurred first.²³ Overall survival was defined as the time to death from any cause.

Statistical Analyses

Sample size was determined on the basis of the primary end point of PSA response, assuming that 30% of men would be AR-V7 positive at baseline. In our prior study,¹¹ enzalutamide- or abiraterone-treated patients showed a difference in PSA response rates between AR-V7-positive and AR-V7-negative patients of 61% (95% CI, 43%-80%). Because we hypothesized here that the impact of AR-V7 status would be smaller in the context of taxane-treated patients compared with enzalutamide- or abiraterone-treated patients, we sought a much smaller difference in PSA response rates such that the upper bound of the 95% CI for the difference was less than 61% (the point estimate from our previous study). Accordingly, a sample size of 36 patients produced a 2-sided 95% CI for the difference in PSA response rates between AR-V7-positive and AR-V7-negative patients with an upper bound of 60%, when the observed absolute difference is 30% (45% PSA response rate in AR-V7-negative men and 15% in AR-V7-positive men).

Clinical outcomes in taxane-treated men were compared between AR-V7-positive and AR-V7-negative patients. The PSA response rates were compared using the Fisher exact test. Time-to-event outcomes (PSA PFS, PFS, OS) were evaluated using Kaplan-Meier analysis, and survival time differences were

compared using the log-rank test. Univariate and multivariable logistic regression analyses (for PSA response) and Cox regression analyses (for time-to-event end points) were used to assess the effect of AR-V7 status in predicting clinical outcomes. Because of the small sample size and limited number of events, each multivariable model included only 3 covariates (AR-V7 status, AR-FL expression levels, and prior use of abiraterone and/or enzalutamide). These 3 variables were strongly associated with clinical outcomes in our prior study of AR-V7.¹¹

We then incorporated updated data on PSA responses, PSA PFS, PFS, and OS from our prior study of enzalutamide- or abiraterone-treated patients ($n = 62$) to compare the impact of AR-V7 status (ie, its ability to differentiate patients with a poor prognosis from those with a good prognosis) in the context of taxane chemotherapy vs AR-directed therapy. Specifically, we tested the interaction between AR-V7 status (positive or negative) and treatment type (taxane vs enzalutamide or abiraterone) with respect to PSA responses, PSA PFS, PFS, and OS. Univariate and multivariable Cox regression analyses were used to assess the interaction of AR-V7 status and treatment type with respect to the time-to-event outcomes; each multivariable model included 6 covariates (AR-V7 status, treatment type, AR-FL expression levels, prior use of chemotherapy, prior use of enzalutamide or abiraterone, and the interaction of AR-V7 status and treatment type).

After observing significant results from the interaction tests, we performed subgroup analyses to evaluate the efficacy of different treatment types (taxane vs abiraterone or enzalutamide) in AR-V7-positive and AR-V7-negative men separately. Univariate and multivariable Cox regression analyses were used to assess the independent effect of treatment type within each AR-V7 subgroup. Multivariable models (constructed separately for each AR-V7 subgroup) included 3 covariates: treatment type, AR-FL expression levels, and prior use of enzalutamide or abiraterone.

All statistical tests were 2-sided, and $P \leq .05$ was considered significant. Statistical analyses were performed using the R software, version 2.15.1.

The clinical investigators were blinded to the AR-V7 data. The laboratory investigators were blinded to the clinical information when determining AR-V7 status. The study statisticians were the first to unblind the data, after at least 36 patients had been enrolled.

Results

Patients

From January 2013 to July 2014, we prospectively enrolled 37 CTC-positive patients; 30 received docetaxel and 7 received cabazitaxel. Forty-three patients were screened to identify 37 men with detectable CTCs (86% yield; CTC-negative patients were excluded from further analysis). At the data cutoff date (September 1, 2014), median (range) follow-up among all taxane-treated patients was 7.7 (0.7-19.0) months. Seventeen (46%) of the 37 men had detectable AR-V7 in their baseline CTC samples. In these patients, the median (range) AR-V7/AR-FL

Table 1. Baseline Characteristics of the 37 Taxane-Treated Patients

Baseline Characteristic	All Patients (n = 37)	AR-V7 Negative (n = 20)	AR-V7 Positive (n = 17)	P Value ^a
Age, median (range), y	67 (46-82)	68 (46-82)	64 (50-77)	.11
Race, No. (%) ^b				
White	32 (86)	16 (80)	16 (94)	.35
Nonwhite	5 (14)	4 (20)	1 (6)	
Time since diagnosis, median (range), y	5 (1-12)	5 (1-12)	4 (1-11)	.60
Tumor stage at diagnosis, No. (%)				
T1/T2	14 (38)	7 (35)	7 (41)	.75
T3/T4	23 (62)	13 (65)	10 (59)	
Gleason sum at diagnosis, No. (%)				
≤7	6 (17)	4 (22)	2 (12)	.66
≥8	29 (83)	14 (78)	15 (88)	
Type of local treatment, No. (%)				
Surgery	14 (38)	7 (35)	7 (41)	.99
Radiation therapy	9 (24)	5 (25)	4 (24)	
None	14 (38)	8 (40)	6 (35)	
Current taxane therapy, No. (%)				
Docetaxel	30 (81)	15 (75)	15 (88)	.42
Cabazitaxel	7 (19)	5 (25)	2 (12)	
No. of prior hormonal therapies, median (range)	4 (2-7)	4 (2-7)	4 (2-6)	.92
Prior use of abiraterone, No. (%)				
Yes	29 (78)	14 (70)	15 (88)	.25
No	8 (22)	6 (30)	2 (12)	
Prior use of enzalutamide, No. (%)				
Yes	15 (41)	7 (35)	8 (47)	.52
No	22 (59)	13 (65)	9 (53)	
Prior use of docetaxel, No. (%)				
Yes	7 (19)	5 (25)	2 (12)	.42
No	30 (81)	15 (75)	15 (88)	
Presence of bone metastases, No. (%)				
Yes	35 (95)	18 (90)	17 (100)	.49
No	2 (5)	2 (10)	0	
No. of bone metastases, No. (%)				
≤5	6 (16)	5 (25)	1 (6)	.19
≥6	31 (84)	15 (75)	16 (94)	
Presence of visceral metastases, No. (%)				
Yes	13 (35)	7 (35)	6 (35)	.99
No	24 (65)	13 (65)	11 (65)	
ECOG performance status, No. (%)				
0	20 (54)	8 (40)	12 (71)	.10
1 or 2	17 (46)	12 (60)	5 (29)	
Baseline PSA level, median (range), ng/mL	126 (0.1-2270)	102 (5-534)	189 (0.1-2270)	.07
Baseline alkaline phosphatase level, median (range), U/L	161 (53-1243)	111 (53-930)	291 (53-1243)	.07
Baseline AR-FL level, copy number, median (range)	16 (0-4567)	4 (0-55)	88 (4-4567)	<.01

Abbreviations: AR-FL, full-length androgen receptor; AR-V7, androgen receptor splice variant 7; ECOG, Eastern Cooperative Oncology Group; PSA, prostate-specific antigen.

SI conversion factors: To convert PSA level to micrograms per liter, multiply by 1.0; to convert alkaline phosphatase to microkats per liter, multiply by 0.017.

^a P values are based on Fisher exact test and Wilcoxon Mann-Whitney test for categorical and continuous variables, respectively.

^b Race was self-reported by participants (although options were defined by the investigators).

ratio was 23% (range, 3%-69%) (eFigure in the [Supplement](#)). The prevalence of AR-V7 was influenced by prior use of enzalutamide or abiraterone: in men who had not previously received enzalutamide or abiraterone, AR-V7 was detected in 2 (25%) of 8 cases; in men who had received either enzalutamide or abiraterone, AR-V7 was detected in 7 (50%) of 14 cases; and in men who had received both enzalutamide and abiraterone, AR-V7 was detected in 8 (53%) of 15 cases.

Table 1 shows baseline characteristics for the taxane-treated population as a whole, and separated by AR-V7 status. The AR-V7-positive men were more likely to have younger age, Gleason score at least 8, prior enzalutamide or abiraterone treatment, at least 6 bone metastases, higher PSA levels, higher alkaline phosphatase levels, and higher AR-FL levels (although most of these differences were not statistically significant).

Table 2. Comparison of Baseline Characteristics of the 37 Taxane-Treated Patients and the 62 Enzalutamide- or Abiraterone-Treated Patients

Baseline Characteristic	Taxane-Treated Patients (n = 37)	Enzalutamide- or Abiraterone-Treated Patients (n = 62)	P Value ^a
Age, median (range), y	67 (46-82)	69 (48-84)	.32
Race, No. (%) ^b			
White	32 (86)	51 (82)	.78
Nonwhite	5 (14)	11 (18)	
Time since diagnosis, median (range), y	5 (1-12)	5 (1-21)	.59
Tumor stage at diagnosis, No. (%)			
T1/T2	14 (38)	29 (47)	.41
T3/T4	23 (62)	33 (53)	
Gleason sum at diagnosis, No. (%)			
≤7	6 (17)	20 (33)	.10
≥8	29 (83)	40 (67)	
Type of local treatment, No. (%)			
Surgery	14 (38)	27 (44)	.68
Radiation therapy	9 (24)	17 (27)	
None	14 (38)	18 (29)	
No. of prior hormonal therapies, median (range)	4 (2-7)	3 (2-6)	<.01
Prior use of enzalutamide or abiraterone, No. (%)			
Yes	29 (78)	24 (39)	<.01
No	8 (22)	38 (61)	
Prior use of docetaxel, No. (%)			
Yes	7 (19)	25 (40)	.04
No	30 (81)	37 (60)	
Presence of bone metastases, No. (%)			
Yes	35 (95)	52 (84)	.20
No	2 (5)	10 (16)	
No. of bone metastases, No. (%)			
≤5	6 (16)	37 (60)	<.01
≥6	31 (84)	25 (40)	
Presence of visceral metastases, No. (%)			
Yes	13 (35)	18 (29)	.66
No	24 (65)	44 (71)	
ECOG performance status, No. (%)			
0	20 (54)	47 (76)	.03
1 or 2	17 (46)	15 (24)	
Baseline PSA level, median (range), ng/mL	126 (0.1-2270)	42 (2.2-3204)	<.01
Baseline alkaline phosphatase level, median (range), U/L	161 (53-1243)	111 (58-1348)	.04
Baseline AR-FL level, copy number, median (range)	16 (0-4567)	7 (0-734)	.05

Abbreviations: AR-FL, full-length androgen receptor; ECOG, Eastern Cooperative Oncology Group; PSA, prostate-specific antigen.

SI conversion factors: To convert PSA level to micrograms per liter, multiply by 1.0; to convert alkaline phosphatase to microkats per liter, multiply by 0.017.

^a P values are based on Fisher exact test and Wilcoxon Mann-Whitney test for categorical and continuous variables, respectively.

^b Race was self-reported by participants (although options were defined by the investigators).

Table 2 compares baseline characteristics of the 37 taxane-treated patients and the 62 enzalutamide- or abiraterone-treated patients incorporated from our prior study.¹¹ These 62 men were enrolled between December 2012 and September 2013, and their clinical outcomes were updated using the cut-off date of September 1, 2014. In this updated analysis, median (range) follow-up among all enzalutamide- or abiraterone-treated patients was 13.0 (1.4-19.8) months. Eighteen (29%) of these 62 men had detectable AR-V7 at baseline. Compared with taxane-treated patients, enzalutamide- or abiraterone-treated men were more likely to have Gleason scores less than

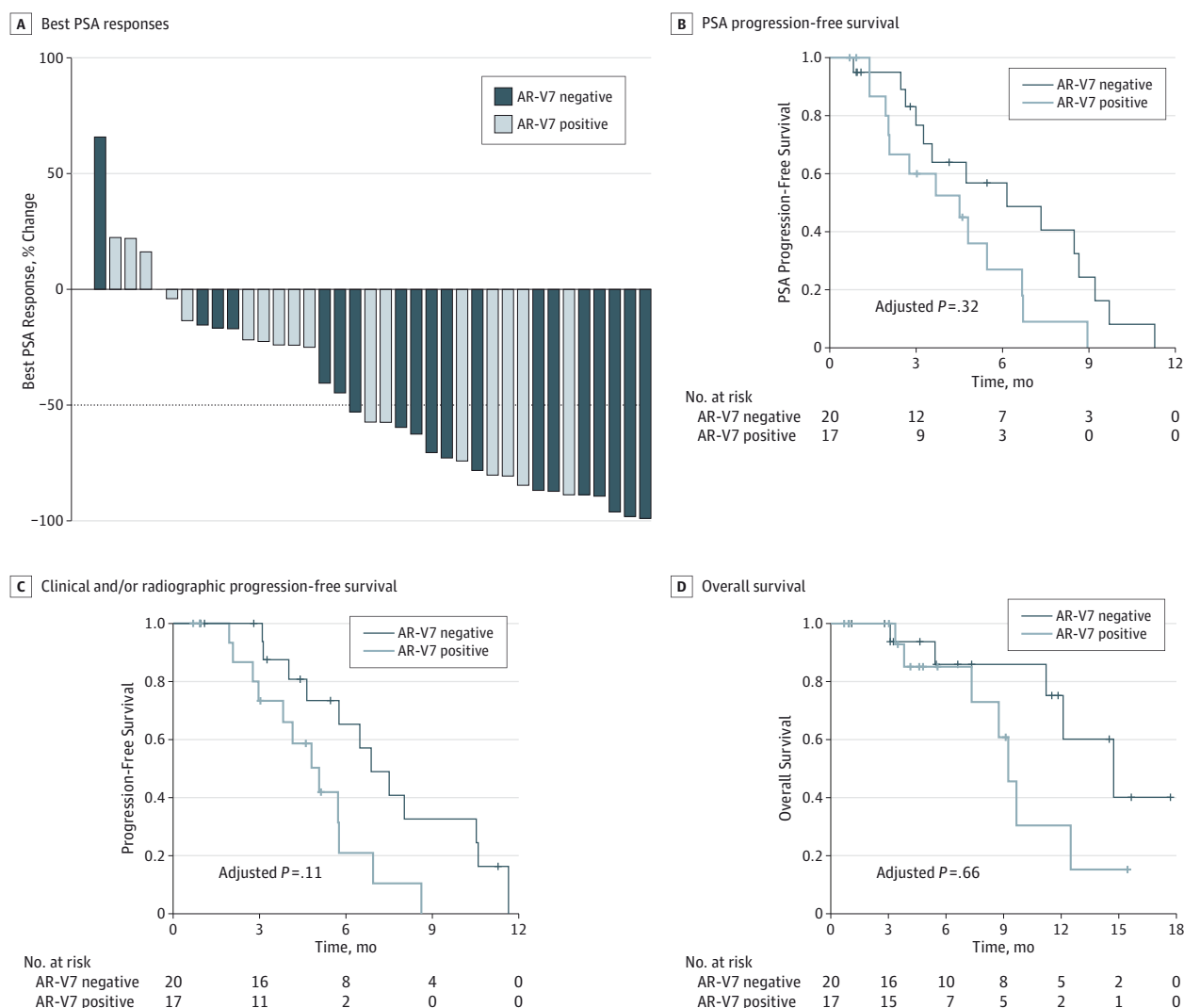
or equal to 7, fewer prior hormonal therapies, no more than 5 bone metastases, Eastern Cooperative Oncology Group (ECOG) performance status of 0, lower PSA levels, lower alkaline phosphatase levels, and lower AR-FL levels (although not all of these differences were statistically significant).

Clinical Outcomes in Taxane-Treated Patients According to AR-V7 Status

PSA Responses

The overall proportion of patients who achieved a PSA response during taxane treatment was 54% (20 of 37 men; 95%

Figure 1. Clinical Outcomes in 37 Taxane-Treated Patients, According to Circulating Tumor Cell Androgen Receptor Splice Variant 7 (AR-V7) Status



A, Waterfall plot depicting best prostate-specific antigen (PSA) responses, according to AR-V7 status. The dotted line shows the threshold for defining a PSA response ($\geq 50\%$ PSA reduction from baseline). Among patients who achieved a PSA response, 35% (7 of 20 men) were AR-V7 positive, whereas in those patients without a PSA response, 59% (12 of 17 men) were AR-V7 positive.

B, Kaplan-Meier curves showing PSA progression-free survival in 37 taxane-treated patients, according to AR-V7 status. C, Kaplan-Meier curves showing clinical and/or radiographic progression-free survival in taxane-treated patients, according to AR-V7 status. D, Kaplan-Meier curves showing overall survival in taxane-treated patients, according to AR-V7 status.

CI, 37%-71%), and there was no significant difference according to AR-V7 status. The PSA response rates were 41% (7 of 17 men; 95% CI, 18%-67%) in AR-V7-positive patients and 65% (13 of 20 men; 95% CI, 41%-85%) in AR-V7-negative patients, a non-significant difference of 24% ($P = .19$; 95% CI for the difference, -13% to 60%). Best PSA responses according to AR-V7 status are depicted in Figure 1A. In multivariable logistic regression modeling, AR-V7 status remained nonpredictive for PSA response (odds ratio, 0.39 [95% CI, 0.06-2.32]; $P = .31$) after adjusting for AR-FL expression and previous use of enzalutamide or abiraterone.

PSA PFS

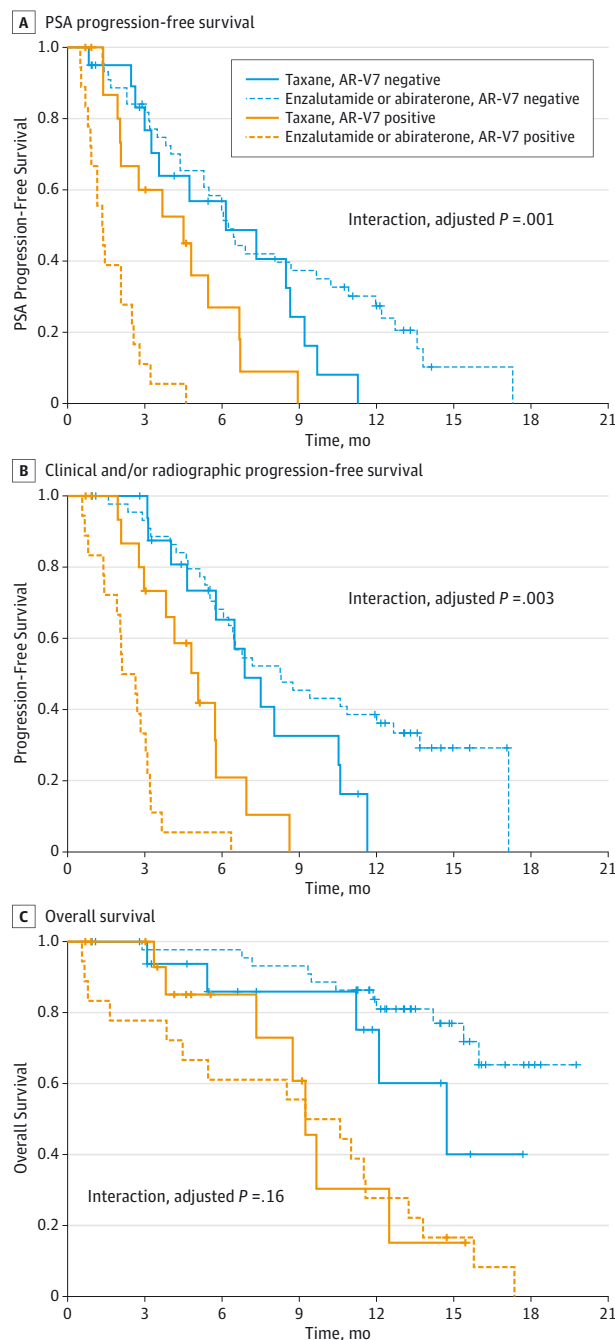
Prostate-specific antigen PFS did not differ significantly according to AR-V7 status. Median PSA PFS was 4.5 months in

AR-V7-positive men and 6.2 months in AR-V7-negative men (hazard ratio [HR], 2.1 [95% CI, 0.9-4.9]; $P = .06$). In a multivariable Cox model adjusting for AR-FL expression and prior enzalutamide or abiraterone use, AR-V7 status remained non-significant in its ability to predict PSA PFS (HR, 1.7 [95% CI, 0.6-5.0]; $P = .32$) (Figure 1B); AR-FL levels (HR, 1.0 [95% CI, 0.9-1.2]) and previous enzalutamide or abiraterone use (HR, 1.4 [95% CI, 0.4-4.2]) were also nonpredictive of PSA PFS in this multivariable analysis.

PFS

Clinical and/or radiographic PFS also did not differ significantly depending on AR-V7 status. Median PFS was 5.1 months in AR-V7-positive men and 6.9 months in AR-V7-negative men (HR, 2.8 [95% CI, 1.2-6.9]; $P = .02$). Although this difference ap-

Figure 2. Interaction Between Androgen Receptor Splice Variant 7 (AR-V7) Status and Treatment Type, After Including Data From Enzalutamide- or Abiraterone-Treated Patients



Kaplan-Meier analysis in 37 taxane-treated patients and 62 enzalutamide- or abiraterone-treated patients, separated according to AR-V7 status. A, Prostate-specific antigen (PSA) progression-free survival. A positive interaction between AR-V7 status and treatment type was observed (adjusted $P = .001$). B, Clinical and/or radiographic progression-free survival. A positive interaction between AR-V7 status and treatment type was observed (adjusted $P = .003$). C, Kaplan-Meier analysis showing overall survival in taxane-treated patients and enzalutamide- or abiraterone-treated patients, according to AR-V7 status. A significant interaction between AR-V7 status and treatment type was not observed (adjusted $P = .16$).

peared significant, in a multivariable Cox model adjusting for AR-FL expression and prior enzalutamide or abiraterone use, AR-V7 status lost its ability to predict PFS (HR, 2.7 [95% CI, 0.8-8.8]; $P = .11$) (Figure 1C); AR-FL levels (HR, 1.0 [95% CI, 0.9-1.1]) and previous enzalutamide or abiraterone use (HR, 1.7 [95% CI, 0.5-6.2]) were also nonpredictive of PFS.

OS (Exploratory)

Overall survival also did not differ significantly according to AR-V7 status. Median OS was 9.2 months in AR-V7-positive men and 14.7 months in AR-V7-negative men (HR, 2.5 [95% CI, 0.8-8.1]; $P = .11$). In a multivariable Cox model adjusting for AR-FL expression, AR-V7 status remained nonsignificant in its ability to predict OS (HR, 0.7 [95% CI, 0.1-3.8]; $P = .66$) (Figure 1D); AR-FL levels were also nonpredictive of OS (HR, 1.3 [95% CI, 0.9-1.8]).

Differential Effect of AR-V7 in Men Treated With Taxanes vs AR-Directed Therapies

PSA Responses

A significant interaction between AR-V7 status and treatment type was observed in the unadjusted linear model ($P = .002$). In an adjusted model also accounting for AR-FL levels, prior chemotherapy use, and prior enzalutamide or abiraterone use, the interaction remained significant ($P = .006$).

PSA PFS

A significant interaction between AR-V7 status and treatment type was observed in the unadjusted Cox model ($P < .001$). In an adjusted model also accounting for AR-FL levels, prior chemotherapy, and prior use of enzalutamide or abiraterone, the interaction remained significant ($P = .001$) (Figure 2A).

PFS

A significant interaction between AR-V7 status and treatment type was observed in the unadjusted Cox model ($P < .001$). In the adjusted model, the interaction remained significant ($P = .003$) (Figure 2B).

OS (Exploratory)

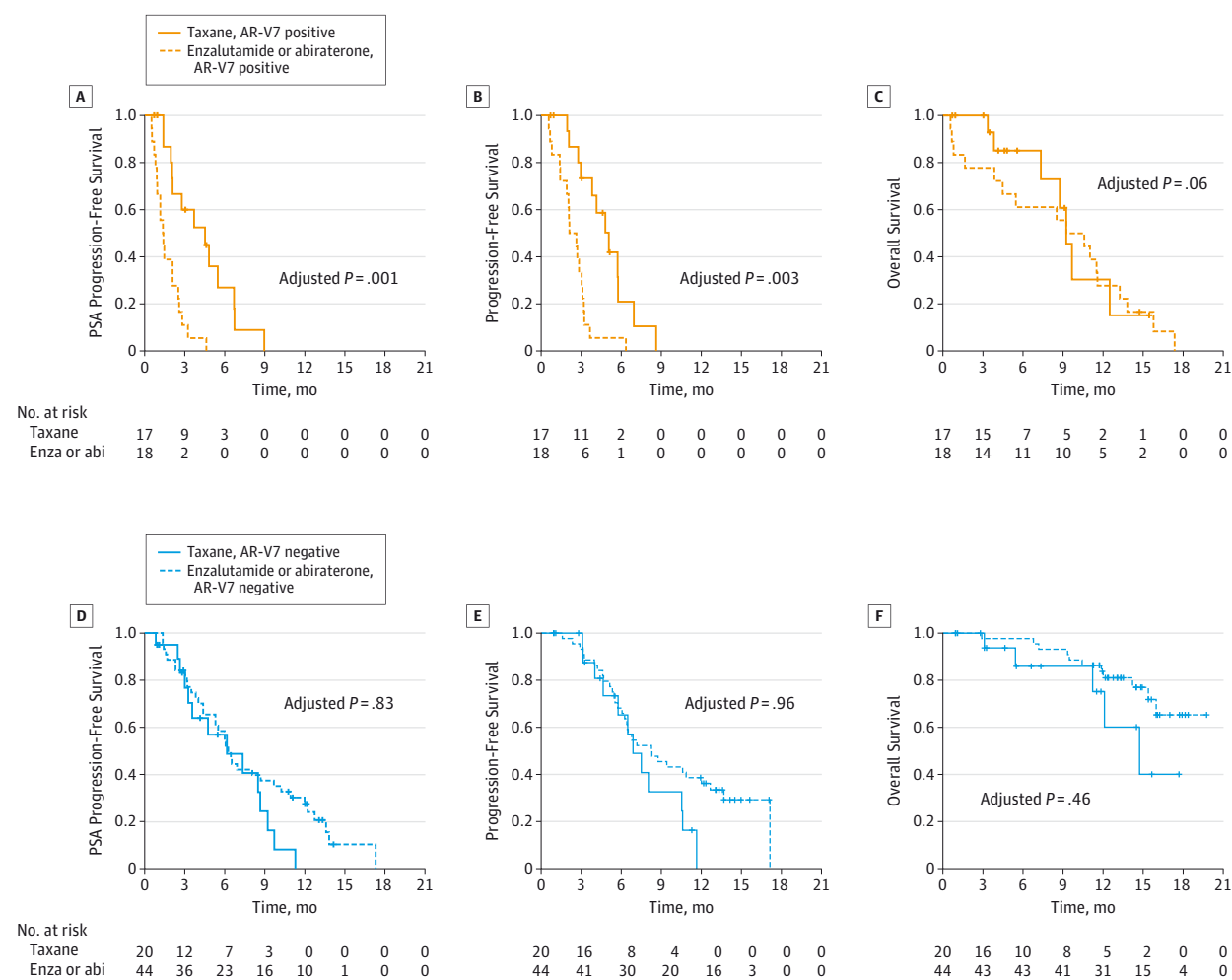
A significant interaction between AR-V7 status and treatment type was not observed either in the unadjusted Cox model ($P = .18$) or the adjusted model ($P = .16$) (Figure 2C).

Clinical Outcomes With Taxanes vs AR-Directed Therapies According to AR-V7 Status

AR-V7-Positive Patients

Treatment with taxanes appeared superior to AR-directed therapy in AR-V7-positive men. The PSA responses were 41% (7 of 17) in taxane-treated patients and 0% (0 of 18) in enzalutamide- or abiraterone-treated patients ($P < .001$). In a multivariable linear model adjusting for AR-FL level, prior chemotherapy, and prior enzalutamide or abiraterone, treatment with taxanes remained superior to enzalutamide or abiraterone ($P < .001$). Moreover, median PSA PFS was longer in taxane-treated men compared with enzalutamide- or abiraterone-treated men (HR, 0.22 [95% CI, 0.09-0.53]; $P < .001$). In a multivariable Cox model adjusting for AR-FL level and prior

Figure 3. Clinical Outcomes With Taxanes vs Androgen Receptor–Directed Therapies for Androgen Receptor Splice Variant 7 (AR-V7)–Positive and AR-V7–Negative Patients



Kaplan-Meier analyses comparing taxane-treated patients vs enzalutamide (enza)- or abiraterone (abi)-treated patients. A, Prostate-specific antigen progression-free survival, focusing only on AR-V7–positive men. B, Clinical and/or radiographic progression-free survival, focusing only on AR-V7–positive men. C, Overall survival, focusing only on AR-V7–positive men.

D, Prostate-specific antigen progression-free survival, focusing only on AR-V7–negative men. E, Clinical and/or radiographic progression-free survival, focusing only on AR-V7–negative men. F, Overall survival, focusing only on AR-V7–negative men.

enzalutamide or abiraterone therapy, taxane therapy remained superior to AR-directed therapy (HR, 0.19 [95% CI, 0.07–0.52]; $P = .001$) (Figure 3A). Similarly, median PFS was longer in taxane-treated compared with enzalutamide- or abiraterone-treated men (HR, 0.26 [95% CI, 0.11–0.59]; $P = .001$). In a multivariable Cox model adjusting for AR-FL level and prior enzalutamide or abiraterone therapy, taxane therapy remained superior (HR, 0.21 [95% CI, 0.07–0.59]; $P = .003$) (Figure 3B). Finally, median OS (exploratory) was numerically superior in taxane-treated compared with enzalutamide- or abiraterone-treated patients (HR, 0.83 [95% CI, 0.34–2.00]; $P = .76$). In a multivariable Cox model adjusting for AR-FL level and prior use of enzalutamide or abiraterone, there was numerically superior survival with taxane therapy (HR, 0.28 [95% CI, 0.07–1.00]; $P = .06$) (Figure 3C).

AR-V7–Negative Patients

There were no significant differences between taxane treatment and AR-directed therapy with respect to any clinical outcomes in AR-V7–negative men. Prostate-specific antigen responses were 65% (13 of 20) in taxane-treated patients and 64% (28 of 44) in enzalutamide- or abiraterone-treated patients ($P = .60$); this difference remained nonsignificant after adjusting for AR-FL level, prior chemotherapy, and prior enzalutamide or abiraterone treatment in a multivariable linear model ($P = .36$). Median PSA PFS was not significantly different in taxane-treated patients compared with enzalutamide- or abiraterone-treated patients (HR, 1.61 [95% CI, 0.84–3.06]; $P = .15$), even after adjusting for AR-FL level and prior enzalutamide or abiraterone treatment in the multivariable Cox model (HR, 1.09 [95% CI, 0.51–2.31]; $P = .83$) (Figure 3D). Similarly, median

PFS was not significantly different in taxane-treated compared with enzalutamide- or abiraterone-treated patients (HR, 1.68 [95% CI, 0.84-3.33]; $P = .14$), even after adjusting for AR-FL and prior enzalutamide or abiraterone treatment in multivariable Cox analysis (HR, 1.02 [95% CI, 0.46-2.25]; $P = .96$) (Figure 3E). Finally, median OS (exploratory) was not significantly different between the 2 treatment groups, either in the univariate (HR, 2.26 [95% CI, 0.78-6.62]; $P = .13$) or the multivariable (HR, 1.55 [95% CI, 0.49-4.95]; $P = .46$) analyses (Figure 3F).

AR-V7 Conversions at Taxane Progression

Twenty-one taxane-treated patients had paired CTC samples collected at baseline and at the time of progression that were evaluable for AR-V7. Among men with initially undetectable AR-V7 ($n = 9$), 1 patient (11%) subsequently converted to AR-V7 positive during the course of taxane treatment whereas 8 patients (89%) remained AR-V7 negative at progression. Conversely, among men with detectable AR-V7 at baseline ($n = 12$), 7 patients (58%) converted to AR-V7 negative during taxane therapy whereas 5 patients (42%) remained AR-V7 positive at progression. The clinical significance of these conversions in AR-V7 status is currently unknown.

Discussion

Although there are multiple available therapies for men with metastatic CRPC, there are currently no molecular biomarkers to help guide optimal treatment choices in these patients. We have previously shown that detection of AR-V7 is associated with primary resistance to abiraterone and enzalutamide therapy, as manifested by inferior PSA responses, shorter PFS, and shorter OS.¹¹ Here we show that men with detectable AR-V7 retain sensitivity to taxane chemotherapies, that the impact of AR-V7 is greater in the context of AR-directed therapies than with chemotherapies, and that taxanes may have superior efficacy to enzalutamide or abiraterone in AR-V7-positive men (but not in AR-V7-negative men). The present study represents the first prospective analysis of AR-V7 in patients receiving taxane chemotherapy, and the totality of our data suggests that AR-V7 may represent a treatment selection marker in CRPC.

Although the principal mechanism of action of taxane agents is the disruption of microtubules, inducing mitotic arrest, it is increasingly understood that taxanes may also mediate their antitumor effects in CRPC by disrupting cytoplasmic-to-nuclear trafficking of AR along the microtubule network,¹⁷⁻²⁰ while other mechanisms have also been postulated.^{25,26} Therefore, some degree of cross-resistance has been suggested between AR-targeting therapies and taxane chemotherapies, although this cross-resistance may be less substantial with cabazitaxel than with docetaxel.²⁷ Recently, work on a particular mouse model of CRPC has also suggested that certain AR-Vs may be associated with sensitivity to taxanes whereas others may mediate taxane resistance.²² To this end, AR-V7 was shown to result in taxane resistance in at least 1 preclinical model, due to deletion

of the AR hinge region that is thought to be necessary for microtubule binding.²² However, our clinical data do not recapitulate the observations from this mouse model. In fact, we show here that in AR-V7-positive patients, PSA response rates to taxanes are 41% and median PFS is 5.1 months. Although clinical outcomes to taxanes may appear inferior in AR-V7-positive compared with AR-V7-negative men, these differences were not statistically significant after multivariable adjustments. More importantly, we demonstrate that AR-V7 detection is not associated with primary resistance to taxane agents (as seen with abiraterone and enzalutamide¹¹).

An important observation from our present study is the suggestion that taxane therapy may be more efficacious than AR-directed therapy for men with AR-V7-positive CRPC. Conversely, clinical outcomes did not seem to differ significantly on the basis of the type of therapy used among AR-V7-negative patients. If these results are confirmed by additional prospective biomarker-stratified clinical trials, this observation might suggest that AR-V7-positive men may fare better receiving taxanes than AR-targeting therapies, whereas in AR-V7-negative men both treatment approaches might be reasonable. However, our study has important limitations. Because of the small sample size, we were unable to perform a comprehensive multivariable analysis to determine the independent contribution of AR-V7 status to prognosis, and we were not able to define subpopulations in which the utility of the biomarker may be greatest. It remains possible that AR-V7 is simply a marker of more advanced disease or higher disease burden. Second, the comparison of clinical outcomes between taxane-treated and enzalutamide- or abiraterone-treated patients is confounded by the fact that treatment selection was not randomly assigned and that baseline patient characteristics (including numbers and types of prior therapies received) were different in the 2 cohorts. Confirmation of these findings will require larger biomarker-driven studies randomizing patients to taxane chemotherapy vs AR-directed therapy. To this end, prospective validation of the AR-V7 biomarker will be pursued in the PRIMCAB study (NCT02379390), a multicenter randomized phase 2 trial of abiraterone or enzalutamide vs cabazitaxel therapy in men with primary resistance to prior enzalutamide or abiraterone therapy.

Finally, an intriguing finding from our study was the fact that certain patients with detectable AR-V7 at baseline converted to AR-V7-negative status during the course of taxane therapy. Notably, in our prior analysis of AR-V7 in enzalutamide- or abiraterone-treated patients, all men with detectable AR-V7 at baseline remained AR-V7 positive throughout treatment with abiraterone and enzalutamide.¹¹ Biologically, a conversion from AR-V7-positive to AR-V7-negative status might imply decreased selection pressure on the AR axis exerted by taxanes, allowing a resumption of canonical AR signaling and a lack of requirement for aberrant AR-V-mediated signaling. An alternative hypothesis is that effective taxane therapy may have decreased the burden of CTCs, thereby making it more difficult to detect AR-V7 present in low abundance. The clinical significance of these AR-V7 conversions remains unclear and is the subject of ongoing investigations.

Conclusions

Our findings suggest that detection of AR-V7 in CTCs from men with CRPC is not associated with primary resistance to

taxane chemotherapy and that AR-V7-positive patients may respond better to taxanes than to AR-targeting drugs. If confirmed in larger-scale clinical trials, AR-V7 status could emerge as the first treatment selection biomarker for CRPC.

ARTICLE INFORMATION

Accepted for Publication: April 10, 2015.

Published Online: June 4, 2015.

doi:10.1001/jamaoncol.2015.1341.

Author Contributions: Drs Antonarakis and Luo had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Antonarakis, Lubner, Wang, Carducci, Eisenberger, Luo.

Acquisition, analysis, or interpretation of data: All authors.

Drafting of the manuscript: Antonarakis, Lubner, Chen, Nakazawa, Carducci, Luo.

Critical revision of the manuscript for important intellectual content: Antonarakis, Lu, Wang, Nadal, Paller, Denmeade, Carducci, Eisenberger, Luo.

Statistical analysis: Antonarakis, Lubner, Wang, Luo.

Obtained funding: Antonarakis, Carducci, Luo.

Administrative, technical, or material support: Antonarakis, Lu, Chen, Nakazawa, Paller, Denmeade, Carducci, Luo.

Study supervision: Antonarakis, Eisenberger, Luo.

Conflict of Interest Disclosures: Dr Antonarakis has served as a paid consultant/advisor for Janssen, Astellas, Sanofi, Dendreon, Essa, and Medivation; received research funding from Janssen, Johnson & Johnson, Sanofi, Dendreon, Exelixis, Genentech, Novartis, and Tokai; and is a co-inventor of a technology that has been licensed to Tokai. Dr Luo has served as a paid consultant/advisor for Astellas, has been a speaker for Sanofi and Gilead, has received research funding from Sanofi and Mirati, and is also a co-inventor of a technology that has been licensed to Tokai. Relevant disclosures have been reviewed and approved by Johns Hopkins University in accordance with its conflict of interest policies. No other disclosures are reported.

Funding/Support: This research was supported by the Prostate Cancer Foundation, the Department of Defense Prostate Cancer Research Program grant W81XWH-12-1-0605, the Patrick C. Walsh Fund, the Johns Hopkins Prostate SPOR grant P50 CA058236, and National Institutes of Health grant P30 CA006973.

Role of the Funder/Sponsor: The funders had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Additional Contributions: We thank the patients and their families who participated in this study.

REFERENCES

1. Basch E, Loblaw DA, Oliver TK, et al. Systemic therapy in men with metastatic castration-resistant prostate cancer: American Society of Clinical Oncology and Cancer Care Ontario clinical practice guideline. *J Clin Oncol*. 2014;32(30):3436-3448.
2. de Bono JS, Logothetis CJ, Molina A, et al; COU-AA-301 Investigators. Abiraterone and

increased survival in metastatic prostate cancer.

N Engl J Med. 2011;364(21):1995-2005.

3. Scher HI, Fizazi K, Saad F, et al; AFFIRM Investigators. Increased survival with enzalutamide in prostate cancer after chemotherapy. *N Engl J Med*. 2012;367(13):1187-1197.

4. Tannock IF, de Wit R, Berry WR, et al; TAX 327 Investigators. Docetaxel plus prednisone or mitoxantrone plus prednisone for advanced prostate cancer. *N Engl J Med*. 2004;351(15):1502-1512.

5. de Bono JS, Oudard S, Ozguroglu M, et al; TROPIC Investigators. Prednisone plus cabazitaxel or mitoxantrone for metastatic castration-resistant prostate cancer progressing after docetaxel treatment: a randomised open-label trial. *Lancet*. 2010;376(9747):1147-1154.

6. Kantoff PW, Higano CS, Shore ND, et al; IMPACT Study Investigators. Sipuleucel-T immunotherapy for castration-resistant prostate cancer. *N Engl J Med*. 2010;363(5):411-422.

7. Parker C, Nilsson S, Heinrich D, et al; ALSYMPCA Investigators. Alpha emitter radium-223 and survival in metastatic prostate cancer. *N Engl J Med*. 2013;369(3):213-223.

8. Seruga B, Ocana A, Tannock IF. Drug resistance in metastatic castration-resistant prostate cancer. *Nat Rev Clin Oncol*. 2011;8(1):12-23.

9. Karantanos T, Evans CP, Tombal B, Thompson TC, Montironi R, Isaacs WB. Understanding the mechanisms of androgen deprivation resistance in prostate cancer at the molecular level. *Eur Urol*. 2015;67(3):470-479.

10. Armstrong AJ, Eisenberger MA, Halabi S, et al. Biomarkers in the management and treatment of men with metastatic castration-resistant prostate cancer. *Eur Urol*. 2012;61(3):549-559.

11. Antonarakis ES, Lu C, Wang H, et al. AR-V7 and resistance to enzalutamide and abiraterone in prostate cancer. *N Engl J Med*. 2014;371(11):1028-1038.

12. Dehm SM, Schmidt LJ, Heemers HV, Vessella RL, Tindall DJ. Splicing of a novel androgen receptor exon generates a constitutively active androgen receptor that mediates prostate cancer therapy resistance. *Cancer Res*. 2008;68(13):5469-5477.

13. Hu R, Dunn TA, Wei S, et al. Ligand-independent androgen receptor variants derived from splicing of cryptic exons signify hormone-refractory prostate cancer. *Cancer Res*. 2009;69(1):16-22.

14. Hu R, Lu C, Mostaghel EA, et al. Distinct transcriptional programs mediated by the ligand-dependent full-length androgen receptor and its splice variants in castration-resistant prostate cancer. *Cancer Res*. 2012;72(14):3457-3462.

15. Mostaghel EA, Marck BT, Plymate SR, et al. Resistance to CYP17A1 inhibition with abiraterone in castration-resistant prostate cancer: induction of

steroidogenesis and androgen receptor splice variants. *Clin Cancer Res*. 2011;17(18):5913-5925.

16. Li Y, Chan SC, Brand LJ, Hwang TH, Silverstein KA, Dehm SM. Androgen receptor splice variants mediate enzalutamide resistance in castration-resistant prostate cancer cell lines. *Cancer Res*. 2013;73(2):483-489.

17. Gan L, Chen S, Wang Y, et al. Inhibition of the androgen receptor as a novel mechanism of taxol chemotherapy in prostate cancer. *Cancer Res*. 2009;69(21):8386-8394.

18. Zhu ML, Horbinski CM, Garzotto M, Qian DZ, Beer TM, Kyprianou N. Tubulin-targeting chemotherapy impairs androgen receptor activity in prostate cancer. *Cancer Res*. 2010;70(20):7992-8002.

19. Darshan MS, Loftus MS, Thadani-Mulero M, et al. Taxane-induced blockade to nuclear accumulation of the androgen receptor predicts clinical responses in metastatic prostate cancer. *Cancer Res*. 2011;71(18):6019-6029.

20. Thadani-Mulero M, Nanus DM, Giannakakou P. Androgen receptor on the move: boarding the microtubule expressway to the nucleus. *Cancer Res*. 2012;72(18):4611-4615.

21. Kirby BJ, Jodari M, Loftus MS, et al. Functional characterization of circulating tumor cells with a prostate-cancer-specific microfluidic device. *PLoS One*. 2012;7(4):e35976.

22. Thadani-Mulero M, Portella L, Sun S, et al. Androgen receptor splice variants determine taxane sensitivity in prostate cancer. *Cancer Res*. 2014;74(8):2270-2282.

23. Scher HI, Halabi S, Tannock I, et al; Prostate Cancer Clinical Trials Working Group. 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*. 2008;26(7):1148-1159.

24. Eisenhauer EA, Therasse P, Bogaerts J, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *Eur J Cancer*. 2009;45(2):228-247.

25. de Leeuw R, Berman-Booty LD, Schiewer MJ, et al. Novel actions of next-generation taxanes benefit advanced stages of prostate cancer. *Clin Cancer Res*. 2015;21(4):795-807.

26. Plymate SR, Bhatt RS, Balk SP. Taxane resistance in prostate cancer mediated by AR-independent GATA2 regulation of IGF2. *Cancer Cell*. 2015;27(2):158-159.

27. van Soest RJ, de Morree ES, Kweldam CF, et al. Targeting the androgen receptor confers in vivo cross-resistance between enzalutamide and docetaxel, but not cabazitaxel, in castration-resistant prostate cancer. *Eur Urol*. 2015; 67(6):981-985.

Serial blood-based analysis of AR-V7 in men with advanced prostate cancer

M. Nakazawa¹, C. Lu¹, Y. Chen¹, C. J. Paller², M. A. Carducci², M. A. Eisenberger², J. Luo^{1*} & E. S. Antonarakis²

Departments of ¹Urology, ²Oncology, Johns Hopkins University School of Medicine, Baltimore, USA

Received 6 May 2015; revised 15 June 2015; accepted 18 June 2015

Background: We previously showed that pretreatment detection of androgen receptor splice variant-7 (AR-V7) in circulating tumor cells (CTCs) from men with castration-resistant prostate cancer is associated with resistance to abiraterone and enzalutamide, but not to taxane chemotherapies. Here, we conducted serial measurements of AR-V7 and evaluated patterns of longitudinal AR-V7 dynamics over the course of multiple sequential therapies.

Patients and methods: Metastatic prostate cancer patients treated at Johns Hopkins with AR-directed therapies or taxane chemotherapies underwent serial liquid biopsies for CTC-based AR-V7 analysis at baseline, during therapy, and at progression. We used a CTC enrichment platform followed by multiplexed reverse-transcription polymerase chain reaction analysis to detect full-length androgen receptor and AR-V7 transcripts. Patients selected for inclusion in this report were those who provided ≥ 4 CTC samples, at least one of which was AR-V7 positive, over the course of ≥ 2 consecutive therapies.

Results: We identified 14 patients who received a total of 37 therapies and contributed 70 CTC samples for AR-V7 analysis during a median follow-up period of 11 months. Three patients remained AR-V7 positive during the entire course of therapy. The remainder underwent transitions in AR-V7 status: there were eight instances of ‘conversions’ from AR-V7-negative to -positive status (during treatment with first-line androgen deprivation therapy, abiraterone, enzalutamide, and docetaxel), and six instances of ‘reversions’ from AR-V7-positive to -negative status (during treatment with docetaxel and cabazitaxel).

Conclusions: AR-V7 is a dynamic marker, and transitions in AR-V7 status may reflect selective pressures on the tumor exerted by therapeutic interventions. While ‘conversions’ to AR-V7-positive status were observed with both AR-directed therapies and taxane chemotherapies, ‘reversions’ to AR-V7-negative status only occurred during taxane therapies. Serial blood-based AR-V7 testing is feasible in routine clinical practice, and may provide insights into temporal changes in tumor evolution.

Key words: AR-V7, splice variant, androgen receptor, circulating tumor cell, prostate cancer

Introduction

Prostate cancer is the most common noncutaneous malignancy in the United States and is the second leading cause of cancer deaths among males, claiming $\sim 30\,000$ lives each year [1]. While prognosis is favorable for early-stage disease, most men with castration-resistant prostate cancer (CRPC) eventually die from their illness. CRPC often remains androgen-dependent and AR-driven. Several mechanisms of castration resistance have been identified, many of which contribute to sustained AR signaling: production of adrenal and intratumoral androgens [2–4], AR amplification or overexpression [5, 6], AR activation through alternative pathways

[7, 8], nontraditional ligand synthesis pathways [9, 10], and activating AR mutations allowing promiscuous signaling [11, 12].

Adding to this complexity, AR splice variants (AR-Vs) also play a significant role in therapeutic resistance. AR-Vs are truncated forms of the AR lacking portions of the ligand-binding domain, resulting in constitutively active functions [13, 14]. Androgen receptor splice variant-7 (AR-V7) is one such variant, capable of being activated without ligand binding [15, 16]. This quality, along with its relative abundance, increased expression in CRPC tissues, and its ability to encode a detectable protein product, is suggestive of its clinical significance [16–18]. Indeed, several preclinical studies have demonstrated that AR-V7 confers resistance to novel AR-directed therapies, abiraterone and enzalutamide [19, 20]. Recently, our group has shown that AR-V7 is associated with clinical resistance to these two agents; patients

*Correspondence to: Dr Jun Luo, Department of Urology, The James Buchanan Brady Urological Institute, Johns Hopkins University School of Medicine, 600 N. Wolfe St, Baltimore, MD 21287, USA. Tel: +1-443-287-5625; E-mail: jluo1@jhmi.edu

with detectable AR-V7 in circulating tumor cells (CTCs) had inferior prostate-specific antigen (PSA) responses as well as worse progression-free and overall survival compared with their AR-V7-negative counterparts [21]. However, AR-V7 does not appear to be a predominant mechanism of resistance to taxane chemotherapies (docetaxel and cabazitaxel) [22].

In this study, we assessed the feasibility of performing serial blood-based AR-V7 sampling across the contemporary treatment landscape of metastatic prostate cancer, and to better understand AR-V7 marker dynamics that may be subject to influences by the different therapies. We describe temporal changes in CTC tumor marker dynamics in men undergoing sequential therapies using AR-targeting agents and taxane chemotherapies with a focus on AR-V7 status. Given the large number of agents now FDA-approved for CRPC, serial AR-V7 analysis might yield important insights into optimal sequencing strategies which are currently unknown [23]. This is particularly relevant in an era of increasing cross-resistance between abiraterone and enzalutamide [24–25], as well as cross-resistance between AR-targeting therapies and taxane chemotherapies [26–27]. Sequential sampling of CTCs for AR-V7 analysis could therefore provide conceptual insights on tumor evolution in response to multiple treatments in a noninvasive manner.

methods

patients

study population. We prospectively enrolled men with metastatic prostate cancer beginning standard-of-care therapy with AR-targeting agents [androgen deprivation therapy (ADT) (luteinizing hormone releasing hormone agonists/antagonists ± first-generation antiandrogen), enzalutamide, and abiraterone] or taxane chemotherapies (docetaxel and cabazitaxel) under an institutional review board-approved protocol allowing sequential collection of liquid biopsies. Both hormone-sensitive and castration-resistant patients were eligible. Peripheral blood samples for CTC-specific AR-V7 analysis were collected at up to three time-points for each therapy received. Specifically, samples were collected before therapy initiation, during the course of therapy, and at the time of clinical/radiographic progression. Subjects were excluded if they planned to receive concurrent immunotherapies, radiopharmaceutical drugs, or other investigational agents; there were no restrictions on prior anticancer therapies. Patients provided written informed consent before each therapy.

study design and assessments. We aimed to prospectively evaluate the dynamics of AR-V7 status in response to AR-directed and taxane therapies administered in sequential fashion across the treatment continuum. Only men who provided ≥4 CTC samples across the course of ≥2 consecutive therapies were included in the present analysis (see selection criteria, supplementary Figure S1, available at *Annals of Oncology* online). Patients had PSA evaluations every 1–2 months and underwent imaging with a computed tomography and bone scan every 3–4 months; treatment was continued until disease progression or unmanageable toxicity. For simplistic purposes, patients were defined as ‘responders’ if they exhibited a ≥50% PSA decline at any time on therapy, maintained for ≥4 weeks.

materials

analysis of CTCs for AR-V7. We used a modified version of the AdnaTest platform for CTC analysis, as previously described [21]. Briefly, CTCs were enriched from peripheral blood using the ProstateCancerSelect kit.

mRNA expression analysis was conducted using the ProstateCancerDetect kit, along with multiplexed reverse-transcription polymerase chain reaction analyses using custom primers to detect full-length AR (AR-FL) mRNA and AR-V7 mRNA. The relative abundance of AR-V7 was reported as a ratio of AR-V7 to AR-FL transcripts [21].

results

patient characteristics

From December 2012 to March 2015, we enrolled 225 men with metastatic prostate cancer beginning therapy with AR-targeting agents or taxane chemotherapies at the Johns Hopkins Sidney Kimmel Comprehensive Cancer Center. After applying our selection criteria (supplementary Figure S1, available at *Annals of Oncology* online), we identified a total of 25 men who had consented to CTC collections over the course of ≥2 consecutive treatments and provided ≥4 total CTC samples, of which 14 patients had ≥1 sample positive for AR-V7. The characteristics of the remaining 11 patients, whose CTCs remained AR-V7 negative throughout the treatments assessed, are summarized in supplementary Table S1, available at *Annals of Oncology* online. A proportion of these patients have been analyzed as part of our previous studies [21, 22], but only in the context of one single therapy at a time. Supplementary Table S2 summarizes the baseline characteristics of all patients.

The present analysis focuses on the 14 CTC-positive patients at baseline with at least one AR-V7-positive CTC sample during follow-up. Table 1 shows baseline characteristics (at the time of study entry) for these patients, who had variable disease burden and prior treatment exposures. Two men, patients 8 and 9, were hormone-sensitive, whereas 12 men were castration-resistant at the time of study entry. Common sites of metastatic disease included bones and lymph nodes. At the first CTC collection, six patients (43%) had detectable AR-V7. In total, 37 therapies were administered [mean 2.6 (range 2–4) therapies per subject], and 70 CTC AR-V7 tests carried out [mean 4.9 (range 4–8) tests per subject], during a median follow-up of 11 [range 6–18] months. The type and duration of each therapy, the PSA response status, and the serial blood-based AR-V7 measurement results are summarized in Figure 1.

AR-V7 conversions and reversions

Given our selection criteria, all 14 patients had at least one instance of detectable AR-V7 during their follow-up. Three patients (7, 10, and 12) were AR-V7 positive during the entire follow-up period. Of the remaining 11 patients, 5 underwent ‘conversions’ from AR-V7 negative to AR-V7 positive during the course of therapy, 1 underwent a ‘reversion’ from AR-V7 positive back to AR-V7 negative, and 5 men experienced both a conversion and a reversion at different points along their treatment trajectory. Two patients (patients 5 and 6) experienced conversions off therapy (during which time they only received ADT). These transitions in AR-V7 status are summarized according to treatment type in Table 2.

We observed eight instances of ‘conversions’ in AR-V7 status (i.e. negative to positive transitions) during the course of 15 treatments (53.5%) (Table 2, A), and six ‘reversions’ (i.e. positive to negative transitions) during the course of 22 treatments

Table 1. Characteristics of the 14 selected patients at the time of baseline CTC sampling

	Age	Years since Dx	Gleason score	Metastatic sites	Previous treatments	PSA (ng/ml)	ALK (U/l)	ECOG PS	AR-V7 status
1	64	1.2	9	Bone, LN, liver	L, B	7.5	112	0	Negative
2	77	2.4	10	Bone	L, B, D	2.2	91	0	Negative
3	61	4.9	9	Bone, LN, liver	L, B, S	25.3	315	0	Positive
4	56	9.1	9	LN	L, B, N, K, A	22.2	70	0	Negative
5	69	8.0	10	Bone	L, B, N, K, E	23.3	68	0	Positive
6	73	5.0	9	Bone, LN	L, B	63.5	53	0	Positive
7	66	0.8	10	Bone, LN	L, B, A	534	486	1	Positive
8	50	0.1	9	Bone, LN	None	314	270	0	Negative
9	57	0.6	9	Bone, LN	None	365	142	0	Negative
10	70	1.1	9	Bone	L, B	157	189	1	Positive
11	60	2.1	10	Bone, LN, liver, lung, adrenals	L, B, A	75.0	101	1	Negative
12	58	3.4	9	Bone, LN	L, B	58.7	838	0	Positive
13	68	5.0	9	Bone	L, B, A	50.7	112	1	Negative
14	82	1.1	Unknown	Bone	L, B, A	895	464	1	Negative

L, LHRH agonist/antagonist; B, bicalutamide; N, nilutamide; K, ketoconazole; A, abiraterone; E, enzalutamide; D, docetaxel; S, sipuleucel-T; ECOG PS, Eastern Cooperative Oncology Group performance status.

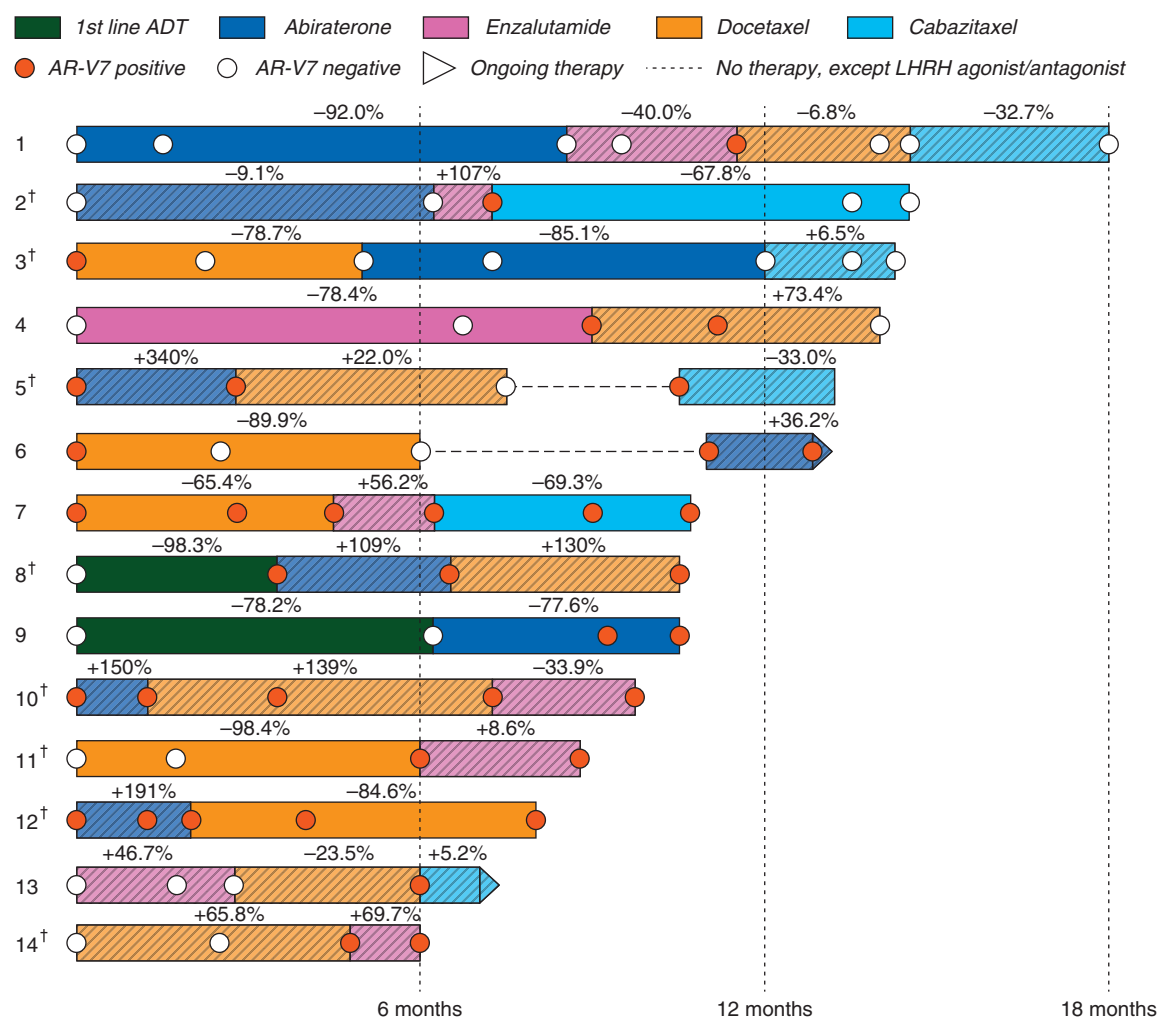


Figure 1. Swimmer plot indicating treatments that patients received, along with timing (and AR-V7 status) of CTC sampling, and whether or not PSA responses occurred during each therapy. Shaded boxes indicate failure to achieve PSA response; unshaded boxes indicate achievement of 50% PSA reduction on therapy. Percentage values indicate best PSA response. Daggers indicate deceased patient. Thirteen of 14 patients have previously been included in our prior publications, but only in the context of a single therapy.

Table 2. Conversions and reversions in AR-V7 status during treatment with AR-directed therapies and taxane chemotherapies

(A) AR-V7 negative at baseline (N = 15)			
Treatment	Remained AR-V7 negative	'Conversions' to AR-V7 positive	Unknown ^a
First-line ADT (n = 2)	1	1	0
Abiraterone (n = 4)	3	1	0
Enzalutamide (n = 4)	1	3	0
Docetaxel (n = 3)	0	3	0
Cabazitaxel (n = 2)	2	0	0
Total (n = 15)	7	8	0
(B) AR-V7 positive at baseline (N = 22)			
Treatment	Remained AR-V7 positive	'Reversions' to AR-V7 negative	Unknown ^a
First-line ADT (n = 0)	0	0	0
Abiraterone (n = 5)	4	0	1
Enzalutamide (n = 4)	4	0	0
Docetaxel (n = 9)	4	5	0
Cabazitaxel (n = 4)	1	1	2
Total (n = 22)	13	6	3
(A) Number of baseline AR-V7-negative individuals that either remained AR-V7 negative or converted to AR-V7 positive during treatment.			
(B) Number of baseline AR-V7-positive individuals that either remained AR-V7 positive or reverted back to AR-V7 negative during treatment.			
^a Patient had no sample collected at progression, or progression has not yet occurred.			

(27.3%) (Table 2, B). Conversions occurred in patients treated with first-line ADT, abiraterone, enzalutamide, and docetaxel. No AR-V7 conversions occurred in patients receiving cabazitaxel (Table 2, A). In contrast, all six incidences of reversions occurred during treatment with taxane chemotherapies (Table 2, B). Notably, no AR-V7-positive patient receiving an AR-targeting agent (abiraterone or enzalutamide) demonstrated a reversion in AR-V7 status back to negative during the AR-directed treatment (Table 2, B).

In three patients (1, 2, and 4), we observed both a conversion and a reversion in AR-V7 status during the treatment trajectory. In all three cases, the conversion occurred during AR-directed therapy, and the reversion occurred during taxane treatment. The longitudinal PSA data for these three patients, as well as the time-points for CTC sampling and corresponding AR-V7 status, are shown in supplementary Figure S2, available at *Annals of Oncology* online, for illustrative purposes.

temporal changes in relative AR-V7 abundance

In the 14 total instances of AR-V7 conversions (n = 8) or reversions (n = 6), we examined the ratio of AR-V7 to AR-FL transcript to quantify relative changes in AR-V7 expression across time (Figure 2). Among patients who experienced conversions or reversions in AR-V7 status during the second sample collected, AR-V7/AR-FL ratios increased further in the third sample in

men with conversions (patients 4 and 9) and decreased further before disappearing in the patient who reverted back to negative (patient 4).

discussion

This is a descriptive study examining temporal changes in AR-V7 status among men undergoing sequential AR-directed therapies and/or taxane chemotherapies for metastatic prostate cancer. While it is possible to remain AR-V7 negative throughout multiple lines of therapy, we were especially interested in those patients who displayed AR-V7 positivity at least once during their follow-up, and thus selected 14 patients based on this additional criterion. We have focused specifically on AR-V7 analysis because of its relative abundance and its established significance in mediating therapy resistance in CRPC. While there are multiple context-specific mechanisms of treatment resistance in CRPC, the emergence of AR-V7 is likely to be important in the setting of AR-directed therapies, where we have previously established the association between blood-based AR-V7 detection and resistance to abiraterone and enzalutamide [21], but not to taxane chemotherapies [22]. In the current study, we demonstrate again that all patients receiving AR-directed therapies with a baseline-positive AR-V7 sample did not exhibit a PSA response; conversely, PSA responses were observed among AR-V7-positive patients receiving taxane agents. We further confirm that AR-V7 is an abundant AR variant (sometimes reaching levels of >50% relative to AR-FL), and AR-V7 appears to always coexist with AR-FL in prostate cancer patients. In addition to these confirmatory findings, this study established the feasibility of serial noninvasive CTC sampling for AR-V7 analysis in routine clinical practice. Indeed, 8 of 14 patients provided ≥5 samples during the course of their therapies.

We also show that AR-V7 is a dynamic marker. In this study, we observed eight instances of conversions from AR-V7-negative to -positive status, and six instances of reversions from AR-V7 positive to negative. Notably, five of eight conversions that occurred were with AR-directed therapies (ADT, abiraterone, enzalutamide), while all six instances of reversions occurred during docetaxel or cabazitaxel treatment. Although AR-V7 conversions can certainly occur during taxane therapy (patients 11, 13, 14—all of whom received docetaxel), reversions have never been observed with AR-targeting drugs. Although similar instances of conversions and reversions occurred in our previous studies examining the role of AR-V7 in predicting resistance to abiraterone/enzalutamide [21] and taxanes [22], the current analysis represent the first study on dynamic changes of AR-V7 over the course of sequential therapies, further demonstrating potentially differential effects of the therapies on detection of AR-V7 in blood.

Conversions from AR-V7-negative to -positive status could reflect adaptive AR-V7 induction as well as selective pressure following potent inhibition of canonical AR signaling by abiraterone and enzalutamide. Reversions from AR-V7-positive to -negative status may reflect the relaxation of inhibition on the AR-signaling axis (rather than a direct effect of taxane treatment *per se*), relieving the selective pressure for AR-V7 expression, thereby decreasing detectable AR-V7. Additionally, the cytotoxic properties of taxanes may also decrease the number of

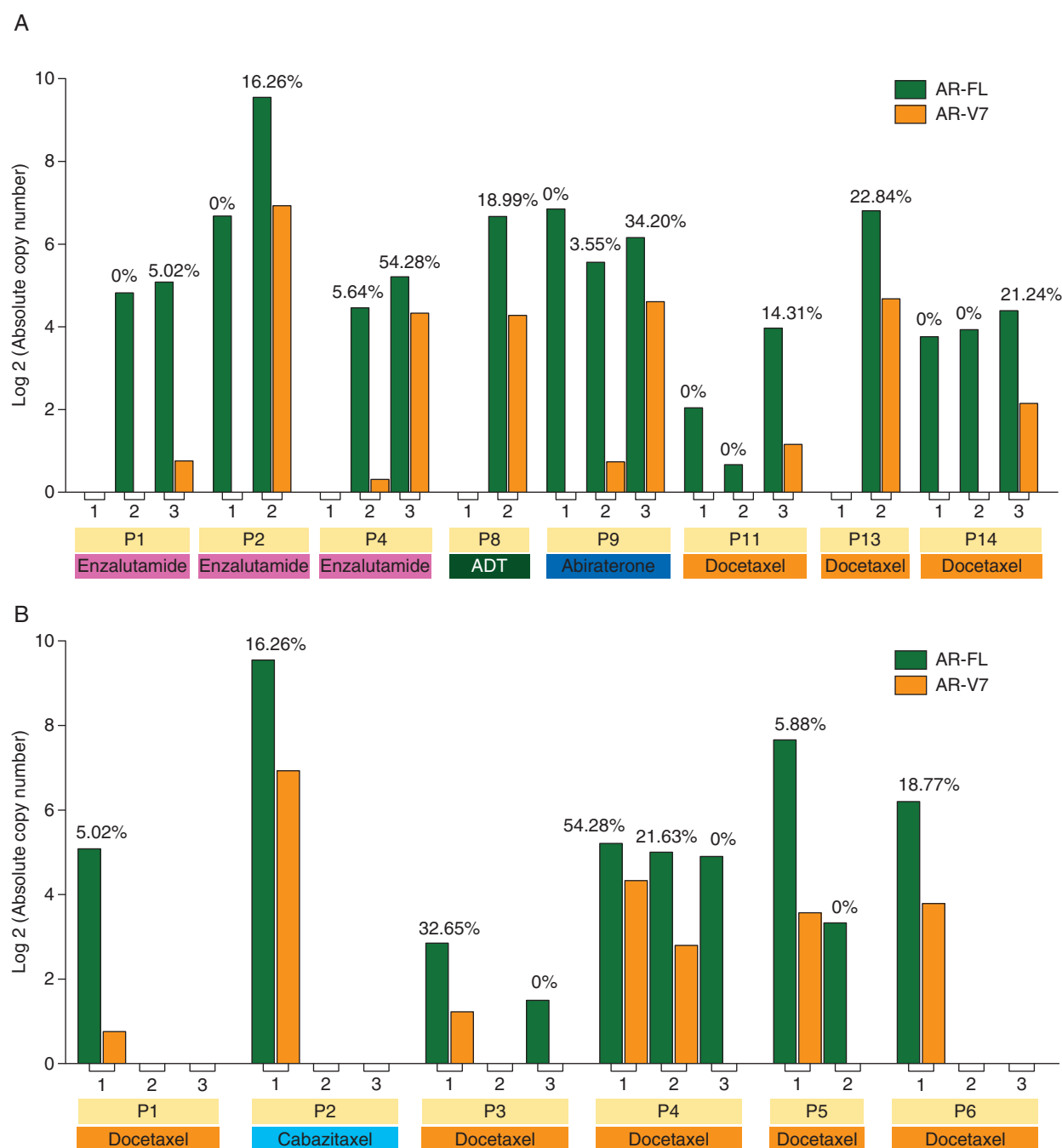


Figure 2. Quantification of AR-V7/AR-FL ratios in patients with conversions and reversions in AR-V7 status with AR-directed therapies or taxane chemotherapies. (A) Patients undergoing conversions from AR-V7-negative to -positive status during treatment with AR-directed therapies (ADT, abiraterone, and enzalutamide) as well as docetaxel chemotherapy. Percentage values indicate ratio of AR-V7 to AR-FL transcripts. Note that positive AR-FL values may not appear due to Log conversion of AR-FL transcript copy numbers. (B) Patients undergoing reversions from AR-V7-positive to -negative status during treatment with taxane chemotherapy (docetaxel or cabazitaxel). Percentage values indicate ratio of AR-V7 to AR-FL transcripts. Note that positive AR-FL values may not appear due to Log conversion of AR-FL transcript copy numbers.

CTCs in blood beyond our detection limit for AR-V7. Of note, 14 of the 70 sampling time-points assessed were negative for CTCs, and many of these CTC-negative samples corresponded to samples taken during clinical responses to therapy. Considering the results from quantitative analysis of AR-V7 and AR-FL copy numbers (Figure 2), we observe that a subset of patients experiencing reversions (patients 1, 2, and 6) had undetectable AR-V7

and AR-FL during treatment and at progression, reflecting the depletion of CTCs. We note that a limitation of this study is that the AdnaTest platform does not permit CTC enumeration, and thus we are unable to assess the AR-V7 positive-to-negative transitions in light of quantitative changes in CTC number.

The clinical significance of these changes in AR-V7 status remains unknown. There is a theoretical possibility that taxanes

may sensitize AR-V7-positive patients to subsequent AR-directed therapies if the AR-V7 status reverts from positive to negative during taxane therapy. This scenario is exemplified by patient 3, who subsequently exhibited a favorable response to abiraterone lasting 7 months. Another example is patient 2, who converted to AR-V7 positive upon progression on enzalutamide, but subsequently reverted to AR-V7 negative during cabazitaxel therapy; he was then re-treated with abiraterone which resulted in a transient PSA reduction of 37%, as shown in supplementary Figure S2B, available at *Annals of Oncology* online (note: the second abiraterone treatment is not included in Figure 1 because he did not re-enroll in the study during abiraterone re-treatment). Interestingly, although the threshold of 50% PSA reduction was not reached, this patient achieved a greater PSA reduction during his second course of abiraterone treatment (compared with the first), which lasted 3 months before further progression occurred. If this anecdotal trend is substantiated by larger patient numbers, taxane treatment could be entertained following progression on abiraterone/enzalutamide for AR-V7-positive individuals who may then become re-sensitized to further AR-directed therapies. However, not all AR-V7-positive patients undergoing taxane treatment revert to negative, and some AR-V7-negative patients even experience conversions to positive status with taxanes. Furthermore, AR-V7 reversions did not necessarily correlate with PSA responses to taxane treatments.

All abiraterone/enzalutamide-treated patients who were AR-V7 positive at baseline remained positive at follow-up; AR-V7 detection thus appears to persist during therapy with AR-targeting agents. Coupled with observations from our previous study [21], we have yet to report an instance where reversions from AR-V7 positive to negative occurred during treatment with an AR-targeting agent, supporting the hypothesis that potent AR-signaling inhibition may promote the induction and maintenance of the AR-V7-positive phenotype.

The present analysis should be interpreted with caution. Our cohort represents a population with very advanced prostate cancer, and is not reflective of the general CRPC population. While our overall CTC collection protocol also enrolled patients with lower risk features, the inclusion criteria for the current analysis [i.e. ≥ 4 CTC samples (≥ 1 AR-V7 positive) across ≥ 2 therapies] invariably selected for patients with more lethal disease (and is therefore biased), as evidenced by higher Gleason scores and higher PSA at baseline (supplementary Table S2, available at *Annals of Oncology* online). In addition, the criteria for the current analysis selected for patients regularly monitored at our institution who agreed to longitudinal follow-up and periodic blood donations. Although the clinical utility of AR-V7 testing might be greatest in high-risk patient populations, further elucidation of subpopulations that may benefit from AR-V7 analysis will require additional studies.

conclusion

This descriptive analysis suggests that AR-V7 can be reliably detected from peripheral blood CTCs, providing a noninvasive means of serially probing AR-V7 in advanced prostate cancer patients. Importantly, we show that AR-V7 is a dynamic marker: patients may exhibit transitions in AR-V7 status as a result of different treatments. Conversions from AR-V7 negative to positive most often occur in patients undergoing AR-directed

therapies, whereas reversions from AR-V7 positive to negative seem to occur only with taxane chemotherapies. Sequential CTC sampling could provide insights into disease evolution, and follow-up studies will shed further light on the clinical significance of these AR-V7 transitions.

acknowledgements

We thank the study participants and their families.

funding

This research was supported by the Prostate Cancer Foundation (PCF), the Department of Defense (DOD) Prostate Cancer Research Program grant (W81XWH-12-1-0605), the Patrick C. Walsh Fund, the Johns Hopkins Prostate SPORE grant (P50 CA058236), and NIH grant (P30 CA006973).

disclosure

ESA has served as a paid consultant/advisor for Janssen, Astellas, Sanofi, Dendreon, Essa, and Medivation; he has received research funding from Janssen, Johnson & Johnson, Sanofi, Dendreon, Exelixis, Genentech, Novartis, and Tokai; he is a co-inventor of a technology that has been licensed to Tokai. JL has served as a paid consultant/advisor for Astellas, has been a speaker for Sanofi and Gilead, has received research funding from Sanofi, Mirati, Orion, Astellas, and Tokai, and is a co-inventor of a technology that has been licensed to Tokai. Relevant disclosures have been reviewed and approved by Johns Hopkins University in accordance with its conflict of interest policies. All remaining authors have declared no conflicts of interest.

references

1. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2013. *CA Cancer J Clin* 2013; 63: 11–30.
2. Titus MA, Schell MJ, Lih FB et al. Testosterone and dihydrotestosterone tissue levels in recurrent prostate cancer. *Clin Cancer Res* 2005; 11: 4653–4657.
3. Zhu H, Garcia JA. Targeting the adrenal gland in castration-resistant prostate cancer: a case for orteronel, a selective CYP-17 17,20-lyase inhibitor. *Curr Oncol Rep* 2013; 15: 105–112.
4. Chen CD, Welsbie DS, Tran C et al. Molecular determinants of resistance to antiandrogen therapy. *Nat Med* 2004; 10: 33–39.
5. Koivisto P, Nononen J, Palmberg C et al. Androgen receptor gene amplification: a possible molecular mechanism for androgen deprivation therapy failure in prostate cancer. *Cancer Res* 1997; 57: 314–319.
6. Visakorpi T, Hyytinen E, Koivisto P et al. In vivo amplification of the androgen receptor gene and progression of human prostate cancer. *Nat Genet* 1995; 9: 401–406.
7. Culig Z, Hobisch A, Cronauer MV et al. Androgen receptor activation in prostatic tumor cell lines by insulin-like growth factor-I, keratinocyte growth factor, and epidermal growth factor. *Cancer Res* 1994; 54: 5474–5478.
8. Yeh S, Miyamoto H, Shima H et al. From estrogen to androgen receptor: a new pathway for sex hormones in prostate. *Proc Natl Acad Sci USA* 1998; 95: 5527–5532.
9. Locke JA, Guns ES, Lubik AA et al. Androgen levels increase by intratumoral de novo steroidogenesis during progression of castration-resistant prostate cancer. *Cancer Res* 2008; 68: 6407–6415.
10. Chang K-H, Li R, Papari-Zareei M et al. Dihydrotestosterone synthesis bypasses testosterone to drive castration-resistant prostate cancer. *Proc Natl Acad Sci* 2011; 108: 13728–13733.

11. Joseph JD, Lu N, Qian J et al. A clinically relevant androgen receptor mutation confers resistance to 2nd generation anti-androgens enzalutamide and ARN-509. *Cancer Discov* 2013; 3: 1020–1029.
12. Taplin ME, Bubley GJ, Shuster TD et al. Mutation of the androgen-receptor gene in metastatic androgen-independent prostate cancer. *N Engl J Med* 1995; 332: 1393–1398.
13. Nakazawa M, Antonarakis ES, Luo J. Androgen receptor splice variants in the era of enzalutamide and abiraterone. *Horm Cancer* 2014; 5: 265–273.
14. Dehm SM, Tindall DJ. Alternatively spliced androgen receptor variants. *Endocr Relat Cancer* 2011; 18: R183–R196.
15. Hu R, Dunn TA, Wei S et al. Ligand-independent androgen receptor variants derived from splicing of cryptic exons signify hormone-refractory prostate cancer. *Cancer Res* 2009; 69: 16–22.
16. Plymate SR, Luo J. The expression signature of androgen receptor splice variants and their distinctive transcriptional activities in castration-resistant prostate cancer. In *Androgen-Responsive Genes in Prostate Cancer*. New York: Springer 2013; 201–213.
17. Guo Z, Yang X, Sun F et al. A novel androgen receptor splice variant is up-regulated during prostate cancer progression and promotes androgen depletion-resistant growth. *Cancer Res* 2009; 69: 2305–2313.
18. Hu R, Isaacs WB, Luo J. A snapshot of the expression signature of androgen receptor splicing variants and their distinctive transcriptional activities. *Prostate* 2011; 71: 1656–1667.
19. Li Y, Chan SC, Brand LJ et al. Androgen receptor splice variants mediate enzalutamide resistance in castration-resistant prostate cancer cell lines. *Cancer Res* 2013; 73: 483–489.
20. Mostaghel EA, Marck BT, Plymate SR et al. Resistance to CYP17A1 inhibition with abiraterone in castration-resistant prostate cancer: induction of steroidogenesis and androgen receptor splice variants. *Clin Cancer Res* 2011; 17: 5913–5925.
21. Antonarakis ES, Lu C, Wang H et al. AR-V7 and resistance to enzalutamide and abiraterone in prostate cancer. *N Engl J Med* 2014; 371: 1028–1038.
22. Antonarakis ES, Lu C, Luber B et al. Androgen receptor splice variant 7 and efficacy of taxane chemotherapy in patients with metastatic castration-resistant prostate cancer. *JAMA Oncol* 2015 June 4 [pub ahead of print], doi:10.1001/jamaoncol.2015.1341.
23. Lorente D, Mateo J, Perez-Lopez R et al. Sequencing of agents in castration-resistant prostate cancer. *Lancet Oncol* 2015; 16: e279–e292.
24. Bianchini D, Lorente D, Rodriguez-Vida A et al. Antitumour activity of enzalutamide (MDV3100) in patients with metastatic castration-resistant prostate cancer (CRPC) pre-treated with docetaxel and abiraterone. *Eur J Cancer* 2014; 50: 78–84.
25. Loriot Y, Bianchini D, Ileana E et al. Antitumour activity of abiraterone acetate against metastatic castration-resistant prostate cancer progressing after docetaxel and enzalutamide (MDV3100). *Ann Oncol* 2013; 24: 1807–1812.
26. Nadal R, Zhang Z, Rahman H et al. Clinical activity of enzalutamide in docetaxel-naïve and docetaxel-pretreated patients with metastatic castration-resistant prostate cancer. *Prostate* 2014; 74: 1560–1568.
27. van Soest RJ, de Morree ES, Kweldam CF et al. Targeting the androgen receptor confers in vivo cross-resistance between enzalutamide and docetaxel, but not cabazitaxel, in castration-resistant prostate cancer. *Eur Urol* 2015; 67: 981–985.

Annals of Oncology 26: 1865–1870, 2015
doi:10.1093/annonc/mdv254
Published online 2 June 2015

Treatment outcome and patterns of relapse following adjuvant carboplatin for stage I testicular seminomatous germ-cell tumour: results from a 17-year UK experience

C. Chau^{1,2,3}, R. Cathomas⁴, M. Wheeler³, D. Klingbiel⁵, M. Fehr⁶, J. Bennett⁷, H. Markham⁸, C. Lee^{1,3}, S. J. Crabb^{1,3} & T. Geldart^{7*}

¹Cancer Sciences Unit, University of Southampton Faculty of Medicine, Southampton; ²NIHR Wellcome Trust Clinical Research Facility, University of Southampton, Southampton; ³Department of Medical Oncology, University Hospital Southampton NHS Foundation Trust, Southampton, UK; ⁴Department of Medical Oncology, Kantonsspital Graubünden, Chur; ⁵Swiss Group for Clinical Cancer Research Coordinating Center, Bern; ⁶Department of Medical Oncology, Kantonsspital St Gallen Hospital, St Gallen, Switzerland; ⁷Dorset Cancer Centre, Poole Hospital NHS Foundation Trust, Poole; ⁸Department of Histopathology, University Hospital Southampton NHS Foundation Trust, Southampton, UK

Received 4 February 2015; revised 19 May 2015; accepted 22 May 2015

Background: Following inguinal orchidectomy, management options for patients with stage I seminoma include initial surveillance or treatment with adjuvant radiotherapy or chemotherapy. The anticipated relapse rate for patients followed by surveillance alone is ~15%, with adjuvant treatment this risk is reduced to ~4%–5% at 5 years. After carboplatin treatment, follow-up strategies vary and there are no validated, predictive markers of relapse.

Patients and methods: We conducted a retrospective analysis of all patients presenting with stage I seminoma who received a single cycle of adjuvant carboplatin in South Central England between 1996 and 2013. We report on outcome and the results of univariate and multivariate analysis evaluating possible risk factors for post carboplatin relapse.

*Correspondence to: Dr Thomas Geldart, Dorset Cancer Centre, Poole Hospital NHS Foundation Trust, Poole BH15 2JB, UK. Tel: +44-1202-665511; E-mail: tom.geldart@rbch.nhs.uk