Association of AR-V7 on Circulating Tumor Cells as a Treatment-Specific Biomarker With Outcomes and Survival in Castration-Resistant Prostate Cancer

Howard I. Scher, MD; David Lu, PhD; Nicole A. Schreiber, BA; Jessica Louw, BS; Ryon P. Graf, PhD; Hebert A. Vargas, MD; Ann Johnson, MS; Adam Jendrisak, MBA; Richard Bambury, MB, BCH, BAO; Daniel Danila, MD; Brigit McLaughlin, BS; Justin Wahl, BS; Stephanie B. Greene, PhD; Glenn Heller, PhD; Dena Marrinucci, PhD; Martin Fleisher, PhD; Ryan Dittamore, MBA

IMPORTANCE A critical decision in the management of metastatic castration-resistant prostate cancer (mCRPC) is when to administer an androgen receptor signaling (ARS) inhibitor or a taxane.

OBJECTIVE To determine if pretherapy nuclear androgen-receptor splice variant 7 (AR-V7) protein expression and localization on circulating tumor cells (CTCs) is a treatment-specific marker for response and outcomes between ARS inhibitors and taxanes.

DESIGN, SETTING, AND PARTICIPANTS For this cross-sectional cohort study at Memorial Sloan Kettering Cancer Center, 265 men with progressive mCRPC undergoing a change in treatment were considered; 86 were excluded because they were not initiating ARS or taxane therapy; and 18 were excluded for processing time constraints, leaving 161 patients for analysis. Between December 2012 and March 2015, blood was collected and processed from patients with progressive mCRPC immediately prior to new line of systemic therapy. Patients were followed up to 3 years.

MAIN OUTCOMES AND MEASURES Prostate-specific antigen (PSA) response, time receiving therapy, radiographic progression-free survival (rPFS), and overall survival (OS).

RESULTS Overall, of 193 prospectively collected blood samples from 161 men with mCRPC, 191 were evaluable (128 pre-ARS inhibitor and 63 pretaxane). AR-V7–positive CTCs were found in 34 samples (18%), including 3% of first-line, 18% of second-line, and 31% of third- or greater line samples. Patients whose samples had AR-V7–positive CTCs before ARS inhibition had resistant posttherapy PSA changes (PTPC), shorter rPFS, shorter time on therapy, and shorter OS than those without AR-V7–positive CTCs. Overall, resistant PTPC were seen in 65 of 112 samples (58%) without detectable AR-V7–positive CTCs prior to ARS inhibition. There were statistically significant differences in OS but not in PTPC, time on therapy, or rPFS for patients with or without pretherapy AR-V7–positive CTCs treated with a taxane. A multivariable model adjusting for baseline factors associated with survival showed superior OS with taxanes relative to ARS inhibitors when AR-V7–positive CTCs were detected pretherapy (hazard ratio, 0.24; 95% CI, 0.10-0.57; \( P = .035 \)).

CONCLUSIONS AND RELEVANCE The results validate CTC nuclear expression of AR-V7 protein in men with mCRPC as a treatment-specific biomarker that is associated with superior survival on taxane therapy over ARS-directed therapy in a clinical practice setting. Continued examination of this biomarker in prospective studies will further aid clinical utility.
Patients with progressive, metastatic castration-resistant prostate cancer (mCRPC) are often classified on the basis of prior chemotherapy exposure, considered by many to provide modest clinical benefit relative to the overall burden of treatment. Consequently, many patients who might benefit from chemotherapy never receive it, while others are only offered chemotherapy as a last resort when tolerance and overall response rates are poor. Multiple approved therapeutic options with diverse mechanisms of action proven to prolong life are currently available—at issue is how best to use them to maximize benefit for individual patients, decisions that are often empirically rather than scientifically based. Simply reviewing the data from registration trials can be misleading because the eligibility criteria are optimized for success and by the fact that patients treated on clinical protocols often experience outcomes superior to those treated in a clinical setting. Further, although line of therapy and sequence of administration do matter, patterns of cross-sensitivity and drug resistance are not predictable from patient to patient.

This dilemma led the Prostate Cancer Working Group (PCWG3) to reclassify the clinical states of mCRPC based on the order individual treatments are administered, regardless of type. Validated predictive biomarkers are needed to guide therapeutic decisions.

Circulating tumor cells (CTCs) are a potential source of tumor for profiling that can be serially obtained with minimal patient discomfort. Studies using a range of platforms in multiple tumor types have shown that prognosis is worse in patients with detectable CTCs vs those without. Serial biologic characterization of CTCs can provide insights into drivers of tumor growth in patients, allowing the pharmacodynamic effects of targeted therapies to be assessed, potentially enabling the prediction of sensitivity to a specific treatment as the disease evolves over time. The promise offered by these analyses in research contrasts sharply with their use in practice. Needed in both cases, however, are validated assays for predictive biomarkers to inform the selection of a specific therapy for a specific patient at a specific point in time.

Prostate cancer is an androgen-dependent disease. Even tumors that are resistant to castration remain androgen receptor (AR) dependent. Androgen receptor splice variants lack the C-terminal ligand-binding domain but retain the N-terminal transcriptional elements that can activate AR signaling (ARS) independent of ligand. In a recent report, detection of the androgen-receptor splice variant 7 (AR-V7) messenger RNA (mRNA) transcript in pooled epithelial cell adhesion molecule (EpCAM)-positive CTCs of men with progressive mCRPC was associated with resistance to the ARS inhibitors abiraterone and enzalutamide. The same group later demonstrated that the presence of AR-V7 mRNA in CTCs did not predict response to taxanes. This finding was validated by an independent group using a similar assay that found no association between the presence of AR-V7 transcripts and response to cabazitaxel. Together, the results suggest that AR-V7 could represent a biomarker to guide treatment selection in mCRPC.

Herein, we report on the analytical and clinical validation of an AR-V7 protein immunofluorescent assay run on the Epic Sciences non-EpCAM-based CTC detection platform. The context of use is the clinical decision point at which a change in systemic therapy is needed. The focus was the association between the pretherapy detection of AR-V7-positive CTCs with line of therapy and objective clinical outcomes following treatment with the most frequently used, approved drug classes for management of mCRPC: ARS inhibitors and taxanes.

Key Points

**Question** Can the measurement of the nuclear androgen-receptor splice variant 7 (AR-V7) protein in circulating tumor cells (CTCs) be a treatment-specific biomarker in metastatic castration-resistant prostate cancer (mCRPC) at therapeutic decision points in the first-line, second-line, or third or greater line setting?

**Findings** In this cross-sectional cohort study, mCRPC patients with pre-androgen receptor signaling (ARS) inhibitor AR-V7-positive CTCs had resistant prostate-specific antigen responses, shorter time on therapy, shorter radiographic progression-free survival, and inferior overall survival. In a multivariable model incorporating line of therapy and other clinical features, AR-V7 status showed significant treatment-specific interaction with taxane administration.

**Meaning** Pretherapy CTC nuclear expression of AR-V7 protein in men with mCRPC is a treatment-specific biomarker predicting superior overall survival for taxane therapy over ARS inhibitors in a clinical practice setting, warranting prospective validation.

**Methods**

**Patient Selection**

Between December 2012 and March 2015, 265 patients with histologically confirmed mCRPC undergoing a change in systemic therapy for progressive disease were treated at Memorial Sloan Kettering Cancer Center (MSKCC). Of these, 104 were excluded because they were not starting therapy with abiraterone acetate, enzalutamide, ARN-590, docetaxel, cabazitaxel, or paclitaxel, or owing to constraints on processing time, leaving 161 evaluable samples from 161 unique patients for analysis (eFigure 1 in the Supplement).

All patients underwent a history evaluation that included stage at diagnosis, initial management and all subsequent systemic therapies, a physical examination, and laboratory studies including complete blood cell count, chemistry panel (albumin, alkaline phosphatase, lactate dehydrogenases, and creatinine), and serum testosterone to confirm castration status (<50 ng/dL [to convert to nmol/L, multiply by 0.0347]). Documentation of disease progression required a minimum of 2 rising prostate-specific antigen (PSA) levels 1 or more weeks apart, new lesions by bone scintigraphy, and/or new or enlarging soft tissue lesions by computed tomography (CT) or magnetic resonance imaging (MRI), per the Prostate Cancer Clinical Trials Working Group 2 (PCWG2) guidelines. All patients signed consent forms based on an institutional review board–approved protocol, and blood samples
Posttreatment Outcomes

For each treatment course, antitumor effects were assessed by the posttherapy PSA changes (PTPC). For AR inhibitors, “sensitive” was defined as a 50% or greater decline from baseline at 12 weeks, but for taxane treatment, 12 weeks or more was used because the maximal decline may occur later.18 For both therapies, “resistant” was defined as the failure to achieve a 50% or greater decline. Radiographic progression-free survival (rPFS), time receiving therapy, and overall survival (OS) were also reported. Time receiving therapy was calculated from initiation of therapy until date of drug discontinuation for any reason. Radiographic progression was determined by independent blinded review of available radionuclide bone scans, CTs, or MRIs, using the PCWG2 criteria,17 and calculated from therapy initiation until radiologically confirmed progression or death owing to any cause within 60 days of stopping treatment. Patients without evidence of radiologic progression at the time of last stable scan or end of therapy, whichever occurred later, were right censored. Overall survival was calculated from initiation of therapy to death from any cause. Patients still alive at time of last follow-up were right-censored.

CTC Collection

Blood (7.5 mL) from each participant was collected in Streck tubes and processed at MSKCC or shipped to Epic Sciences and processed within 48 hours. Red blood cells were lysed, and approximately 3 million nucleated blood cells were dispensed.
onto 10-16 glass microscope slides (25.3 mm × 75.3 mm) and placed at −80°C for long-term storage as previously described. Sample processing and testing were conducted in laboratories following Clinical Laboratory Improvement Amendments (CLIA) regulations.

**Analytical Validation: Specificity of AR-V7 Detection**

As per common practice for verifying the accuracy of diagnostic-grade antibodies, the AR-V7 antibody was comprehensively tested via tissue microarrays containing malignant, tumor-adjacent, and healthy tissue samples (eFigure 2F in the Supplement). An independent pathologist scored the samples for background staining and cross-reactivity. AR-V7-positive and AR-V7-negative mCRPC patient tissue were screened and included as part of the tissue microarray panel as positive and negative controls, respectively.

**CTC Immunofluorescent Staining and Analysis**

Slides created from healthy donor blood samples spiked with prostate cancer cell line cells (controls), or from mCRPC patient samples, underwent automated immunofluorescent staining for DNA, cytokeratins (CK), CD45 (lymphocyte common antigen), and AR-V7 (Figure 1), as previously described. 15,16 A rabbit monoclonal anti-AR-V7 antibody (EPR15656; Abcam) was used for all AR-V7 applications herein described. Separate slides from patient samples were tested with a second automated immunofluorescent assay, staining for DNA, CK, CD45, and the AR N-terminal domain. Up to 2 slides were evaluated per blood sample per assay. Fluorescent scanners and morphology algorithms were used to identify CTCs, CTC clusters, apoptotic CK-positive cells, and CK-negative CTCs. A more thorough description of CTC types has been published previously. 16 Clinical laboratory scientists (licensed in California) conducted final quality control of CTC subpopulation classification and subcellular biomarker localization.

AR-V7 and AR N-terminal positivity were defined by protein expression level above a threshold intensity (eFigure 2 in the Supplement). The expression threshold was defined by signal quantitation above background relative to AR-V7-negative or AR N-terminal-negative control cell lines, as appropriate. Nuclear localization was also required to classify
CTCs as AR-V7 positive. Apoptotic CTCs were not included or reported in subsequent analyses, as nuclear fragmentation precludes protein localization analysis.

The specificity of AR-V7 protein detection in single prostate cancer cell line cells spiked into whole blood was corroborated by single-cell mRNA analyses (eFigure 2A-D in the Supplement). The requirement for AR-V7 protein signal localization in the nucleus is consistent with AR-V7-mediated ligand-independent proliferation in preclinical models.\(^{20,21}\) AR-V7 localization in human solid tumor tissue,\(^{10,22,23}\) and previously validated AR-V7 prognostic tissue scoring criteria.\(^{23}\) Sample-level specificity of AR-V7-positive staining in CTCs was established by staining up to 2 additional slides per sample with a separate AR N-terminal immunofluorescent assay. Samples with at least 1 AR-V7-positive or AR N-terminal-positive CTC were considered positive for the respective biomarker (Figure 2).

**Statistical Analyses**

Patient demographics and clinical characteristics at the time of blood draw were evaluated by descriptive statistics: overall, by line of therapy, and by drug administered. Fisher exact and Wilcoxon rank sum tests were used to compare treatment groups for categorical and continuous characteristics, respectively.

The association of AR-V7 status (positive or negative) with resistant or sensitive PTPC was evaluated using univariable logistic regression. Time-to-event outcomes were evaluated using the Kaplan-Meier method. Differences in time-to-event outcomes between patient samples with AR-V7-positive and AR-V7-negative CTCs were evaluated using the log-rank test. The association of AR-V7 status with time-to-event outcomes was evaluated with hazard ratios (HRs) estimated from univariable and multivariable Cox proportional hazards regression methods. The pretherapy predictors evalu-
ated for the multivariable Cox proportional hazards models included line of therapy, presence of liver and/or lung metastases, lactate dehydrogenase levels, patient age, hemoglobin levels, type of therapy, AR-V7 status, PSA levels, albumin levels, and alkaline phosphatase levels. Using a best subset selection method based on the global score $\chi^2$ statistic, pre-therapy PSA, albumin, and alkaline phosphatase were excluded from the final model. To address unique patients having exposures to more than 1 therapy, the robust sandwich estimate for the covariance matrix was implemented for all Cox proportional hazards models to correct for possible underestimation of variance. All statistical analyses were 2-sided and performed at the 5% significance level using SAS version 9.4 statistical software (SAS Institute Inc).

Results

Clinical Characteristics of the Patient Population
A cohort of 161 patients with mCRPC who received a total of 193 treatments between September 2012 and March 2015 were evaluated. Of these, 130 patients (80.8%) had a single therapy; 30 (18.6%), 2 therapies (60 samples); and 1 (0.6%), 3 therapies. Patient and treatment characteristics at the time of sample collection are detailed in the Table.

Analytical Validation: Specificity of AR-V7 Detection
Tissue microarrays containing representative cancer, near-tumor adjacent tissue, and healthy tissues alongside positive and negative control samples were used to test assay specificity in an immunohistochemical format akin to the immunofluorescent CTC assay. An independent pathologist assessed the TMA slides and found no significant off-target staining or background signal on the tissue sections. Importantly, the tissues on the array most pertinent as potential sources of CTCs, such as liver, lymph nodes, and bone, showed no appreciable specific or off-target staining, indicative of a highly AR-V7-specific antibody (eFigure 2F in the Supplement).

Specificity of AR-V7 in Patient Samples
Parallel AR assays (AR N-terminal and AR-V7) run on sister slides revealed greater incidence of AR N-terminal-positive CTCs relative to AR-V7-positive CTCs (eFigure 2F in the Supplement), consistent with previous studies on AR-V7 prevalence in relation to total AR gene product. Of the 193 samples tested, 191 (99%) were evaluable (2 samples were invaluable for subcellular localization): 72 samples (38%) were AR N-terminal positive and AR-V7 negative; 4 samples (2%) were AR N-terminal negative and AR-V7 positive; 30 samples (16%) were AR N-terminal positive and AR-V7 positive; and 85 samples (44%) were AR N-terminal negative and AR-V7 negative.

AR-V7 Is Expressed in Multiple CTC Subtypes
AR-V7 expression was found on a variety of CTC subtypes, including traditional CK-positive single CTCs (Figure 1A), CTC clusters (Figure 1B), and CK-negative CTCs (Figure 1C). Hereafter, all 3 subtypes will collectively be referred to as CTCs.

Prevalence and Frequency of AR-V7 CTC Positivity Increases by Line of Therapy
The majority of the CTCs detected were AR-V7 negative (Figure 2D). AR-V7-positive CTCs were detected in 34 samples with AR-V7-positive CTC burden ranging from 0.74/mL to 105/mL (median 2.4/mL), exhibiting a wide range of subclonal contribution to total CTCs (median [range], 22% [0.3%-100%]) (Figure 2). AR-V7-positive CTC detection frequency increased by line of therapy (Figure 2A-C), ranging from 3% (2 of 67 samples) prior to first-line therapy, 18% (9 of 50 samples) prior to second-line therapy, and 31% (23 of 74 samples) prior to third or subsequent lines of therapy (Figure 2D; eTable in the Supplement) ($P < .001$).

Presence of AR-V7-Positive CTCs Predicts Posttherapy PSA Change, rPFS, Time Receiving Therapy, and OS With ARS Inhibitors
Of the 128 samples from patients treated with ARS inhibitors, 47 (37%) showed sensitive and 81 (63%) had resistant PTPC. None of the 47 with sensitive PTPC had AR-V7-positive CTCs (0%; 95% CI, 0.0%-9.41%). In contrast, 16 of the 81 with resistant PTPC (20%; 95% CI, 12.1%-30.4%) had AR-V7-positive CTCs prior to therapy (Figure 3A). Three of these 16 samples had AR-V7 expression exclusively in CK-negative CTCs. A subset analysis of the pre-ARS therapy samples with PSA-resistant profiles ($n = 81$) showed dramatically worse OS with pretherapy AR-V7-positive CTCs relative to those without (median, 4.6 months vs not reached; $P < .001$) (eFigure 4 in the Supplement).

Pre-ARS inhibitor samples with AR-V7-positive CTCs were associated with worse outcomes in all time-to-event measures: rPFS (median, 2.3 vs 14.5 months; $P < .001$), time on therapy (median, 2.1 vs 6.8 months; $P < .001$), and OS (median, 4.6 months vs not reached; $P < .001$) (Figure 3; eFigure 3 in the Supplement). This was not the case for pretaxane samples, where time on therapy (median, 3.0 vs 3.7 months; $P = .23$) and rPFS (median, 5.3 vs 6.6 months; $P = .46$) were not significantly different by AR-V7 status. There was a significant difference in OS for pretaxane AR-V7-positive vs AR-V7-negative samples (median, 8.9 vs 19.8 months; $P < .001$). However, this difference is best interpreted in the multivariable setting.

Patients Harboring Pretherapy AR-V7-Positive CTCs Experience Better OS With Taxanes Than With ARS Inhibitors After Adjusting for Clinical Measures
Patients with AR-V7-positive CTCs had longer median survival with taxanes relative to ARS inhibitors (median, 8.9 vs 4.6 months) even though taxanes tended to be administered later (Figure 3A and B) when disease burdens were greater. To adjust for this imbalance, a Cox proportional hazards model incorporating line of therapy, presence of visceral metastases, lactate dehydrogenase, patient age, hemoglobin, therapy type, and AR-V7 status was developed. Results showed that AR-V7 status remained the most significant factor ($P < .001$) among all pretreatment clinical measures (Figure 4A), and that patients who were AR-V7 positive had more favorable survival times with taxanes relative to ARS inhibitors (HR, 0.24;
95% CI, 0.10-0.57; \( P = .035 \), while patients who were AR-V7 negative did not (HR, 0.92; 95% CI, 0.44-1.95) (Figure 4B).

When applied to AR-N-terminal-positive CTCs (inclusive of full-length AR and most splice variants), the same analysis showed a trend for improved survival with taxanes relative to ARS inhibitors (HR, 0.59; 95% CI, 0.32-1.08) (eFigure 5 in the Supplement). However, this effect was not statistically significant (\( P = .11 \)).

**Discussion**

The goal of a treatment-specific predictive biomarker is to determine patients likely to have a poor outcome to a particular drug or drug class and simultaneously identify a potentially effective, already approved alternative therapy. Focusing on the context of use of predicting response to either ARS inhibi-
mRNA transcript detection approaches. In contrast, no associations of others, suggest that patients in whom AR-V7–positive CTCs were resistant to treatment with ARS inhibitors. Every patient harboring AR-V7–positive cells were resistant to treatment with ARS inhibitors (eFigure 4 in the Supplement). The clinical implication is that, within each line of therapy, once AR-V7–positive cells are detected, the preferred choice of therapy is a taxane rather than an ARS inhibitor.

### Conclusions

The treatment-specific effect for taxane therapy in the setting of AR-V7 positivity was shown when baseline factors associated with survival were accounted for in a multivariable Cox proportional hazards model: patients who had AR-V7–positive CTCs treated with taxanes had a much lower risk of death than those on ARS inhibitors (HR, 0.24; 95% CI, 0.10-0.57; P = .035). This level of evidence has not been achieved with other AR-V7 testing modalities. Given the magnitude of stratification and outcome specificity of the nuclear-specific AR-V7 protein test in CTCs, a diagnostic-grade test that informs the selection of ARS inhibitors or taxanes has the potential to significantly improve outcomes, by enabling patients to receive treatments to which they are most likely to respond while avoiding the toxic effects and costs associated with an ineffective treatment. Prospective trials to validate these findings and further elucidate clinical utility are currently in development.

---

**ARTICLE INFORMATION**

**Accepted for Publication:** April 21, 2016.

**Correction:** This article was corrected online September 29, 2016, for incorrect author affiliations and to add missing table headers to eTable 1 in the Supplement and was corrected on November 10, 2016, for an error in the caption of Figure 2.

**Published Online:** June 4, 2016. doi:10.1001/jamaoncol.2016.1828

---

**Open Access:** This article is published under JAMA Oncology’s open access model and is free to read on the day of publication.

**Author Affiliations:** Genitourinary Oncology Service, Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, New York (Scher, Schreiber, Bambury, Danila, McLaughlin); Department of Medicine, Weill Cornell Medical College, New York, New York (Scher, Danila); Epic Sciences, La Jolla, California (Lu, Louw, Graf, Johnson, Jendrisak, Wahl, Greene, Marrinucci, Dittamore); Body Imaging Service, Department of Radiology, Memorial Sloan Kettering Cancer Center, New York, New York (Vargas); Department of Radiology, Weill Cornell Medical College, New York, New York (Vargas); Cancer Services, Department of Medical Oncology, Cork University Hospital, Wilton, Cork, Ireland (Bambury); Biostatistics Service, Department of Epidemiology and Biostatistics, Memorial Sloan Kettering Cancer Center, New York, New York.
AR-V7 Expression in Circulating Tumor Cells and Castration-Resistant Prostate Cancer Outcomes

Original Investigation Research

AR-V7ExpressioninCirculatingTumorCellsandCastration-ResistantProstateCancerOutcomes

November 2016 Volume 2, Number 11 JAMA Oncology

New York (Heller); Clinical Chemistry Service, Department of Laboratory Medicine, Memorial Sloan Kettering Cancer Center, New York, New York (Fleisher).

Author Contributions: Dr Scher had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Scher, Bambury, Danila, Marrinucci, Fleisher, Dittamore.

Acquisition, analysis, or interpretation of data: Scher, Lu, Schreiber, Louw, Graf, Vargas, Johnson, Jendrisak, Bambury, Danila, McLaughlin, Wahl, Greene, Heller, Dittamore.

Drafting of the manuscript: Scher, Lu, Schreiber, Graf, Johnson, Jendrisak, Danila, Dittamore.

Critical revision of the manuscript for important intellectual content: Scher, Lu, Schreiber, Louw, Graf, Vargas, Jendrisak, Bambury, Danila, McLaughlin, Wahl, Greene, Heller, Marrinucci, Fleisher, Dittamore.

Statistical analysis: Louw, Graf, Johnson, Jendrisak, Heller.

 Obtained funding: Scher.

 Administrative, technical, or material support: Scher, Lu, Schreiber, Louw, Graf, Vargas, Bambury, Danila, McLaughlin, Marrinucci, Fleisher, Dittamore.

Study supervision: Scher, Graf, Vargas, Marrinucci, Fleisher, Dittamore.

Assay development: Lu.

Patient sample testing: Lu.

Conflict of Interest Disclosures: Dr Scher reports nonfinancial support from Janssen and Medivation, personal fees from Astellas and Sanofi Aventis, and research funding from Janssen Diagnostics, Janssen Pharmaceuticals, and Medivation. Dr Danila reports financial support from Janssen and Medivation. Astellas. Drs Lu, Graf, Greene, Marrinucci, Ms Louw, Ms Johnson; and Mr Jendrisak, Mr Wahl, and Mr Dittamore are employees of Epic Sciences. No other disclosures are reported.

Funding/Support: This study was supported by equal distributions of funds from the National Institutes of Health (grant no. NIH/NCI P50 CA076271, SPORE in Prostate Cancer; and NIH/NCI Cancer Center Support Grant P30 CA008748); the Department of Defense Prostate Cancer Research Program (grants PC071610 and PC121111), the Prostate Cancer Foundation Challenge Award, and the David H. Koch Fund for Prostate Cancer Research.

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

Previous Presentation: This study was presented at the 2016 American Society of Clinical Oncology (ASCO) meeting, June 4; 2016, Chicago, Illinois.

Additional Contributions: We would like to thank the patients and their families taking part in this study; the clinical and laboratory staff at MSKCC and Epic Sciences. We would additionally like to thank Kyle Botsch, BS, and Connie Landaverde, PhD, from Epic Sciences for technical laboratory support for the analyses used in the Supplemental Data, and Margaret McPartland, BA, an independently supported editor at MSKCC, for editorial assistance. Compensation was not received for their contributions and written permission was obtained for their inclusion in the Acknowledgments.

REFERENCES


Downloaded From: https://oncology.jamanetwork.com/ by a Non-Human Traffic (NHT) User on 07/21/2019