

AWARD NUMBER:
W81XWH-17-1-0124

TITLE:
Identifying Genomic Drivers of Prostate Cancer Progression and Drug Resistance in Tumors and Circulating DNA

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REPORT DATE:
SEPTEMBER 2022

TYPE OF REPORT:
FINAL

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

DISTRIBUTION STATEMENT: Approved for Public Release;
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REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
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1. REPORT DATE SEPTEMBER 2022		2. REPORT TYPE FINAL		3. DATES COVERED 22May2017-21May2022	
4. TITLE AND SUBTITLE Identifying Genomic Drivers of Prostate Cancer Progression and Drug Resistance in Tumors and Circulating DNA				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER W81XWH-17-1-0124	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) WASSIM ABIDA MD PHD E-Mail: abidam@mskcc.org				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) SLOAN KETTERING INSTITUTE 1275 YORK AVENUE NEW YORK, NY 10065				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Development 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 The landscape of genomic alterations in early and advanced prostate cancer has been well established. However, the association of genomic changes with disease progression and clinical outcomes and their application to clinical decision-making remain unclear. In my initial application, I proposed to leverage evolving large genomic datasets to identify genomic drivers of prostate cancer progression and drug resistance. Since the start of the project, I have accomplished the major goals outlined in the application: I led the publication of a large multi-institutional study correlating advanced prostate cancer genomics with clinical outcomes (the SU2C-PCF dataset) and published several large studies including the characterization of microsatellite instability-high prostate cancer, CDK12 mutations across solid tumors, DNA repair alterations and responses to PARP inhibition and platinum chemotherapy, genomic associations with outcomes in newly diagnosed metastatic prostate cancer, and racial genomic disparities.					
15. SUBJECT TERMS Prostate Cancer, Genomics, Drug Resistance					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT Unclassified	18. NUMBER OF PAGES 117	19a. NAME OF RESPONSIBLE PERSON USAMRMC
a. REPORT Unclassified	b. ABSTRACT Unclassified	c. THIS PAGE Unclassified			19b. TELEPHONE NUMBER (include area code)

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1. INTRODUCTION:

In this project, I proposed to identify genomic drivers of prostate cancer progression and drug resistance using prostate cancer tumor genomic datasets and cell free DNA (cfDNA) analysis. I have close involvement in large genomic projects (SU2C-PCF International Prostate Cancer Dream Team Project, MSK-IMPACT tumor profiling), and mentorship by world-experts in the field (Drs Charles Sawyers and Dr Howard Scher), that have allowed me to achieve the aims of the project. The ultimate goal of the project is to develop biomarkers and therapies for mCRPC based on the understanding of genomic drivers of resistance.

2. **KEYWORDS:** Genomics, Prostate Cancer, Androgen Receptor Targeting, Drug resistance, Molecular Profile, Clinical Outcomes, Clonality

3. ACCOMPLISHMENTS:

a. What were the major goals of the project?

The major goals of the project are to:

- Determine genomic features associated with resistance to enzalutamide and abiraterone using a large multi-institutional genomic dataset (SU2C-PCF). This goal is 100% complete, in line with the approved SOW (Major Task 1).
- Investigate genomic drivers of disease progression using matched tumors from patients who developed metastatic prostate cancer. This goal is approximately 100% complete, in line with the approved SOW (Major Task 2).
- Identify genomic drivers of disease progression and resistance to enzalutamide and abiraterone acetate in cell free DNA. This goal is approximately 100% complete, in line with the approved SOW (Major Task 3).

b. What was accomplished under these goals?

The aims outlined in the initial application have been accomplished, following the timeline indicated in the SOW and extended for 1 year with an NCE.

AIM1: Determine genomic features associated with resistance to abiraterone and enzalutamide

Major Activities:

As reported in the 2019 Annual Report (submitted JUNE 2019), and in line with the proposed in the SOW, work for this aim is complete. We completed clinical annotation for a dataset of 444 tumors from 429 patients with mCRPC who underwent tumor sequencing as part of the SU2C-PCF multi-institutional project. I led the clinical annotation and analysis correlating tumors genomics with clinical outcomes in this large dataset, the largest reported to date for mCRPC, and finalized the principal analysis outlined in the proposal (Aim 1), leading to a publication in *PNAS*.

Specific objectives:

The objectives proposed in the SOW were: 1- complete clinical annotation for subject who are part of the SU2C-PCF mCRPC genomic dataset, 2- perform initial analyses correlating genomics with outcomes, 3- expand the dataset size and 4- finalize the analyses

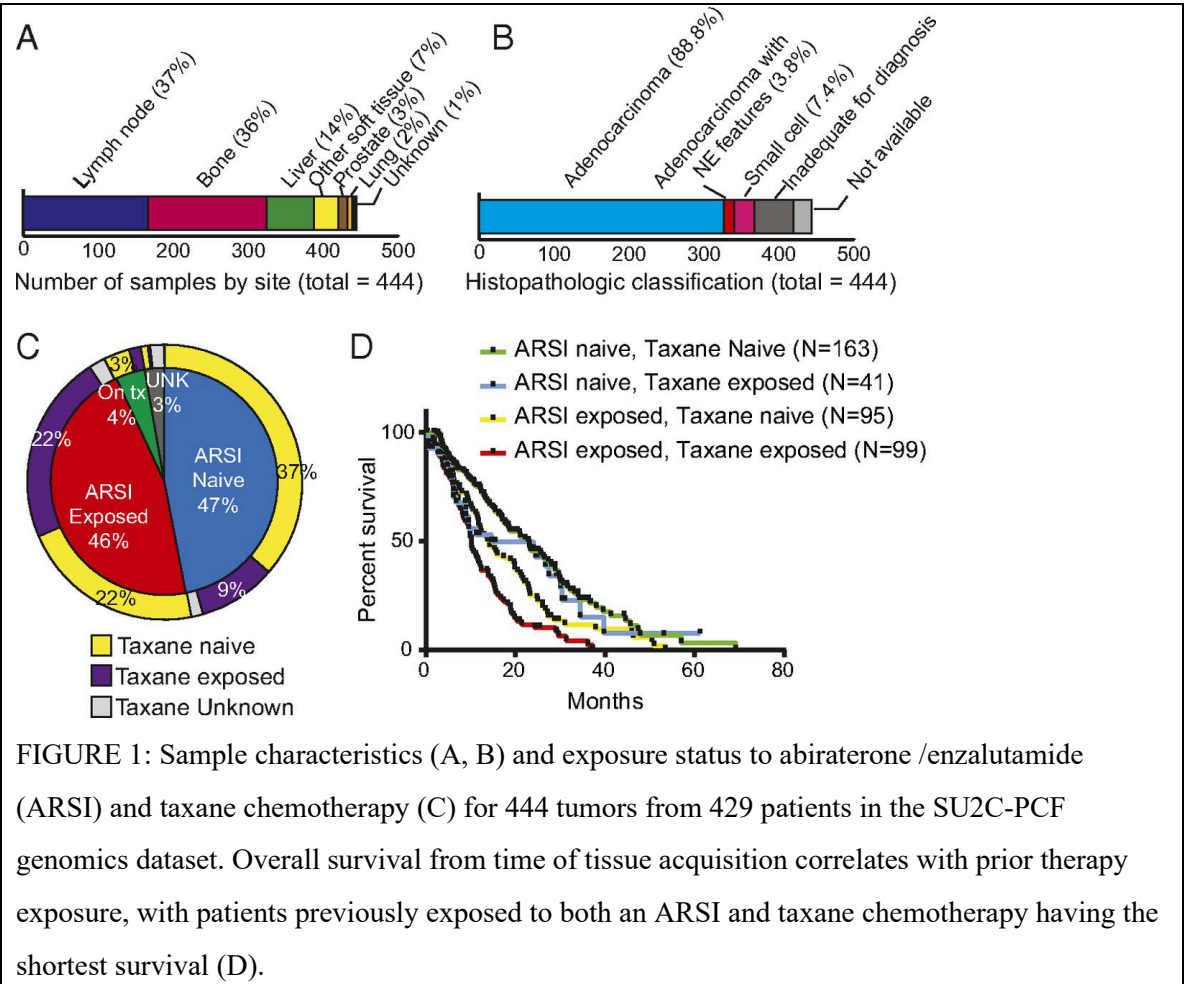
Significant results/key outcomes:

As reported in the 2019 Annual Report (submitted JUNE 2019), all objectives have been met following the timeline set in the SOW. Specifically, clinical annotation was completed for 444 tumors from 429 subjects with mCRPC who have undergone tumor sequencing, and analysis was performed correlating tumor genomics with clinical outcomes. While the SOW proposed to expand to 500 subjects, it has been difficult to reach this number due to sequencing failure, and 429 subjects was deemed to be a sufficiently high number of subjects to establish an analysis freeze and proceed with final analyses. This is the largest dataset of mCRPC genomics, integrating whole exome and RNA sequencing, and now includes annotation of clinical outcomes, namely survival and time on treatment with abiraterone and enzalutamide.

Final sample characteristics and exposure status to prior standard mCRPC therapies are shown in FIGURE 1, as well as overall survival from the time of tissue acquisition, which expectedly correlates with prior treatment exposure.

We explored the co-occurrence and mutual exclusivity of genomic alterations in the dataset to identify genomic interactions that may be biological relevant. Several interesting interactions emerged: namely the co-occurrence of alterations in TP53 and

RB1, CDK12 and CCND1, and the mutual exclusivity of alterations in CDKN1B and AR (FIGURE 2).



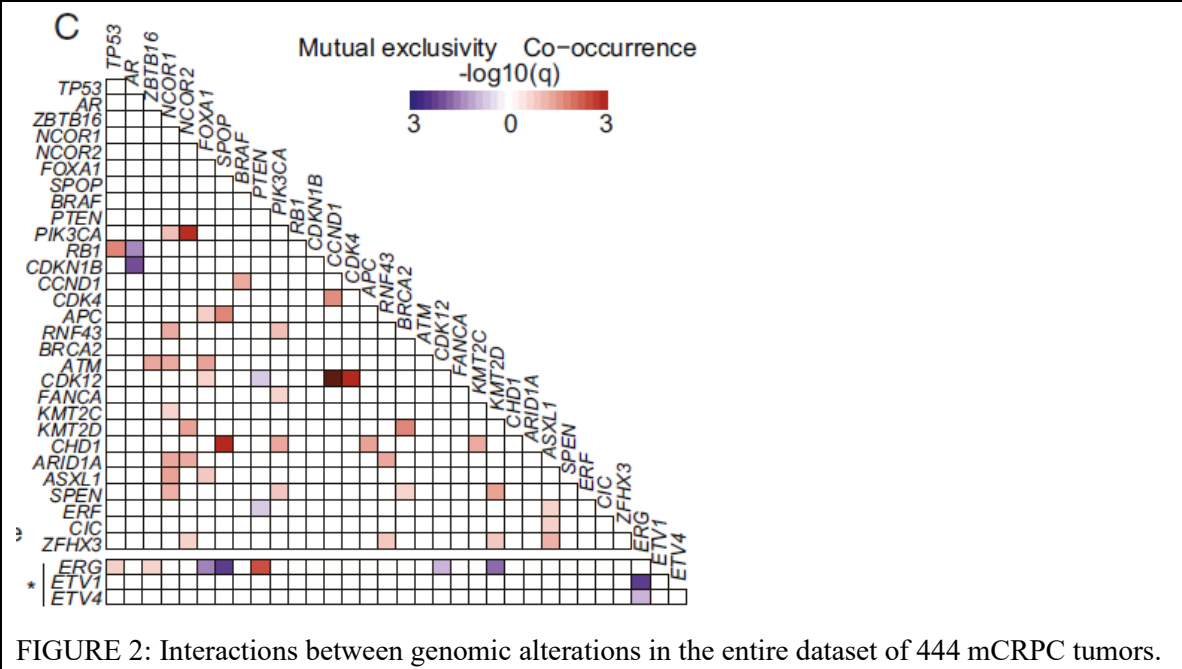


FIGURE 2: Interactions between genomic alterations in the entire dataset of 444 mCRPC tumors.

Given a question in the field regarding the emergence of a neuroendocrine phenotype following resistance to novel AR-targeted therapies, we performed integrative analysis that includes genomic loss of tumors suppressors that have been associated with the neuroendocrine phenotype (TP53 and RB1), expression analysis for AR and neuroendocrine prostate cancer (NEPC) signatures, and histologic analysis for neuroendocrine features, and found that while genomic loss of TP53/RB1, NEPC signatures and histologic neuroendocrine features are generally correlated, there is incomplete concordance (FIGURE 3A). We found enrichment of the histologic neuroendocrine phenotype post-exposure to ARSIs among patients who received an ARSI during their disease course (FIGURE 3B,C).

As proposed in the SOW, we explored genomic features that were associated with response or resistance to abiraterone and enzalutamide, as well as with overall survival in mCRPC. In univariate analysis incorporating the most common genomic alterations, RB1 genomic alterations were the most significantly associated with survival and time on treatment with first-line abiraterone and enzalutamide (FIGURE 4). Genomic alterations in TP53 and AR were also associated with time on treatment, but not with overall survival (Table 1). RB1 genomic alteration was significantly associated with outcome in multivariate analysis as well (Table 2).

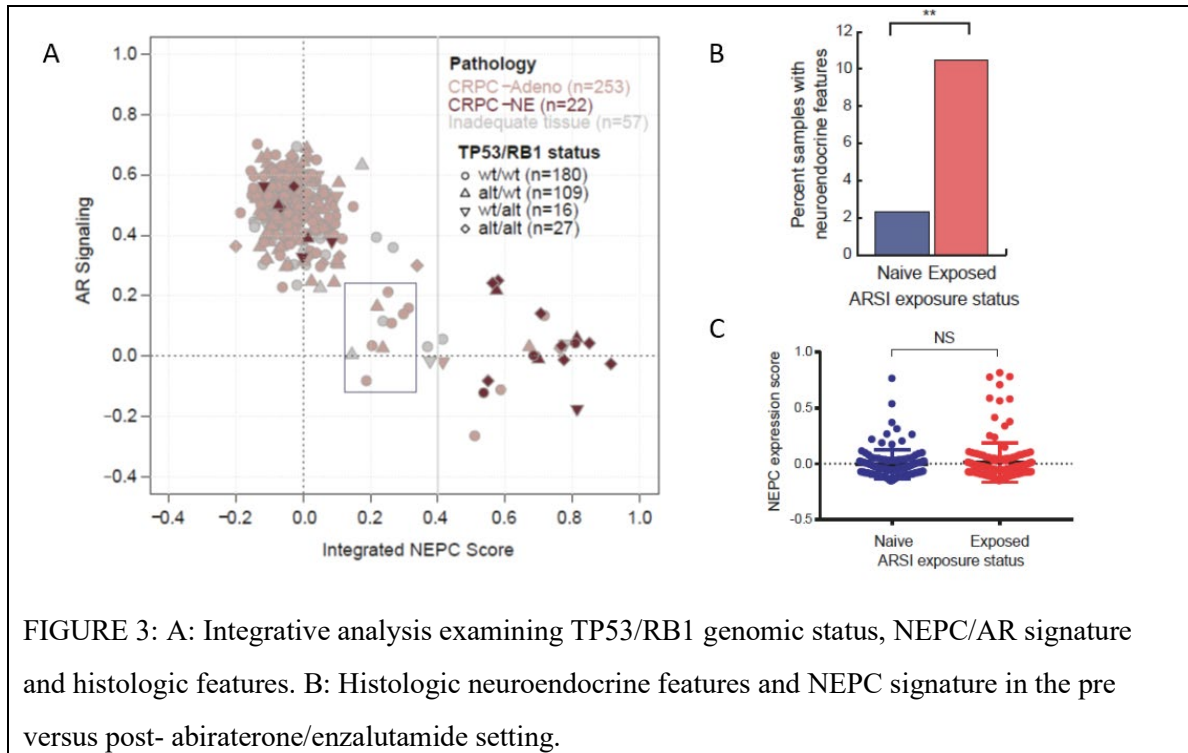


FIGURE 3: A: Integrative analysis examining TP53/RB1 genomic status, NEPC/AR signature and histologic features. B: Histologic neuroendocrine features and NEPC signature in the pre versus post- abiraterone/enzalutamide setting.

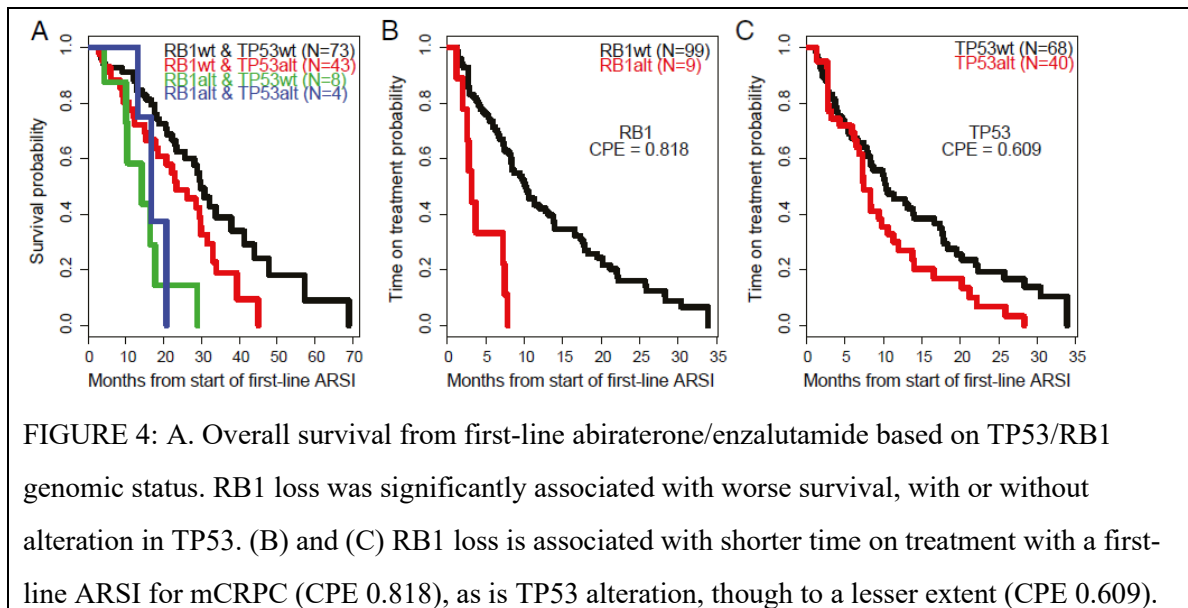


FIGURE 4: A. Overall survival from first-line abiraterone/enzalutamide based on TP53/RB1 genomic status. RB1 loss was significantly associated with worse survival, with or without alteration in TP53. (B) and (C) RB1 loss is associated with shorter time on treatment with a first-line ARSI for mCRPC (CPE 0.818), as is TP53 alteration, though to a lesser extent (CPE 0.609).

Of note, we explored the association of alterations in the PI3K pathway, WNT pathway, and homologous recombination (HR) DNA repair genes, and there was no statistically significant association between genomic alterations in these pathways and worse survival or worse response to abiraterone or enzalutamide. The finding for HR DNA repair genes is particularly relevant given recent conflicting data suggesting both worse and better

response to abiraterone and enzalutamide for BRCA2, BRCA1 and ATM-altered tumors (Table 1).

Interestingly, expression of AR splice variants in tumor, including AR-V7, was not associated with clinical outcomes, contrary to data from circulating tumor cells. A commonly used neuroendocrine prostate cancer expression score was also not associated with outcome, whereas expression scores associated with RB1 loss (RB1 loss score and cell cycle progression (CCP) score) were significantly associated with both survival and time on treatment, consistent with the critical prognostic role of RB1 loss (Table 1).

Gene/pathway alteration	Univariate <i>P</i> value for survival from first-line ARSI (CPE, <i>n</i> = 128 or as indicated)	Univariate <i>P</i> value for time on treatment with first-line ARSI (CPE, <i>n</i> = 108 or as indicated)
RB1	0.002 (CPE 0.768)	<0.001 (CPE 0.818)
TP53	0.072 (CPE 0.605)	0.046 (CPE 0.609)
WNT pathway	0.115	0.153
ETS fusion	0.159	0.206
APC	0.255	0.167
CTNNB1	0.274	0.448
ATM	0.331	0.850
BRCA2	0.327	0.418
BRCA2/BRCA1/ATM	0.495	0.611
AKT1	0.558	0.053
RNF43	0.614	0.844
AR	0.658	0.005 (CPE 0.651)
PTEN	0.676	0.412
PI3K pathway	0.699	0.138
PIK3CA	0.716	0.165
PIK3R1	0.752	0.892
PIK3CB	0.799	0.277
BRCA1	0.809	0.998
NEPC score	0.218 (<i>n</i> = 99)	0.930 (<i>n</i> = 80)
AR signaling score	0.847 (<i>n</i> = 99)	0.847 (<i>n</i> = 80)
RB1 loss score	<0.001 (<i>n</i> = 99)	0.014 (<i>n</i> = 80)
CCP score	0.002 (<i>n</i> = 99)	0.045 (<i>n</i> = 80)
AR-V7 SRPM	0.524 (<i>n</i> = 75)	0.329 (<i>n</i> = 56)
AR-V7/ARpromoter1-2	0.475 (<i>n</i> = 75)	0.378 (<i>n</i> = 56)
AR-V3 SRPM	0.444 (<i>n</i> = 75)	0.077 (<i>n</i> = 56)

Table 1: Association of common genomic alterations with overall survival and time on treatment with first-line ARSI

Clinical outcome	Gene alteration(s)	Multivariate relative risk (95% CI for RR)
Overall survival from start of first-line ARSI	RB1–	1
	RB1+	3.31 (1.64, 6.67)
Time on treatment with first-line ARSI	RB1– and AR–	1
	RB1– and AR+	1.86 (1.18, 2.95)
	RB1+	6.56 (2.94, 14.62)

Common genomic alterations listed in Table 1 were included in this analysis. Only significant associations are shown. CI, confidence interval; RR, relative risk.

Table 2: Multivariate analysis evaluating the association of common genomic alterations with overall survival and time on treatment with first-line ARSI

The goals proposed in the SOW have been met following the timeline initially proposed, except for the expansion to 500 subjects, though the final 429 subjects used in the analysis were adequate to reach our significant conclusions, which are now published in *PNAS* (see Appendix)

This is the largest dataset of mCRPC genomics, integrating whole exome and RNA sequencing, and now includes annotation of clinical outcomes, namely survival and time on treatment with abiraterone and enzalutamide. Detailed findings, and a copy of the publication in *PNAS* were provided with the 2019 report (manuscript included again in the appendix, Ref a.)

There were no unmet goals proposed in the SOW for this aim.

AIM2: Investigate genomic drivers of disease progression in matched tumors from patients with metastatic prostate cancer

Major Activities:

As described in the SOW, major activities have included the molecular profiling of matched tumors from individual patients in order to identify genomic drivers of disease progression. We have reported on the genomics and evolution of alteration in DNA mismatch repair deficient prostate cancer (dMMR/MSI-H – 2019 REPORT) and CDK12 (2020 REPORT). In addition, we have performed an analysis correlating genomics at diagnosis of metastatic prostate cancer (metastatic castration-sensitive prostate cancer,

mCSPC) with clinical outcome, as an alternate method of identifying genomic drivers of disease progression, and have published the findings of our analysis, the largest to date, in *Clinical Cancer Research* as reported in the 2020 progress report.

As reported in the 2021 report, we have expanded our analysis to identify genomic disparities among men of different racial origin (now published in Clin Cancer Res)

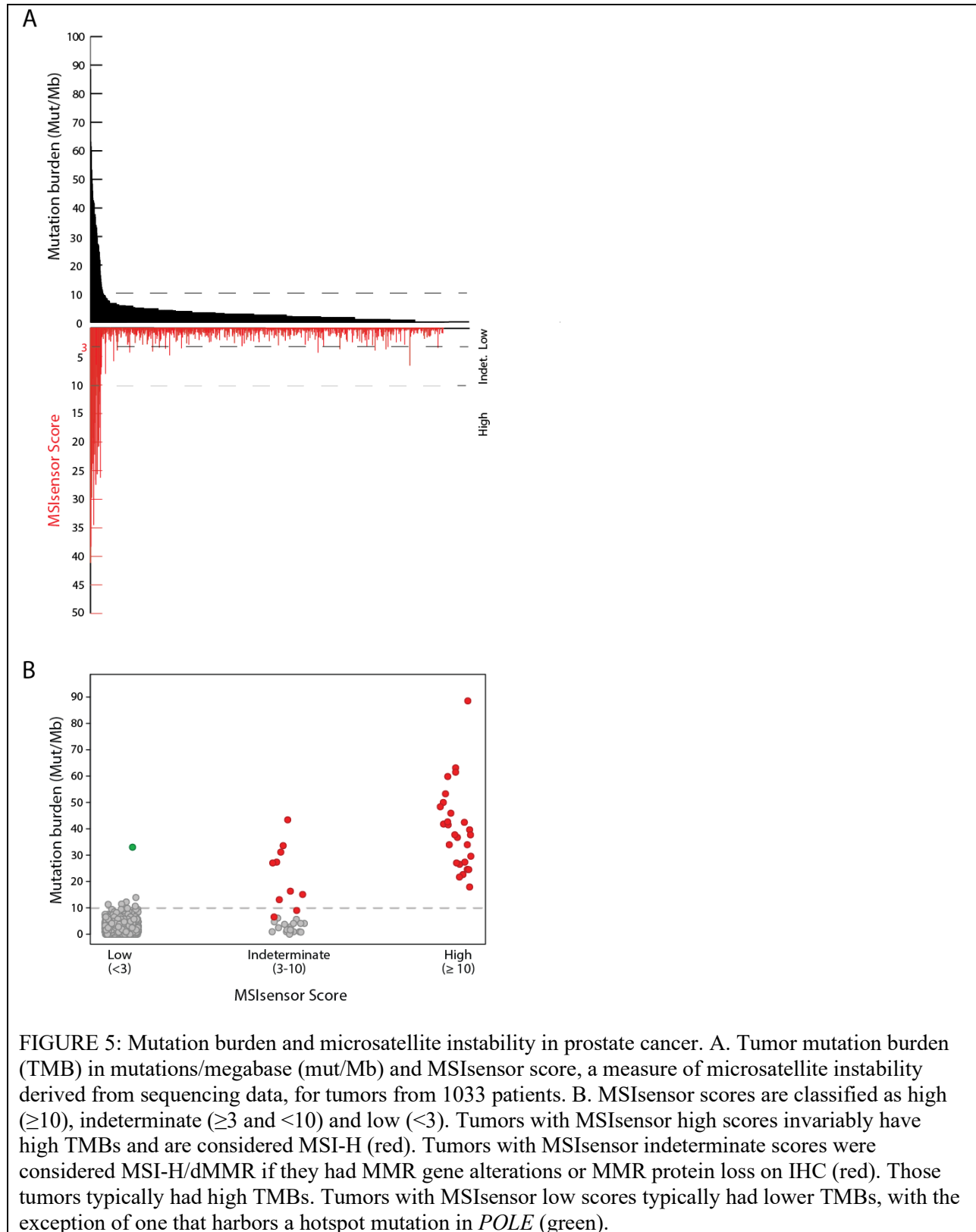
Specific objectives:

The objectives proposed in the SOW were: 1- Identify patients who underwent sequencing of metastatic tumors that had genomic alterations of interest, including patients with neuroendocrine features, 2- perform sequencing of matched tumors from these patients and 3- Perform analyses examining the evolution of genomic alterations in these tumors to identify drivers of disease progression.

Significant results/key outcomes:

To date, 3500 tumors from 3000 patients with prostate cancer have undergone sequencing with the MSK-IMPACT assay, with over 500 patients having had two or more tumors sequenced. Subsets of these patients harbor hotspot alterations in genes of interest (e.g. TP53, BRCA2, ATM).

In the 2019 report, we described the genomics and evolution of the MSI-high/dMMR phenotype in a subset of prostate cancers. These patients typically have tumors that harbor high mutation burdens, and often have alterations in MMR genes MSH2, MSH6, MLH1 and PMS2. We aimed to identify these patients in the dataset and examine the evolution of their disease through matched tumors analysis, within the major goals of this Aim. We performed integrative analysis that included assessment of MSI sensor score (an algorithmic score derived from sequencing data that is associated with microsatellite instability), mutation burden, presence of genomic alterations in MMR genes, and mutational signatures in a set of 1033 patients, and found that 32 (3.1%) had MSI-H/dMMR tumors (FIGURE 5).



Importantly, we examined the evolution of MSI/MMR in a subset of these patients through matched tumor analysis. Of 6 patients who had matched tumors analyzed, two had evidence of acquired MSI/dMMR later during their disease evolution. This was not associated with the acquisition of mutations in MMR genes, suggesting an epigenetic mechanisms. One patient had a germline alteration in *PMS2*, likely a driver event that led to the development of his malignancy, and two patients had evidence of genomic alterations in MMR genes in all of their matched tumors, suggesting early somatic events that led to the development of MSI/dMMR (FIGURE 6). The finding of acquired MSI/dMMR in a subset of cases is particularly relevant because MSI is typically not considered to be an acquired phenotype during disease evolution, and suggests that a recent metastatic tumor should be profiled whenever possible for the identification of MSI/dMMR patients who may benefit from immune checkpoint blockade, given that an earlier archival tumor may not display the phenotype.

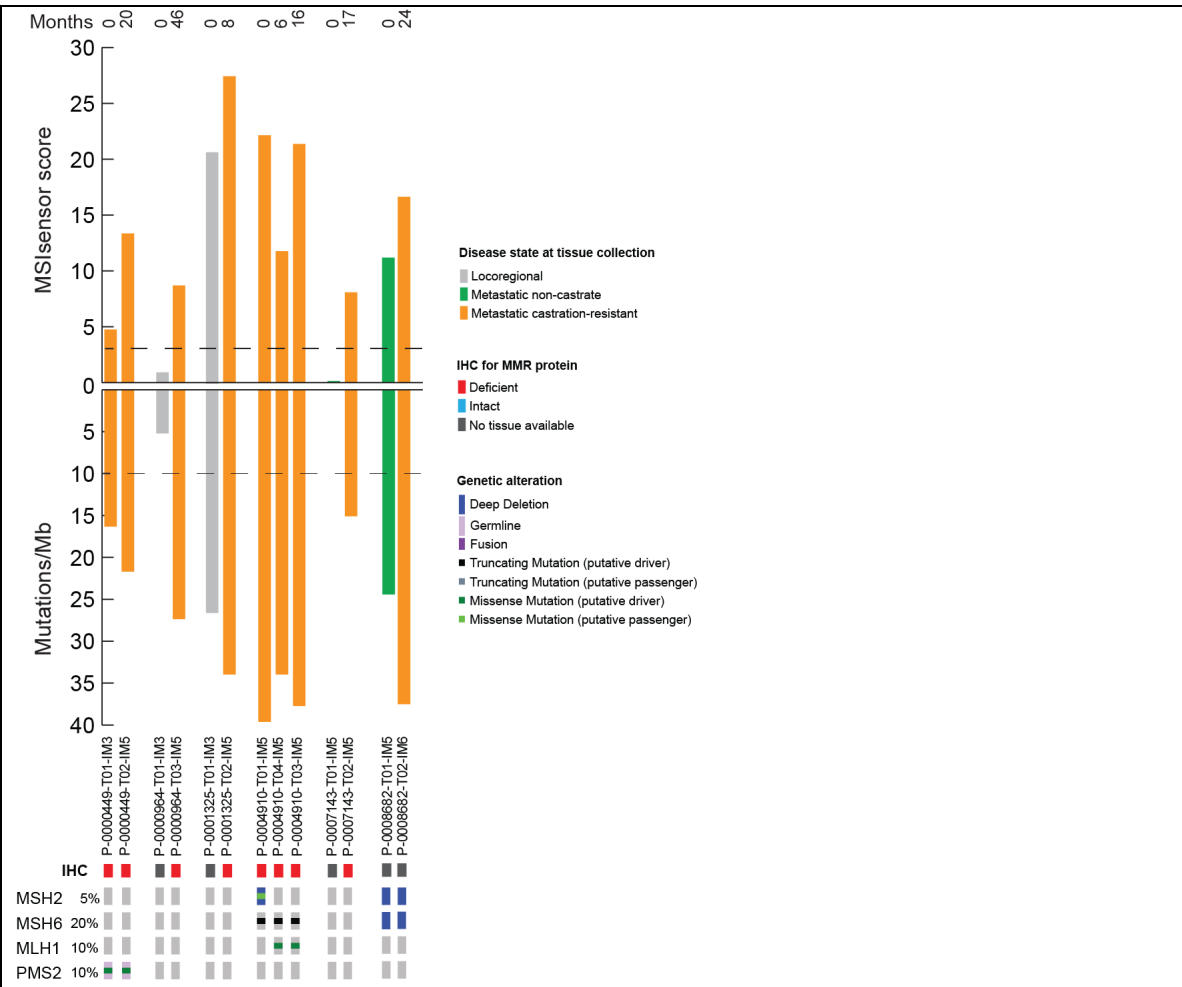
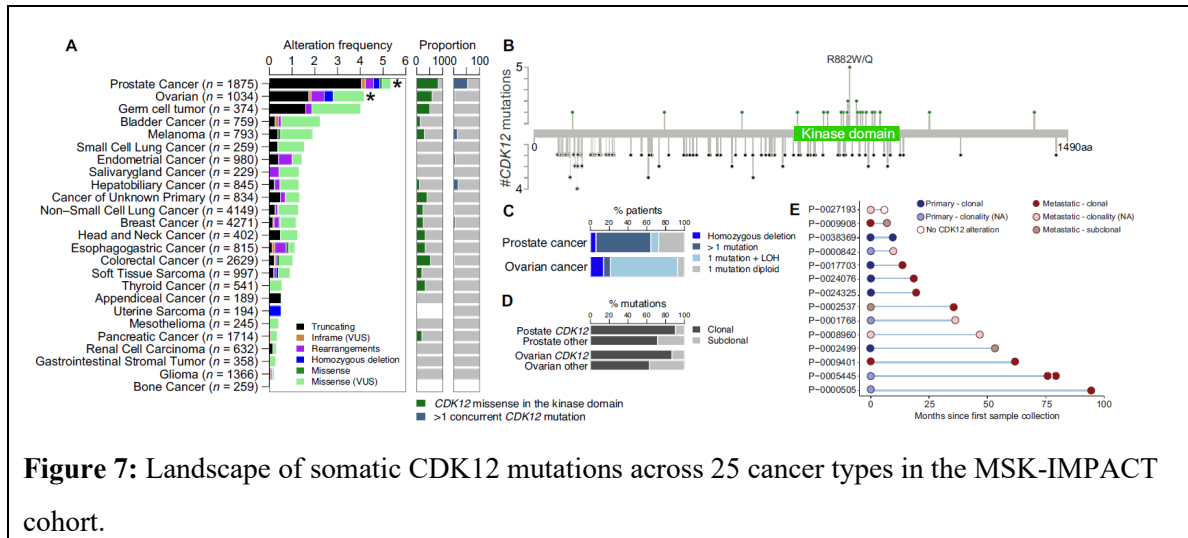


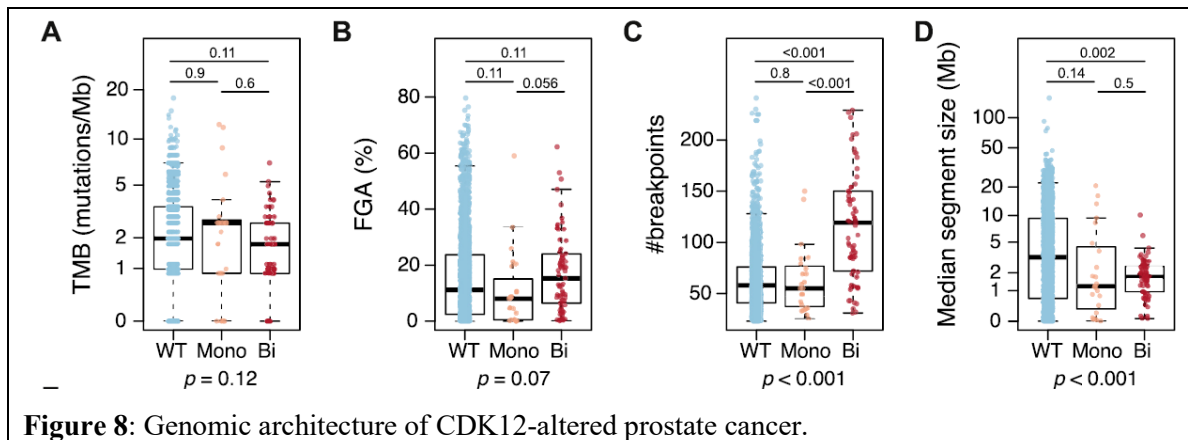
FIGURE 6: Longitudinal assessment of MSI-H/dMMR in matched tumors. Six patients with MSI-H/dMMR prostate cancer had more than one tumor profiled. The time of acquisition of these tumors (in months) is indicated at top, relative to the time of acquisition of the first profiled tumors (Month 0). Two of the six patients (P-0000964 and P-0007143) displayed somatically acquired MSI in the latter mCRPC tumors. For patient P-0007143, the earlier tumor was a prostate sample acquired at his diagnosis with de novo metastatic non-castrate disease. This tumor had very low MSI sensor score and TMB. One patient (P-0000449) with a germline *PMS2* mutation displayed MSI in both his matched tumors.

This work was published in JAMA Oncology (Appendix, Ref b.)

In the JULY2020 report, I described our analysis of *CDK12*-mutated prostate cancer and other solid tumors. *CDK12* alterations occur in ~6% of advanced prostate cancers, and represent a relevant clinical subset that may benefit from immune checkpoint blockade. We initially performed a pan-cancer analysis of *CDK12* alterations in a dataset of >20,000 tumors and found that *CDK12* alterations occur most frequently in prostate, followed by ovarian cancer (Figure 7A). In prostate cancer, the majority of alterations are truncating alterations that are predicted to inactivate the protein product (Figure 7B), and 80% of *CDK12*-altered prostate cancers display biallelic *CDK12* loss of function alterations (Figure 7C), which typically occur clonally (Figure 7D). Importantly, we performed matched tumors analysis for patients with *CDK12* alterations who had multiple tumors profiled during their disease course, and found that the *CDK12* alteration occurred early during their disease course, implying that *CDK12* loss is an early oncogenic event in a subset of prostate cancers (Figure 7E).



We showed that cases with biallelic *CDK12* loss were enriched with features of genomic instability, including increased number of breakpoints and genomic loss/gain of small size fragments (Figure 8), concordant with the tandem duplicator phenotype that was previously described. Furthermore, *CDK12* loss in prostate cancer was associated with genomic alterations in the cell cycle pathway, including amplification of *CCND1* (figure not shown), suggesting that targeting of *CDK4/6* may be explored in this disease subset.



Lastly, we explored the association of *CDK12* loss with clinical outcome. We found that patients with *CDK12* loss had more aggressive disease features at diagnosis, including higher diagnostic Gleason scores and a higher rate of metastasis at diagnosis, and had shorter overall survival and shorter time to castration resistance compared with *CDK12*-

wild type patients (Figure 9). The findings of this work were published in *European Urology* (Appendix Ref. c.).

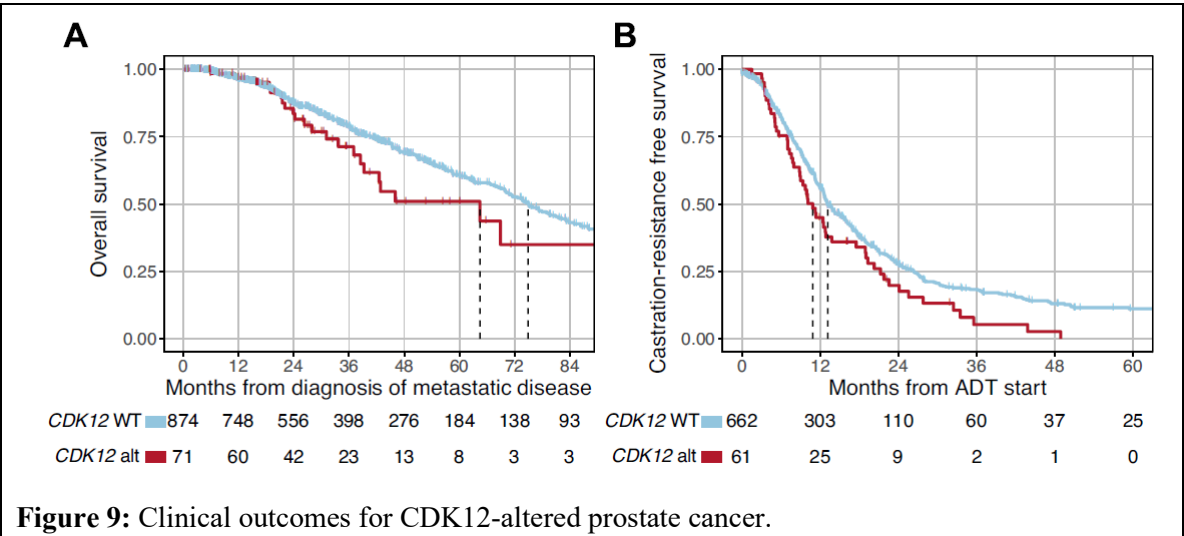
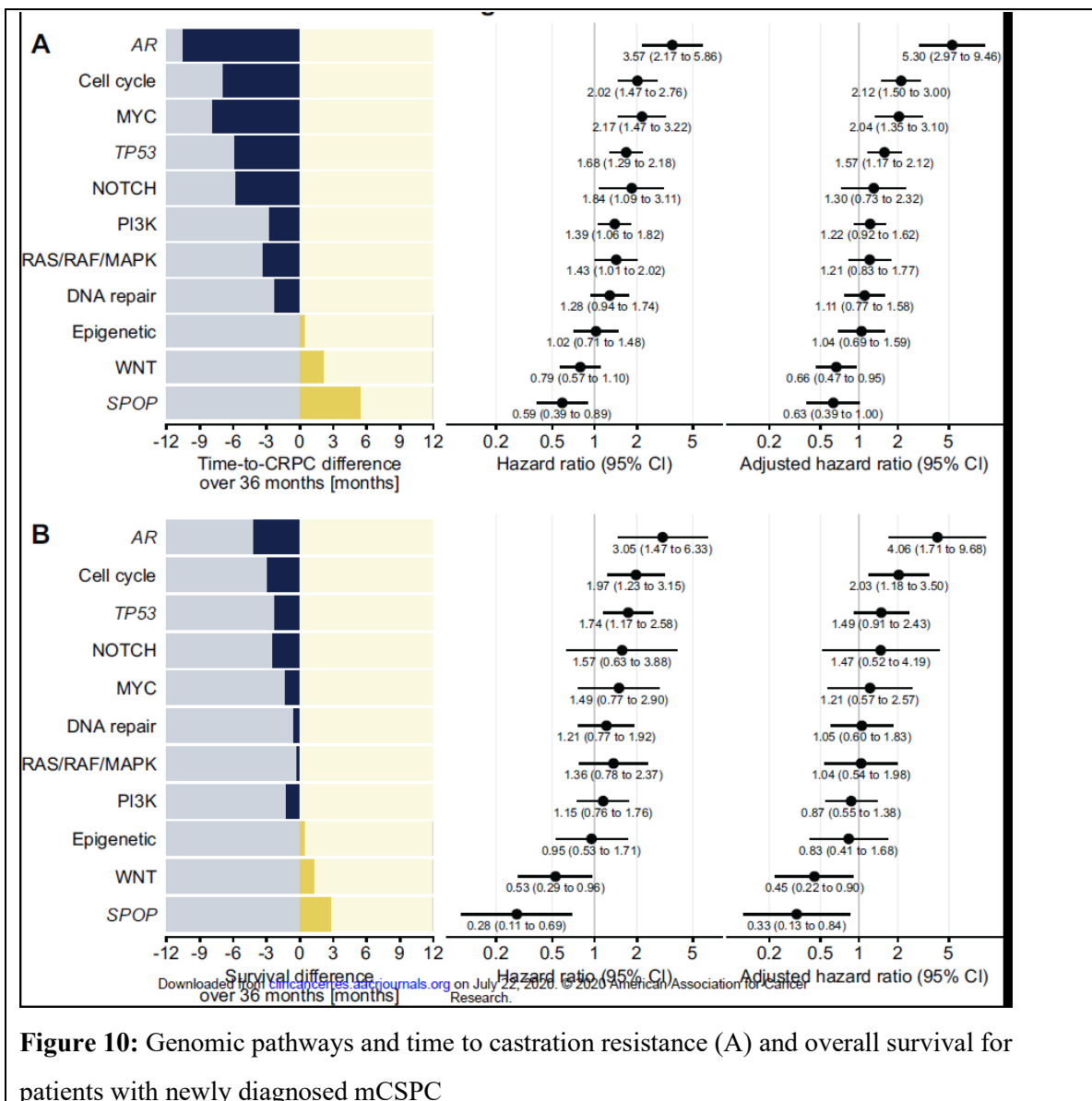


Figure 9: Clinical outcomes for CDK12-altered prostate cancer.

As an alternate method of analyzing matched tumors to investigate drivers of disease progression and aggressive prostate cancer, and as reported in the 2020 report, we investigated the association of genomics at the diagnosis of metastatic disease, in the metastatic castration-sensitive state (mCSPC), with clinical outcomes. We found that fraction of genomic alteration (FGA) in the tumor was associated with worse outcomes and with higher volume of metastatic disease at diagnosis, and that genomic alterations in the cell cycle pathway, *TP53* and *MYC* were associated with shorter time to castration resistance and worse overall survival (Figure 10). The findings of this work were published in *Clinical Cancer Research* (Appendix Ref d.).



Also reported in the 2020 report, we explored the association of genomic alterations in several genes associated with DNA damage repair (DDR) deficiency with response to PARP inhibition in the context of the TRITON2 prospective study of rucaparib in mCRPC. We found that response rates to rucaparib were low for patients with alterations in *ATM*, *CDK12* and *CHEK2*, while responses were observed in less-commonly altered genes including *PALB2* and *FANCA*, shedding light on genomic alterations that confer synthetic lethality with PARP inhibition. These findings were published in *Clinical Cancer Research* (Appendix Ref. e) and the study has led to the FDA approval of rucaparib for the treatment of mCRPC with *BRCA2* and *BRCA1* alterations (Appendix

Ref. f.). Along the same lines, we explored the association of DDR gene alterations with response to platinum-based chemotherapy and found that DDR alterations are associated with improved PSA response, though numbers were limited to identify an association with other outcomes. This work was published in *JCO Precision Oncology* (Appendix Ref g.)

As reported in the 2021 report, we also performed an analysis of genomic differences between patients from different self-reported racial backgrounds (Figure 11), as well as genomically-inferred ancestry (Figure 12). This is a clinically relevant problem given the disparities in clinical outcomes observed in racial minority groups, and could highlight genomic drivers of disease and new genomically-targeted therapeutic pathways to improve on these disparities. We found that tumors from Black men harbored fewer *PTEN* mutations (14%, vs. 20% in White men; adjusted difference, -7 percentage points; 95% CI, -12 to -1) and more *AR* alterations (18%) than those from White men (12%; adjusted difference, 5 percentage points; 95% CI, 1 to 8). Tumors from Asian men had more *FOXA1* mutations (27%, vs. 13% in White men; adjusted difference, 15 percentage points; 95% CI, 6 to 26) and more *ZFHX3* alterations (17%) than White men (7%; adjusted difference, 8 percentage points; 95% CI, 1 to 17). Despite fewer *TP53* mutations (19%, vs. 30% in White men; adjusted difference, -10 percentage points; 95% CI, -3 to -17), tumors from Black men had more aneuploidy, particularly chromosome arm 8q gains (49%, vs. 37% in White men; adjusted difference, 11 percentage points; 95% CI, 4 to 18), an adverse prognostic factor. These genomic difference persisted after adjusting for clinical factors.

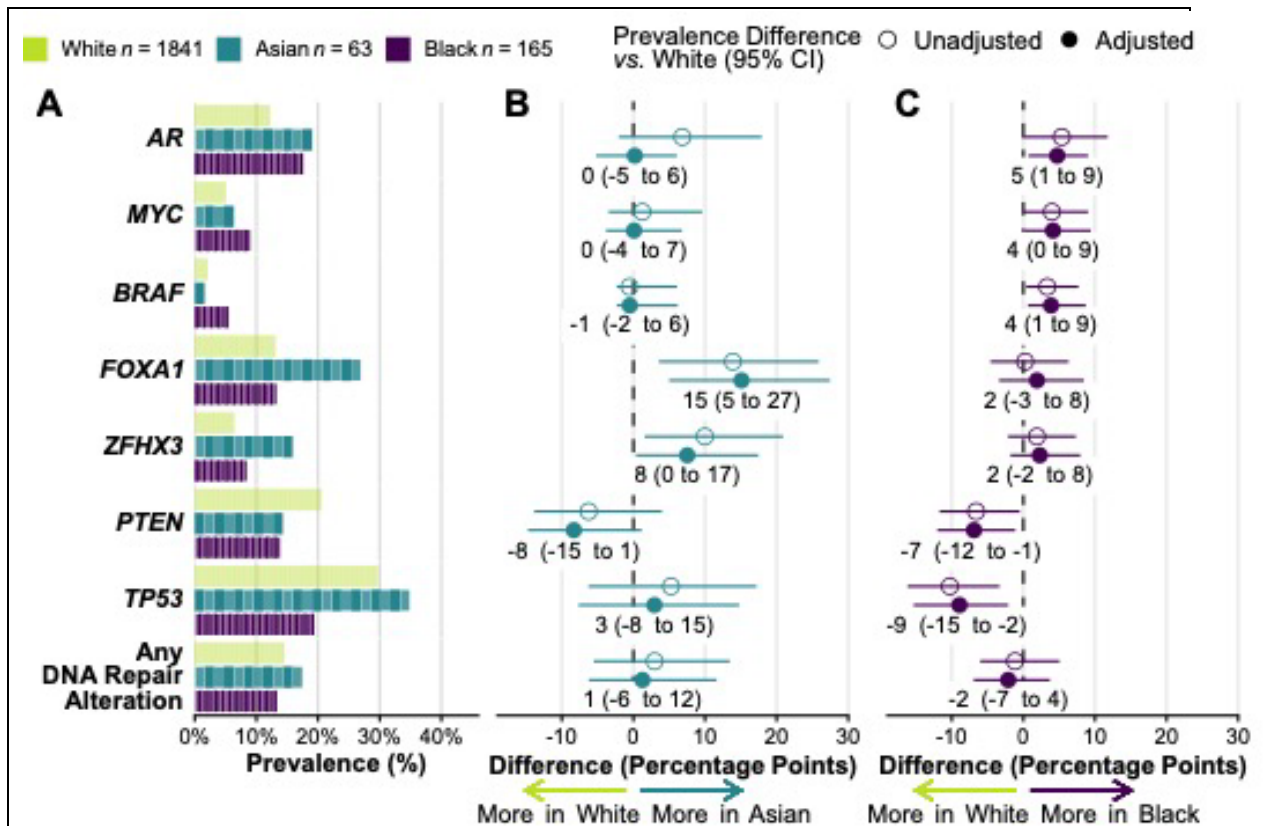
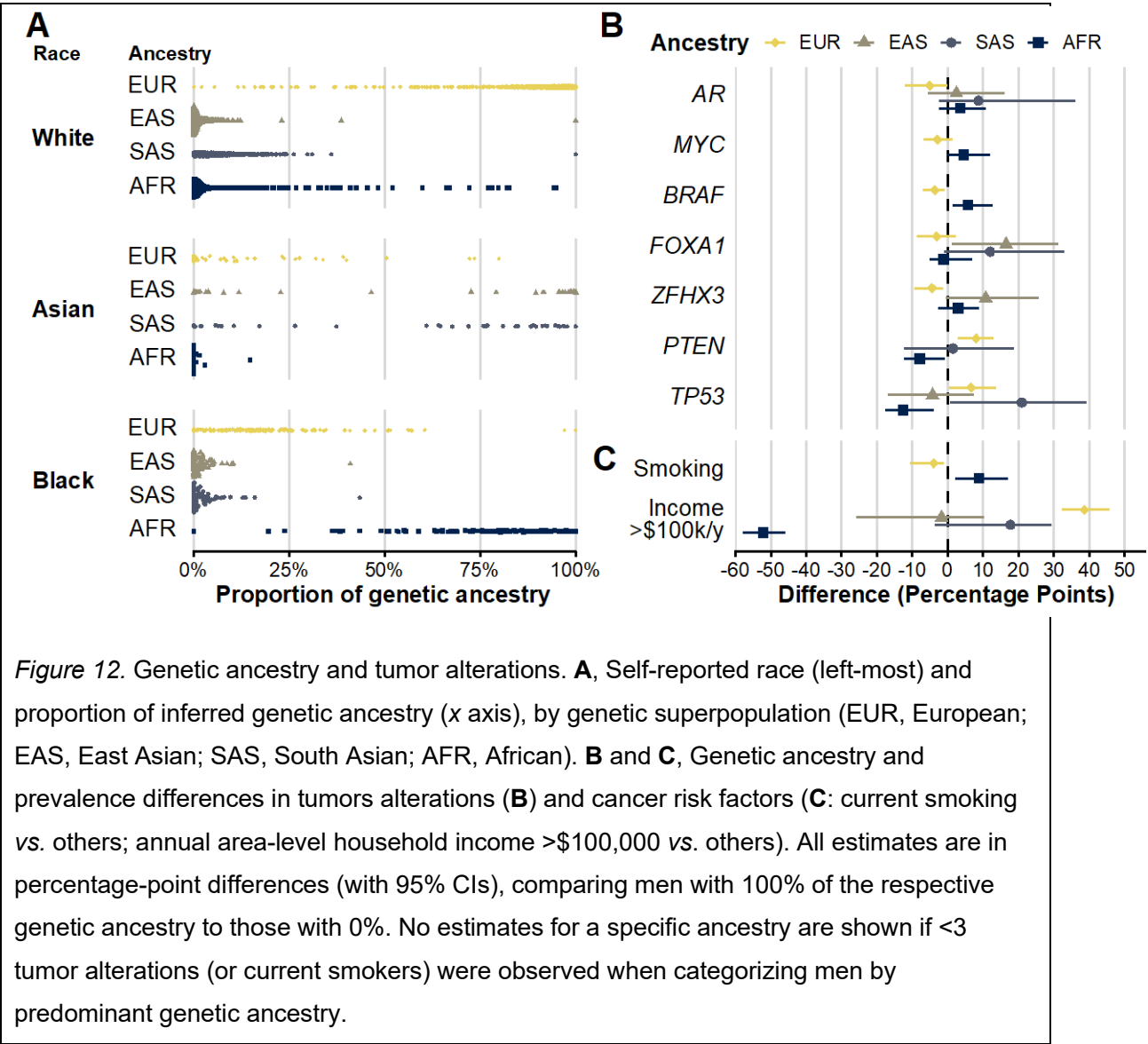


Figure 11: Self-reported race and tumor alterations. **A**, Prevalence of oncogenic alterations by self-reported race. Prevalence is a proportion; each “brick” represents one patient with an alteration (not visible for White men because of the high number). **B**, Differences in alteration prevalence, comparing Asian men vs. White men. **C**, Differences in alteration prevalence, comparing Black men vs. White men. Panels **B** and **C** show absolute differences in percentage points. Adjusted models include age at sample (linear), disease extent (localized, regional nodes, metastatic hormone-sensitive, or castration-resistant), prostate-specific antigen (PSA) levels at diagnosis ($\log_e$ -transformed, mean-imputed), stage at diagnosis (*de novo* metastatic vs. non-metastatic), and sample type (prostate, lymph node, bone, or visceral). Individual genes are ordered by prevalence difference in Black men vs. White men. Only 1,924 samples (93%) were profiled for *ZFHX3* due to its inclusion in a later panel version only. For comparability with ⁸, DNA repair alterations encompass *ERCC5*, *MRE11*, *TP53BP1*, *POLE*, *RAD21*, *MSH2*, *MSH6*, *BRCA1*, *BRCA2*, *ATR*, and *ATM*.

Genetic ancestry was associated with similar tumor alterations as self-reported race, but also with modifiable cancer risk factors (Figure 5).



In summary, we found that tumor genomics differed by race even after accounting for clinical characteristics. Equalizing access to care alone may not fully eliminate such differences, and therapies for genomic alterations more common in racial minorities are needed. Tumor genomic differences should not be assumed to be entirely due to germline genetics. This work has now been published in Clin Cancer Res (Appendix Ref h.)

There are no unmet goals proposed in the SOW for this aim.

AIM3: Identify genomic drivers of resistance to abiraterone and enzalutamide in cfDNA

Major Activities:

As described in the SOW, major activities include performing a pilot assay establishing the sensitivity of cfDNA sequencing relative to tumor, and identifying patients with banked plasma/cfDNA samples that will undergo sequencing to identify genomic drivers of resistance to abiraterone/enzalutamide. These activities were completed.

Specific objectives:

The objectives proposed in the SOW were: 1- to perform pilot assays to determine the sensitivity of cfDNA sequencing relative to tumor, 2- To identify patients with banked samples that will undergo sequencing of cfDNA, 3- To perform sequencing of these samples and 4- To perform analysis correlating genomic findings with response and resistance to abiraterone and enzalutamide.

Significant results/key outcomes:

As reported in the 2019 and 2020 reports, for the pilot assay, we identified 21 patients with banked plasma samples who had undergone sequencing of their tumors using whole exome sequencing (Figure 13). The banked plasma samples were collected within 60 days of tumor acquisition, without intervening therapy (so to as to ensure that a potential response to therapy would not affect the cfDNA sequencing result). The patients were selected to reflect a variety of genomic findings. cfDNA was extracted from the banked plasma (median 1.5mL, range 0.5-2.6mL plasma volume). Median cfDNA yield per sample was 9.3 ng, with a range of 1.8-327ng (median 6.3ng/mL, range 1.8-234ng/mL). We proceeded to perform cfDNA sequencing using the MSK-IMPACT targeted sequencing assay described in the project proposal. Mean exonic coverage was ~500x, as expected for this assay. There was a clear correlation between mean exonic coverage and total input DNA, where samples with low DNA input below 5ng total cfDNA had lower mean exonic coverage.

Overall, we found that 1/3 of samples (7/21) had identification of all tumor mutations in cfDNA, 1/3 of samples (7/21) had identification of some tumor mutations in cfDNA, and 1/3 of samples (7/21) had no mutations called in cfDNA (failed samples) (TABLE 3).

cfDNA sequencing success correlated again with input DNA, with samples with < 5ng total input cfDNA having a high failure rate (FIGURE 14). Interestingly, in 3 of 21 patients, cfDNA sequencing identified ‘novel’ mutations, not identified in tumors, likely reflecting disease heterogeneity. Some of these variants were ‘hotspot’ mutations in AR (e.g. H875Y and T878A), suggesting biologically-meaningful genomic heterogeneity that can impact response to therapy.

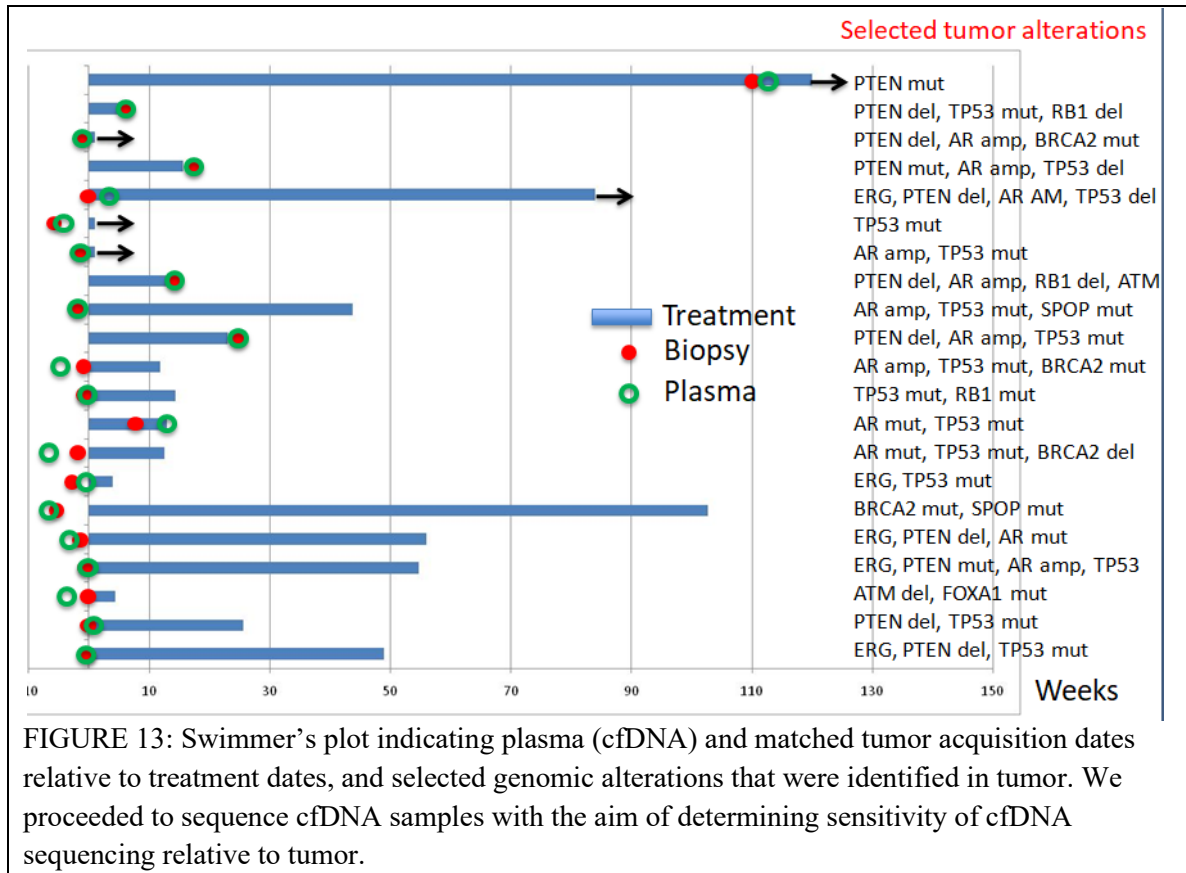
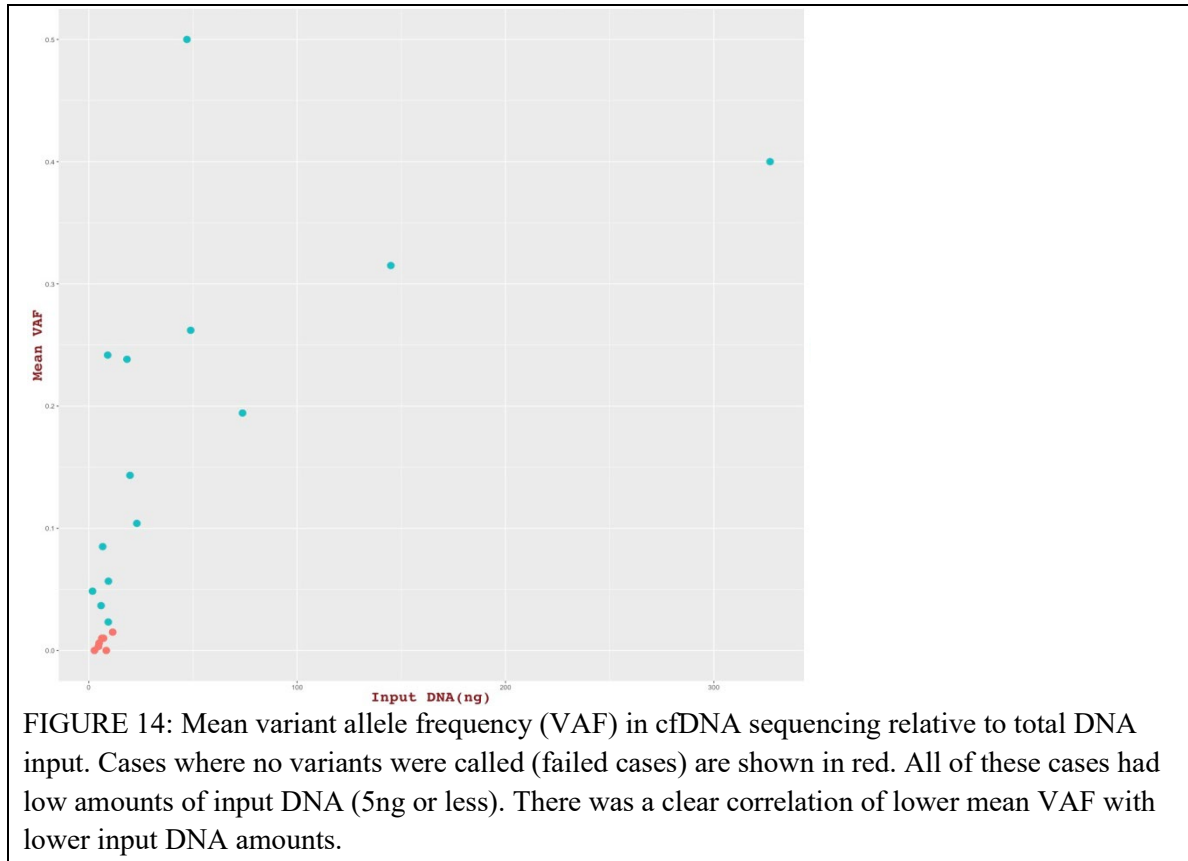


FIGURE 13: Swimmer's plot indicating plasma (cfDNA) and matched tumor acquisition dates relative to treatment dates, and selected genomic alterations that were identified in tumor. We proceeded to sequence cfDNA samples with the aim of determining sensitivity of cfDNA sequencing relative to tumor.

	WES	cfDNA	% cfDNA/tumor
Samples with variants called (relative to tumor)	21	14	66%
- All variants called		7	33%
- Some variants called		7	33%
- No variants called		7	33%
Samples with variants detected (relative to tumor)	21	19	90%

TABLE 3: cfDNA mutation detection using tumor sequencing as a reference. In total, we sequenced cfDNA from 21 patients who also had matched tumor sequencing. cfDNA variants were called if allele frequency was at least 0.05 or 0.02 to 0.04 for hotspot mutations. cfDNA

variants were ‘detected’ in cases where allele frequency was at least 0.01 in cases where the mutation was also identified in the tumor.



As reported in 2020 and following goal 2 in the SOW, we moved forward with identifying ~60 patients with matched baseline and progression (as well as an on-treatment) banked plasma samples and proceeded with DNA extraction for sequencing. Based on the 1/3 failure rate identified in the pilot project previously described using the MSK-IMPACT assay, we shifted our plan from using the MSK-IMPACT assay (targeted to 460 genes at mean 500x coverage), to a new ctDNA-specific assay developed at MSKCC called MSK-ACCESS targeting exonic regions from ~130 genes at higher depth of sequencing, resulting in improved sensitivity to detect tumor alterations in plasma. To maximize sensitivity, we limited the analysis to 30 patients with matched samples where DNA yield was at least 10ng in total. Concordance between cfDNA and tissue

sequencing (when available) using the MSK-ACCESS assay was high as anticipated, with common alterations for mCRPC identified in cfDNA (Figure 15).

We observed declines in mutation allele fractions early during treatment in the on-treatment sample, correlating with response to therapy, as well as the emergence of *AR* alterations at resistance to abiraterone/prednisone, as previously reported (Figure 16). We have not identified any new drivers of resistance to abiraterone or enzalutamide beyond what we identified in the analysis described in Aim 1 (Appendix Ref. a.)

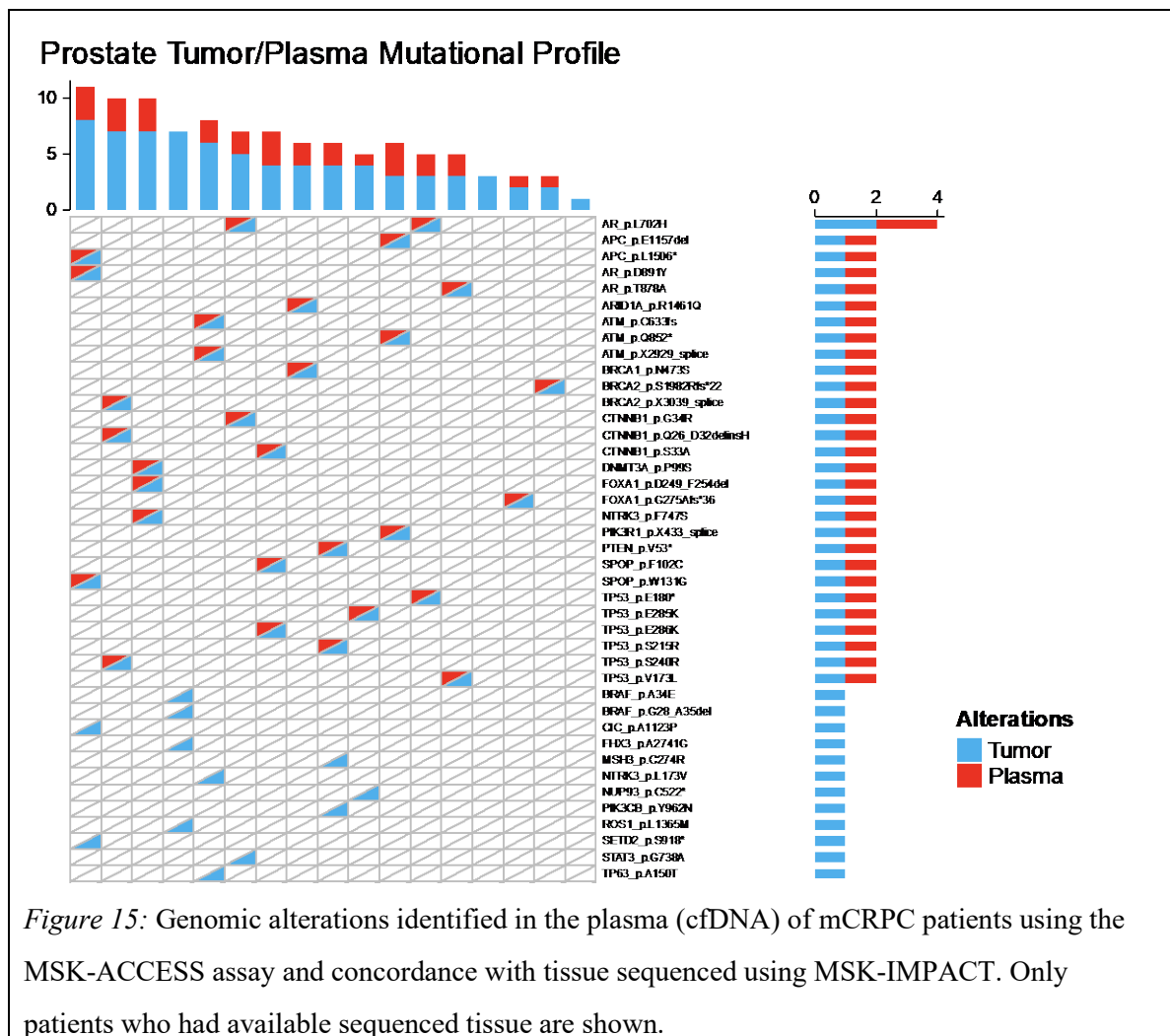
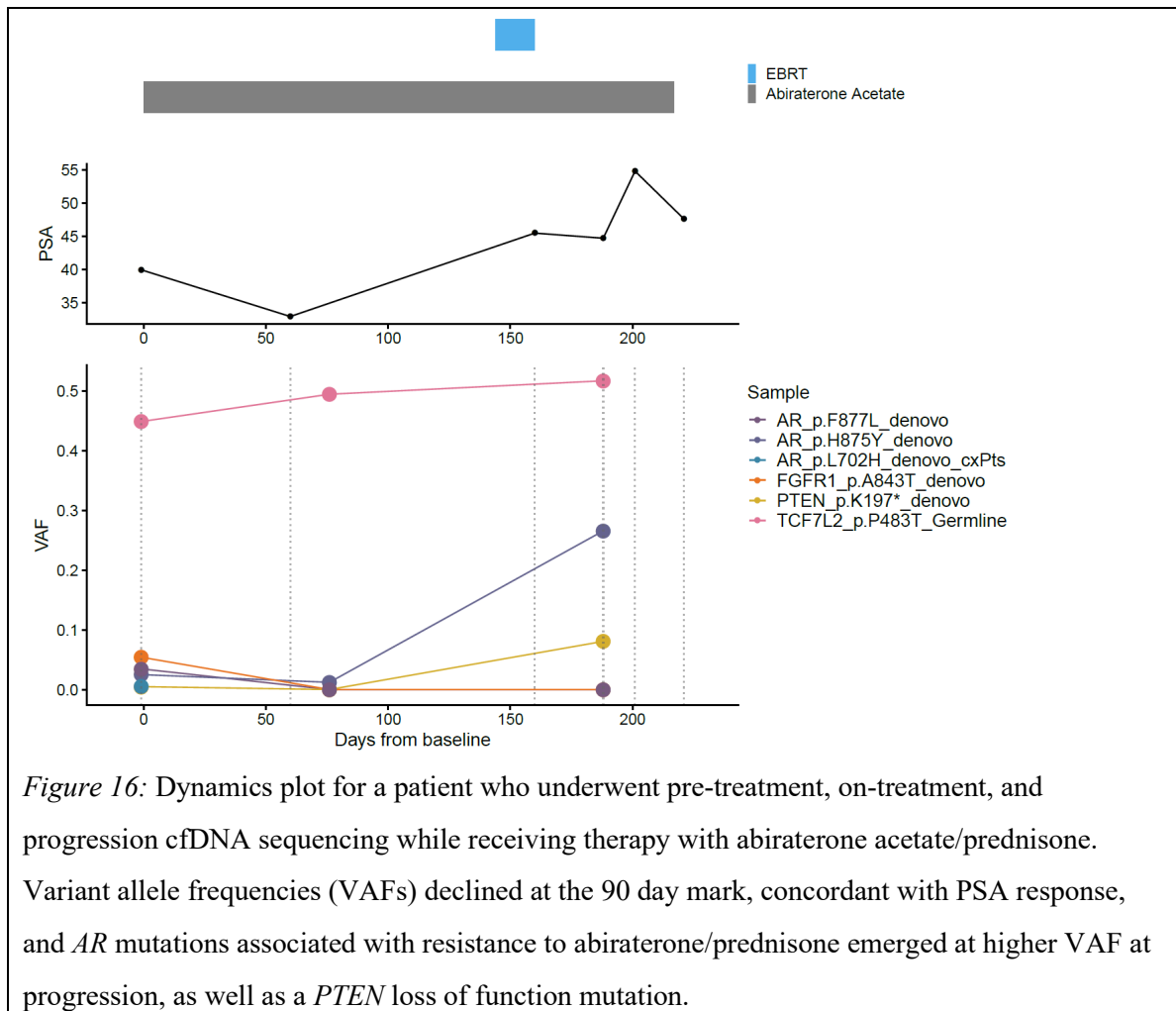


Figure 15: Genomic alterations identified in the plasma (cfDNA) of mCRPC patients using the MSK-ACCESS assay and concordance with tissue sequenced using MSK-IMPACT. Only patients who had available sequenced tissue are shown.



There are no unmet goals proposed in the SOW for this aim.

c. What opportunities for training and professional development has the project provided?

Progress has been made towards training and professional development of the PI of the project as proposed in the initial application:

- The applicant continued to meet regularly with his mentors Dr. Charles Sawyers and Dr. Howard Scher to discuss progress on the proposed project as well as future directions
- The applicant has presented his research internally at MSKCC at the Prostate Cancer Translational meeting, as well as within the Genitourinary Oncology Service and to the Prostate Disease Management Team at MSKCC, that includes urologists and radiation oncologists. During the course of the grant, he presented his work at multiple venues

including national meetings (ASCO, GU ASCO, LUGPA urological association), and to professional media outlets including OncLive and Medscape.

- The applicant has continued to present his work on multi-institutional SU2C-PCF project telephone calls and Prostate Cancer Foundation meetings.
- The applicant continues to attend national meetings (GU ASCO, ASCO) virtually during the pandemic and in-person.

d. How were the results disseminated to communities of interest?

The work performed has been published in multiple publications as described above, and as appended.

e. What do you plan to do during the next reporting period to accomplish the goals?

Nothing to report – this is a final report.

4. IMPACT:

a. What was the impact on the development of the principal discipline(s) of the project?

An initial publication describing results of matched tumor analysis has been published in JCO Precision Oncology. In the prior reporting period, we have published the findings of our MSI/dMMR analysis in JAMA Oncology. This publication has shed light on the frequency of the MSI-H/dMMR phenotype in prostate cancer, and importantly, has shown that MSI-H can develop later during disease evolution based on the analysis of matched tumors, arguing for profiling of a recent metastatic sample whenever possible, given the clinical relevance of the MSI-H phenotype for treatment of patients with immune checkpoint blockade. This dataset is the largest known to date describing MSI-H prostate cancer and response to immunotherapy in this molecularly-defined subset of the disease.

Also, we have published the largest single genomic dataset of advanced mCRPC totaling 444 tumors, with associated clinical outcomes, in PNAS. We performed analysis identifying RB1 loss as a key prognostic factor in mCRPC, and critically, we have made the genomic and clinical data publicly available as a resource to the research community, such that the relation of any genomic alteration with clinical outcome can be interrogated.

We have published the largest dataset of *CDK12* altered prostate cancer in *European Urology* with an accompanying commentary. This publication has shed light on the genomic

and clinical characteristics of an important subset (~6%) of advanced prostate cancers, which we have separately shown in another publication in *Clinical Cancer Research* does not respond to PARP inhibitors as previously hypothesized.

We have also published the largest dataset of genomically-characterized mCSPC, which has generally not been well described in the literature, and identified genomic features associated with more aggressive behavior, including shorter time to castration resistance.

We have published studies investigating the association of alterations in DNA repair genes with response to PARP inhibition and platinum chemotherapy, which is particularly relevant given the increased importance of targeted molecularly-selected therapies in the clinic and the recent FDA approval of rucaparib and olaparib for the treatment of mCRPC.

We have also published on genomic differences by self-reported race. We found that tumor genomics differed by race even after accounting for clinical characteristics. Equalizing access to care alone may not fully eliminate such differences, and therapies for genomic alterations more common in racial minorities are needed.

b. What was the impact on other disciplines?

Nothing to report

c. What was the impact on technology transfer?

Nothing to report at this time.

d. What was the impact on society beyond science and technology?

Nothing to report.

5. CHANGES/PROBLEMS:

a. Changes in approach and reasons for change

There are no significant changes in the approach initially proposed for this project.

b. Actual or anticipated problems or delays and actions or plans to resolve them

There have been no significant problems or delays at this point in the course of performing the proposed tasks in the SOW, beyond delays from the pandemic that justified a 1-year cost extension to finalize some analyses and the publication of a manuscript (now complete).

c. Changes that had a significant impact on expenditures

There are no changes that have had a significant impact on expenditures.

d. Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

e. Significant changes in use or care of human subjects

There are no significant deviations, unexpected outcomes, or changes in approved protocols.

The IRB's last review was in May 2022 and no comments were made. A copy of the IRB approval letter was forwarded to the Department of Defense.

f. Significant changes in use or care of vertebrate animals.

Not applicable.

g. Significant changes in use of biohazards and/or select agents

Not applicable.

6. PRODUCTS:

i. Publications, conference papers, and presentations

ii. Journal publications. (all journal publications co-authored by PI listed)

Sautois B, Loehr A, Watkins SP, Schroeder H, Abida W. A Case Study of Clinical Response to Rucaparib in a Patient with Metastatic Castration-Resistant Prostate Cancer and a *RAD51B* Alteration. *Curr Oncol*. 2022 Jun 8;29(6):4178-4184. doi: 10.3390/curroncol29060333. PMID: 35735442; PMCID: PMC9221801.

Gounder MM, Agaram NP, Trabucco SE, Robinson V, Ferraro RA, Millis SZ, Krishnan A, Lee J, Attia S, Abida W, Drilon A, Chi P, Angelo SP, Dickson MA, Keohan ML, Kelly CM, Agulnik M, Chawla SP, Choy E, Chugh R, Meyer CF, Myer PA, Moore JL, Okimoto RA, Pollock RE, Ravi V, Singh AS, Somaiah N, Wagner AJ, Healey JH, Frampton GM, Venstrom JM, Ross JS, Ladanyi M, Singer S, Brennan MF, Schwartz GK, Lazar AJ, Thomas DM, Maki RG, Tap WD, Ali SM, Jin DX. Clinical genomic profiling in the management of patients with soft tissue and bone sarcoma. *Nat Commun*. 2022 Jun 15;13(1):3406. doi: 10.1038/s41467-022-30496-0. PMID: 35705558; PMCID: PMC9200814.

Chakraborty G, Nandakumar S, Hirani R, Nguyen B, Stopsack KH, Kreitzer C, Rajanala SH, Ghale R, Mazzu YZ, Pillarsetty NVK, Lee GM, Scher HI, Morris MJ, Traina T, Razavi P, Abida W, Durack JC, Solomon SB, Vander Heiden MG, Mucci LA, Wibmer AG, Schultz N, Kantoff PW. The Impact of PIK3R1 Mutations and Insulin- PI3K-Glycolytic Pathway Regulation in Prostate Cancer. *Clin Cancer Res*. 2022 Aug 15;28(16):3603-3617. doi: 10.1158/1078-0432.CCR-21-4272. PMID: 35670774; PMCID: PMC9438279.

Weiner AB, Siebert AL, Fenton SE, Abida W, Agarwal N, Davis ID, Dorff TB, Gleave M, James ND, Poon DMC, Suzuki H, Sweeney CJ. First-line Systemic Treatment of Recurrent Prostate Cancer After Primary or Salvage Local Therapy: A Systematic Review of the Literature. *Eur Urol Oncol*. 2022 Aug;5(4):377-387. doi: 10.1016/j.euo.2022.04.009. Epub 2022 May 28. PMID: 35641398.

Tang F, Xu D, Wang S, Wong CK, Martinez-Fundichely A, Lee CJ, Cohen S, Park J, Hill CE, Eng K, Bareja R, Han T, Liu EM, Palladino A, Di W, Gao D, Abida W, Beg S, Puca L, Meneses M, de Stanchina E, Berger MF, Gopalan A, Dow LE, Mosquera JM, Beltran H, Sternberg CN, Chi P, Scher HI, Sboner A, Chen Y, Khurana E. Chromatin profiles classify castration-resistant prostate cancers suggesting therapeutic targets. *Science*. 2022 May 27;376(6596):eabe1505. doi: 10.1126/science.abe1505. Epub 2022 May 27. PMID: 35617398; PMCID: PMC9299269.

Keegan NM, Vasselmann SE, Barnett ES, Nweji B, Carbone EA, Blum A, Morris MJ, Rathkopf DE, Slovin SF, Danila DC, Autio KA, Scher HI, Kantoff PW, Abida W, Stopsack KH. Clinical annotations for prostate cancer research: Defining data elements, creating a reproducible analytical pipeline, and assessing data quality. *Prostate*. 2022 Aug;82(11):1107-1116. doi: 10.1002/pros.24363. Epub 2022 May 10. PMID: 35538298; PMCID: PMC9246896.

Shrestha Bhattarai T, Shamu T, Gorelick AN, Chang MT, Chakravarty D, Gavrilu EI, Donoghue MTA, Gao J, Patel S, Gao SP, Reynolds MH, Phillips SM, Soumerai T, Abida W, Hyman DM, Schram AM, Solit DB, Smyth LM, Taylor BS. AKT mutant allele- specific activation dictates pharmacologic sensitivities. *Nat Commun*. 2022 Apr 19;13(1):2111. doi: 10.1038/s41467-022-29638-1. PMID: 35440569; PMCID: PMC9018718.

Labadie BW, Morris DS, Bryce AH, Given R, Zhang J, Abida W, Chowdhury S, Patnaik A. Guidelines for Management of Treatment-Emergent Adverse Events During Rucaparib Treatment of Patients with Metastatic Castration-Resistant Prostate Cancer. *Cancer Manag Res*. 2022 Feb 17;14:673-686. doi: 10.2147/CMAR.S335962. PMID: 35210863; PMCID: PMC8860352.

Nguyen B, Fong C, Luthra A, Smith SA, DiNatale RG, Nandakumar S, Walch H, Chatila WK, Madupuri R, Kundra R, Bielski CM, Mastrogiacomio B, Donoghue MTA, Boire A, Chandarlapaty S, Ganesh K, Harding JJ, Iacobuzio-Donahue CA, Razavi P, Reznik E, Rudin CM, Zamarin D, Abida W, Abou-Alfa GK, Aghajanian C, Cercek A, Chi P, Feldman D, Ho AL, Iyer G, Janjigian YY, Morris M, Motzer RJ, O'Reilly EM, Postow MA, Raj NP, Riely GJ, Robson ME, Rosenberg JE, Safonov A, Shoushtari AN, Tap W, Teo MY, Varghese AM, Voss M, Yaeger R, Zauderer MG, Abu-Rustum N, Garcia- Aguilar J, Bochner B, Hakimi A, Jarnagin WR, Jones DR, Molena D, Morris L, Rios- Doria E, Russo P, Singer S, Strong VE, Chakravarty D, Ellenson LH, Gopalan A, Reis-Filho JS, Weigelt B, Ladanyi M, Gonen M, Shah SP, Massague J, Gao J, Zehir A, Berger MF, Solit DB, Bakhoun SF, Sanchez-Vega F, Schultz N. Genomic characterization of metastatic patterns from prospective clinical sequencing of 25,000 patients. *Cell*. 2022 Feb 3;185(3):563-575.e11. doi: 10.1016/j.cell.2022.01.003. PMID: 35120664; PMCID: PMC9147702.

Maxwell KN, Cheng HH, Powers J, Gulati R, Ledet EM, Morrison C, Le A, Hausler R, Stopfer J, Hyman S, Kohlmann W, Naumer A, Vagher J, Greenberg SE, Naylor L, Laurino M, Konnick EQ, Shirts BH, AlDubayan SH, Van Allen EM, Nguyen B, Vijai J, Abida W, Carlo MI, Dubard-Gault M, Lee DJ, Maese LD, Mandelker D, Montgomery B, Morris MJ, Nicolosi P, Nussbaum RL, Schwartz LE, Stadler Z, Garber JE, Offit K, Schiffman JD, Nelson PS, Sartor O, Walsh MF, Pritchard CC. Inherited TP53 Variants and Risk of Prostate Cancer. *Eur Urol*. 2022 Mar;81(3):243-250. doi: 10.1016/j.eururo.2021.10.036. Epub 2021 Dec 1. PMID: 34863587; PMCID: PMC8891030.

Stopsack KH, Nandakumar S, Arora K, Nguyen B, Vasselman SE, Nweji B, McBride SM, Morris MJ, Rathkopf DE, Slovin SF, Danila DC, Autio KA, Scher HI, Mucci LA, Solit DB, Gönen M, Chen Y, Berger MF, Schultz N, Abida W*, Kantoff PW*. Differences in Prostate Cancer Genomes by Self-reported Race: Contributions of Genetic Ancestry, Modifiable Cancer Risk Factors, and Clinical Factors. *Clin Cancer Res*. 2022 Jan 15;28(2):318-326. doi: 10.1158/1078-0432.CCR-21-2577. Epub 2021 Oct 19. PMID: 34667026; PMCID: PMC8776579. *co-corresponding author

Loehr A, Patnaik A, Campbell D, Shapiro J, Bryce AH, McDermott R, Sautois B, Vogelzang NJ, Bambury RM, Voog E, Zhang J, Piulats JM, Hussain A, Ryan CJ, Merseburger AS, Daugaard G, Heidenreich A, Fizazi K, Higano CS, Krieger LE, Sternberg CN, Watkins SP, Despain D, Simmons AD, Dowson M, Golsorkhi T, Chowdhury S, Abida W. Response to Rucaparib in BRCA-Mutant Metastatic Castration-Resistant Prostate Cancer Identified by Genomic Testing in the TRITON2 Study. *Clin Cancer Res*. 2021 Dec 15;27(24):6677-6686. doi: 10.1158/1078-0432.CCR-21-2199. Epub 2021 Oct 1. PMID: 34598946; PMCID: PMC8678310.

Mateo J, McKay R, Abida W, Aggarwal R, Alumkal J, Alva A, Feng F, Gao X, Graff J, Hussain M, Karzai F, Montgomery B, Oh W, Patel V, Rathkopf D, Rettig M, Schultz N, Smith M, Solit D, Sternberg C, Van Allen E, VanderWeele D, Vinson J, Soule HR, Chinnaiyan A, Small E, Simons JW, Dahut W, Miyahira AK, Beltran H. Accelerating precision medicine in metastatic prostate cancer. *Nat Cancer*. 2020 Nov;1(11):1041-1053. doi: 10.1038/s43018-020-00141-0. Epub 2020 Nov 17. PMID: 34258585; PMCID: PMC8274325.

Rose Brannon A, Jayakumaran G, Diosdado M, Patel J, Razumova A, Hu Y, Meng F, Haque M, Sadowska J, Murphy BJ, Baldi T, Johnson I, Ptashkin R, Hasan M, Srinivasan P, Rema AB, Rijo I, Agarunov A, Won H, Perera D, Brown DN, Samoila A, Jing X, Gedvilaite E, Yang JL, Stephens DP, Dix JM, DeGroat N, Nafa K, Syed A, Li A, Lebow ES, Bowman AS, Ferguson DC, Liu Y, Mata DA, Sharma R, Yang SR, Bale T, Benhamida JK, Chang JC, Dogan S, Hameed MR, Hechtman JF, Mounq C, Ross DS, Vakiani E, Vanderbilt CM, Yao J, Razavi P, Smyth LM, Chandarlapaty S, Iyer G, Abida W, Harding JJ, Krantz B, O'Reilly E, Yu HA, Li BT, Rudin CM, Diaz L, Solit DB, Arcila ME, Ladanyi M, Loomis B, Tsui D, Berger MF, Zehir A, Benayed R. Enhanced specificity of clinical high-sensitivity tumor mutation profiling in cell-free DNA via paired normal sequencing using MSK-ACCESS. *Nat Commun*. 2021 Jun 18;12(1):3770. doi: 10.1038/s41467-021-24109-5. PMID: 34145282; PMCID: PMC8213710.

Kim DH, Sun D, Storck WK, Welker Leng K, Jenkins C, Coleman DJ, Sampson D, Guan X, Kumaraswamy A, Rodansky ES, Urrutia JA, Schwartzman JA, Zhang C, Beltran H, Labrecque

MP, Morrissey C, Lucas JM, Coleman IM, Nelson PS, Corey E, Handelman SK, Sexton JZ, Aggarwal R, Abida W, Feng FY, Small EJ, Spratt DE, Bankhead A 3rd, Rao A, Gesner EM, Attwell S, Lakhota S, Campeau E, Yates JA, Xia Z, Alumkal JJ. BET Bromodomain Inhibition Blocks an AR-Repressed, E2F1-Activated Treatment-Emergent Neuroendocrine Prostate Cancer Lineage Plasticity Program. *Clin Cancer Res*. 2021 Sep 1;27(17):4923-4936. doi: 10.1158/1078-0432.CCR-20-4968. Epub 2021 Jun 18. PMID: 34145028; PMCID: PMC8416959.

Stadler ZK, Maio A, Chakravarty D, Kemel Y, Sheehan M, Salo-Mullen E, Tkachuk K, Fong CJ, Nguyen B, Erakky A, Cadoo K, Liu Y, Carlo MI, Latham A, Zhang H, Kundra R, Smith S, Galle J, Aghajanian C, Abu-Rustum N, Varghese A, O'Reilly EM, Morris M, Abida W, Walsh M, Drilon A, Jayakumaran G, Zehir A, Ladanyi M, Ceyhan-Birsoy O, Solit DB, Schultz N, Berger MF, Mandelker D, Diaz LA Jr, Offit K, Robson ME. Therapeutic Implications of Germline Testing in Patients With Advanced Cancers. *J Clin Oncol*. 2021 Aug 20;39(24):2698-2709. doi: 10.1200/JCO.20.03661. Epub 2021 Jun 16. PMID: 34133209; PMCID: PMC8376329.

Tsui DWY, Cheng ML, Shady M, Yang JL, Stephens D, Won H, Srinivasan P, Huberman K, Meng F, Jing X, Patel J, Hasan M, Johnson I, Gedvilaite E, Houck- Loomis B, Socci ND, Selcuklu SD, Seshan VE, Zhang H, Chakravarty D, Zehir A, Benayed R, Arcila M, Ladanyi M, Funt SA, Feldman DR, Li BT, Razavi P, Rosenberg J, Bajorin D, Iyer G, Abida W, Scher HI, Rathkopf D, Viale A, Berger MF, Solit DB. Tumor fraction-guided cell-free DNA profiling in metastatic solid tumor patients. *Genome Med*. 2021 May 31;13(1):96. doi: 10.1186/s13073-021-00898-8. PMID: 34059130; PMCID: PMC8165771.

Tukachinsky H, Madison RW, Chung JH, Gjoerup OV, Severson EA, Dennis L, Fendler BJ, Morley S, Zhong L, Graf RP, Ross JS, Alexander BM, Abida W, Chowdhury S, Ryan CJ, Fizazi K, Golsorkhi T, Watkins SP, Simmons A, Loehr A, Venstrom JM, Oxnard GR. Genomic Analysis of Circulating Tumor DNA in 3,334 Patients with Advanced Prostate Cancer Identifies Targetable BRCA Alterations and AR Resistance Mechanisms. *Clin Cancer Res*. 2021 Jun 1;27(11):3094-3105. doi: 10.1158/1078-0432.CCR-20-4805. Epub 2021 Feb 8. PMID: 33558422; PMCID: PMC9295199.

Chakraborty G, Patail NK, Hirani R, Nandakumar S, Mazzu YZ, Yoshikawa Y, Atiq M, Jehane LE, Stopsack KH, Lee GM, Abida W, Morris MJ, Mucci LA, Danila D, Kantoff PW. Attenuation of SRC Kinase Activity Augments PARP Inhibitor-mediated Synthetic Lethality in *BRCA2*-altered Prostate Tumors. *Clin Cancer Res*. 2021 Mar 15;27(6):1792-1806. doi: 10.1158/1078-0432.CCR-20-2483. Epub 2020 Dec 17. PMID: 33334906; PMCID: PMC7956224.

Wise DR, Schneider JA, Armenia J, Febles VA, McLaughlin B, Brennan R, Thoren KL, Abida W, Sfanos KS, De Marzo AM, Yegnasubramanian S, Fox JJ, Haas M, Heath H, Kagey MH, Newman W, Sirard CA, Fleisher M, Morris MJ, Chen Y, Larson SM, Haffner MC, Nelson PS, Schultz N, Garabedian MJ, Scher HI, Logan SK, Sawyers CL; International SU2C/PCF Prostate Cancer Dream Team. Dickkopf-1 Can Lead to Immune Evasion in Metastatic Castration-Resistant Prostate Cancer. *JCO Precis Oncol*. 2020 Sep 29;4:PO.20.00097. doi: 10.1200/PO.20.00097. PMID: 33015525; PMCID: PMC7529521.

Aggarwal RR, Schweizer MT, Nanus DM, Pantuck AJ, Heath EI, Campeau E, Attwell S, Norek K, Snyder M, Bauman L, Lakhotia S, Feng FY, Small EJ, Abida W, Alumkal JJ. A Phase Ib/IIa Study of the Pan-BET Inhibitor ZEN-3694 in Combination with Enzalutamide in Patients with Metastatic Castration-resistant Prostate Cancer. *Clin Cancer Res*. 2020 Oct 15;26(20):5338-5347. doi: 10.1158/1078-0432.CCR-20-1707. Epub 2020 Jul 21. PMID: 32694156; PMCID: PMC7572827.

Leibold J, Ruscetti M, Cao Z, Ho YJ, Baslan T, Zou M, Abida W, Feucht J, Han T, Barriga FM, Tsanov KM, Zamechek L, Kulick A, Amor C, Tian S, Rybczyk K, Salgado NR, Sánchez-Rivera FJ, Watson PA, de Stanchina E, Wilkinson JE, Dow LE, Abate-Shen C, Sawyers CL, Lowe SW. Somatic Tissue Engineering in Mouse Models Reveals an Actionable Role for WNT Pathway Alterations in Prostate Cancer Metastasis. *Cancer Discov*. 2020 Jul;10(7):1038-1057. doi: 10.1158/2159-8290.CD-19-1242. Epub 2020 May 6. PMID: 32376773; PMCID: PMC7334089.

Karthaas WR, Hofree M, Choi D, Linton EL, Turkecul M, Bejnood A, Carver B, Gopalan A, Abida W, Laudone V, Biton M, Chaudhary O, Xu T, Masilionis I, Manova K, Mazutis L, Pe'er D, Regev A, Sawyers CL. Regenerative potential of prostate luminal cells revealed by single-cell analysis. *Science*. 2020 May 1;368(6490):497-505. doi: 10.1126/science.aay0267. PMID: 32355025; PMCID: PMC7313621.

Abida W*, Patnaik A, Campbell D, Shapiro J, Bryce AH, McDermott R, Sautois B, Vogelzang NJ, Bambury RM, Voog E, Zhang J, Piulats JM, Ryan CJ, Merseburger AS, Daugaard G, Heidenreich A, Fizazi K, Higano CS, Krieger LE, Sternberg CN, Watkins SP, Despain D, Simmons AD, Loehr A, Dowson M, Golsorkhi T, Chowdhury S; TRITON2 investigators. Rucaparib in Men With Metastatic Castration-Resistant Prostate Cancer Harboring a *BRCA1* or *BRCA2* Gene Alteration. *J Clin Oncol*. 2020 Nov 10;38(32):3763-3772. doi: 10.1200/JCO.20.01035. Epub 2020 Aug 14. PMID: 32795228

Stopsack KH, Nandakumar S, Wibmer AG, Haywood S, Weg ES, Barnett ES, Kim CJ, Carbone EA, Vasselmann SE, Nguyen B, Hullings MA, Scher HI, Morris MJ, Solit DB, Schultz N, Kantoff PW, Abida W*. Oncogenic Genomic Alterations, Clinical Phenotypes, and Outcomes in Metastatic Castration-Sensitive Prostate Cancer. *Clin Cancer Res*. 2020 Jul 1;26(13):3230-3238. doi: 10.1158/1078-0432.CCR-20-0168. Epub 2020 Mar 27. PubMed PMID: 32220891; PubMed Central PMCID: PMC7334067. *Corresponding author

Mota J, Barnett E, Nauseef J, Nguyen B, Stopsack K, Wibmer A, Flynn J, Heller G, Danila D, Rathkopf D, Slovin S, Kantoff P, Scher H, Morris M, Schultz N, Solit D, Abida W*. Platinum-Based Chemotherapy in Metastatic Prostate Cancer With DNA Repair Gene Alterations. *JCO Precision Oncology*. 2020/07; (4):355-366. doi: 10.1200/PO.19.00346. *Corresponding author

Zhang Z, Karthaas WR, Lee YS, Gao VR, Wu C, Russo JW, Liu M, Mota JM, Abida W, Linton E, Lee E, Barnes SD, Chen HA, Mao N, Wongvipat J, Choi D, Chen X, Zhao H, Manova-Todorova K, de Stanchina E, Taplin ME, Balk SP, Rathkopf DE, Gopalan A, Carver BS, Mu P, Jiang X, Watson PA, Sawyers CL. Tumor Microenvironment-Derived NRG1 Promotes

Antiandrogen Resistance in Prostate Cancer. *Cancer Cell*. 2020 Jun 23;. doi: 10.1016/j.ccell.2020.06.005. [Epub ahead of print] PubMed PMID: 32679108.

Abida W*, Campbell D, Patnaik A, Shapiro JD, Sautois B, Vogelzang NJ, Voog EG, Bryce AH, McDermott R, Ricci F, Rowe J, Zhang J, Piulats JM, Fizazi K, Merseburger AS, Higano CS, Krieger LE, Ryan CJ, Feng FY, Simmons AD, Loehr A, Despain D, Dowson M, Green F, Watkins SP, Golsorkhi T, Chowdhury S. Non-BRCA DNA Damage Repair Gene Alterations and Response to the PARP Inhibitor Rucaparib in Metastatic Castration-Resistant Prostate Cancer: Analysis From the Phase II TRITON2 Study. *Clin Cancer Res*. 2020 Jun 1;26(11):2487-2496. doi: 10.1158/1078-0432.CCR-20-0394. Epub 2020 Feb 21. PubMed PMID: 32086346.

*Corresponding author

Karthauss WR, Hofree M, Choi D, Linton EL, Turkecul M, Bejnood A, Carver B, Gopalan A, Abida W, Laudone V, Biton M, Chaudhary O, Xu T, Masionis I, Manova K, Mazutis L, Pe'er D, Regev A, Sawyers CL. Regenerative potential of prostate luminal cells revealed by single-cell analysis. *Science*. 2020 May 1;368(6490):497-505. doi: 10.1126/science.aay0267. PubMed PMID: 32355025; PubMed Central PMCID: PMC7313621.

Nguyen B, Mota JM, Nandakumar S, Stopsack KH, Weg E, Rathkopf D, Morris MJ, Scher HI, Kantoff PW, Gopalan A, Zamarin D, Solit DB, Schultz N, Abida W*. Pan-cancer Analysis of CDK12 Alterations Identifies a Subset of Prostate Cancers with Distinct Genomic and Clinical Characteristics. *Eur Urol*. 2020 Apr 19;. doi: 10.1016/j.eururo.2020.03.024. [Epub ahead of print] PubMed PMID: 32317181. *Corresponding author

Chakraborty G, Armenia J, Mazzu YZ, Nandakumar S, Stopsack KH, Atiq MO, Komura K, Jehane L, Hirani R, Chadavada K, Yoshikawa Y, Khan NA, Chen Y, Abida W, Mucci LA, Lee GM, Nanjangud GJ, Kantoff PW. Significance of BRCA2 and RB1 Co-loss in Aggressive Prostate Cancer Progression. *Clin Cancer Res*. 2020 Apr 15;26(8):2047-2064. doi: 10.1158/1078-0432.CCR-19-1570. Epub 2019 Dec 3. PubMed PMID: 31796516.

Zhang Z, Zhou C, Li X, Barnes SD, Deng S, Hoover E, Chen CC, Lee YS, Zhang Y, Wang C, Metang LA, Wu C, Tirado CR, Johnson NA, Wongvipat J, Navrazhina K, Cao Z, Choi D, Huang CH, Linton E, Chen X, Liang Y, Mason CE, de Stanchina E, Abida W, Lujambio A, Li S, Lowe SW, Mendell JT, Malladi VS, Sawyers CL, Mu P. Loss of CHD1 Promotes Heterogeneous Mechanisms of Resistance to AR-Targeted Therapy via Chromatin Dysregulation. *Cancer Cell*. 2020 Apr 13;37(4):584-598.e11. doi: 10.1016/j.ccell.2020.03.001. Epub 2020 Mar 26. PubMed PMID: 32220301; PubMed Central PMCID: PMC7292228.

Gupta S, Fine SW, Chang J, Tickoo SK, Chen YB, Al-Ahmadie HA, Sirintrapun SJ, Abida W, Ladanyi M, Reuter VE, Gopalan A. Morphologic and Immunohistochemical Assessment of CDH1 Loss of Function Alterations in Prostatic Adenocarcinoma. *Am J Surg Pathol*. 2020 Mar;44(3):420-422. doi: 10.1097/PAS.0000000000001427. PubMed PMID: 31876541.

Gupta S, Vanderbilt C, Abida W, Fine SW, Tickoo SK, Al-Ahmadie HA, Chen YB, Sirintrapun SJ, Chadalavada K, Nanjangud GJ, Bialik A, Morris MJ, Scher HI, Ladanyi M, Reuter VE, Gopalan A. Immunohistochemistry-based assessment of androgen receptor status and the AR-null phenotype in metastatic castrate resistant prostate cancer. *Prostate Cancer Prostatic Dis*. 2020 Feb 24;. doi: 10.1038/s41391-020-0214-6. [Epub ahead of print] PubMed PMID: 32094488

Razavi P, Li BT, Brown DN, Jung B, Hubbell E, Shen R, Abida W, Juluru K, De Bruijn I, Hou C, Venn O, Lim R, Anand A, Maddala T, Gnerre S, Vijaya Satya R, Liu Q, Shen L, Eattock N, Yue J, Blocker AW, Lee M, Sehnert A, Xu H, Hall MP, Santiago-Zayas A, Novotny WF, Isbell JM, Rusch VW, Plitas G, Heerdt AS, Ladanyi M, Hyman DM, Jones DR, Morrow M, Riely GJ, Scher HI, Rudin CM, Robson ME, Diaz LA Jr, Solit DB, Aravanis AM, Reis-Filho JS. High-intensity sequencing reveals the sources of plasma circulating cell-free DNA variants. *Nat Med*. 2019 Dec;25(12):1928-1937. doi: 10.1038/s41591-019-0652-7. Epub 2019 Nov 25. PubMed PMID: 31768066; PubMed Central PMCID: PMC7061455.

Woo S, Suh CH, Eastham JA, Zelefsky MJ, Morris MJ, Abida W, Scher HI, Sidlow R, Becker AS, Wibmer AG, Hricak H, Vargas HA. Comparison of Magnetic Resonance Imaging-stratified Clinical Pathways and Systematic Transrectal Ultrasound-guided Biopsy Pathway for the Detection of Clinically Significant Prostate Cancer: A Systematic Review and Meta-analysis of Randomized Controlled Trials. *Eur Urol Oncol*. 2019 Nov;2(6):605-616. doi: 10.1016/j.euo.2019.05.004. Epub 2019 Jun 14. PubMed PMID: 31204311;

Jonsson P, Bandlamudi C, Cheng ML, Srinivasan P, Chavan SS, Friedman ND, Rosen EY, Richards AL, Bouvier N, Selcuklu SD, Bielski CM, Abida W, Mandelker D, Birsoy O, Zhang L, Zehir A, Donoghue MTA, Baselga J, Offit K, Scher HI, O'Reilly EM, Stadler ZK, Schultz N, Socci ND, Viale A, Ladanyi M, Robson ME, Hyman DM, Berger MF, Solit DB, Taylor BS. Tumour lineage shapes BRCA-mediated phenotypes. *Nature*. 2019 Jul;571(7766):576-579. doi: 10.1038/s41586-019-1382-1. Epub 2019 Jul 10. PubMed PMID: 31292550; PubMed Central PMCID: PMC7048239.

Abida W, Cyrta J, Heller G, Prandi D, Armenia J, Coleman I, Cieslik M, Benelli M, Robinson D, Van Allen EM, Sboner A, Fedrizzi T, Mosquera JM, Robinson BD, De Sarkar N, Kunju LP, Tomlins S, Wu YM, Nava Rodrigues D, Loda M, Gopalan A, Reuter VE, Pritchard CC, Mateo J, Bianchini D, Miranda S, Carreira S, Rescigno P, Filipenko J, Vinson J, Montgomery RB, Beltran H, Heath EI, Scher HI, Kantoff PW, Taplin ME, Schultz N, deBono JS, Demichelis F, Nelson PS, Rubin MA, Chinnaiyan AM, Sawyers CL. Genomic correlates of clinical outcome in advanced prostate cancer. *Proc Natl Acad Sci U S A*. 2019 Jun 4;116(23):11428-11436.

Stopsack KH, Gerke T, Tyekucheva S, Mazzu YZ, Lee GM, Chakraborty G, Abida W, Mucci LA, Kantoff PW. Low expression of the androgen-induced tumor suppressor gene PLZF and lethal prostate cancer. *Cancer Epidemiol Biomarkers Prev*. 2019. ePub ahead of print. PMID: 30602500

Abida W*, Cheng ML, Armenia J, Middha S, Autio KA, Vargas HA, Rathkopf D, Morris MJ, Danila DC, Slovin SF, Carbone E, Barnett ES, Hullings M, Hechtman JF, Zehir A, Shia J, Jonsson P, Stadler ZK, Srinivasan P, Laudone VP, Reuter V, Wolchok JD, Socci ND, Taylor BS, Berger MF, Kantoff PW, Sawyers CL, Schultz N, Solit DB, Gopalan A, Scher HI. Analysis of the Prevalence of Microsatellite Instability in Prostate Cancer and Response to Immune Checkpoint Blockade. *JAMA Oncol.* 2018. December 27. PMID: 30589920. *Corresponding author

Carlo MI, Giri VN, Paller CJ, Abida W, Alumkal JJ, Beer TM, Beltran H, George DJ, Heath EI, Higano CS, McKay RR, Morgans AK, Patnaik A, Ryan CJ, Schaeffer EM, Stadler WM, Taplin ME, Kauff ND, Vinson J, Antonarakis ES, Cheng HH. Evolving Intersection Between Inherited Cancer Genetics and Therapeutic Clinical Trials in Prostate Cancer: A White Paper From the Germline Genetics Working Group of the Prostate Cancer Clinical Trials Consortium. *JCO Precis Oncol.* Epub 2018 Aug 16. PMID: 30761386

Nava Rodrigues D, Rescigno P, Liu D, Yuan W, Carreira S, Lambros MB, Seed G, Mateo J, Riisnaes R, Mullane S, Margolis C, Miao D, Miranda S, Dolling D, Clarke M, Bertan C, Crespo M, Boysen G, Ferreira A, Sharp A, Figueiredo I, Keliher D, Aldubayan S, Burke KP, Sumanasuriya S, Fontes MS, Bianchini D, Zafeiriou Z, Teixeira Mendes LS, Mouw K, Schweizer MT, Pritchard CC, Salipante S, Taplin ME, Beltran H, Rubin MA, Cieslik M, Robinson D, Heath E, Schultz N, Armenia J, Abida W, Scher H, Lord C, D'Andrea A, Sawyers CL, Chinnaiyan AM, Alimonti A, Nelson PS, Drake CG, Van Allen EM, de Bono JS. Immunogenomic analyses associate immunological alterations with mismatch repair defects in prostate cancer. *J Clin Invest.* 2018 Oct 1;128(10):4441-4453

Hieronimus H, Murali R, Tin A, Yadav K, Abida W, Moller H, Berney D, Scher H, Carver B, Scardino P, Schultz N, Taylor B, Vickers A, Cuzick J, Sawyers CL. Tumor copy number alteration burden is a pan-cancer prognostic factor associated with recurrence and death. *Elife.* 2018 Sep 4;7.

Armenia J, Wankowicz SAM, Liu D, Gao J, Kundra R, Reznik E, Chatila WK, Chakravarty D, Han GC, Coleman I, Montgomery B, Pritchard C, Morrissey C, Barbieri CE, Beltran H, Sboner A, Zafeiriou Z, Miranda S, Bielski CM, Penson AV, Tolonen C, Huang FW, Robinson D, Wu YM, Lonigro R, Garraway LA, Demichelis F, Kantoff PW, Taplin ME, Abida W, *et al.*. The long tail of oncogenic drivers in prostate cancer. *Nat Genet.* 2018 Apr 2. [Epub ahead of print]

Giri VN, Knudsen KE, Kelly WK, Abida W, *et al.* Role of Genetic Testing for Inherited Prostate Cancer Risk: Philadelphia Prostate Cancer Consensus Conference 2017. *J Clin Oncol.* 2018 Feb 1; 36(4):414-424

Shah N, Wang P, Wongvipat J, Karthaus WR, Abida W, Armenia J, Rockowitz S, Drier Y, Bernstein BE, Long HW, Freedman ML, Arora VK, Zheng D, Sawyers CL. Regulation of the

glucocorticoid receptor via a BET-dependent enhancer drives antiandrogen resistance in prostate cancer. *Elife*. 2017 Sep 11

Mandelker D, Zhang L, Kemel Y, Stadler ZK, Joseph V, Zehir A, Pradhan N, Arnold A, Walsh MF, Li Y, Balakrishnan AR, Syed A, Prasad M, Nafa K, Carlo MI, Cadoo KA, Sheehan M, Fleischut MH, Salo-Mullen E, Trottier M, Lipkin SM, Lincoln A, Mukherjee S, Ravichandran V, Cambria R, Galle J, Abida W, Arcila ME, *et al*. Mutation Detection in Patients With Advanced Cancer by Universal Sequencing of Cancer-Related Genes in Tumor and Normal DNA vs Guideline-Based Germline Testing. *JAMA* 2017, August, 318(9):825-835

Abida W, Armenia J, Gopalan A, Brennan R, Walsh M, Barron D, Danila D *et al*. Prospective Genomic Profiling of Prostate Cancer Across Disease States Reveals Germline and Somatic Alterations that May Affect Clinical Decision Making. *JCO Precision Oncol*. 2017, June, 1: 1-16

iii. **Books or other non-periodical, one-time publications.**

None

b. **Other publications, conference papers, and presentations.**

None

c. **Technologies or techniques**

None

d. **Inventions, patent applications, and/or licenses**

None

e. **Other Products**

None

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

a. **What individuals have worked on the project?**

- i. Only the PI has worked at least one-person-month per year on this project

Name:	Wassim Abida, MD, PhD
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	NA
Nearest person month worked:	7
Contribution to Project:	The PI oversees the project and performs major analyses

Funding Support:	DoD PCRP Physician Research Award Prostate Cancer Foundation Young Investigator Award
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- b. **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

Nothing to report

- c. **What other organizations were involved as partners?**

1. **Organization Name:** Prostate Cancer Foundation
2. **Location of Organization:** Santa Monica, CA
3. **Partner's contribution to the project** The Prostate Cancer Foundation has provided extension funding for the multi-institutional SU2C-PCF project that is a component of AIM
 1. This funding is related to sequencing and management of the overall project, and not directly related / does not overlap with the work funded by DoD to the PI of this grant.
 - a. **Financial support:** As above.
 - b. **In-kind support** None
 - c. **Facilities** None
 - d. **Collaboration** None
 - e. **Personnel exchanges** None
 - f. **Other.** None

8. **SPECIAL REPORTING REQUIREMENTS**

- a. **COLLABORATIVE AWARDS:** Not applicable
- b. **QUAD CHARTS:** Not applicable

9. **APPENDICES: JOURNAL PUBLICATIONS** (please note – only copies of journal

publications co-authored by PI for work performed under the scope of this grant are attached)

- a. Abida W, Cyrta J, Heller G, Prandi D, Armenia J, Coleman I, Cieslik M, Benelli M, Robinson D, Van Allen EM, Sboner A, Fedrizzi T, Mosquera JM, Robinson BD, De Sarkar N, Kunju LP, Tomlins S, Wu YM, Nava Rodrigues D, Loda M, Gopalan A, Reuter VE, Pritchard CC, Mateo J, Bianchini D, Miranda S, Carreira S, Rescigno P, Filipenko J, Vinson J, Montgomery RB, Beltran H, Heath EI, Scher HI, Kantoff PW, Taplin ME, Schultz N, deBono JS, Demichelis F, Nelson PS, Rubin MA, Chinnaiyan AM, Sawyers CL. Genomic correlates of clinical outcome in advanced prostate cancer. Proc Natl Acad Sci U S A. 2019 Jun 4;116(23):11428-11436.

- b. Abida W*, Cheng ML, Armenia J, Middha S, Autio KA, Vargas HA, Rathkopf D, Morris MJ, Danila DC, Slovin SF, Carbone E, Barnett ES, Hullings M, Hechtman JF, Zehir A, Shia J, Jonsson P, Stadler ZK, Srinivasan P, Laudone VP, Reuter V, Wolchok JD, Socci ND, Taylor BS, Berger MF, Kantoff PW, Sawyers CL, Schultz N, Solit DB, Gopalan A, Scher HI. Analysis of the Prevalence of Microsatellite Instability in Prostate Cancer and Response to Immune Checkpoint Blockade. *JAMA Oncol.* 2018. December 27. PMID: 30589920.
*Corresponding author
- c. Nguyen B, Mota JM, Nandakumar S, Stopsack KH, Weg E, Rathkopf D, Morris MJ, Scher HI, Kantoff PW, Gopalan A, Zamarin D, Solit DB, Schultz N, Abida W*. Pan-cancer Analysis of CDK12 Alterations Identifies a Subset of Prostate Cancers with Distinct Genomic and Clinical Characteristics. *Eur Urol.* 2020 Apr 19;. doi: 10.1016/j.eururo.2020.03.024. [Epub ahead of print] PubMed PMID: 32317181.
*Corresponding author
- d. Stopsack KH, Nandakumar S, Wibmer AG, Haywood S, Weg ES, Barnett ES, Kim CJ, Carbone EA, Vasselmann SE, Nguyen B, Hullings MA, Scher HI, Morris MJ, Solit DB, Schultz N, Kantoff PW, Abida W*. Oncogenic Genomic Alterations, Clinical Phenotypes, and Outcomes in Metastatic Castration-Sensitive Prostate Cancer. *Clin Cancer Res.* 2020 Jul 1;26(13):3230-3238. doi: 10.1158/1078-0432.CCR-20-0168. Epub 2020 Mar 27. PubMed PMID: 32220891; PubMed Central PMCID: PMC7334067. *Corresponding author
- e. Abida W*, Campbell D, Patnaik A, Shapiro JD, Sautois B, Vogelzang NJ, Voog EG, Bryce AH, McDermott R, Ricci F, Rowe J, Zhang J, Piulats JM, Fizazi K, Merseburger AS, Higano CS, Krieger LE, Ryan CJ, Feng FY, Simmons AD, Loehr A, Despain D, Dowson M, Green F, Watkins SP, Golsorkhi T, Chowdhury S. Non-BRCA DNA Damage Repair Gene Alterations and Response to the PARP Inhibitor Rucaparib in Metastatic Castration-Resistant Prostate Cancer: Analysis From the Phase II TRITON2 Study. *Clin Cancer Res.* 2020 Jun 1;26(11):2487-2496. doi: 10.1158/1078-0432.CCR-20-0394. Epub 2020 Feb 21. PubMed PMID: 32086346. *Corresponding author
- f. Abida W*, Patnaik A, Campbell D, Shapiro J, Bryce AH, McDermott R, Sautois B, Vogelzang NJ, Bambury RM, Voog E, Zhang J, Piulats JM, Ryan CJ, Merseburger AS, Daugaard G, Heidenreich A, Fizazi K, Higano CS, Krieger LE, Sternberg CN, Watkins SP, Despain D, Simmons AD, Loehr A, Dowson M, Golsorkhi T, Chowdhury S; TRITON2 investigators. Rucaparib in Men With Metastatic Castration-Resistant Prostate Cancer Harboring a *BRCA1* or *BRCA2* Gene Alteration. *J Clin Oncol.* 2020 Nov 10;38(32):3763-3772. doi: 10.1200/JCO.20.01035. Epub 2020 Aug 14. PMID: 32795228 *Corresponding author
- g. Mota J, Barnett E, Nauseef J, Nguyen B, Stopsack K, Wibmer A, Flynn J, Heller G, Danila D, Rathkopf D, Slovin S, Kantoff P, Scher H, Morris M, Schultz N, Solit D, Abida W*. Platinum-Based Chemotherapy in Metastatic Prostate Cancer With DNA Repair Gene Alterations. *JCO Precision Oncology.* 2020/07; (4):355-366. doi: 10.1200/PO.19.00346.
*Corresponding author

- h. Stopsack KH, Nandakumar S, Arora K, Nguyen B, Vasselmann SE, Nweji B, McBride SM, Morris MJ, Rathkopf DE, Slovin SF, Danila DC, Autio KA, Scher HI, Mucci LA, Solit DB, Gönen M, Chen Y, Berger MF, Schultz N, Abida W*, Kantoff PW*. Differences in Prostate Cancer Genomes by Self-reported Race: Contributions of Genetic Ancestry, Modifiable Cancer Risk Factors, and Clinical Factors. Clin Cancer Res. 2022 Jan 15;28(2):318-326. doi: 10.1158/1078-0432.CCR-21-2577. Epub 2021 Oct 19. PMID: 34667026; PMCID: PMC8776579. *co-corresponding author

Genomic correlates of clinical outcome in advanced prostate cancer

Wassim Abida^{a,1}, Joanna Cytra^{b,c,1,2}, Glenn Heller^{d,1}, Davide Prandi^e, Joshua Armenia^{f,3}, Ilsa Coleman^g, Marcin Cieslik^h, Matteo Benelli^{e,4}, Dan Robinson^h, Eliezer M. Van Allen^{ij}, Andrea Sbener^b, Tarcisio Fedrizzi^e, Juan Miguel Mosquera^b, Brian D. Robinson^b, Navonil De Sarkar^g, Lakshmi P. Kunju^h, Scott Tomlins^h, Yi Mi Wu^h, Daniel Nava Rodrigues^{k,l}, Massimo Loda^{b,m}, Anuradha Gopalanⁿ, Victor E. Reuterⁿ, Colin C. Pritchard^g, Joaquin Mateo^{k,l,5}, Diletta Bianchini^{k,l}, Susana Miranda^{k,l}, Suzanne Carreira^{k,l}, Pasquale Rescigno^{k,l}, Julie Filipenko^o, Jacob Vinson^o, Robert B. Montgomery^g, Himisha Beltran^{i,p}, Elisabeth I. Heath^{q,r}, Howard I. Scher^a, Philip W. Kantoff^a, Mary-Ellen Taplin^{i,6}, Nikolaus Schultz^{d,6}, Johann S. deBono^{k,l,6}, Francesca Demichelis^{e,6}, Peter S. Nelson^{g,6,7}, Mark A. Rubin^{b,c,6,7}, Arul M. Chinnaiyan^{h,s,6,7}, and Charles L. Sawyers^{t,6,7}

Contributed by Charles L. Sawyers, March 27, 2019 (sent for review February 19, 2019; reviewed by Samuel Aparicio, John T. Isaacs, and Nandita Mitra)

Heterogeneity in the genomic landscape of metastatic prostate cancer has become apparent through several comprehensive profiling efforts, but little is known about the impact of this heterogeneity on clinical outcome. Here, we report comprehensive genomic and transcriptomic analysis of 429 patients with metastatic castration-resistant prostate cancer (mCRPC) linked with longitudinal clinical outcomes, integrating findings from whole-exome, transcriptome, and histologic analysis. For 128 patients treated with a first-line next-generation androgen receptor signaling inhibitor (ARSI; abiraterone or enzalutamide), we examined the association of 18 recurrent DNA- and RNA-based genomic alterations, including androgen receptor (AR) variant expression, AR transcriptional output, and neuroendocrine expression signatures, with clinical outcomes. Of these, only *RB1* alteration was significantly associated with poor survival, whereas alterations in *RB1*, *AR*, and *TP53* were associated with shorter time on treatment with an ARSI. This large analysis integrating mCRPC genomics with histology and clinical outcomes identifies *RB1* genomic alteration as a potent predictor of poor

outcome, and is a community resource for further interrogation of clinical and molecular associations.

castration-resistant prostate cancer | integrative genomics | clinical outcomes | biomarkers

Several studies have described the genomic landscape of primary and metastatic castration-resistant prostate cancer (mCRPC), revealing distinct genomic subtypes in primary localized disease, including ETS fusion-positive and *SPOP*-mutated prostate cancer (1–5), and subsets of patients with advanced disease who harbor potentially clinically actionable alterations in their tumor or in the germline (4–6). Based on these findings, prospective trials are currently enrolling patients with defined genomic alterations, including PARP inhibitor studies for patients with alterations in *BRCA2/1*, *ATM*, and other DNA repair genes (NCT02952534, NCT02987543, NCT02854436), and AKT inhibitor

Author contributions: W.A., G.H., F.D., P.S.N., M.A.R., A.M.C., and C.L.S. designed research; J.C., N.S., F.D., and M.A.R. performed research; J.M., D.B., S.M., S.C., P.R., R.B.M., H.B., E.I.H., H.I.S., P.W.K., M.-E.T., and J.S.d. contributed new reagents/analytic tools; W.A., G.H., D.P., J.A., I.C., M.C., M.B., D.R., E.M.V.A., A.S., T.F., J.M.M., B.D.R., N.D.S., L.P.K., S.T., Y.M.W., D.N.R., M.L., A.G., V.E.R., C.C.P., J.F., J.V., N.S., F.D., P.S.N., M.A.R., and A.M.C. analyzed data; and W.A., F.D., P.S.N., M.A.R., A.M.C., and C.L.S. wrote the paper.

Reviewers: S.A., BC Cancer Agency and University of British Columbia; J.T.I., Johns Hopkins Oncology Center; and N.M., University of Pennsylvania.

Conflict of interest statement: W.A. has consulted for Clovis Oncology, Janssen, ORIC Pharmaceuticals, and MORE Health; has received honorarium from Caret Healthcare; has received travel funds from Clovis Oncology, ORIC Pharmaceuticals, and GlaxoSmithKline; and has received research funding from AstraZeneca, Clovis Oncology, Zenith Epigenetics, and GlaxoSmithKline. G.H., received research funding from Janssen. J.A. is currently employed at AstraZeneca. E.M.V.A. has consulted for Tango Therapeutics, Genome Medical, Invitae, Illumina, Foresite Capital, and Dynamo; has received research support from Novartis and BMS; has equity in Tango Therapeutics, Genome Medical, Syapse, and Microsoft; has received travel reimbursement from Roche/Genentech; and is a coinventor of institutional patents filed on ERCC2 mutations and chemotherapy response, chromatin mutations and immunotherapy response, and methods for clinical interpretation. H.B. has received research funding from Millennium Pharmaceuticals, AbbVie/Stemcentrx, Astellas, Janssen, and Eli Lilly; and is a consultant/advisor for Sanofi Genzyme and Janssen. S.T. is a coinventor for University of Michigan patents on ETS fusion genes, and diagnostic field of use licensed to Hologic/Gen-Probe Inc., which has sublicensed rights to Roche/Ventana Medical Systems; is a consultant for and has received honoraria from Janssen, AbbVie, Sanofi, Almac Diagnostics, and Astellas/Medivation; has performed sponsored research from Astellas/Medivation and GenomeDx; and is an equity holder in and employee of Strata Oncology. J.M. has consulted for AstraZeneca and Janssen; and has received travel support or speaker fees from Astellas, AstraZeneca, Sanofi, and IPSEN. D.B. has received honoraria and travel support from Janssen. H.I.S. has consulted for Astellas, Ferring Pharmaceuticals, Janssen Biotech, Janssen Research and Development, Sanofi Aventis, Clovis Oncology, Merck, and WCG Oncology; is a member of the board of directors for Asterias Biotherapeutics; and has received research support from Illumina Inc., Innocrin, and Janssen. P.W.K. is a board member for Context Therapeutics LLC, has consulted for BIND Biosciences, BN Immunotherapeutics, DRGT, GE Healthcare, Janssen Pharmaceutica, Metamark, New England Research Institutes, OncoCellMDx, Progenity, Sanofi-Aventis, Seer Biosciences Inc., Tarveda Therapeutics, and Thermo Fisher Scientific; serves on data safety monitoring boards for Genentech/Roche and Merck & Co.; and has investment interest in Context Therapeutics, DRGT, Seer Biosciences, Placon, and Tarveda Therapeutics. M.-E.T. has consulted for Janssen. J.S.d. is an advisory board member for AstraZeneca, Astellas, Bayer, Boehringer Ingelheim, Genentech/Roche, Genmab, GSK, Janssen, Merck Serono, Merck Sharp & Dohme, Menarini/Silicon Biosystems, Orion, Pfizer, Sanofi Aventis, and Taiho; and his institution has received funding or other support from AstraZeneca, Astellas, Bayer, Genentech, GSK, Janssen, Merck Serono, MSD, Menarini/Silicon Biosystems, Orion, Sanofi Aventis, and Taiho. P.S.N. is a compensated advisor to Janssen, Astellas, and Roche. M.A.R. is a coinventor on a patent for Gene Fusion Prostate Cancer (Harvard/Michigan) and E2H2 (Michigan) in the area of diagnostics and therapeutics; is a coinventor on a patent filed by Cornell University on AURKA and SPOP mutations in the field of prostate cancer diagnostics; receives royalties on licensing agreements for these inventions; and receives research support from Janssen, Eli Lilly, Millenium, and Sanofi-Aventis. C.L.S. serves on the board of directors of Novartis; is a cofounder of ORIC Pharmaceuticals and coinventor of enzalutamide and apalutamide; is a science advisor to Agios, Beigene, Blueprint, Column Group, Foghorn, Housley Pharma, Nextech, KSQ, Petra, and PMV; and is a cofounder of Seragon, purchased by Genentech/Roche in 2014.

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Data deposition: The data reported in this paper are available in [Dataset S1](https://www.ncbi.nlm.nih.gov/bioproject/51) and at www.cbiportal.org, and have been deposited in GitHub, https://github.com/cBioPortal/datahub/tree/master/public/prad_su2c_2019.

See Commentary on page 11090.

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⁶M.-E.T., N.S., J.S.d., F.D., P.S.N., M.A.R., A.M.C., and C.L.S. contributed equally to this work.

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This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1902651116/-DCSupplemental.

Published online May 6, 2019.

Significance

The genomic landscape of metastatic castration-resistant prostate cancer (mCRPC) has been well-defined, but the association of genomic findings with patient clinical outcomes and with other characteristics including histology and transcriptional pathway activity remains poorly understood. Here, we describe comprehensive integrative analysis of genomic and transcriptomic profiles, histology, and clinical outcomes for 429 patients with mCRPC. Of all the molecular factors we examined, alterations in *RB1* had the strongest association with poor outcome. Our study identifies molecularly defined groups of patients who may benefit from a more aggressive treatment approach, with the genomic and outcome data made available to the research community for further interrogation.

studies for men with PI3K pathway alterations (NCT02525068, NCT03310541).

In addition, various genomic and histologic features of prostate cancer have been described as conferring a worse prognosis. Among these are the presence of neuroendocrine or small-cell characteristics in tumors, sometimes referred to as aggressive variant prostate cancer or neuroendocrine prostate cancer (7–9), the detection of androgen receptor (AR) splice variant 7 in circulating tumor cells (10, 11), and the presence of genomic alterations in TP53, RB1, DNA repair genes, AR, and PI3K pathway genes in circulating tumor DNA (12, 13). However, studies that comprehensively examine all of these characteristics—histology, genomics, and transcriptomics—and their association with outcomes in mCRPC are lacking.

Here, we expand a foundational genomic resource of mCRPC tumors (5) from 150 to 429 patients (444 tumors), and integrate the analysis of whole-exome sequencing, gene expression, and

histopathology with clinical outcomes, including survival and time on treatment with the next-generation androgen signaling inhibitors (ARSIs) enzalutamide and abiraterone acetate to identify the most important prognostic markers in mCRPC within a single large multiinstitutional genomic dataset, with tumor- and patient-level data made available for additional correlative analyses.

Results

Clinical and Histopathologic Parameters. A total of 429 patients were enrolled at one of seven international consortium centers, all of whom underwent biopsy for the collection of mCRPC tissue as well as collection of blood for matched normal DNA extraction. Whole-exome sequencing was successfully performed on 444 tumors (some patients underwent multiple biopsies), and RNA sequencing (RNA-seq) was successfully performed on a subset of these (332 tumors from 323 patients). Of the 444 biopsies, 37% were lymph node, 36% were bone, and 14% were liver (Fig. 1A). Samples underwent central histopathologic review (Fig. 1B), revealing neuroendocrine (NE) or small-cell features in 11.2% (41 of 366) of evaluable cases, including from patients who were enrolled on a trial of the Aurora kinase A inhibitor alisertib (14). Median age at diagnosis with prostate cancer was 61 y, median age at biopsy of the profiled sample was 67 y, and median overall survival from the time of biopsy was 16 mo (SI Appendix, Table S1). Samples were balanced for exposure status to ARSIs (47% ARSI naive, 46% previously exposed) (Fig. 1C). Sixty-three percent of samples were acquired before exposure to a taxane. Patients who were naive to both ARSI and taxane at the time of biopsy had the longest median overall survival from the date of biopsy, whereas patients previously treated with both an ARSI and a taxane had the shortest (Fig. 1D), consistent with their more advanced disease state at the time of tissue acquisition. Of note, time

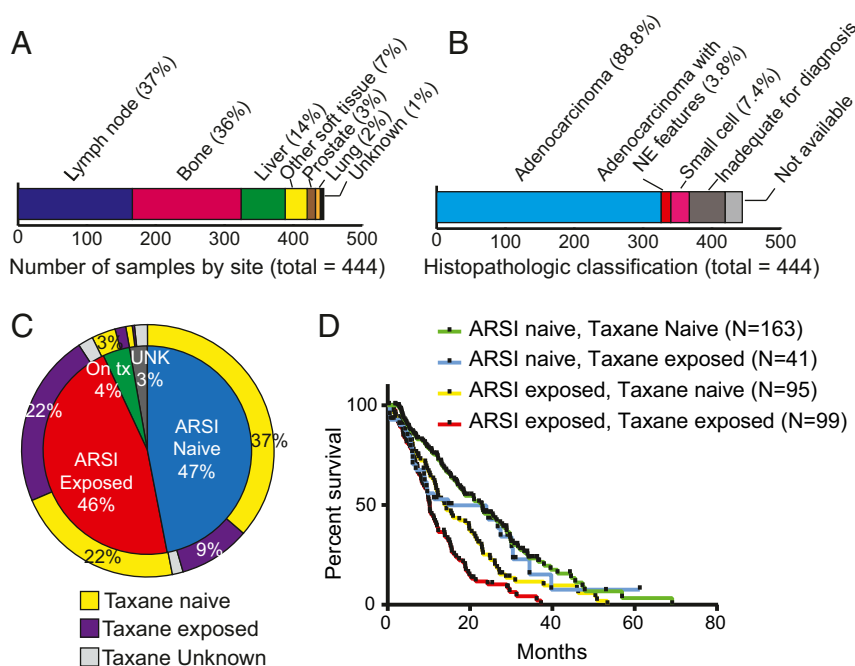


Fig. 1. Overview of sample and patient characteristics for 444 tumors from 429 patients with mCRPC. (A) Site of mCRPC tumors profiled. (B) Histopathologic classification of profiled tumors. Tumors were classified by central review as adenocarcinoma, pure small-cell/neuroendocrine cancer, adenocarcinoma with neuroendocrine features (also included mixed acinar/neuroendocrine carcinoma), or could not be classified due to scant material or no tumor visible on the slides that were available for review despite successful sequencing. (C) Patient exposure status to next-generation AR signaling inhibitors (abiraterone acetate, enzalutamide, or ARN509) and to taxanes at the time of biopsy for the 444 profiled tumors. (D) Overall survival (OS) from the date of biopsy of the profiled tumor. OS was longer for tumors from ARSI- and taxane-naïve patients compared with patients who had received an ARSI before the biopsy ($P < 0.01$, log-rank test). Survival was shortest when the patient had received both an ARSI and taxane chemotherapy at the time of biopsy.

on treatment with a first-line ARSI was highly associated with overall survival from the start of first-line therapy (Kendall's $\tau = 0.65$).

Landscape of Genomic Alterations. The frequency of genomic alterations was similar to that reported in prior cohorts (4, 5, 15), with *AR*, ETS family transcription factors including *ERG* and *ETV1*, *TP53*, and *PTEN* and *RB1* emerging as the most commonly altered genes (Fig. 2A). Likewise, alterations in biological pathways (*SI Appendix, Table S2*) were also consistent with prior reports, with a significant (>20%) subset of patients harboring at least one alteration in a PI3K, cell-cycle, epigenetic, or DNA repair pathway gene. Single-nucleotide variants (SNVs) in the most frequently altered genes were found to be likely oncogenic (16) in the majority of cases (Fig. 2B), with a high fraction of oncogenic mutations in *AR*, *TP53*, *PIK3CA*, *BRCA2*, *PTEN*, *APC*, and *CDK12*. Mutations in *ATM* were predicted to be likely oncogenic in nearly 60% of cases, with the rest being missense mutations of unknown significance.

The large size of the dataset allowed for a comprehensive search for genomic alterations that are co-occurring or mutually exclusive (Fig. 2C). As expected, we found mutual exclusivity between alterations in genes of the ETS family (e.g., *ERG* and *ETV1*), and between alterations in *ERG* and *SPOP* or *FOXA1*, which represent distinct genomic subsets of prostate cancer (1, 3). Alterations in *ERG* and *PTEN* were co-occurring, in line with their synergistic role in promoting oncogenesis in mouse models of prostate cancer (17). We also confirmed co-occurrence between alterations in *TP53* and *RB1*, known to occur at high frequency in neuroendocrine cancers (7, 9), and to confer aggressive behavior in prostate cancer models (18, 19). Interestingly, *RB1* alteration had a tendency toward mutual exclusivity with alterations in *AR*. *CHD1* alterations also tended to co-occur with *SPOP* mutations (3). We found strong co-occurrence of loss-of-function alterations in *CDK12*, a gene implicated in the control of genomic stability (20) whose inactivation in prostate cancer is associated with focal tandem duplications (21–23), with amplification of the cell-cycle genes *CCND1* and *CDK4*, raising the possibility of vulnerability to CDK4/6 inhibitors for *CDK12*-mutated tumors. Conversely, while genomic alterations in *RB1* and *BRCA2*, both

located on chromosome 13q, 16 Mb apart, had a tendency toward co-occurrence, this association did not reach statistical significance.

Association of Genomic Alterations with Clinical Outcomes. A key novelty of this dataset is the opportunity to correlate contemporaneously obtained comprehensive genomic profiles with clinical outcome. We focused our analysis on 18 of the most commonly altered genes and pathways. For clinical outcome, we restricted the analysis to those patients who were taxane-naïve and initiating therapy with a first-line ARSI for mCRPC ($n = 128$).

We examined the association of genomic alterations with overall survival from the start of a first-line ARSI ($n = 128$) and time on treatment with a first-line ARSI (subset of $n = 108$ patients who received the ARSI without another concurrent therapy) in univariate analysis (Table 1). In this analysis, genomic alterations in the PI3K pathway and its component genes (Fig. 3A) were not significantly associated with either time on therapy with ARSIs or with overall survival (Fig. 3B and C), unlike prior cell-free DNA (cfDNA)-based analysis (13). Furthermore, we explored the association of genomic alterations in the DNA repair genes *BRCA2*, *BRCA1*, and *ATM* (Fig. 3D) with clinical outcomes, given prior conflicting reports of prognostic significance of these alterations (13, 24–26). We again found no association between alterations in these genes and time on treatment or overall survival (Fig. 3E and Table 1). Notably, we found an association between *SPOP* mutation and longer time on treatment with a first-line ARSI (*SI Appendix, Fig. S1*), consistent with prior data showing enrichment of *SPOP* mutations in earlier disease relative to mCRPC (1, 4) and favorable prognosis of *SPOP*-mutated tumors (27, 28), though this did not translate into a survival benefit. Alterations in *AR* and *TP53* were associated with a shorter time on an ARSI (Figs. 4D and 5C), though there was no association with overall survival (Table 1). Overall, genomic alterations in *RB1* showed the strongest discrimination for a shorter time on an ARSI and survival, with concordance probability estimates (CPEs) of 0.82 and 0.77, respectively (Fig. 5A and B and Table 1). Aneuploid chromosomal

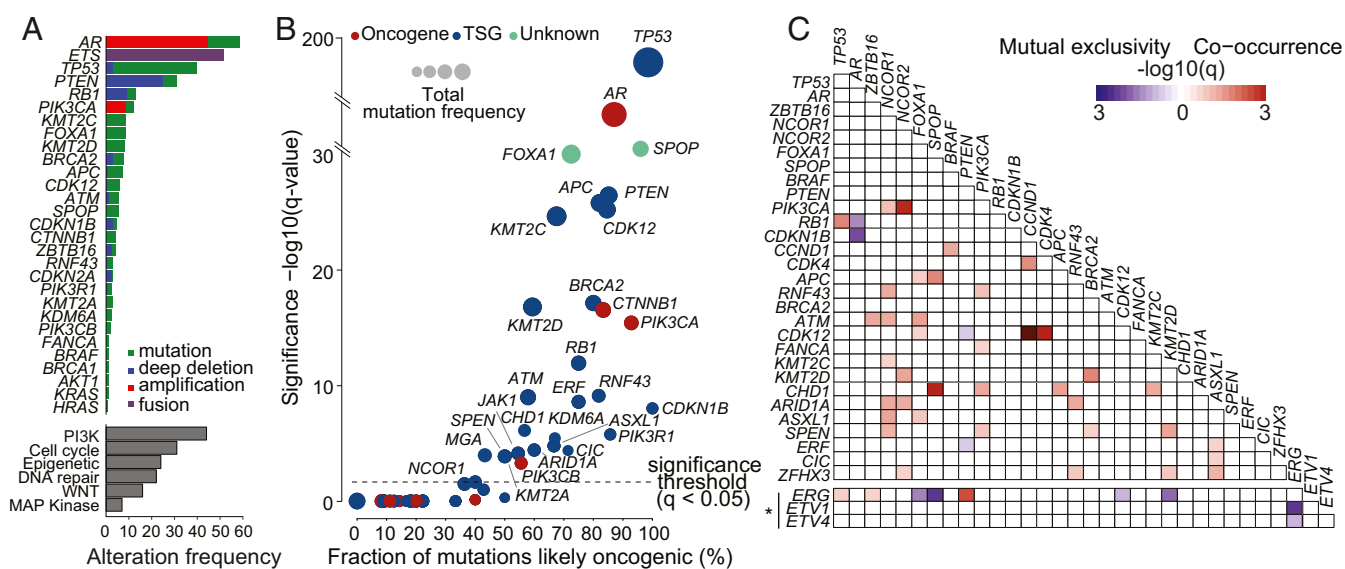


Fig. 2. Landscape of genomic alterations in 444 tumors from 429 patients with mCRPC. (A) Genomic alterations of potential biologic relevance by frequency. (A, Top) Frequency of alteration by gene. Frequency of ETS gene alterations applies to the subset of 323 patients who underwent tumor RNA sequencing, where fusion status could be determined. (A, Bottom) Frequency of alteration by pathway (*SI Appendix, Table S2*). (B) Fraction of SNVs considered to be likely oncogenic for genes harboring mutations. Genes colored in red are putative oncogenes, genes colored in blue are putative tumor suppressor genes (TSGs), and genes colored green are currently unknown. (C) Co-occurrence or mutual exclusivity between the most commonly altered genes. The significance of the relationship is represented by gradient. Relationships with P value < 0.05 with multiple hypothesis correction are shown. Associations involving ETS genes (*) apply only to cases where RNA-sequencing data are available.

Table 1. Association of common genomic alteration with overall survival and time on treatment with first-line ARSI

Gene/pathway alteration	Univariate <i>P</i> value for survival from first-line ARSI (CPE, <i>n</i> = 128 or as indicated)	Univariate <i>P</i> value for time on treatment with first-line ARSI (CPE, <i>n</i> = 108 or as indicated)
RB1	0.002 (CPE 0.768)	<0.001 (CPE 0.818)
TP53	0.072 (CPE 0.605)	0.046 (CPE 0.609)
WNT pathway	0.115	0.153
ETS fusion	0.159	0.206
APC	0.255	0.167
CTNNB1	0.274	0.448
ATM	0.331	0.850
BRCA2	0.327	0.418
BRCA2/BRCA1/ATM	0.495	0.611
AKT1	0.558	0.053
RNF43	0.614	0.844
AR	0.658	0.005 (CPE 0.651)
PTEN	0.676	0.412
PI3K pathway	0.699	0.138
PIK3CA	0.716	0.165
PIK3R1	0.752	0.892
PIK3CB	0.799	0.277
BRCA1	0.809	0.998
NEPC score	0.218 (<i>n</i> = 99)	0.930 (<i>n</i> = 80)
AR signaling score	0.847 (<i>n</i> = 99)	0.847 (<i>n</i> = 80)
RB1 loss score	<0.001 (<i>n</i> = 99)	0.014 (<i>n</i> = 80)
CCP score	0.002 (<i>n</i> = 99)	0.045 (<i>n</i> = 80)
AR-V7 SRPM	0.524 (<i>n</i> = 75)	0.329 (<i>n</i> = 56)
AR-V7/ARpromoter1-2	0.475 (<i>n</i> = 75)	0.378 (<i>n</i> = 56)
AR-V3 SRPM	0.444 (<i>n</i> = 75)	0.077 (<i>n</i> = 56)

Univariate log-rank analysis for association of common genomic alterations with survival from the start of a first-line ARSI for mCRPC (*n* = 128), and with time on treatment with a first-line ARSI for mCRPC (*n* = 108 patients who received a first-line ARSI as monotherapy). Where indicated, analysis was limited to a subset of patients who had RNA-sequencing data either from polyA libraries or both polyA and capture libraries. *P* < 0.05 are highlighted in bold.

status was associated with worse overall survival and time on treatment compared with diploid status (*SI Appendix, Fig. S2*).

Androgen Receptor Alterations. We confirmed a high frequency of genomic alterations in *AR*, namely amplifications and mutations, consistent with prior reports (5, 12) (Fig. 24). Using RNA-seq data, we also identified splice variants in *AR*, most commonly AR splice variant 7 (AR-V7) and variant 3 (AR-V3), both a product of splicing with cryptic exons, similar to our prior report (5) (Fig. 44). Genomic alterations (amplification and mutation) were associated with an increased *AR* expression score, consistent with increased *AR* output (Fig. 4B). Furthermore, genomic alterations in *AR* were detected at higher frequency postexposure to the ARSIs compared with ARSI-naïve tumors (Fig. 4C), suggesting an association with resistance to these next-generation AR-targeting agents. Consistent with this, we found an association of *AR* genomic alterations with a shorter time on treatment with a first-line ARSI (Fig. 4D) but not with overall survival from the start of a first-line ARSI (Table 1). Given prior data showing a strong association between AR-V7 expression in circulating tumor cells (CTCs) and clinical outcomes (10, 11), we examined the association of AR-V7 expression and outcomes in our tumor dataset. AR-V7 levels were increased in tumors exposed to taxanes and to ARSI therapy (*SI Appendix, Fig. S3*). However, there was no association between AR-V7 expression in tumors with either time on a first-line ARSI or overall survival (Fig. 4E

and Table 1). This was true even when deriving an AR-V7 expression cutpoint that produced the maximum log-rank test (AR-V7 cutpoint for survival 1.92, *P* = 0.62). Similar results were observed for time on treatment, AR-V7/AR promoter 1:2 ratio, and AR-V3 (Table 1).

Integrative Analysis of Histopathology, Genomics, and Expression.

There is growing recognition that a subset of CRPC patients have a more fulminant clinical course—typically characterized by rapidly progressive visceral metastasis (versus bone), relatively low serum prostate-specific antigen (PSA) levels, and variable expression of neuroendocrine markers such as synaptophysin or chromogranin (29). However, there is a lack of consensus on how to precisely define this clinical state, particularly since the frequency of histologically defined neuroendocrine prostate cancer varies widely in different cohorts. Furthermore, there is increasing recognition that patients can develop AR-negative (PSA-low) disease that is histologically negative for neuroendocrine marker expression (30). RNA-based expression signatures have been proposed as a potential alternative diagnostic strategy to define this distinct clinical state (8, 30, 31). The availability of matched histology, whole-exome, and RNA-seq data from this cohort provides an opportunity to explore this question through an unbiased integrative approach.

As previously noted, 11.2% of tumors in our dataset had evidence of NE features on histopathologic review (Fig. 1D). Among patients who received treatment with an ARSI during their disease course, tumors with histopathologic NE features were enriched postexposure to an ARSI (10.5%) compared with ARSI-naïve tumors (2.3%) (Fig. 5D). Transcript-based NE score was not significantly different between the two groups, but a subset of tumors in the post-ARSI setting displayed a higher NE expression score (Fig. 5E). Of note, unsupervised gene expression clustering identified a distinct cluster of tumors with higher NE expression score, in line with prior reports and independent of site of metastasis (*SI Appendix, Fig. S4*) (31).

We performed an integrative analysis incorporating histology, expression-based AR signaling and NE scores, and *RB1/TP53* genomic status (7, 32) in all tumors where RNA-sequencing data were available (Fig. 5F). As expected, there was an inverse correlation between cases with a high AR signaling score and cases with a high NE score. We identified three groups based on the expression signatures. The first and largest group, characterized by high AR signaling and low NE score, consisted predominantly (86%) of adenocarcinomas without NE histologic features (Fig. 5G). The second group, demonstrating intermediate NE and AR scores (*n* = 17), included cases classified histologically as adenocarcinoma (59%) and cases that were “inadequate for diagnosis” (41%). The third group, demonstrating high NE score and low AR signaling, consisted predominantly (74%) of tumors harboring histologic NE features (Fig. 5H).

Although there was an association between NE expression score, histologic NE features, and *RB1/TP53* loss (*SI Appendix, Fig. S5*), concordance between these characteristics was imperfect (Fig. 5F). We posited that some cases with discrepancy between pathology and transcriptomic classification may demonstrate a distinct morphology. To address this, a second consensus review of those cases was performed by three study pathologists. We confirmed that all discrepant cases from group 3 (high NE score but showing adenocarcinoma histology) and all cases from group 2 (intermediate AR and NE scores) displayed adenocarcinoma features. However, we noted distinct nuclear features in about half of these cases, including various degrees of nuclear pleomorphism, irregular nuclear membrane contours, and/or high mitotic activity (Fig. 5 I and J). Of note, within group 2, 10 cases (3% of total; Fig. 5F, box) had low AR and low NEPC expression scores. Of these, all histologically evaluable cases

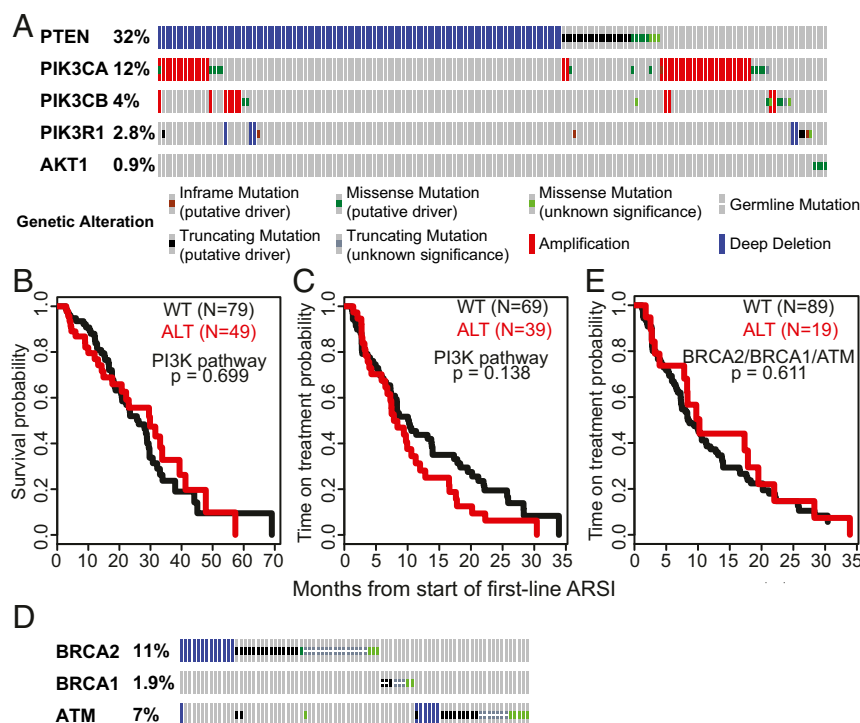


Fig. 3. Alteration in PI3K and homologous recombination repair genes and association with clinical outcomes. (A) Oncoprint of genomic alterations in PI3K pathway genes. (B and C) Kaplan-Meier analysis showing overall survival (B) and time on treatment with a first-line ARSI (C) in PI3K pathway altered (red) versus unaltered (black) tumors. (D) Oncoprint of genomic alterations in BRCA2, BRCA1, and ATM. (E) Kaplan-Meier analysis showing time on treatment with a first-line ARSI in BRCA2/1/ATM-altered (homozygous deletion or somatic or pathogenic germline mutation) (red) versus unaltered (black) tumors.

showed adenocarcinoma histology, and the majority harbored atypical nuclear features.

Given incomplete concordance between these histopathologic, genomic, and expression characteristics, we asked which of these features was most associated with clinical outcome. While there were insufficient histopathologic NE cases to make this determination, NE transcriptional score was not significantly associated with time on a first-line ARSI or overall survival (Table 1 and *SI Appendix*, Fig. S6). Of all molecular characteristics examined in a multivariate analysis, *RB1* alteration emerged as the only variable strongly associated with survival (relative risk 3.31) and with time on treatment with a first-line ARSI (relative risk 6.56) (Table 2). Consistent with this, expression scores for *RB1* loss and cell-cycle progression (CCP) were both associated with worse survival and time on treatment with a first-line ARSI (Table 1 and *SI Appendix*, Fig. S7).

Discussion

The landscape of genomic alterations in mCRPC has been established, with a subset of patients harboring potentially actionable alterations that are currently being explored in targeted prospective clinical trials. However, the majority of genomic alterations in prostate cancer do not yet have clear clinical applicability. Some studies have associated specific genomic or molecular features with clinical outcomes, though this has generally been performed in cell-free DNA or circulating tumor cells. Here, we present an integrative analysis of genomic alterations with expression and histologic assessment in tumors from patients with mCRPC, representing the clinical spectrum of advanced disease, with tissue collected pre- and posttreatment with ARSIs and taxanes.

Importantly, we find that *RB1* loss is the only genomic factor that is significantly associated with both survival and time on ARSI therapy in mCRPC. It is worth noting that the association

was strong despite likely underestimation of *RB1* loss by examining genomic homozygous loss alone, as *RB1* loss has also been shown to occur epigenetically, through structural genomic events like tandem duplication of partial exons, and focally, by immunohistochemistry (33). *TP53* and *AR* alteration were also associated with shorter duration of ARSI therapy, though the association was not as strong as for *RB1* loss and did not extend to survival. The *AR* findings suggest that AR targeting with the ARSIs abiraterone acetate and enzalutamide may be incomplete, and that further targeting of the protein may be clinically beneficial in patients who develop resistance to these agents.

We found no association between alterations in PI3K pathway genes or alterations in the DNA damage repair genes *BRCA2*, *BRCA1*, and *ATM* with overall survival and time on treatment with an ARSI. This is in contrast to a prior study that found an association between alterations in *BRCA2*, *BRCA1*, and *ATM* detected in cfDNA with both response to ARSIs and with survival (13). The differing conclusions may be related to differences between tumor and cfDNA profiling, potential difficulty in detecting homozygous loss in cfDNA relative to a tumor, and a possible bias introduced by requiring detectable cfDNA, though the authors accounted for cfDNA detection in a multivariate analysis. Furthermore, data for the prognostic role for *BRCA2*, *BRCA1*, and *ATM* alterations were previously conflicting, with another study showing better prognosis for tumors with alterations in these genes (25) and a third study showing no impact for the presence of germline DNA repair gene alterations on outcomes in mCRPC (26). Notably, we also found no association between AR-V7 in tumors and clinical outcomes, in contrast to prior CTC-based studies (10, 11). This finding requires further exploration of the concordance between AR-V7 expression in tissue versus CTCs, and the biological significance of AR-V7 RNA detection in tumors may have limited clinical utility. Overall, while profiling of cfDNA and CTCs offers advantages over tumor

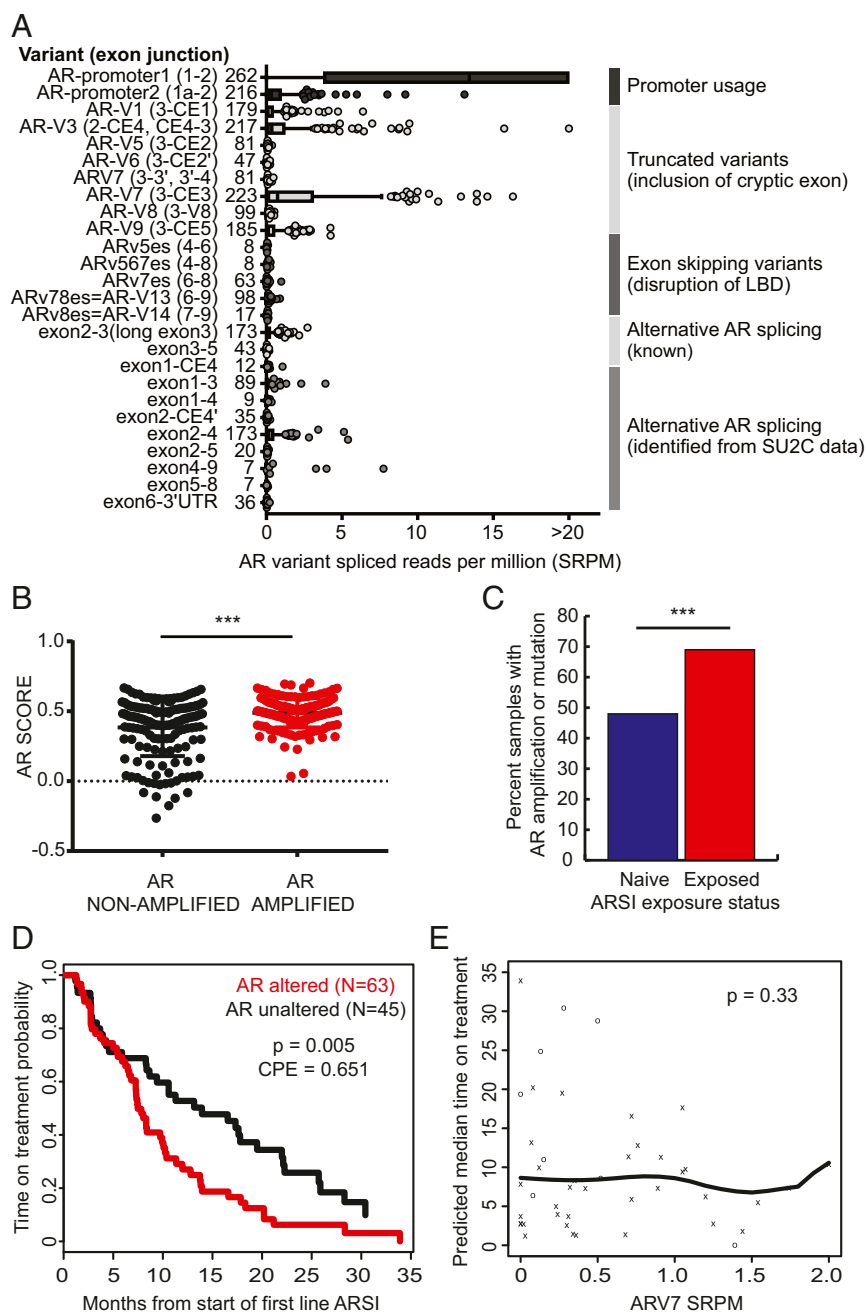


Fig. 4. Androgen receptor alterations and outcome. (A) AR splice variant landscape. LBD, ligand binding domain. (B) AR pathway expression score in AR-amplified ($n = 168$) versus nonamplified ($n = 159$) tumors. $***P < 0.001$. (C) AR amplification frequency in ARSI-naive versus exposed tumors. (D) Kaplan-Meier analysis showing time on treatment with a first-line ARSI in AR-amplified versus nonamplified tumors. (E) Association between ARV7 expression and time on treatment with a first-line ARSI. o, censored event; x, off-treatment event.

profiling, including ease of access and the ability to capture genomic heterogeneity (34, 35), blood-based profiling may be limited by lower sensitivity of detection and may reflect the fact that CTCs and cfDNA are generally detectable in patients with more advanced or aggressive disease. We recognize that association with clinical outcomes was performed in a subset of 128 patients who were initiating first-line therapy for mCRPC with an ARSI, and that a larger cohort could reveal additional associations. Nonetheless, the size of our cohort meets or exceeds previously reported datasets, and it is unclear if smaller differences in outcomes would be considered clinically meaningful.

We found that neuroendocrine histology, generally viewed as conferring more aggressive clinical behavior, is more frequent postexposure to ARSIs (10.5%), though at a lower frequency than recently reported in another study (17%) (8), despite inclusion in our cohort of patients with neuroendocrine features from a clinical trial of alisertib (14). Prostate cancers with histologic neuroendocrine differentiation (36) typically have alterations in *TP53* and/or *RBI*, high neuroendocrine expression score, and low AR signaling score, though concordance is not complete. This is not surprising, given the complexity of defining histologic neuroendocrine differentiation, which relies on the identification of a variety of characteristic features (29). Some

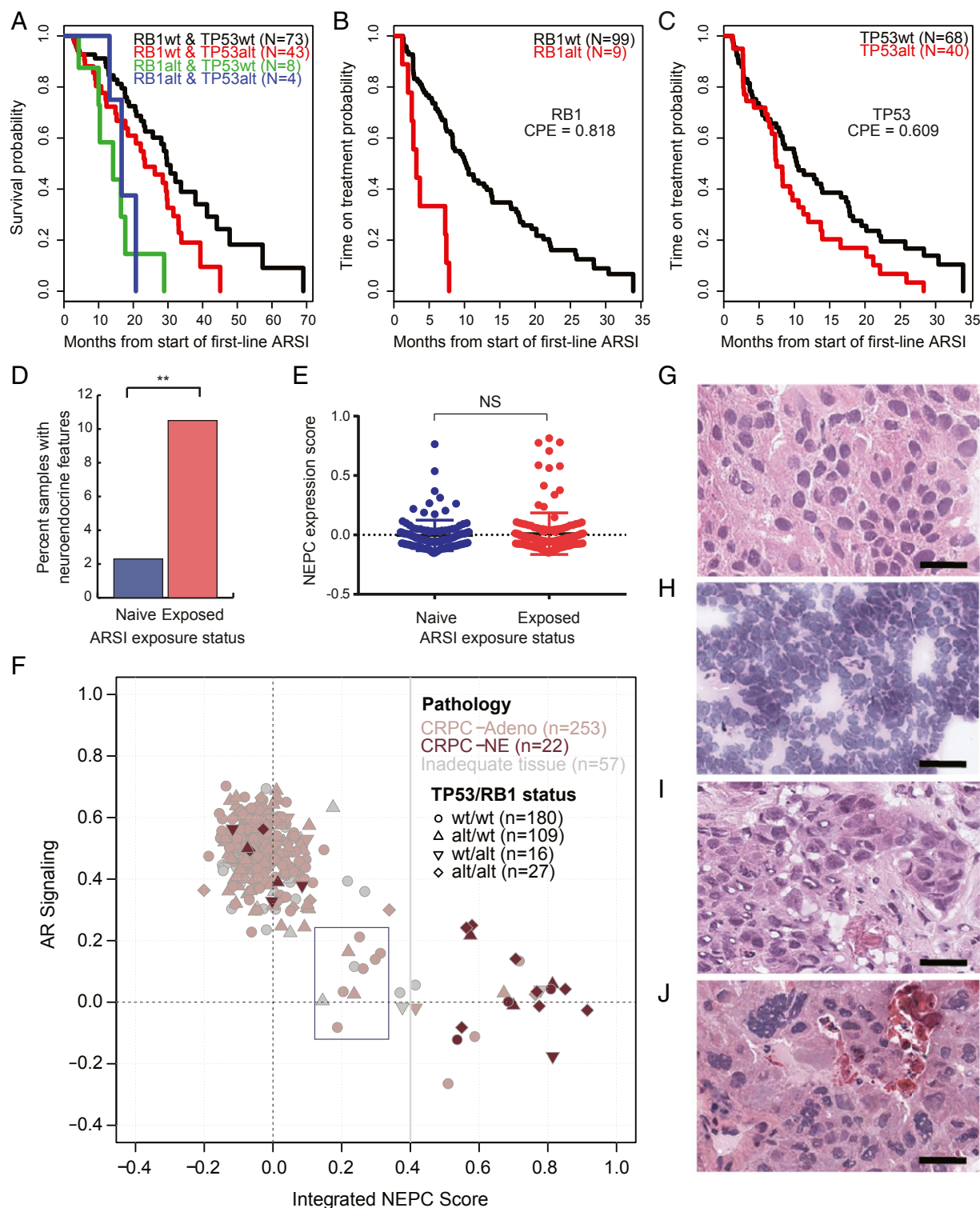


Fig. 5. Integrative analysis incorporating histopathology, transcript-based assessment of AR signaling and NEPC score, TP53 and RB1 genomic status, and clinical outcomes. (A) Kaplan–Meier analysis showing overall survival from the start of a first-line ARSI versus genomic status for TP53 and RB1 in $n = 128$ patients who received a first-line ARSI and underwent tissue profiling at baseline (before or within 90 d of therapy start). (B and C) Kaplan–Meier analysis showing time on treatment with a first-line ARSI by genomic status for RB1 and TP53. P values were generated from the log-rank statistic. (D) Frequency of histopathologic neuroendocrine features in pre- versus post-ARSI samples, among patients who received an ARSI at some point during their treatment history. Patients who were not reported to have received an ARSI at any point were excluded. $**P < 0.01$. (E) NEPC expression score in pre- ($n = 118$) versus post- ($n = 152$) ARSI samples, as in D. NS, not significant. (F) AR and NEPC expression scores, histopathology (CRPC-Adeno, no NE features; CRPC-NE, histopathologic NE features) and TP53/RB1 genomic status (circle, wild type for both; diamond, both altered) for the 332 tumors with RNA-sequencing data. Ten cases (3%, blue box) had low AR and low NEPC expression scores. (G–J) Representative cases of CRPC-Adeno (G), CRPC-NE, small-cell type (H), CRPC-Adeno showing intermediate transcriptomic scores (I), and CRPC-Adeno showing a high NEPC score/low AR signaling score (J). Tumors represented in I and J were noted to have distinct nuclear features, including various degrees of nuclear pleomorphism, irregular nuclear membrane contours, and/or high mitotic activity. (Scale bars, 25 μm .)

Table 2. Multivariate analysis evaluating the association of common genomic alterations with overall survival and time on treatment with first-line ARSI

Clinical outcome	Gene alteration(s)	Multivariate relative risk (95% CI for RR)
Overall survival from start of first-line ARSI	RB1–	1
	RB1+	3.31 (1.64, 6.67)
Time on treatment with first-line ARSI	RB1– and AR–	1
	RB1– and AR+	1.86 (1.18, 2.95)
	RB1+	6.56 (2.94, 14.62)

Common genomic alterations listed in Table 1 were included in this analysis. Only significant associations are shown. CI, confidence interval; RR, relative risk.

tumors with discordant histologic and molecular classification may be in transition from adenocarcinoma to NE differentiation, and displayed distinct nuclear features that may suggest such a transition. In such cases, paraffin-embedded tissue may aid in the classification by allowing for further examination of histomorphologic features and immunohistochemical staining of markers.

The size of our whole-exome sequencing dataset allows for genomic association analysis that was not previously possible. Through this analysis, we identified co-occurrence of alterations in *CDK12*, recently shown to confer immunogenic potential, and alterations in cell-cycle genes *CDK4* and *CCND1* (23), pointing toward a possible role for combination immune checkpoint blockade and CDK4/6 inhibition in clinical trials. Further laboratory studies will be needed to explore this and other potential biological interactions identified through genomic analysis.

In summary, we present an integrative analysis of genomic alterations, gene expression, histopathology, and clinical outcomes in the largest single mCRPC dataset to date, with the data made available to the research community for interrogation of genomic features in relation to outcomes. We find that *RB1* loss is the molecular factor most strongly associated with poor clinical outcomes in a contemporary cohort, highlighting the need for further investigation into mechanisms of resistance to AR therapies induced by loss of RB, and potential therapeutic strategies targeting this mechanism.

Methods

Patients and Samples. Subjects with mCRPC who were receiving standard-of-care therapy or treatment in a clinical trial [including trials combining AR therapies with other agents, a trial of the PARP inhibitor olaparib (37), and a trial of the Aurora kinase A inhibitor alisertib in patients with neuroendocrine features (14)] and who had disease amenable to biopsy under radiographic guidance were considered for inclusion at one of seven SU2C-PCF (Stand Up to Cancer/Prostate Cancer Foundation) International Prostate Cancer Dream Team consortium sites (Dana-Farber Cancer Institute, Karmanos Cancer Institute, Memorial Sloan Kettering Cancer Center, Royal Marsden, University of Michigan, University of Washington, and Weill Cornell Medicine) (5). All subjects included in this study provided written consent for research use of tumor tissue with institutional review board approvals or appropriate waivers (Office of Human Research Studies at the Dana-Farber Cancer Institute, Wayne State University Institutional Review Board, Memorial Sloan Kettering Cancer Center Institutional Review Board/Privacy Board, Royal Marsden Ethics Committee, University of Michigan Medical School Institutional Review Board, University of Washington Institutional Review Board, and Weill Cornell Medicine Institutional Review Board). Clinical data, including treatment history, duration of therapy, and survival, were collected using a web-based electronic data capture. All samples and clinical data were deidentified.

Histopathology. Pathology for all available cases was reviewed centrally by three board-certified pathologists with expertise in prostate cancer pathology, who were blinded to clinical and genomic data. Review was conducted on H&E-stained frozen sections, allowing for review of the exact material that was used for nucleic acid extraction. Each slide was assessed for the ability to make

a diagnosis based on the quality of the sample and presence of tumor cells; cases in which a specific pathology diagnosis could not be called were classified as inadequate for diagnosis. The remaining cases ($n = 366$) were classified according to a previously published system (36), by consensus in the event that all three pathologists did not agree. There was no additional material for paraffin embedding, immunohistochemistry, or other confirmatory studies.

Sequencing and Analysis. Flash-frozen needle biopsies and matched normal samples underwent nucleic acid extraction as previously described (5). Extracted DNA underwent whole-exome library construction and somatic mutation analysis as previously described. BAM files were aligned to the hg19 human genome build. Copy-number aberrations were quantified and reported for each gene as previously described (38, 39). Amplifications and homozygous deletions for a set of 20 genes previously implicated in prostate cancer (*SI Appendix, Table S3*) underwent further confirmatory review of segmentation files. Annotation of known or likely oncogenic SNVs was performed using the OncoKB platform (16).

Transcriptome libraries were prepared as previously described (5), using polyA+ RNA isolation, or captured using Agilent SureSelect Human All Exon V4 reagents, or in some cases using both polyA and capture methods. Library quality assessment and sequencing were performed as previously described. Paired-end transcriptome-sequencing reads were aligned to the human reference genome (GRCh38) using STAR (40). Gene expression as fragments per kilobase of exon per million fragments mapped (FPKM) was determined using featureCounts against protein-coding genes from the GenCode v26 reference. Fusions in ETS genes (*ERG*, *ETV1*, *ETV4*, *ETV5*, *FLI1*) and *RAFI1* *BRAF* were detected using CODAC (41) and assessed manually in all cases where RNA-sequencing data were available. In addition, the presence of AR splice variants was quantified as the number of reads across specific splice junctions in splice reads per million (SRPMs) and as the ratio of reads across a specific splice junction to the sum of AR promoter 1 and promoter 2 reads (a surrogate of total AR expression), separately for polyA and capture libraries.

NEPC and AR signaling scores were computed by the Pearson's correlation coefficient between the log2-transformed FPKM values of each score's gene list and a reference gene expression vector, as previously described (7, 32). CCP and RB loss scores were computed by the average (i.e., mean) Z score-transformed expression levels across each score's gene list, as previously described (42, 43). A high correlation ($R \geq 0.95$, $P < 0.001$, Pearson's correlation test) was noted between scores derived from polyA versus capture RNA-sequencing libraries (*SI Appendix, Fig. S8*), allowing for joint analysis of samples sequenced with either library construction method.

All data from SNV, copy-number, and expression analysis as well as clinical characteristics and outcomes measures (*Dataset S1*) have been made available in cBioPortal (44) (www.cbioportal.org), and have been deposited in GitHub, https://github.com/cBioPortal/datahub/tree/master/public/prad_su2c_2019.

Statistics and Genomic Association with Outcomes. Fisher's exact tests and unpaired *t* tests were performed in R (3.5.0) and GraphPad Prism software as indicated. For the analysis shown in Fig. 2B, enrichment analysis using a binomial distribution test was performed as previously described (45) to identify genes that had a significant fraction of known or likely oncogenic alterations (as defined by OncoKB) among all identified SNVs. Multiple hypothesis test correction was applied using the Benjamini-Hochberg method, with *q* values of <0.05 considered significant for enrichment of oncogenic mutations among all SNVs identified for a gene. Kaplan-Meier analysis was performed from time of biopsy to death for all samples. Overall survival analysis was performed for the $n = 128$ subjects who received an ARSI (abiraterone, enzalutamide, or apalutamide) in the first-line setting before a taxane, either alone or in combination with another agent in a clinical trial, and where the profiled tissue was obtained before the start of therapy or within 90 d after starting first-line therapy. Time on treatment analysis was evaluated for a subset of $n = 108$ patients (of the 128 above), who received an ARSI in the first-line setting without another agent, so as not to confound the interpretation of response to the ARSI. *P* values for individual (univariate) association tests between genomic status and survival/time on treatment were generated from the log-rank statistic. In cases where a data-driven threshold value was used to determine the genomic status, the *P* value was computed from the maximum log-rank statistic. When a genomic class contained a small number of events, the *P* value was produced using a permutation log-rank test. A concordance probability estimate provided a metric to assess the level of separation between the Kaplan-Meier curves and is reported in relevant cases. Multivariate analyses were performed for the association of common genomic characteristics shown in Table 1 with overall survival and time on a first-line ARSI, with relative risk reported based on the Cox proportional hazards model. Kendall's tau, derived from

the Clayton copula, was used to evaluate the level of association between the time on therapy end point and overall survival.

ACKNOWLEDGMENTS. We thank the affected individuals who participated in this study. We thank the following for their support in the conduct of the study: Jyoti Athanikar, Karen Giles, Ritika Kundra, Brigit McLaughlin, and Liangzuo (Tony) Ren. This work was supported by the Prostate Cancer Foundation and by a Stand Up to Cancer Prostate Cancer Dream Team research grant. Stand Up to Cancer is a program of the Entertainment Industry Foundation administered by the American Association for Cancer Research Award SU2C-AACR-DT0712. Additional support was provided by Prostate Cancer Foundation Young Investigator Awards (to W.A., J.M., and

N.S.); Department of Defense Prostate Cancer Research Program Awards W81XWH-17-1-0124 (to W.A.), W81XWH-09-1-0147 (PCCTC), PC170510 and PC170503P2 (to C.C.P.), and W81XWH-17-1-0380 (to N.D.S.); NCI Prostate Cancer SPORE Awards P50CA186786 (University of Michigan), P50CA092629 (MSKCC), P50CA090381 (DFCI), P50CA211024 (WCMC), and P50CA097186 (University of Washington); NCI Cancer Center Award P30 CA008748 (MSKCC); NIH Award R01CA125612 (to M.A.R.); European Research Council Consolidator Grant 648670 (to F.D.); and the Nuovo-Soldati Foundation (J.C.). The RM and ICR team is supported by the Movember Foundation and Prostate Cancer UK, PCF, the ECMC network from Cancer Research UK, the Department of Health in the UK, and BRC grant funding.

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1. Cancer Genome Atlas Research Network (2015) The molecular taxonomy of primary prostate cancer. *Cell* 163:1011–1025.
2. Taylor BS, et al. (2010) Integrative genomic profiling of human prostate cancer. *Cancer Cell* 18:11–22.
3. Barbieri CE, et al. (2012) Exome sequencing identifies recurrent SPOP, FOXA1 and MED12 mutations in prostate cancer. *Nat Genet* 44:685–689.
4. Abida W, et al. (2017) Prospective genomic profiling of prostate cancer across disease states reveals germline and somatic alterations that may affect clinical decision making. *JCO Precis Oncol*, 10.1200/PO.17.00029.
5. Robinson D, et al. (2015) Integrative clinical genomics of advanced prostate cancer. *Cell* 161:1215–1228.
6. Pritchard CC, et al. (2016) Inherited DNA-repair gene mutations in men with metastatic prostate cancer. *N Engl J Med* 375:443–453.
7. Beltran H, et al. (2016) Divergent clonal evolution of castration-resistant neuroendocrine prostate cancer. *Nat Med* 22:298–305.
8. Aggarwal R, et al. (2018) Clinical and genomic characterization of treatment-emergent small-cell neuroendocrine prostate cancer: A multi-institutional prospective study. *J Clin Oncol* 36:2492–2503.
9. Aparicio AM, et al. (2016) Combined tumor suppressor defects characterize clinically defined aggressive variant prostate cancers. *Clin Cancer Res* 22:1520–1530.
10. Antonarakis ES, et al. (2014) AR-V7 and resistance to enzalutamide and abiraterone in prostate cancer. *N Engl J Med* 371:1028–1038.
11. Scher HI, et al. (2017) Nuclear-specific AR-V7 protein localization is necessary to guide treatment selection in metastatic castration-resistant prostate cancer. *Eur Urol* 71:874–882.
12. Conteduca V, et al. (2017) Androgen receptor gene status in plasma DNA associates with worse outcome on enzalutamide or abiraterone for castration-resistant prostate cancer: A multi-institutional correlative biomarker study. *Ann Oncol* 28:1508–1516.
13. Annala M, et al. (2018) Circulating tumor DNA genomics correlate with resistance to abiraterone and enzalutamide in prostate cancer. *Cancer Discov* 8:444–457.
14. Beltran H, et al. (2019) A phase II trial of the Aurora kinase A inhibitor alisertib for patients with castration-resistant and neuroendocrine prostate cancer: Efficacy and biomarkers. *Clin Cancer Res* 25:43–51.
15. Armenia J, et al.; PCF/SU2C International Prostate Cancer Dream Team (2018) The long tail of oncogenic drivers in prostate cancer. *Nat Genet* 50:645–651.
16. Chakravarty D, et al. (2017) OncoKB: A precision oncology knowledge base. *JCO Precis Oncol*, 10.1200/PO.17.00011.
17. Carver BS, et al. (2009) Aberrant ERG expression cooperates with loss of PTEN to promote cancer progression in the prostate. *Nat Genet* 41:619–624.
18. Ku SY, et al. (2017) Rb1 and Trp53 cooperate to suppress prostate cancer lineage plasticity, metastasis, and antiandrogen resistance. *Science* 355:78–83.
19. Mu P, et al. (2017) SOX2 promotes lineage plasticity and antiandrogen resistance in TP53- and RB1-deficient prostate cancer. *Science* 355:84–88.
20. Blazek D, et al. (2011) The Cyclin K/Cdk12 complex maintains genomic stability via regulation of expression of DNA damage response genes. *Genes Dev* 25:2158–2172.
21. Quigley DA, et al. (2018) Genomic hallmarks and structural variation in metastatic prostate cancer. *Cell* 174:758–769.e9, and erratum (2018) 175:889.
22. Viswanathan SR, et al. (2018) Structural alterations driving castration-resistant prostate cancer revealed by linked-read genome sequencing. *Cell* 174:433–447.e19.
23. Wu YM, et al. (2018) Inactivation of CDK12 delineates a distinct immunogenic class of advanced prostate cancer. *Cell* 173:1770–1782.e14.
24. Abida W, Sawyers CL (2018) Targeting DNA repair in prostate cancer. *J Clin Oncol* 36:1017–1019.
25. Hussain M, et al. (2018) Targeting androgen receptor and DNA repair in metastatic castration-resistant prostate cancer: Results from NCI 9012. *J Clin Oncol* 36:991–999.
26. Mateo J, et al. (2018) Clinical outcome of prostate cancer patients with germline DNA repair mutations: Retrospective analysis from an international study. *Eur Urol* 73:687–693.
27. Boysen G, et al. (2018) SPOP-mutated/CHD1-deleted lethal prostate cancer and abiraterone sensitivity. *Clin Cancer Res* 24:5585–5593.
28. Blattner M, et al. (2017) SPOP mutation drives prostate tumorigenesis in vivo through coordinate regulation of PI3K/mTOR and AR signaling. *Cancer Cell* 31:436–451.
29. Beltran H, et al. (2014) Aggressive variants of castration-resistant prostate cancer. *Clin Cancer Res* 20:2846–2850.
30. Bluemn EG, et al. (2017) Androgen receptor pathway-independent prostate cancer is sustained through FGF signaling. *Cancer Cell* 32:474–489.e6.
31. Beltran H, et al. (2011) Molecular characterization of neuroendocrine prostate cancer and identification of new drug targets. *Cancer Discov* 1:487–495.
32. Hieronymus H, et al. (2006) Gene expression signature-based chemical genomic prediction identifies a novel class of HSP90 pathway modulators. *Cancer Cell* 10:321–330.
33. Nava Rodrigues D, et al. (2018) RB1 heterogeneity in advanced metastatic castration resistant prostate cancer. *Clin Cancer Res* 25:687–697.
34. Wyatt AW, et al. (2017) Concordance of circulating tumor DNA and matched metastatic tissue biopsy in prostate cancer. *J Natl Cancer Inst* 110:78–86.
35. Romanel A, et al. (2015) Plasma AR and abiraterone-resistant prostate cancer. *Sci Transl Med* 7:312re10.
36. Epstein JI, et al. (2014) Proposed morphologic classification of prostate cancer with neuroendocrine differentiation. *Am J Surg Pathol* 38:756–767.
37. Mateo J, et al. (2015) DNA-repair defects and olaparib in metastatic prostate cancer. *N Engl J Med* 373:1697–1708.
38. Brastianos PK, et al. (2015) Genomic characterization of brain metastases reveals branched evolution and potential therapeutic targets. *Cancer Discov* 5:1164–1177.
39. Shen R, Seshan VE (2016) FACETS: Allele-specific copy number and clonal heterogeneity analysis tool for high-throughput DNA sequencing. *Nucleic Acids Res* 44:e131.
40. Dobin A, et al. (2013) STAR: Ultrafast universal RNA-seq aligner. *Bioinformatics* 29:15–21.
41. Robinson DR, et al. (2017) Integrative clinical genomics of metastatic cancer. *Nature* 548:297–303.
42. Cuzick J, et al.; Transatlantic Prostate Group (2012) Prognostic value of a cell cycle progression signature for prostate cancer death in a conservatively managed needle biopsy cohort. *Br J Cancer* 106:1095–1099.
43. Ertel A, et al. (2010) RB-pathway disruption in breast cancer: Differential association with disease subtypes, disease-specific prognosis and therapeutic response. *Cell Cycle* 9:4153–4163.
44. Cerami E, et al. (2012) The cBio Cancer Genomics Portal: An open platform for exploring multidimensional cancer genomics data. *Cancer Discov* 2:401–404.
45. Chang MT, et al. (2016) Identifying recurrent mutations in cancer reveals widespread lineage diversity and mutational specificity. *Nat Biotechnol* 34:155–163.

Analysis of the Prevalence of Microsatellite Instability in Prostate Cancer and Response to Immune Checkpoint Blockade

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IMPORTANCE The anti-programmed cell death protein 1 (PD-1) antibody pembrolizumab is approved by the US Food and Drug Administration for the treatment of microsatellite instability-high (MSI-H) or mismatch repair-deficient (dMMR) solid tumors, but the prevalence of MSI-H/dMMR prostate cancer and the clinical utility of immune checkpoint blockade in this disease subset are unknown.

OBJECTIVE To define the prevalence of MSI-H/dMMR prostate cancer and the clinical benefit of anti-PD-1/programmed cell death 1 ligand 1 (PD-L1) therapy in this molecularly defined population.

DESIGN, SETTING, AND PARTICIPANTS In this case series, 1551 tumors from 1346 patients with prostate cancer undergoing treatment at Memorial Sloan Kettering Cancer Center were prospectively analyzed using a targeted sequencing assay from January 1, 2015, through January 31, 2018. Patients had a diagnosis of prostate cancer and consented to tumor molecular profiling when a tumor biopsy was planned or archival tissue was available. For each patient, clinical outcomes were reported, with follow-up until May 31, 2018.

MAIN OUTCOMES AND MEASURES Tumor mutation burden and MSIsensor score, a quantitative measure of MSI, were calculated. Mutational signature analysis and immunohistochemistry for MMR protein expression were performed in select cases.

RESULTS Among the 1033 patients who had adequate tumor quality for MSIsensor analysis (mean [SD] age, 65.6 [9.3] years), 32 (3.1%) had MSI-H/dMMR prostate cancer. Twenty-three of 1033 patients (2.2%) had tumors with high MSIsensor scores, and an additional 9 had indeterminate scores with evidence of dMMR. Seven of the 32 MSI-H/dMMR patients (21.9%) had a pathogenic germline mutation in a Lynch syndrome-associated gene. Six patients had more than 1 tumor analyzed, 2 of whom displayed an acquired MSI-H phenotype later in their disease course. Eleven patients with MSI-H/dMMR castration-resistant prostate cancer received anti-PD-1/PD-L1 therapy. Six of these (54.5%) had a greater than 50% decline in prostate-specific antigen levels, 4 of whom had radiographic responses. As of May 2018, 5 of the 6 responders (5 of 11 total [45.5%]) were still on therapy for as long as 89 weeks.

CONCLUSIONS AND RELEVANCE The MSI-H/dMMR molecular phenotype is uncommon yet therapeutically meaningful in prostate cancer and can be somatically acquired during disease evolution. Given the potential for durable responses to anti-PD-1/PD-L1 therapy, these findings support the use of prospective tumor sequencing to screen all patients with advanced prostate cancer for MSI-H/dMMR. Because not all patients with the MSI-H/dMMR phenotype respond, further studies should explore mechanisms of resistance.

JAMA Oncol. 2019;5(4):471-478. doi:10.1001/jamaoncol.2018.5801
Published online December 27, 2018.

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Immune checkpoint blockade has shown limited benefit in prostate cancer in several studies.¹⁻³ Nonetheless, durable objective responses have been reported, suggesting that patients with molecularly defined subsets of prostate cancer may benefit from this therapeutic approach.⁴⁻⁶ Pembrolizumab, an antibody targeting the programmed cell death protein 1 (PD-1) receptor, recently earned accelerated approval by the US Food and Drug Administration for the treatment of microsatellite instability-high (MSI-H) or mismatch repair deficient (dMMR) solid tumors, independent of site of origin. Detection of MSI thus represents the first clinical indication for prospective tumor profiling in patients with prostate cancer.⁷ However, the optimal method for determining MSI-H/dMMR status in patients with prostate cancer and the clinical implications of broader screening for this phenotype remain unknown.

The prevalence of MSI-H/dMMR prostate cancer is unclear, with frequencies ranging from 1.2% to 12.0% in prior reports.^{8,9} Recent sequencing studies of metastatic castration-resistant prostate cancer (mCRPC) showed that 2% to 3% of tumors have a higher mutation burden that is often associated with genomic alterations in MMR-associated genes, suggesting that tumor sequencing may be an efficient method for identifying MSI-H/dMMR prostate cancer.^{10,11} Herein, we leveraged a prospectively generated genomic data set of 1551 prostate tumors from 1346 patients to define the frequency of MSI-H/dMMR prostate cancer and report outcomes for patients with MSI-H/dMMR mCRPC who were treated with anti-PD-1/programmed cell death 1 ligand 1 (PD-L1) therapy.

Methods

Patients and Samples

From January 1, 2015, through January 31, 2018, 1551 tumors from 1346 patients with prostate cancer treated at Memorial Sloan Kettering Cancer Center, New York, New York, underwent clinical genomic profiling with a hybridization capture-based next-generation sequencing assay (Integrated Mutation Profiling of Actionable Cancer Targets [MSK-IMPACT]).^{12,13} Patients were given the option to consent to secondary germline analysis. Immunohistochemical (IHC) analysis for *MSH2* (OMIM 609309), *MSH6* (OMIM 600678), *MLH1* (OMIM 600678), and *PMS2* (OMIM 600259) was performed on select tumors. Medical record review for patient clinical characteristics and outcomes, with follow-up until May 31, 2018, was performed under a protocol approved by the institutional review board of Memorial Sloan Kettering Cancer Center, with a waiver of consent for the analysis of deidentified data.

Sequencing and Analysis

Tumors and matched normal blood samples were sequenced as previously described.^{10,13} An algorithm for the detection of somatic microsatellite changes using paired tumor-normal sequence data (MSIsensor)^{14,15} was applied to all tumors, yielding a quantitative MSIsensor score. Tumors deemed to have inadequate tumor content or quality (<200 × median exon coverage, <10% median exonic variant allele frequency, or no mutations with ≤20% tumor content

Key Points

Question What is the prevalence of microsatellite instability in prostate cancer and its association with response to immune checkpoint blockade?

Findings In this case series of 1346 patients with prostate cancer who underwent paired tumor and germline sequencing, 32 of 1033 (3.1%) had microsatellite instability-high or mismatch repair deficient disease, of whom 7 (21.9%) carried a germline mutation in a Lynch syndrome-associated gene. Five of 11 patients who received an anti-PD-1/PD-L1 agent had durable clinical benefit.

Meaning The microsatellite instability-high/mismatch repair deficient phenotype is uncommon but clinically important in prostate cancer and can be somatically acquired during disease evolution.

on pathologic review) were flagged, and their MSIsensor scores were excluded from the primary analysis. Tumor mutation burden (TMB) was calculated as mutations per megabase (mut/Mb). Mutational signature analysis^{16,17} (eMethods in the Supplement) was performed for tumors with somatic mutation counts of at least 10. Alterations in MMR genes were considered deleterious and likely oncogenic if they were pathogenic or likely pathogenic alterations present in the germline^{18,19} or if they were somatic truncating mutations or deletions.²⁰ For statistical analysis, comparison of MSI-H/dMMR frequency between disease subsets was performed using a 2-tailed Fisher exact test.

Results

Identification of MSI-H/dMMR in Prostate Cancer Using Next-Generation Sequencing

A total of 1551 tumors from 1346 patients with prostate cancer underwent prospective sequencing.^{10,13} Forty-seven tumors (3.0%) were hypermutated, defined herein as a TMB of at least 10 mut/Mb.^{10,11} To assess for MSI, we calculated MSIsensor scores^{14,15} for all samples. Notably, 384 tumors (24.8% of the total) were excluded from the primary MSIsensor analysis owing to low tumor purity (eTable 1 in the Supplement), which can affect MSIsensor score reliability.¹⁴ Thus, 1167 tumors from 1033 patients (mean [SD] age, 65.6 [9.3] years) were considered adequate for MSIsensor assessment (Figure 1A).

We classified MSIsensor scores as high (≥10), indeterminate (≥3 to <10), or low (<3) based on a large, retrospective pan-cancer analysis comparing MSIsensor score with orthogonal methods¹⁴ (Figure 1B). Twenty-three of 1033 patients (2.2%) had tumors with MSIsensor scores of at least 10 and were thus classified as having MSI-H disease. All MSI-H tumors had a TMB of at least 10 mut/Mb and evidence of MMR protein loss by IHC or a deleterious alteration in an MMR gene with the exception of 1 patient (P-0010034), who had a high TMB (22.6 mut/Mb) but no MMR gene alteration and insufficient tissue for IHC (Figure 2).

An additional 28 patients (2.7%) had tumors with indeterminate MSIsensor scores (eFigure 1A in the Supplement). To

further characterize these tumors, we reviewed each case for deleterious alterations in MMR genes, and in tumors for which adequate tissue was available, we performed IHC to assess for MMR protein loss (Figure 2). Within this subset, tumors were classified as MSI-H/dMMR if they were found to harbor a deleterious germline or somatic alteration in an MMR gene or had MMR protein loss on IHC findings. Of the 28 patients with MSI-sensor-indeterminate scores, 9 met these criteria and were considered to have MSI-H/dMMR prostate cancer. These patients typically had high TMBs and evidence of a predominant MSI/MMR signature on mutational signature decomposition (Figures 1B and 2). All patients who were identified as having MSI-H/dMMR tumors based on a deleterious MMR gene alteration were confirmed to have evidence of biallelic loss (eTable 1 in the Supplement). In total, 32 of 1033 patients (3.1%) met the criteria defined above for MSI-H/dMMR prostate cancer (eFigures 1A and 2 in the Supplement). The disease state at the time of tumor collection and the frequencies of MSI-H/dMMR in disease subsets are shown in eFigure 3 in the Supplement. Of note, 7 of the 1033 patients (0.7%) had a deleterious MMR gene alteration (1 germline and 6 somatic) without evidence of MSI or hypermutation (eTables 1 and 3 in the Supplement).

As outlined above, 384 prostate tumors (24.8%) with quality scores sufficiently high for mutational calling had insufficient sequence coverage or tumor purity for MSIsensor analysis. We reviewed these tumors to assess whether orthogonal measures, such as TMB, IHC, or mutational signature decomposition, could help guide clinical decision making in these cases. In total, 9 of 313 patients (2.9%) with inadequate tissue quality for MSIsensor analysis had high TMB and/or a somatic mutation in an MMR-associated gene. Two patients (P-00021600 and P-0024488) had TMBs greater than 20 mut/Mb and were found to have loss of MMR proteins on IHC analysis, consistent with dMMR (eTable 1 in the Supplement). Based on these results, in cases where tumor purity limits MSIsensor assessment, we now recommend confirmatory IHC for tumors with a high TMB or a deleterious alteration in an MMR gene (eFigure 4 in the Supplement).

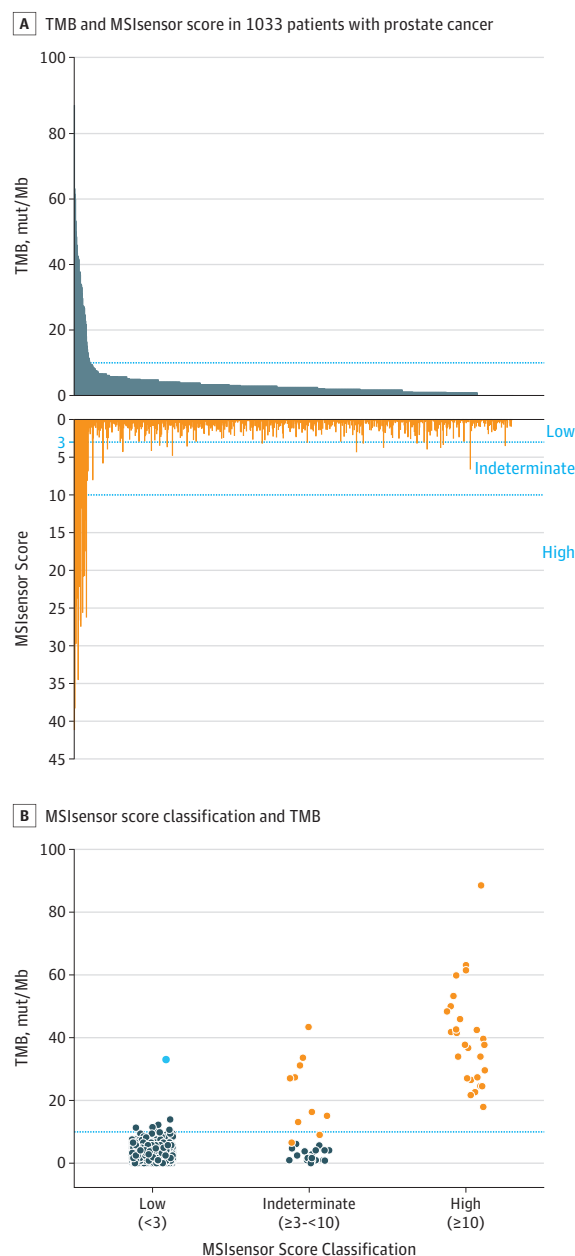
Germline Alterations in MMR Genes and MSI-H/dMMR Status

Mismatch repair-deficient prostate cancer has been reported to occur at increased frequency in Lynch syndrome mutation carriers.²¹ We sought to determine the rate of germline alterations in MMR genes in patients with MSI-H/dMMR prostate cancer who were identified through tumor profiling. Seven of the 32 patients with MSI-H/dMMR disease (21.9%) had a pathogenic or likely pathogenic germline mutation in an MMR-associated gene (eFigure 1B in the Supplement), including 5 in *MSH2*, 1 in *MSH6*, and 1 in *PMS2*. One additional patient had a pathogenic germline mutation in *MSH6* but no evidence of MSI or hypermutation in his tumor, suggesting that the pathogenic *MSH6* mutation did not contribute to the pathogenesis of his prostate cancer (eTable 3 in the Supplement).

Analysis of Longitudinally Profiled Patients

As outlined above, 21.9% of patients with MSI-H/dMMR prostate cancer had a germline MMR gene mutation that presum-

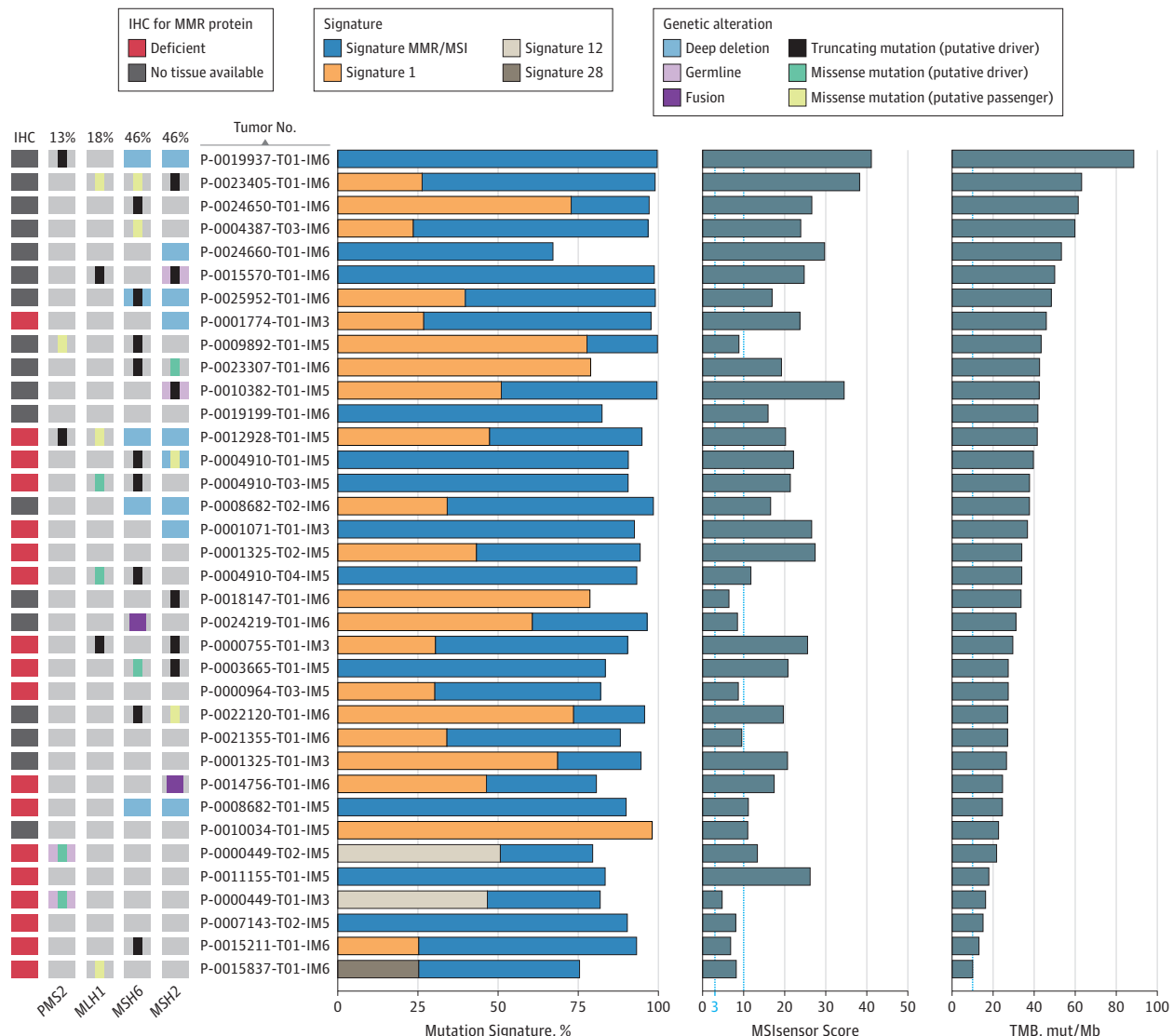
Figure 1. Tumor Mutation Burden (TMB) and Microsatellite Instability (MSI) in Prostate Cancer



A, Tumor mutation burden in mutations per megabase (mut/Mb) and MSIsensor score, a measure of microsatellite instability derived from sequencing data, are given for 1167 tumors from 1033 patients. B, MSIsensor scores were classified as high (≥ 10), indeterminate (≥ 3 to < 10), or low (< 3). Tumors with high MSIsensor scores invariably had high TMB and were considered to have high MSI (MSI-H) (orange). Tumors with MSIsensor indeterminate scores were classified as MSI-H or mismatch repair deficient (dMMR) if they had deleterious MMR gene alterations or MMR protein loss on immunohistochemical analysis (orange). Tumors with MSIsensor low scores typically had lower TMBs, with 1 exception that harbored a hotspot mutation in *POLE* (cyan).

ably played a causative role in the development of their malignant disease. However, most patients with MSI-H/dMMR

Figure 2. Integrative Analysis of Microsatellite Instability (MSI), Tumor Mutation Burden (TMB), Mutational Signature Decomposition, and Mismatch Repair (MMR) Gene and Protein Status



Tumors with the highest TMB (in mutations per megabase [mut/Mb]) and indeterminate (≥ 3 to <10) MSI sensor scores are shown. Mutation signatures with a contribution of at least 20% are shown, including MMR/MSI signatures 6, 15, 20 and 26; signature 1, which is associated with

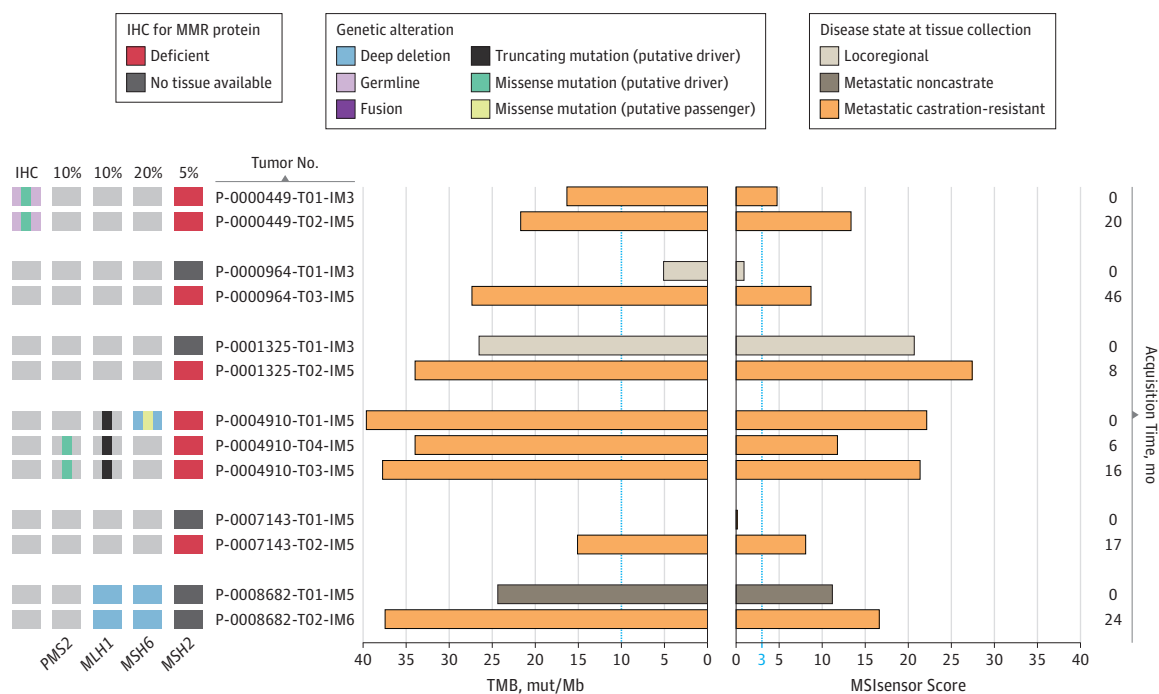
aging; and signatures 12 and 28.^{16,17} The oncoprint (left) shows genomic alterations in MMR genes, and immunohistochemical analysis (IHC) for MMR proteins (*MSH2*, *MSH6*, *MLH1*, and *PMS2*).

prostate cancer were not germline mutation carriers (eFigure 1B in the Supplement). Six of the 32 patients with MSI-H/dMMR disease underwent sequencing of 2 or more tumors acquired during their disease course (Figure 3). Two of these demonstrated somatic acquisition of MSI-H/dMMR status in mCRPC tumors obtained later in their disease course, whereas their earlier tumors showed no evidence of MSI. The earlier tumors had adequate tumor content as quantitated by histopathologic review (50%-70%) and bioinformatically (24%-60%) (eMethods in the Supplement). The remaining 4 patients had evidence of MSI-H/dMMR in all profiled tumors, including 1 patient with a germline *PMS2* mutation.

Clinical Characteristics of Patients With MSI-H/dMMR Prostate Cancer

The clinical characteristics of the 32 patients with MSI-H/dMMR cancer are summarized in eTable 2 in the Supplement. Median age at diagnosis was 64.5 years (range, 39-85 years). One patient had pure small-cell histologic findings. Among the 31 patients with prostate adenocarcinoma, 21 (67.7%) had mCRPC as their last disease state. For those patients, the median time to castration resistance was 8.6 months (range, 1.2-54.2 months) and the median duration of treatment with first-line abiraterone acetate or enzalutamide for mCRPC was 9.9 months (range, 3.0-34.5 months).

Figure 3. Longitudinal Assessment of Microsatellite Instability–High and Mismatch Repair–Deficient Status (MSI-H/dMMR) in Matched Tumors



Six patients with MSI-H/dMMR prostate cancer had more than 1 tumor profiled. The time of acquisition of these tumors is indicated relative to the time of acquisition of the first profiled tumor (month 0). Two of the 6 patients (P-0000964 and P-0007143) had evidence of somatically acquired MSI in the latter metastatic castration-resistant tumors. For patient P-0007143, the earlier

tumor was a prostate sample acquired at his diagnosis, at which time he had de novo metastatic noncastrate disease. This tumor had a very low MSIsensor score and tumor mutation burden (TMB). One patient (P-0000449) with a germline *PM2* mutation displayed MSI in both matched tumors. IHC indicates immunohistochemical analysis.

Responses to Anti-PD-1/PD-L1 Therapy in MSI-H/dMMR mCRPC

As of May 2018, 11 of the 32 patients with MSI-H/dMMR had received an anti-PD-1 or anti-PD-L1 agent for mCRPC as monotherapy or in combination with another immunomodulatory agent (Figure 4). The remaining 21 patients had not received immune checkpoint blockade owing to death before pembrolizumab approval or had not yet experienced disease progression during standard therapies.

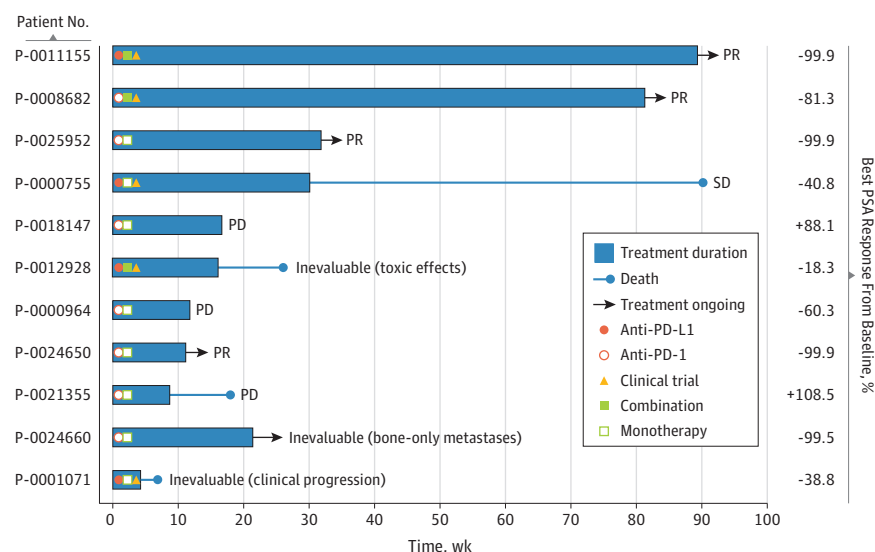
For the 11 patients with mCRPC treated with anti-PD-1/PD-L1 therapy, duration of therapy ranged from 4.6 to 89 weeks or longer (Figure 4). Six (54.5%) achieved a decline in prostate-specific antigen (PSA) levels greater than 50%, including 4 with a greater than 99% decline. Eight patients were evaluable for radiographic response; of these, 4 achieved objective responses (eFigure 5 in the Supplement), 1 had stable disease for approximately 6 months, and 3 had radiographic progression as best response. Disease in the remaining 3 patients was inevaluable for radiographic response, owing to early clinical progression or toxic effects in 2 and bone-only disease in 1, although the latter had a durable decline in PSA levels greater than 99%. In total, 5 of 11 patients with MSI-H/dMMR mCRPC (45.5%) continued to receive therapy with durable clinical benefit, 5 (45.5%) had no benefit, and 1 (9.1%) had stable disease for approximately 6 months.

Discussion

The National Comprehensive Cancer Network guidelines for prostate cancer were recently amended to include consideration of MSI-H/dMMR testing and pembrolizumab treatment for MSI-H/dMMR mCRPC in the second-line setting or beyond.⁷ However, in its approval of pembrolizumab, the US Food and Drug Administration did not define how MSI-H/dMMR status should be evaluated, and detailed guidance is not provided as part of national guidelines. Some next-generation sequencing assays can assess for MSI-H/dMMR status by interrogating microsatellite loci for evidence of MSI, by identifying mutations and copy number alterations in MMR-associated genes, and by inferring TMB, a phenotypic hallmark of MSI-H/dMMR tumors.^{10,11,13} Next-generation sequencing-based tumor genomic profiling may therefore represent a robust and efficient strategy to identify the subset of patients with prostate cancer who may benefit from anti-PD-1/PD-L1 therapy.

Herein, we report the largest experience to date of MSI-H/dMMR characterization in 1346 patients with prostate cancer. Thirty-two (3.1%) of the 1033 patients with evaluable disease had phenotypic evidence of MSI-H/dMMR. We also found that 21.9% of patients with MSI-H/dMMR prostate cancer had a germline mutation in an MMR gene, suggesting that germ-

Figure 4. Responses to Immune Checkpoint Blockade in Microsatellite Instability–High and Mismatch Repair Deficient (MSI-H/dMMR) Prostate Cancer



Eleven patients with MSI-H/dMMR castration-resistant prostate cancer received an anti-programmed cell death protein 1 (PD-1)/programmed cell death ligand 1 (PD-L1) agent. Best prostate-specific antigen (PSA) response from baseline to date, and radiographic responses (partial response [PR], stable disease [SD], progressive disease [PD]) are noted. As of May 31, 2018, 5 patients continued to receive treatment with greater than 50% declines in PSA levels, 4 of whom had

objective radiographic responses (eFigure 5 in the Supplement). One patient (eFigure 5B in the Supplement) had an initial radiographic PR, progressed in the prostate and by PSA, underwent palliative radiotherapy to the prostate, and continued to receive immune therapy with further clinical benefit. One patient had stable disease (SD) for approximately 6 months. Five patients showed no benefit, one of whom died, possibly owing to drug-related toxic effects.

line testing should be considered for all patients with MSI-H/dMMR prostate cancer. We acknowledge that the strict criteria used to define MSI-H/dMMR in our study, including the tumor purity threshold required to report MSIsensor scores, may have resulted in false-negative results in a small number of cases. Our experience suggests that, in clinical practice, it will be of benefit to incorporate information on mutational burden and mutational status of MMR genes in cases in which MSIsensor scores are indeterminate or tumor tissue is of low quality or purity. Sequencing can also identify those patients for whom IHC may be of incremental utility.

A notable finding from our study was that the MSI-H/dMMR phenotype was acquired somatically later during disease evolution in 2 of 6 patients who underwent longitudinal tumor profiling. Although MSI may have been subclonal in the earlier samples and thus missed owing to tumor heterogeneity, this result indicates that it was not a truncal event and would have been missed if only an older archival sample had been profiled. These data suggest that metastatic tissue may represent the optimal material for assessment of MSI status.

Because the MSI-H/dMMR phenotype is uncommon in prostate cancer, data describing responses to immune checkpoint blockade in this disease subset remain limited. Herein, we report clinical outcomes for the largest group of patients with MSI-H/dMMR mCRPC treated with anti-PD-1/PD-L1

therapy. Overall, 45.5% of patients with MSI-H/dMMR mCRPC derived durable clinical benefit, in line with other MSI-H/dMMR malignant neoplasms.⁵ Because approximately half of patients with MSI-H/dMMR had no response to immunotherapy, future studies should explore mechanisms of resistance in this population, which may involve alterations in the tumor antigen-presenting machinery and tumor-extrinsic factors, including inadequate T-cell activation.

Limitations

Our study has several limitations. It is a single-center retrospective analysis, and the number of patients with MSI-H who were treated with anti-PD-1/PD-L1 therapy is limited. Immunohistochemical analysis was not possible in all patients because of limited tumor tissue. Not all patients had tumors of sufficient quality for MSIsensor analysis, which could be addressed in the future with emerging cell-free DNA platforms.

Conclusions

In conclusion, we propose a comprehensive sequencing-based approach to identify patients with MSI-H/dMMR prostate cancer. Our results demonstrate that anti-PD-1/PD-L1 therapy is associated with meaningful clinical benefit in nearly half of MSI-H/dMMR mCRPC.

ARTICLE INFORMATION

Accepted for Publication: October 2, 2018.

Published Online: December 27, 2018.

doi:10.1001/jamaoncol.2018.5801

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Obtained funding: Abida, Sawyers, Solit.

Administrative, technical, or material support:

Abida, Autio, Morris, Danila, Slovin, Hullings, Shia, Socci, Berger, Sawyers, Solit.

Supervision: Abida, Armenia, Autio, Zehir, Kantoff, Sawyers, Schultz, Solit, Gopalan.

Conflict of Interest Disclosures: Dr Abida reported consulting for Clovis Oncology, Janssen Pharmaceutica, and More Health, Inc; receiving honoraria from Caret Healthcare; receiving research funding from Clovis Oncology, Zenith Epigenetics, Ltd, and AstraZeneca; and receiving travel funding from Clovis Oncology, Sanofi, and GlaxoSmithKline. Dr Cheng reported receiving travel and accommodation funding from Allergan, Sanofi, Daiichi Sankyo, Conquer Cancer Foundation, and Prostate Cancer Foundation and research funding from the National Institutes of Health (NIH) and Prostate Cancer Foundation. Dr Armenia reported current employment at AstraZeneca. Dr Autio reported receiving research funding from Pfizer, GlaxoSmithKline, Merck & Co, ARMO Biosciences, CytomX, and Eli Lilly and Co. Dr Vargas reported receiving travel funding from GE Healthcare. Dr Rathkopf reported consulting for Janssen Pharmaceutica and Genentech/Roche and receiving research funding from Janssen Pharmaceutica, Genentech/Roche, Celgene, Ferring Pharmaceuticals, Medivation/Astellas, Pfizer, AstraZeneca, Tracoe Pharmaceuticals, Inc, and Taiho Oncology, Inc. Dr Morris reported consulting

or an advisory role for Advanced Accelerator Applications, Astellas Pharma, Bayer, Blue Earth Diagnostics, Endocyte, Tokai Pharmaceuticals, and Tolmar Pharmaceuticals; receiving research funding from Bayer (Memorial Sloan Kettering Cancer Center [MSKCC]), Corcept Therapeutics (MSKCC), Endocyte (MSKCC), Progenics (MSKCC), Genentech/Roche (MSKCC), and Sanofi (MSKCC); and receiving funds for travel, accommodations, and expenses from Bayer and Endocyte. Dr Danila reported consulting for Angle LLT, Janssen Pharmaceutica Research & Development, Astellas Pharma, Medivation/Astellas, Agensys Corporation, and ScreenCell and research support from the US Department of Defense, American Society of Clinical Oncology, Prostate Cancer Foundation, Stand Up 2 Cancer, Janssen Pharmaceutica Research & Development, Astellas Pharma, Pfizer, Medivation/Astellas, Agensys Corporation, Genentech/Roche, and CreaTV. Dr Hechtman reported consulting for Navigant Consulting and receiving honoraria from Bayer and Loxo Oncology, Inc. Dr Stadler reported consulting in ophthalmology by an immediate family member for Allergan, Adverum Biotechnologies, Alimera Sciences, Biomarin Pharmaceutical, Inc, Fortress Biotech, Inc, Genentech/Roche, Novartis, Optos, Regeneron, Regenxbio, and Spark Therapeutics. Dr Wolchok reported consulting for Adaptive Biotechnologies, Amgen, Apricity, Array BioPharma, Ascentage Pharma, BeiGene, Bristol-Myers Squibb, Celgene, Chugai Pharmaceutical Co, Elucida Oncology, Inc, Eli Lilly and Co, F Star Biotechnology, Ltd, Genentech/Roche, Imvax Therapeutics Corp, Kleo Pharmaceuticals, MedImmune, Merck & Co, Neon Therapeutics, Ono Pharmaceutical Co, Ltd, Polaris Pharmaceuticals, Polynoma, PsiOxus, PureTech Health, Recepta, Trieza, SELLAS Life Sciences, Seramatrix, Surface Oncology, and Syndax Pharmaceuticals, Inc; receiving research support from Bristol-Myers Squibb, MedImmune, Merck Pharmaceuticals, and Genentech/Roche; and having equity in Potenza Therapeutics, Tizona Therapeutics, Inc, Adaptive Biotechnologies, Elucida Oncology, Inc, Imvax Therapeutics Corp, BeiGene, and TriEnza. Dr Berger reported consulting for Roche and receiving research support from Illumina. Dr Kantoff reported consulting for BIND Biosciences, BN Immunotherapeutics, DRGT, GE Healthcare, Janssen Pharmaceutica, Metamark, New England Research Institutes, OncoCellMDx, Progenity, Sanofi, Seer Biosciences, Inc, Tarveda Therapeutics, and Thermo Fisher Scientific; company board membership for Context Therapeutics; serving on data safety monitoring boards for Genentech/Roche and Merck & Co; and investment interest in Context Therapeutics, DRGT, Seer Biosciences, Placon, and Tarveda Therapeutics. Dr Sawyers reported serving on the board of directors of Novartis; cofounding ORIC Pharmaceuticals, Inc; coinventing enzalutamide and apalutamide; serving as science advisor to Agios Pharmaceuticals, BeiGene, Blueprint, Column Group, FogHorn Systems, Housey Pharmaceuticals, Inc, Nextech, KSQ, Petra, and PMV Pharma; and cofounding Seragon Pharmaceuticals, purchased by Genentech/Roche in 2014. Dr Solit reported consulting for Pfizer, Loxo Oncology, Inc, Intezyme, and Illumina. Dr Scher reported consulting for Astellas Pharma, Ferring Pharmaceuticals, Janssen Biotech, Inc, Janssen Pharmaceutica Research and Development, Sanofi, Clovis Oncology, Merck & Co,

and WCG Oncology; serving on the board of directors for Asterias Biotherapeutics; and receiving research support from Illumina, Innocrin, and Janssen Pharmaceutica. No other disclosures were reported.

Funding/Support: This study was supported by Challenge and Young Investigator awards from the Prostate Cancer Foundation (Drs Abida, Cheng, Schultz, and Scher); Cancer Center Support grant P30 CA008748 from the National Cancer Institute (NCI), NIH; Prostate Specialized Program of Research Excellence grant P50CA092629-16 from the NCI; Prostate Cancer Research Program awards W81XWH-17-1-0124 (Dr Abida), and PC071610 and PC121111 (Dr Scher) from the Department of Defense; the David H. Koch Fund for Prostate Cancer Research; the Marie-Josée and Henry R. Kravis Center for Molecular Oncology; and the Robertson Foundation (Dr Schultz).

Role of the Funder/Sponsor: The funding organizations 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; or the decision to submit the manuscript for publication.

REFERENCES

1. Beer TM, Kwon ED, Drake CG, et al. Randomized, double-blind, phase III trial of ipilimumab versus placebo in asymptomatic or minimally symptomatic patients with metastatic chemotherapy-naïve castration-resistant prostate cancer. *J Clin Oncol*. 2017;35(1):40-47. doi:10.1200/JCO.2016.69.1584
2. Kwon ED, Drake CG, Scher HI, et al; CA184-043 Investigators. Ipilimumab versus placebo after radiotherapy in patients with metastatic castration-resistant prostate cancer that had progressed after docetaxel chemotherapy (CA184-043): a multicentre, randomised, double-blind, phase 3 trial. *Lancet Oncol*. 2014;15(7):700-712. doi:10.1016/S1470-2045(14)70189-5
3. Topalian SL, Hodi FS, Brahmer JR, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *N Engl J Med*. 2012;366(26):2443-2454. doi:10.1056/NEJMoa1200690
4. Graff JN, Alumkal JJ, Drake CG, et al. Early evidence of anti-PD-1 activity in enzalutamide-resistant prostate cancer. *Oncotarget*. 2016;7(33):52810-52817. doi:10.18632/oncotarget.10547
5. Le DT, Durham JN, Smith KN, et al. Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. *Science*. 2017;357(6349):409-413. doi:10.1126/science.aan6733
6. Hansen AR, Massard C, Ott PA, et al. Pembrolizumab for advanced prostate adenocarcinoma: findings of the KEYNOTE-028 study. *Ann Oncol*. 2018;29(8):1807-1813. doi:10.1093/annonc/mdy232
7. National Comprehensive Cancer Network. Prostate cancer (version 3). https://www.nccn.org/professionals/physician_gls/pdf/prostate.pdf. Accessed June 25, 2018.
8. Guedes LB, Antonarakis ES, Schweizer MT, et al. MSH2 loss in primary prostate cancer. *Clin Cancer Res*. 2017;23(22):6863-6874. doi:10.1158/1078-0432.CCR-17-0955
9. Pritchard CC, Morrissey C, Kumar A, et al. Complex MSH2 and MSH6 mutations in hypermutated microsatellite unstable advanced

prostate cancer. *Nat Commun*. 2014;5:4988. doi:10.1038/ncomms5988

10. Abida W, Armenia J, Gopalan A, et al. Prospective genomic profiling of prostate cancer across disease states reveals germline and somatic alterations that may affect clinical decision making [published online May 31, 2017]. *JCO Precis Oncol*. 2017;2017. doi:10.1200/PO.17.00029

11. Robinson D, Van Allen EM, Wu YM, et al. Integrative clinical genomics of advanced prostate cancer. *Cell*. 2015;161(5):1215-1228. doi:10.1016/j.cell.2015.05.001

12. Cheng DT, Mitchell TN, Zehir A, et al. Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT): a hybridization capture-based next-generation sequencing clinical assay for solid tumor molecular oncology. *J Mol Diagn*. 2015;17(3):251-264. doi:10.1016/j.jmoldx.2014.12.006

13. Zehir A, Benayed R, Shah RH, et al. Mutational landscape of metastatic cancer revealed from prospective clinical sequencing of 10 000 patients. *Nat Med*. 2017;23(6):703-713. doi:10.1038/nm.4333

14. Middha S, Zhang L, Nafa K, et al. Reliable pan-cancer microsatellite instability assessment by using targeted next-generation sequencing data. *JCO Precis Oncol*. 2017;2017. doi:10.1200/PO.17.00084

15. Niu B, Ye K, Zhang Q, et al. MSIsensor: microsatellite instability detection using paired tumor-normal sequence data. *Bioinformatics*. 2014;30(7):1015-1016. doi:10.1093/bioinformatics/btt755

16. Alexandrov LB, Nik-Zainal S, Wedge DC, et al; Australian Pancreatic Cancer Genome Initiative; ICGC Breast Cancer Consortium; ICGC MML-Seq Consortium; ICGC PedBrain. Signatures of mutational processes in human cancer [published correction appears in *Nature*. 2013;502(7470):258]. *Nature*. 2013;500(7463):415-421. doi:10.1038/nature12477

17. Alexandrov LB, Nik-Zainal S, Wedge DC, Campbell PJ, Stratton MR. Deciphering signatures of mutational processes operative in human cancer. *Cell Rep*. 2013;3(1):246-259. doi:10.1016/j.celrep.2012.12.008

18. Mandelker D, Zhang L, Kemel Y, et al. Mutation detection in patients with advanced cancer by universal sequencing of cancer-related genes in tumor and normal DNA vs guideline-based germline testing. *JAMA*. 2017;318(9):825-835. doi:10.1001/jama.2017.11137

19. Pritchard CC, Mateo J, Walsh MF, et al. Inherited DNA-repair gene mutations in men with metastatic prostate cancer. *N Engl J Med*. 2016;375(5):443-453. doi:10.1056/NEJMoa1603144

20. Chakravarty D, Gao J, Phillips SM, et al. OncoKB: a precision oncology knowledge base [published online May 16, 2017]. *JCO Precis Oncol*. 2017;2017. doi:10.1200/PO.17.00011

21. Dominguez-Valentin M, Joost P, Therkildsen C, Jonsson M, Rambech E, Nilbert M. Frequent mismatch-repair defects link prostate cancer to Lynch syndrome. *BMC Urol*. 2016;16:15. doi:10.1186/s12894-016-0130-1

Invited Commentary

Microsatellite Instability as an Emerging Biomarker for Checkpoint Inhibitor Response in Advanced Prostate Cancer

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In addition to surgery, radiotherapy, chemotherapy, and targeted therapy, immunotherapy is now the fifth pillar of oncology treatment. Despite this status, determinants of immunotherapy response in most tumors are still unknown. One



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phenotype that was hypothesized to enhance immunogenicity is microsatellite instability (MSI), or the accumulation of errors in repetitive sequences due to loss of function of mismatch repair (MMR) genes. To test this hypothesis, Le et al¹ treated 41 patients with colorectal and other cancers with the anti-programmed cell death protein 1 (PD-1) checkpoint inhibitor pembrolizumab and determined that patients whose tumors harbored MSI were more likely to respond. Building on these results, Le et al² conducted a phase 2 trial of pembrolizumab in patients whose advanced solid tumors harbored MSI (86 patients representing 12 different tumor histologic types). The objective response rate was 53%, and complete responses were achieved in 21% of patients. Based on those results, in 2017, the US Food and Drug Administration granted approval for pembrolizumab for the treatment of solid tumors harboring MSI—the first tumor-agnostic oncology drug approval.

Microsatellite instability is found in approximately 3% of patients with metastatic castration-resistant prostate cancer.³ However, the role of MSI in anti-PD-1 checkpoint inhibitor response in prostate cancer was largely unknown because only 1 patient with prostate cancer was included in the trial by

Le et al.² More recent work^{4,5} demonstrates that pembrolizumab leads to responses or stable disease in subsets of patients with castration-resistant prostate cancer, including those whose tumor biopsies harbor programmed cell death 1 ligand 1 (PD-L1)-expressing cells. However, molecular features that contribute to response are currently unknown.

In this issue of *JAMA Oncology*, Abida et al⁶ examined the frequency of MSI in 1551 tumor specimens from 1346 patients with prostate cancer who had undergone germline and tumor sequencing at Memorial Sloan Kettering Cancer Center, New York, New York. Of the 1033 patients who underwent analysis, 32 (3.1%) had MSI/MMR-deficient tumors, of whom 7 (21.9%) had germline mutations in a Lynch syndrome-associated gene. Importantly, a subset of these patients (n = 11) with MSI-positive tumors underwent treatment with anti-PD-1 or anti-PD-L1 checkpoint inhibitors, and 5 of these patients (45.5%) had durable clinical benefit. Thus, the retrospective analysis by Abida et al⁶ confirms the findings from Le et al^{1,2} and demonstrates that treatment with an anti-PD-1 or anti-PD-L1 checkpoint inhibitor—either Food and Drug Administration-approved pembrolizumab or another anti-PD-1 or anti-PD-L1 checkpoint inhibitor through a clinical trial—should be considered in patients with prostate cancer whose tumors harbor MSI. However, several questions remain about anti-PD-1 or anti-PD-L1 checkpoint inhibitor treatment in prostate cancer, including in patients whose tumors harbor MSI.



European Association of Urology



Platinum Priority – Prostate Cancer

Editorial by Ariel E. Marciscano and Christopher E. Barbieri on pp. 680–681 of this issue

Pan-cancer Analysis of *CDK12* Alterations Identifies a Subset of Prostate Cancers with Distinct Genomic and Clinical Characteristics

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Article info

Associate Editor: T Morgan

Keywords:

CDK12
Clinical sequencing
Genomics
Prostate cancer
Tumor biology

Abstract

Background: *CDK12* genomic alterations occur in several tumor types, but little is known about their oncogenic role and clinical significance.

Objective: To describe the landscape of *CDK12* alterations across solid cancers and the clinical features of *CDK12*-altered prostate cancer.

Design, setting, and participants: A single-center retrospective study of 26743 patients across 25 solid tumor types who underwent tumor sequencing was performed. Clinicopathologic features and outcomes were assessed in prostate cancer.

Outcome measurements and statistical analysis: *CDK12* alterations and their association with genomic characteristics are described. For prostate cancer patients, overall survival and time to castration resistance were assessed using univariable and multivariable Cox regression analysis.

Results and limitations: *CDK12* alterations were identified in 404/26743 patients (1.5%) overall, but were most frequent in prostate (100/1875, 5.3%) and ovarian cancer (43/1034, 4.2%), in which they were associated with a high prevalence of truncating variants and biallelic inactivation. *CDK12* alterations defined a genomic subtype of prostate cancer with a unique copy-number alteration profile and involvement of distinct oncogenic pathway alterations, including cell-cycle pathway genes. *CDK12*-altered prostate cancer was associated with somewhat more aggressive clinical features and shorter overall survival (median 64.4 vs 74.9 mo; $p = 0.032$) independent of standard clinical factors and tumor copy-number alteration burden (adjusted hazard ratio 1.80, 95% confidence interval 1.12–2.89; $p = 0.024$). The study is limited by its retrospective nature.

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Conclusions: *CDK12* alteration is a rare event across solid cancers but defines a clinically distinct molecular subtype of prostate cancer associated with unique genomic alterations and slightly more aggressive clinical features.

Patient summary: *CDK12* gene alterations occur rarely across tumor types, but more frequently in prostate cancer, where they are associated with genomic instability, cell-cycle pathway gene alterations, and somewhat worse clinical outcomes, warranting further investigation of therapeutic targeting of this disease subset.

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1. Introduction

Genomic alterations in *CDK12* have been observed in various cancer types [1–4] but their oncogenic function and clinical relevance are poorly understood. *CDK12* encodes a cyclin-dependent serine/threonine kinase involved in the regulation of the cell cycle and of DNA repair by homologous recombination (HR). Specifically, it has been shown that *CDK12* loss of function suppresses the expression of several HR genes, at least in part, via intronic polyadenylation [5–8]. Growing evidence suggests that biallelic *CDK12* loss determines a distinct phenotype of ovarian and prostate cancer characterized by high genomic instability and tandem duplications [9–11].

It has been reported that *CDK12* alterations occur in 4–11% of prostate cancer cases and are more frequent in the metastatic castration-resistant setting (mCRPC) [3,9,12]. Despite preclinical work suggesting that *CDK12* loss or inhibition could impair genes in the HR repair pathway, recent evidence has shown that PARP inhibitors have limited efficacy in mCRPC patients harboring a *CDK12* alteration [13,14]. However, Wu and colleagues [9] recently demonstrated that *CDK12* loss determines a novel molecular subtype of prostate cancer associated with a higher proportion of fusion neoantigens and potentially high immune infiltration and response to anti-PD1/PD-L1 agents.

In this study we sought to survey the landscape of *CDK12* mutations across different cancer types. We identified prostate cancer as the cancer type with the highest prevalence of *CDK12* oncogenic alterations and the largest proportion of truncating variants, with biallelic inactivation occurring frequently, and characterized the genomic architecture of *CDK12*-altered prostate carcinomas. We describe clinicopathologic characteristics and clinical outcomes for patients with prostate cancers harboring *CDK12* alterations. Our work adds to recent publications [15,16] by integrating pan-cancer genomic analysis from a clinical sequencing assay, identifying distinct genomic alterations associated with *CDK12*-altered prostate cancer, and describing clinical features of the largest set of prostate cancer patients with *CDK12* alterations identified using a single assay.

2. Patients and methods

2.1. Study design and patients

All patients in the study underwent tumor sequencing performed using the Memorial Sloan Kettering Integrated Molecular Profiling of

Actionable Cancer Targets (MSK-IMPACT) clinical sequencing assay, a hybridization capture-based, next-generation sequencing platform, with 341, 410, or 468 genes (all panels included *CDK12*) [17,18]. After excluding 1965 cases with high microsatellite instability (MSI) cases, defined as an MSI sensor [19] score >10, and/or high tumor mutation burden (TMB) cases, defined as TMB $\geq$ 20 mutations/Mb, because of the high likelihood of passenger alterations in *CDK12*, a total of 26743 patients across 25 solid tumor types (minimum number of patients 100 per cancer type) who had MSK-IMPACT sequencing between July 2014 and April 12, 2019 were assessed for the presence of *CDK12* alterations. For patients with multiple sequenced samples, one sample was selected according to the following hierarchy: tumor purity (highest tumor purity as assessed by pathologist), gene panels (most recent), and coverage (highest sample sequencing coverage). Chart review was performed to extract clinical and pathologic data for prostate cancer patients. Data collection and retrospective analysis were performed with approval from the MSKCC institutional review board.

2.2. Genomic analysis

TMB was calculated as the total number of nonsynonymous mutations excluding *CDK12*, divided by the number of bases sequenced. Recurrent oncogenic alterations were defined as oncogenic according to OncoKB [20] (version August 28, 2019) and present in at least 1% in the whole cohort. Canonical oncogenic pathway-level alterations were computed using curated pathway templates [21]. MutSigCV v1.4 was used to determine significantly mutated genes (false discovery rate [FDR] <0.1) [22]. Segmented copy-number data were processed using the CNtools package v1.4. The fraction of genome altered (FGA) was calculated for each sample as the percentage of the genome with log2 copy ratios >0.2 or <-0.2. Thresholds for copy-number alteration gain and loss were set at log2 copy ratios of >0.2 and <-0.2, respectively. *CDK12* biallelic (*CDK12*-Bi) inactivation was defined as either deep deletion, two or more deleterious mutations in the same tumor sample, one mutation with concurrent heterozygous loss of the wild-type (WT) allele, or one mutation with concurrent copy-neutral loss-of-heterozygosity (CN-LOH) as computed using the FACETS algorithm [23]. FACETS is an allele-specific copy-number analysis pipeline providing accurate, purity- and ploidy-corrected, integer DNA copy-number calls and cancer cell fraction (CCF) from sequencing data. Integer copy-number calls were used to determine the presence of *CDK12*-Bi inactivation due to heterozygous loss of the WT allele or CN-LOH. FACETS was also used to determine clonality. Mutations were defined as clonal if the upper bound of the 95% confidence interval (CI) for CCF was $\geq$ 0.8, and all other mutations were called subclonal. Of note, samples with *CDK12* rearrangement events were excluded in the biallelic analysis.

2.3. Prostate cancer outcomes

Baseline clinical characteristics and outcomes were available for a subset of patients with histologically confirmed prostate cancer who underwent tumor genomic profiling. Outcomes assessed were overall survival

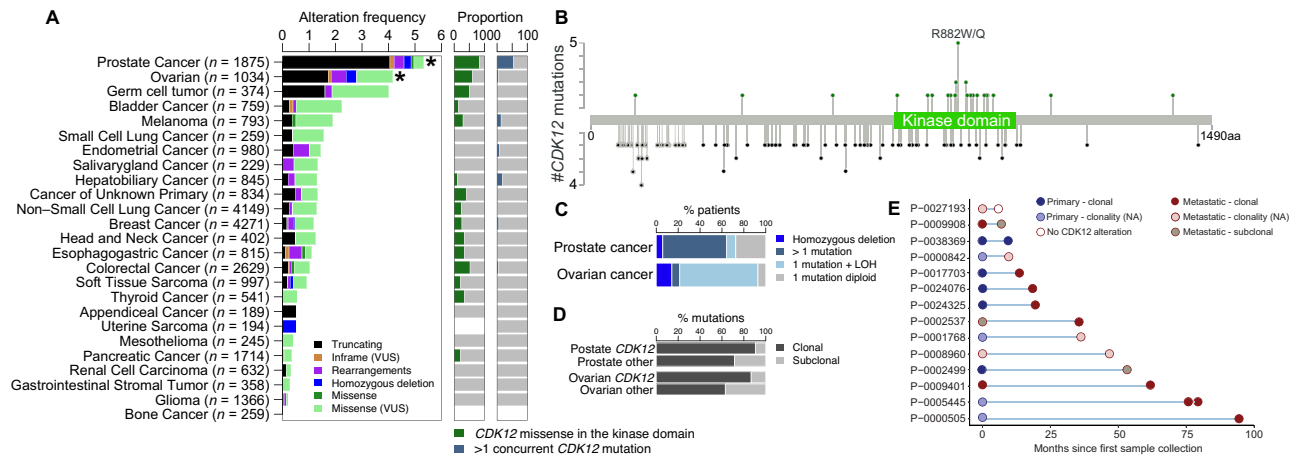


Fig. 1 – Landscape of somatic CDK12 mutations across 25 cancer types in the MSK-IMPACT cohort. (A) Somatic CDK12 alteration prevalence (left panel), proportion of kinase domain mutations among all missense mutations (middle panel), and proportion of cases with >1 CDK12 mutation in an individual tumor (right panel) across 26743 tumors from 25 different cancer types. CDK12 is significantly mutated in prostate and ovarian cancer only. * Significantly mutated according to MutSigCV. (B) Distribution of missense (upper green lollipop) and truncating mutations (lower black lollipop) in CDK12 in prostate cancer. (C) Prevalence and type of biallelic inactivation of CDK12 in prostate and ovarian cancer. (D) Prevalence of CDK12 clonal mutations compared to all other mutations in prostate and ovarian cancer. (E) CDK12 alterations in matched tumors in the prostate CDK12 alteration cohort, including localized primaries and later metastases and other matched tumors from the same patients. LOH = loss of heterozygosity; NA = not evaluable.

(OS), time to castration resistance, and time on treatment with first-line abiraterone acetate or enzalutamide for castration-resistant disease (Supplementary Fig. 1). For OS, follow-up started at the time of first metastasis and ended with patient death, with censorship occurring at last patient contact. For time to castration-resistance, follow-up started at the time of start of continuous androgen deprivation therapy (ADT) and ended at the development of castration-resistant disease as documented in the medical record, with censorship occurring at last patient assessment. For time-on-treatment analysis, follow-up started at the time of starting the agent as first-line therapy for CRPC and ended with discontinuation of the agent, with censorship occurring at last patient assessment. Median follow-up time was calculated for patients without the event only.

2.4. Statistical analysis

The Kruskal-Wallis test was used to evaluate differences in TMB, FGA, number of breakpoints, median segment size, tumor purity, and sequencing coverage among the three groups defined by CDK12 allelic status (CDK12-WT vs CDK12 monoallelic [CDK12-Mono] vs CDK12-Bi). We used a pairwise post-hoc Mann-Whitney test corrected for multiple comparisons using the FDR method. Fisher's exact test (adjusted for multiple comparisons) was used to evaluate differences in the prevalence of copy-number gain and copy-number loss, and the prevalence of oncogenic pathway alterations based on CDK12 allelic status. We used a binomial test to determine whether the ratio of missense mutations present in the kinase domain of CDK12 differed significantly from the proportion of nucleic acids present in the kinase domain of CDK12. We used the Wilcoxon signed-rank test to determine whether TMB, FGA, and the number of breakpoints differed significantly between paired samples collected at two different time points. Time-to-event outcomes were analyzed using the Kaplan-Meier method and compared via the log-rank test. The association between CDK12 alteration and survival was evaluated using univariable and multivariable Cox proportional-hazards regression models. Multivariable analysis was adjusted for standard clinical prognostic factors: age at diagnosis (continuous), Gleason score at diagnosis (<8 vs ≥8), prostate-specific

antigen (PSA) at diagnosis (continuous), visceral metastasis at diagnosis (presence vs absence), de novo metastatic status at diagnosis (presence vs absence), and FGA. Multivariable tests were performed using analysis of variance to compare the models with and without the extra term. The *p* values reported are two-tailed. When applicable, multiple testing correction was performed using the FDR method and FDR < 0.05 was considered significant. All analyses were performed using R v3.5.2 (www.R-project.org) and Bioconductor v3.4.

3. Results

3.1. Landscape of CDK12 alterations across cancer types

We assessed the prevalence of CDK12 alterations among 26743 patients across 25 solid cancer types profiled using the MSK-IMPACT clinical sequencing test. A total of 404 patients (1.5%) had tumors with at least one CDK12 alteration. Prostate (*n* = 100/1875, 5.3%) and ovarian (*n* = 43/1034, 4.2%) cancers were the types with the highest frequencies of CDK12 oncogenic alterations (Fig. 1A), including somatic point mutations, rearrangements, and deep deletions. Using MutSigCV [22], we found that CDK12 was significantly recurrently mutated only in prostate and ovarian cancers compared with background mutation rates (FDR < 0.001 and FDR = 0.056, respectively; Fig. 1A). The majority of CDK12 alterations in prostate cancer were truncating mutations, followed by missense mutations, rearrangements, and deep deletions (Supplementary Fig. 2A,B). Most truncating mutations were located within or upstream of the kinase domain (101/105, 96%), whereas missense mutations were more frequently clustered within the kinase domain (25/30, 83%), which was higher than expected by chance (*p* < 0.001; Fig. 1B), suggesting that these mutations probably have a deleterious effect on

CDK12 function. Moreover, in prostate cancer the majority of patients with missense mutations in the kinase domain of *CDK12* (18/24, 75%) had a concurrent truncating mutation suggestive of *CDK12*-Bi inactivation, which was not observed in other cancer types. In total, we identified 61/84 (73%) and 26/28 patients (93%) with putative *CDK12*-Bi inactivation in prostate and ovarian cancer, respectively (Fig. 1C). The principal genomic event responsible for *CDK12*-Bi inactivation in prostate cancer was multiple concurrent mutations of *CDK12* (49/61, 75%), whereas in ovarian cancer it was LOH (20/26, 77%; Fig. 1C). We observed a higher prevalence of clonal *CDK12* mutations than non-*CDK12* mutations in prostate and ovarian cancer (105/116 [91%] vs 140/196 [71%]; $p < 0.001$; and 26/30 [87%] vs 82/130 [63%]; $p = 0.016$, respectively; Fig. 1D, Supplementary Fig. 2C).

Of 14 prostate cancer patients with more than one tumor sequenced longitudinally, 13 showed shared *CDK12* alterations among all matched tumors, with one patient having samples collected up to 7.8 yr apart (Fig. 1E). Of note, the

only patient who did not have shared *CDK12* alterations in their matched tumors had a *CDK12* rearrangement known to be prone to false-negative results in hybrid capture DNA sequencing. FGA and the number of breakpoints increased over time, whereas TMB did not change significantly between paired samples (Supplementary Fig. 2D). The frequency of *CDK12*-Bi inactivation did not differ between primary (34/48, 70%) and metastatic tumors (27/36, 75%) in which zygosity could be determined. These analyses suggest that *CDK12* alteration is an early event in the genomic makeup of *CDK12*-altered metastatic prostate cancer.

3.2. Genomic architecture of *CDK12*-altered prostate cancer

Of 84/100 *CDK12*-altered prostate cancers for which zygosity could be determined, 23 had *CDK12* monoallelic inactivation (*CDK12*-Mono) and 61 had biallelic inactivation (*CDK12*-Bi). There was no difference in tumor purity or sequencing coverage by *CDK12* alteration status (Supple-

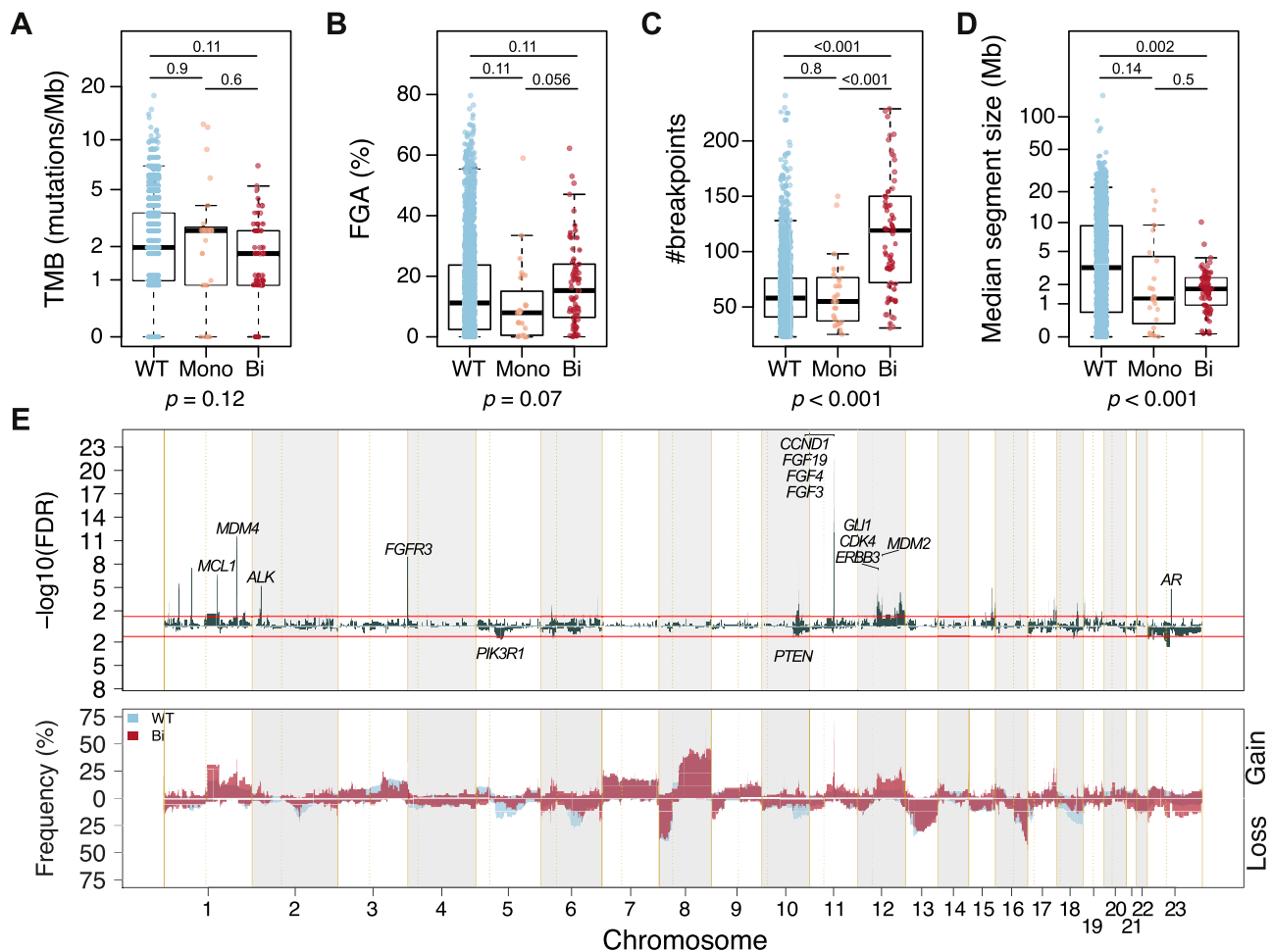


Fig. 2 – Genomic architecture of *CDK12*-altered prostate cancer. (A–D) Comparison of (A) tumor mutational burden (TMB), (B) fraction of genome altered (FGA), (C) number of breakpoints, and (D) median size of copy number alterations in prostate cancers with wild-type *CDK12* (WT, blue), *CDK12* with monoallelic inactivation (Mono, orange), or *CDK12* with biallelic inactivation (Bi). The p values are derived from the Kruskal-Wallis H test. The q value (adjusted for multiple comparison) derived from the pairwise Mann-Whitney test is shown above each comparison. (E) Prevalence plot (bottom) and corresponding $-\log_{10}(\text{FDR})$ value derived from Fisher's exact test (top) comparing copy-number gains and losses between *CDK12*-WT (blue) and *CDK12*-Bi (red) prostate cancer. Selected oncogenic genes within affected chromosomal regions are shown. Red lines indicate FDR = 0.05. FDR = false discovery rate.

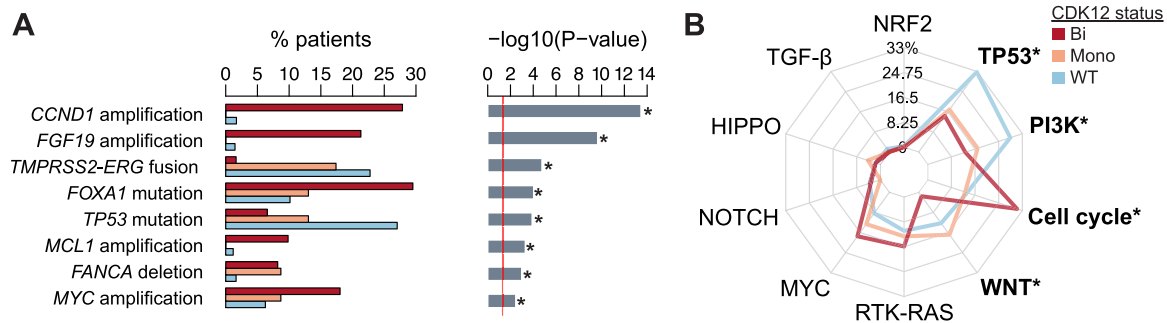


Fig. 3 – Oncogenic alterations associated with CDK12-altered prostate cancer. (A) Prevalence of recurrent oncogenic alteration in prostate cancer with CDK12 biallelic (Bi, red) compared with CDK12 monoallelic (Mono, orange) inactivation or CDK12 wild-type tumors (WT, blue). The *p* values are derived from Fisher's exact test (* FDR < 0.05). **(B)** Radar plots showing the percentage of patients with alterations in the corresponding canonical oncogenic signaling pathways according to CDK12 alterations. Statistically significant pathways associated with CDK12 alteration are indicated with an asterisk (* FDR < 0.05). FDR = false discovery rate.

mentary Fig. 3A,B). TMB and overall FGA were similar between CDK12-WT, CDK12-Mono, and CDK12-Bi (Fig. 2A, B), but we observed a higher fraction of genome gain in CDK12-Bi prostate cancer (Supplementary Fig. 3C). Of note, CDK12-Bi prostate cancer was associated with a higher number of breakpoints and smaller median size of copy-number-altered segments (Fig. 2C,D and Supplementary Fig. 3E,F). We compared copy-number alteration patterns and found that 13% of the evaluated genome was differentially affected between CDK12-WT and CDK12-Bi cases (Fig. 2E and Supplementary Table 1). This highly fragmented genome with a pattern of narrow spikes in copy-number-altered segments distributed along the genome (Fig. 2E) is suggestive of a tandem duplicator phenotype [9]. Chromosome 11q13.3, a locus that includes *CCND1*, *FGF19*, *FGF4*, and *FGF3*, was more frequently affected by copy-number gain in CDK12-Bi than in CDK12-WT tumors. Overall, these results suggest that CDK12-Bi inactivation may shape the genomic architecture of prostate cancer.

3.3. Oncogenic alterations associated with CDK12-altered prostate cancer

We investigated differences in the prevalence of 53 recurrent oncogenic alterations (Methods 2.2 and Supplementary Table 2) by CDK12 allelic status. We found eight recurrent oncogenic alterations that differed in prevalence by CDK12 status (FDR < 0.05; Fig. 3A and Supplementary Table 2): *CCND1*, *MCL1*, and *MYC* were more frequently amplified, *FANCA* was more frequently lost, and *FOXA1* was more frequently mutated in the CDK12-Bi group. *TP53* mutations and *TMPRSS2-ERG* fusions were less frequently observed in the CDK12-Bi group. Next, we interrogated the alteration prevalence of ten canonical oncogenic signaling pathways: cell cycle, Hippo, Myc, Notch, Nrf2, PI-3-Kinase/Akt, RTK-RAS, TGFβ signaling, p53, and β-catenin/Wnt [21]. The cell cycle pathway was more frequently altered in CDK12-Bi than in CDK12-WT tumors, whereas alterations in the p53, PI-3-Kinase/Akt, and β-catenin/Wnt pathways were less frequently observed in CDK12-Bi than in CDK12-WT tumors (Fig. 3B).

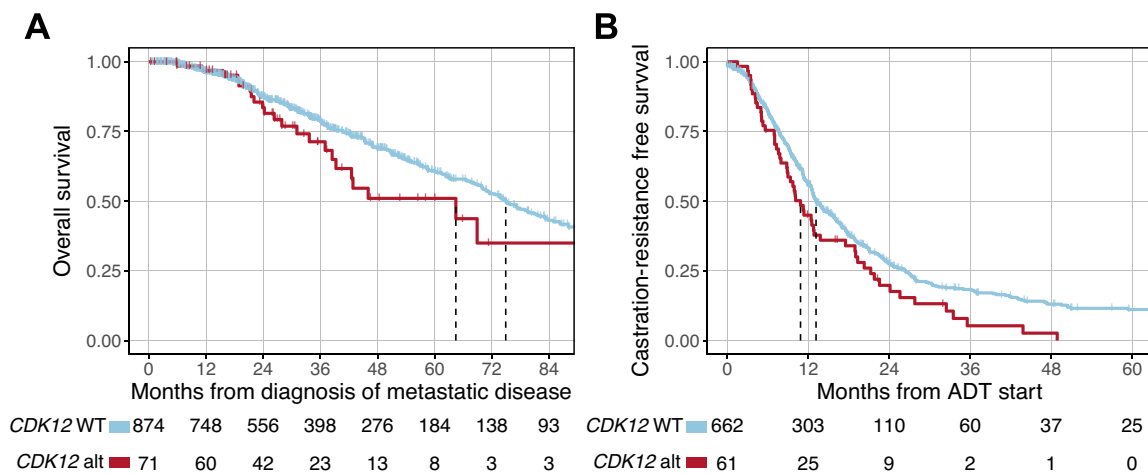
3.4. Clinicopathologic features and outcomes for CDK12-altered prostate cancer

A subset of 1465 patients with prostate cancer were evaluable for clinical outcomes, of whom 100 had CDK12 alterations and 1365 were CDK12-WT (Supplementary Fig. 1). Baseline clinicopathologic characteristics are shown in Table 1. Patients with CDK12-altered tumors had clinical features associated with worse prognosis, including a higher prevalence of de novo metastatic disease (40% vs 26%), higher PSA at diagnosis (median 14.8 vs 9.0 ng/mL), and a higher prevalence of Gleason score ≥8 (80% vs 57%). Patients with CDK12-Bi inactivation had the worst baseline characteristics (Supplementary Table 4). Clinical status at the time of tissue collection for molecular profiling is summarized in Supplementary Table S4 and shown in cBioPortal [26] (www.cbioportal.org/study?id=prad_cdk12_mskcc_2020). We interrogated the association between CDK12 status and OS. There were a total of 315 deaths (CDK12-WT: 290/874 evaluable patients; CDK12-altered: 25/71) and the median follow-up time for censored patients was 30.2 mo. Compared to CDK12-WT patients, patients in the CDK12-altered group had shorter OS from diagnosis of metastatic disease (median 64.4 vs 74.9 mo; Fig. 4A). This difference was maintained after adjustment for known prognostic factors, including FGA [24] (adjusted hazard ratio [aHR] 1.80, 95% CI 1.12–2.89; *p* = 0.024). We also examined time to castration resistance from the start of ADT. There were a total of 522 events (CDK12-WT: 468/662 evaluable patients; CDK12-altered: 54/61) and the median follow-up time for censored patients was 12.2 mo. Patients in the CDK12-altered group had a shorter time to the development of castration-resistant disease (median 10.8 vs 13.1 mo; aHR 1.49, 95% CI 1.09–2.03; *p* = 0.017; Fig. 4B). Finally, among patients who received first-line abiraterone or enzalutamide for castration-resistant disease, those with CDK12-altered tumors had a similar time on treatment compared to CDK12-WT patients (median 9.7 vs 8.7 mo; aHR 1.08, 95% CI 0.57–1.51; *p* = 0.8; Supplementary Fig. 4). Collectively, these results suggest that CDK12 oncogenic alterations define a subset of somewhat more aggressive

Table 1 – Clinical and pathological characteristics of patients with prostate cancer with and without *CDK12* alterations.

	<i>CDK12</i> status	
	Altered	Wild type
Patients (n)	100	1365
Median age at diagnosis, yr (interquartile range)	61 (54–67)	63 (56–68)
Histology at diagnosis, n (% of known cases)		
Adenocarcinoma	94 (97)	1216 (99)
Adenocarcinoma with neuroendocrine features	2 (2)	12 (1)
Other	1 (1)	6 (0)
Unknown	3	131
Median prostate-specific antigen at diagnosis, ng/mL (interquartile range)	14.8 (7.7–77.8)	9.0 (5.3–24.1)
Gleason score, n (% of known cases)		
6	7 (8)	108 (9)
7	10 (12)	435 (34)
≥8	68 (80)	727 (57)
Not evaluable	15	95
Metastatic disease at diagnosis, n (% of known cases)		
Yes	39 (40)	346 (26)
Unknown	2	23
Sites of metastasis at tissue collection, n (%) ^a		
Bone	48 (60)	528 (57)
Nonregional lymph nodes	44 (55)	380 (41)
Liver	5 (6)	93 (10)
Lung	4 (5)	101 (11)
Visceral	7 (9)	165 (18)

^a As a percentage of the number of patients with metastatic disease.

**Fig. 4 – Clinical outcomes for *CDK12*-altered prostate cancer. (A) Overall survival from diagnosis of metastatic disease. (B) Time to development of castration-resistant prostate cancer from ADT initiation. ADT = androgen deprivation therapy.**

prostate cancer that progresses slightly more rapidly to castration resistance and is associated with slightly worse survival.

4. Discussion

We analyzed the prevalence and types of somatic *CDK12* alterations across >26000 tumors spanning 25 cancer types and found that prostate and ovarian cancers were the malignancies with the highest prevalence of *CDK12* alterations, similar to a recent report [15]. The overall prevalence of *CDK12* alterations in prostate cancer was 5.3%, which falls

within the previously reported range [3,15]. The majority of *CDK12* alterations in these cancers were oncogenic and clonal and resulted in biallelic inactivation, suggesting that *CDK12* loss of function has an important biological role in a subset of prostate and ovarian cancers. In prostate cancer in particular, the main event presumed to be responsible for *CDK12*-Bi inactivation is the co-occurrence of two deleterious mutations, whereas in *CDK12*-altered ovarian cancer it is an LOH event, which can span a larger chromosomal segment and may not be specific to *CDK12*. While we were not able to definitively demonstrate the existence of *CDK12* homozygous inactivation via copy-number alteration only,

we included *CDK12* deep deletion in the definition of the *CDK12*-altered group. Future studies are needed to determine whether complete loss of *CDK12* can occur through deletion alone in prostate cancer. *CDK12*-Bi inactivation in prostate cancer was associated with a higher prevalence of small-fragment copy-number alterations and a higher number of breakpoints across the genome, consistent with the previously described tandem duplicator phenotype [9,25], as tandem duplications are difficult to characterize directly using targeted panel sequencing assays. We found that *CDK12* mutations occur clonally in metastatic prostate cancer, and were identified in primary matched tumors from patients who developed metastatic disease, suggesting that *CDK12* loss of function is an early event in the pathogenesis of *CDK12*-altered metastatic prostate cancer. Our study differs from the pan-cancer analysis by Sokol et al. [15] in that we identified early occurrence of *CDK12* alterations in patients with matched tumors, and include a description of clinicopathologic features and clinical outcomes for prostate cancer patients, all within the same data set. Furthermore, our analysis of copy-number alterations in prostate tumors with *CDK12*-Bi inactivation compared with *CDK12*-WT tumors revealed numerous differentially altered regions. This includes amplification of 11q13.3, a region that encompasses the oncogene *CCND1*, and enrichment of alterations in cell-cycle pathway genes in patients with *CDK12* loss of function. We did not identify a significant co-occurrence of alterations in *CDK12* with DNA-repair gene alterations.

Identifying vulnerabilities of *CDK12*-altered prostate cancer is particularly important given our finding of somewhat more aggressive clinicopathologic features associated with *CDK12* alteration, including more aggressive disease at diagnosis, slightly shorter OS, and shorter time to castration resistance. A study in an independent cohort of 46 patients with *CDK12*-altered prostate cancer by Reimers et al. [16] recently revealed similar outcomes with regard to time to castration resistance and time on treatment with first-line abiraterone and enzalutamide. Our larger study differs in that it also showed shorter OS for patients with *CDK12*-altered cancer, and differences in outcomes were maintained after adjusting for common clinical prognostic variables, probably facilitated by the larger sample size, all within a single institutional data set using a single clinical tumor-sequencing assay. We noted a similar rate of Gleason 8–10 prostate cancer at diagnosis for patients with *CDK12*-altered disease in comparison to the Reimers study (80% vs 88%). One vulnerability of *CDK12*-altered prostate cancer may be a higher sensitivity to immune checkpoint blockade owing to the higher neoantigen burden resulting from the tandem duplicator phenotype associated with *CDK12* loss of function [9], a hypothesis that is currently being tested in a multi-institutional phase 2 study combining nivolumab with ipilimumab (NCT03570619). Of note, the slightly worse clinical outcomes we identified for *CDK12*-altered prostate cancer were unaffected by adjustment for FGA, suggesting that *CDK12* loss of function impacts disease behavior through a mechanism independent of broad copy-number changes,

possibly by promoting genomic alterations in specific pathways, or by impacting gene expression through increased intronic polyadenylation [7]. Limitations of our study include its retrospective nature, the use of a targeted panel assay for genomic profiling, which can miss genomic rearrangements, and potential selection bias arising from the inclusion of patients only from a tertiary referral center.

5. Conclusions

In summary, our results suggest that *CDK12* loss of function defines a distinct subset of disease associated with unique genomic alterations and somewhat worse clinical outcomes, and highlights the need for novel therapies targeting *CDK12*-altered prostate cancer.

Author contributions: Wassim Abida had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Acquisition of data: Nguyen, Mota, Nandakumar, Stopsack, Weg, Rathkopf, Morris, Scher, Zamarin, Abida.

Analysis and interpretation of data: Nguyen, Mota, Nandakumar, Stopsack, Kantoff, Gopalan, Zamarin, Solit, Schultz, Abida.

Drafting of the manuscript: Nguyen, Mota, Stopsack, Schultz, Abida.

Critical revision of the manuscript for important intellectual content: All authors.

Statistical analysis: Nguyen, Stopsack, Schultz.

Obtaining funding: Scher, Solit, Abida.

Administrative, technical, or material support: Morris, Scher, Kantoff, Schultz, Abida.

Supervision: Schultz, Abida.

Other: None.

Financial disclosures: Wassim Abida certifies that all conflicts of interest, including specific financial interests and relationships and affiliations relevant to the subject matter or materials discussed in the manuscript (eg, employment/affiliation, grants or funding, consultancies, honoraria, stock ownership or options, expert testimony, royalties, or patents filed, received, or pending), are the following: Howard I. Scher is an uncompensated consultant/advisory board member for Amgen, ESSA Pharma Inc., Janssen Research & Development LLC, Janssen Biotech Inc., and Menarini Silicon; a compensated consultant/advisory board member for Ambry Genetics Corporation, Konica Minolta Inc., OncLive Insights, Physicians Education Resource, and WCG Oncology; has received research funding from Epic Sciences, Illumina Inc., Janssen, Menarini Silicon, and ThermoFisher; and has received travel/accommodation expenses from Amgen, Clovis Oncology, ESSA Pharma Inc., Menarini Silicon, OncLive Insights, Physician Education Resource, Prostate Cancer Foundation, and WCG Oncology. Philip W. Kantoff holds investments in Context Therapeutics LLC, DRGT, Placon, Seer Biosciences, and Tarveda Therapeutics; is a company board member for Context Therapeutics LLC; is a consultant/scientific advisory board member for Bavarian Nordic Immunotherapeutics, DRGT, GE Healthcare, Janssen, New England Research Institutes Inc., OncoCellMDX, Progenity, Sanofi, Seer Biosciences, Tarveda Therapeutics, and Thermo Fisher; and sits on data safety monitoring boards for Genentech/Roche and Merck. Michael J. Morris is an unpaid consultant for Astellas, Bayer, and Endocyte; is a paid consultant for Advanced

Accelerator Applications, Blue Earth Diagnostics, Tokai, Tolmar, and Oric; and has received institutional research funding from Bayer, Sanofi, Endocyte, Progenics, Corcept, and Roche/Genentech. Dmitriy Zamarin has received consulting fees from Western Oncolytics, Agenus Inc., Tesaro, and Merck, and has received research funding from Merck. David B. Solit has received consulting fees or honoraria from Pfizer, Loxo Oncology, Lilly Oncology, Illumina, and Vivideon Therapeutics. Wassim Abida has received consulting fees from Clovis Oncology, Janssen Pharmaceutica, MORE Health, and ORIC Pharmaceuticals; research funding from AstraZeneca, Zenith Epigenetics, Clovis Oncology, and GlaxoSmithKline; and travel expenses from Clovis Oncology, GlaxoSmithKline, ORIC Pharmaceuticals, and the Prostate Cancer Foundation. Dana Rathkopf has received research funding from Janssen, Celgene, Ferring, Novartis, Taiho, Tracoon, AstraZeneca, and Genentech/Roche, and has been an uncompensated consultant for Janssen, Tracoon, AstraZeneca, Genentech, and Bayer. The remaining authors have nothing to disclose.

Funding/Support and role of the sponsor: This study was supported by Young Investigator and Challenge Awards from the Prostate Cancer Foundation (W.A., N.S., K.H.S.); Department of Defense Prostate Cancer Research Program Physician Research AwardW81XWH-17-1-0124 (W.A.) and Early Investigator Research AwardW81XWH-18-1-0330 (K.H.S.); and National Cancer Institute (NCI) cancer center support grant P30CA008748, and NCI prostate cancer SPORE grant P50CA092629. The sponsors played no direct role in the study.

CRedit authorship contribution statement

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.eururo.2020.03.024>.

References

- [1] Bell D, Berchuck A, Birrer M, et al. Integrated genomic analyses of ovarian carcinoma. *Nature* 2011;474:609–15. <http://dx.doi.org/10.1038/nature10166>.
- [2] Abeshouse A, Ahn J, Akbani R, et al. The tancer. *Cell* 2015;163:1011–25. <http://dx.doi.org/10.1016/j.cell.2015.10.025>.
- [3] Robinson D, Van Allen EM, Wu YM, et al. Integrative clinical genomics of advanced prostate cancer. *Cell* 2015;161:1215–28. <http://dx.doi.org/10.1016/j.cell.2015.05.001>.
- [4] Armenia J, Wankowicz SAM, Liu D, et al. The long tail of oncogenic drivers in prostate cancer. *Nat Genet* 2018;50:645–51. <http://dx.doi.org/10.1038/s41588-018-0078-z>.
- [5] Ekumi KM, Paculova H, Lenasi T, et al. Ovarian carcinoma CDK12 mutations misregulate expression of DNA repair genes via deficient formation and function of the Cdk12/CycK complex. *Nucleic Acids Res* 2015;43:2575–89. <http://dx.doi.org/10.1093/nar/gkv101>.
- [6] Blazek D, Kohoutek J, Bartholomeeusen K, et al. The cyclin K/Cdk12 complex maintains genomic stability via regulation of expression of DNA damage response genes. *Genes Dev* 2011;25:2158–72. <http://dx.doi.org/10.1101/gad.16962311>.
- [7] Dubbury SJ, Boutz PL, Sharp PA. CDK12 activates HRR by suppressing intronic polyadenylation, 2018. *Nature* 2018;564:141–5. <http://dx.doi.org/10.1038/s41586-018-0758-y>.
- [8] Krajewska M, Dries R, Grasseti AV, et al. CDK12 loss in cancer cells affects DNA damage response genes through premature cleavage and polyadenylation. *Nat Commun* 2019;10:1757. <http://dx.doi.org/10.1038/s41467-019-09703-y>.
- [9] Wu Y-M, Cie M, Lonigro RJ, et al. Inactivation of CDK12 delineates a distinct immunogenic class of advanced prostate cancer article inactivation of CDK12 delineates a distinct immunogenic class of advanced prostate cancer. *Cell* 2018;173:1770–82. <http://dx.doi.org/10.1016/j.cell.2018.04.034>.
- [10] Popova T, Manié E, Boeva V, et al. Ovarian cancers harboring inactivating mutations in CDK12 display a distinct genomic instability pattern characterized by large tandem duplications. *Cancer Res* 2016;76:1882–91. <http://dx.doi.org/10.1158/0008-5472.CAN-15-2128>.
- [11] Quigley DA, Dang HX, Zhao SG, et al. Genomic hallmarks and structural variation in metastatic prostate cancer. *Cell* 2018;174:758–769.e9. <http://dx.doi.org/10.1016/j.cell.2018.06.039>.
- [12] Abida W, Armenia J, Gopalan A, et al. Prospective genomic profiling of prostate cancer across disease states reveals germline and somatic alterations that may affect clinical decision making. *JCO Precis Oncol* 2017;1:1–16.
- [13] Non-BRCA DNA Damage Repair Gene Alterations and Response to the PARP Inhibitor Rucaparib in Metastatic Castration-Resistant Prostate Cancer: analysis from the phase 2 TRITON2 study Wassim Abida, David Campbell, Akash Patnaik, Jeremy D Shapiro, Brieuc Sautois, Nicholas J Vogelzang, Eric G Voog, Alan H. Bryce, Ray McDermott, Francesco Ricci, Julie Rowe, Jingsong Zhang, Josep Maria Piulats, Karim Fizazi, Axel S Merseburger, Celestia S. Higano, Laurence E. Krieger, Charles J Ryan, Felix Y. Feng, Andrew D Simmons, Andrea Loehr, Darrin Despain, Melanie Dowson, Foad Green, Simon P. Watkins, Tony Golsorkhi and Simon Chowdhury *Clin Cancer Res* February 21 2020 <https://doi.org/10.1158/1078-0432.CCR-20-0394>.
- [14] Mateo J, Porta N, McGovern UB, et al. TOPARP-B: a phase II randomized trial of the poly(ADP)-ribose polymerase (PARP) inhibitor olaparib for metastatic castration resistant prostate cancers (mCRPC) with DNA damage repair (DDR) alterations. *J Clin Oncol* 2019;37(15 Suppl):5005. http://dx.doi.org/10.1200/jco.2019.37.15_suppl.5005.

- [15] Sokol ES, Pavlick D, Frampton GM, et al. Pan-cancer analysis of CDK12 loss-of-function alterations and their association with the focal tandem-duplicator phenotype. *Oncologist* 2019;24:1526–33. <http://dx.doi.org/10.1634/theoncologist.2019-0214>.
- [16] Reimers MA, Yip SM, Zhang L, et al. Clinical outcomes in cyclin-dependent kinase 12 mutant advanced prostate cancer. *Eur Urol* 2020;77:333–41. <http://dx.doi.org/10.1016/j.eururo.2019.09.036>.
- [17] Zehir A, Benayed R, Shah RH, et al. Mutational landscape of metastatic cancer revealed from prospective clinical sequencing of 10,000 patients. *Nat Med* 2017;23:703–13. <http://dx.doi.org/10.1038/nm.4333>.
- [18] Cheng DT, Mitchell T, Zehir A, et al. MSK-IMPACT: a hybridization capture-based next-generation sequencing clinical assay for solid tumor molecular oncology. *J Mol Diagn* 2015;17:251–64. <http://dx.doi.org/10.1016/j.jmoldx.2014.12.006>.
- [19] Niu B, Ye K, Zhang Q, et al. MSIsensor: microsatellite instability detection using paired tumor-normal sequence data. *Bioinformatics* 2014;30:1015–6. <http://dx.doi.org/10.1093/bioinformatics/btt755>.
- [20] Chakravarty D, Gao J, Phillips S, Kundra R. OncoKB: a precision oncology knowledge base. *Precis Oncol* 2017;1:1–16. <http://dx.doi.org/10.1200/PO.17.00011>.
- [21] Sanchez-Vega F, Mina M, Armenia J, et al. Oncogenic signaling pathways in The Cancer Genome Atlas. *Cell* 2018;173:321–337. e10. <http://dx.doi.org/10.1016/j.cell.2018.03.035>.
- [22] Lawrence MS, Stojanov P, Polak P, et al. Mutational heterogeneity in cancer and the search for new cancer-associated genes. *Nature* 2013;499:214–8. <http://dx.doi.org/10.1038/nature12213>.
- [23] Shen R, Seshan VE. FACETS: allele-specific copy number and clonal heterogeneity analysis tool for high-throughput DNA sequencing. *Nucleic Acids Res* 2016;44:1–9. <http://dx.doi.org/10.1093/nar/gkw520>.
- [24] Hieronymus H, Schultz N, Gopalan A, et al. Copy number alteration burden predicts prostate cancer relapse. *Proc Natl Acad Sci U S A* 2014;111:11139–44. <http://dx.doi.org/10.1073/pnas.1411446111>.
- [25] Viswanathan SR, Ha G, Hoff AM, et al. Structural alterations driving castration-resistant prostate cancer revealed by linked-read genome sequencing. *Cell* 2018;174:433–447.e19. <http://dx.doi.org/10.1016/j.cell.2018.05.036>.
- [26] Cerami E, Gao J, Dogrusoz U, et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. *Cancer Discov* 2012;2(5):401–4. <http://dx.doi.org/10.1158/2159-8290.CD-12>.

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Oncogenic Genomic Alterations, Clinical Phenotypes, and Outcomes in Metastatic Castration-Sensitive Prostate Cancer

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ABSTRACT

Purpose: The genomic underpinning of clinical phenotypes and outcomes in metastatic castration-sensitive prostate cancer is unclear.

Experimental Design: In patients with metastatic castration-sensitive prostate cancer at a tertiary referral center, clinical-grade targeted tumor sequencing was performed to quantify tumor DNA copy number alterations and alterations in predefined oncogenic signaling pathways. Disease volume was classified as high volume (≥ 4 bone metastases or visceral metastases) versus low volume.

Results: Among 424 patients (88% white), 213 (50%) had high-volume disease and 211 (50%) had low-volume disease, 275 (65%) had *de novo* metastatic disease, and 149 (35%) had metastatic recurrence of nonmetastatic disease. Rates of castration resistance [adjusted hazard ratio, 1.84; 95% confidence interval (CI), 1.40–2.41] and death (adjusted hazard ratio, 3.71; 95% CI, 2.28–6.02) were higher

in high-volume disease. Tumors from high-volume disease had more copy number alterations. The NOTCH, cell cycle, and epigenetic modifier pathways were the highest-ranking pathways enriched in high-volume disease. *De novo* metastatic disease differed from metastatic recurrences in the prevalence of *CDK12* alterations but had similar prognosis. Rates of castration resistance differed 1.5-fold to 5-fold according to alterations in *AR*, *SPOP* (inverse), and *TP53*, and the cell cycle, WNT (inverse), and MYC pathways, adjusting for disease volume and other genomic pathways. Overall survival rates differed 2-fold to 4-fold according to *AR*, *SPOP* (inverse), WNT (inverse), and cell-cycle alterations. PI3K pathway alterations were not associated with prognosis once adjusted for other factors.

Conclusions: This study identified genomic features associated with prognosis in metastatic castration-sensitive disease that may aid in molecular classification and treatment selection.

Introduction

Treatment options for metastatic castration-sensitive prostate cancer are expanding (1). Both docetaxel and next-generation androgen receptor axis-directed therapies prolong overall survival (2–8). However, it is not clear which patients benefit most from intensified therapies, which increase toxicity and cost, or how clinical

cal disease phenotypes can be used to find a drug class more likely to benefit a patient (1, 9). Some but not all trials show more favorable outcomes with docetaxel for patients with high-volume disease, characterized by the presence of visceral metastases or high-risk bone metastases (2, 10, 11). Similarly, it has been suggested that patients who initially presented with *de novo* metastases and those who developed metastatic recurrence after a diagnosis with localized disease may have differences in benefit from therapy (2, 12).

The genomic underpinning of clinical phenotypes in metastatic castration-sensitive prostate cancer has not been elucidated to date. It is also incompletely understood which tumor DNA alterations are drivers of aggressiveness in prostate cancer (13, 14), especially in castration-sensitive disease (1).

We set out to assess oncogenic alterations in tumor signaling pathways as the mechanistic basis of clinical phenotypes defined by disease volume and by timing of metastasis (*de novo* vs. metastatic recurrence). We further aimed to determine which genomic alterations are associated with clinical outcomes in metastatic castration-sensitive prostate cancer. We addressed these questions using clinical-grade-targeted tumor sequencing in a large cohort of patients with metastatic castration-sensitive prostate cancer.

Materials and Methods

Patient population and clinical characteristics

This study included patients with prostate cancer who underwent institutional review board-approved paired tumor–blood sequencing at a tertiary referral center after providing written informed consent. The study was conducted in accordance with the U.S. Common Rule. Patients had metastatic castration-sensitive disease at the time a

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (<http://clincancerres.aacrjournals.org/>).

Genomic and clinical data are available at https://www.cbiportal.org/study/summary?id=prad_mscpc_mskcc_2020.

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Clin Cancer Res 2020;26:3230–8

doi: 10.1158/1078-0432.CCR-20-0168

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Translational Relevance

The genomic landscape of metastatic castration-sensitive prostate cancer is not well defined, and disease stratification for the purpose of initial treatment selection has primarily relied on clinical phenotypes, including volume of disease at the time of metastasis. Here, we describe tumor genomics in a large cohort of patients with metastatic castration-sensitive prostate cancer and show genomic features that are associated with clinical phenotypes, including with disease volume. We identify genomic alterations that are associated with prognosis in metastatic castration-sensitive disease (overall survival and time to castration resistance), demonstrating that alterations in *AR*, *TP53*, and the cell cycle and *MYC* pathways occur in tumors with worse prognosis, while alterations in *SPOP* and the *WNT* pathway occur in tumors with better prognosis. Our findings may aid in molecular classification of metastatic castration-sensitive prostate cancer, and pathways that are prognostically relevant could be targeted in studies of intensified upfront therapy.

specimen for genomic profiling was obtained. Disease extent had to be metastatic and before a potential future date of castration resistance. We first identified patients who had a tumor sample genomically profiled that had been confirmed as a primary or metastasis from prostate cancer by a pathologist. We set up a structured clinical research database for patient and sample characteristics, treatments, and clinical outcomes, including pilot testing for reliability and validity of team-based annotations. Data sources included pathology reports (primarily from rereview; if missing, external reports); patients' self-reported race, ethnicity, and smoking status; and the medical record, drawing from medical oncology, urology, or radiation subspecialty documentation from the initial presentation at the cancer center as well as visits before and after obtaining a sample for genomic profiling. Castration resistance and metastasis were determined by the primary treating physicians; in cases of discrepancies, the record was reviewed by more than one team member. Extent of disease, sites of disease, and bone disease volume were reviewed by the study radiologist (A.G. Wibmer) based primarily on bone scans and computed tomography. For high-volume disease, we used a modified definition, requiring presence of visceral metastases or at least four bone metastases, without requirements for extra-axial metastases unlike prior clinical trials (2, 6, 7); others were classified as low-volume disease. Additional imaging that had been obtained clinically was also reviewed for ambiguous cases.

Genomic profiling

For genomic profiling, both archival specimens or newly obtained specimens were formalin-fixed, and a peripheral blood sample was drawn as a source of germline DNA. Analysis of the germline sample was only performed in patients who signed a specific consent (15). The MSK-IMPACT panel, a hybridization capture-based clinical assay authorized by the FDA, was used to detect single-nucleotide variations, small insertions and deletions, copy number alterations, and structural rearrangements in 341 to 468 genes, depending on assay version (16). Clonality and allele-specific copy numbers were estimated using FACETS (17). Samples that had low tumor purity of 20% or less on histologic assessment or FACETS estimations, no detected somatic mutations, and no copy number alterations were excluded (6.7% overall; 4.1% of samples from metastatic castration-sensitive disease).

If repeat samples during progression of metastatic castration-sensitive disease had been obtained (5% of patients), only the first sample was included.

As a measure of genomic instability, the burden of copy number alterations was inferred (fraction genome altered), also leveraging evenly distributed probes that tile common single nucleotide polymorphisms, and corrected for tumor purity (17). Tumor mutation burden was calculated as mutations per megabase. Potential actionability of tumor genomic alterations was annotated using OncoKB as of August 1, 2019 (18). Oncogenic alterations detected in the tumor and/or germline sample were grouped by major signaling pathways from pan-cancer analyses (19, 20), excluding the HIPPO and TGF β pathways that were altered in <5% of samples within clinical subgroups. *TP53* was not grouped with DNA repair genes and considered separately, as were two individual genes relevant in prostate cancer (*AR*, *SPOP*).

Analysis

The primary prespecified analysis of clinical phenotype (low-volume disease vs. high-volume disease and *de novo* vs. metastatic recurrence) compared fraction genome altered and prevalence of oncogenic alterations in key signaling pathways. Prevalence differences and ratios were estimated using binomial regression, convergence-assisted by Poisson models (21). We adjusted for predefined factors, including disease volume (in analyses of timing of metastases) or timing of metastases (in analyses of disease volume), origin of sample (prostate in the setting of metastatic disease vs. metastasis), age at sampling, time from metastasis to sample, and continuous exposure to androgen deprivation therapy. Our goal was valid quantification of genomic differences between clinical phenotypes, not decision making; confidence intervals (CI) are not means for testing null hypotheses of no difference.

In a not hypothesis-driven exploratory analysis to probe for individual genes missed by pathway groupings, we compared prevalence of oncogenic gene alterations between phenotypes for genes that were altered in at least 5% of samples in any of the four clinical phenotype groups, using the Fisher exact test for binomial proportions with a Benjamini-Hochberg false-discovery rate for multiple-testing correction.

To assess associations of clinical phenotypes (22, 23) and pathway alterations with time to castration resistance as well as overall survival, we estimated hazard ratio (HR) using Cox proportional hazards regression and differences in three-year restricted mean survival times (24). Follow-up started when the genomically profiled sample was obtained via biopsy, and patients without event were censored at the last clinic visit (for castration resistance) or at last contact (for overall survival). In a sensitivity analysis of pathways and prognosis, the top two most frequently altered genes per pathway (if altered in >2.5% of samples) were assessed individually.

Results

Study population

From 424 patients (median age at sample collection, 66 years; interquartile range, 59–72), a sample obtained while the patient had metastatic castration-sensitive disease was sequenced between May 2015 and September 2018 (Table 1). The majority of patients had *de novo* metastatic disease ($n = 275$, 65%). Similar numbers of patients had low-volume disease and high-volume disease. As expected, patients with metastatic recurrences of initially nonmetastatic disease, compared to those with *de novo* metastatic disease, tended to be different in terms of age at sampling, prostate-specific antigen (PSA)

Table 1. Patient and sample characteristics, by disease volume at genomic profiling and by timing of metastases.

	All N = 424	By disease volume		By timing of metastases	
		Low-volume disease n = 211	High-volume disease n = 213	Metastatic recurrence n = 149	De novo metastatic n = 275
Age at sampling (years)	65.6 (59.0–71.8)	65.3 (57.3–71.1)	65.8 (60.7–72.6)	68.3 (63.2–74.4)	64.2 (57.1–70.0)
Race					
Asian	12 (4%)	7 (4%)	5 (3%)	1 (1%)	11 (5%)
Black/African American	27 (8%)	13 (7%)	14 (8%)	13 (10%)	14 (6%)
White	312 (88%)	155 (88%)	157 (88%)	111 (88%)	201 (89%)
Other	2 (0%)	1 (0%)	1 (0%)	1 (1%)	1 (0%)
(Missing)	71	35	36	23	48
Timing of metastases					
Metastatic recurrence	149 (35%)	80 (38%)	69 (32%)	149 (100%)	0 (0%)
De novo metastatic	275 (65%)	131 (62%)	144 (68%)	0 (0%)	275 (100%)
Disease volume					
Low-volume disease	211 (50%)	211 (100%)	0 (0%)	80 (54%)	131 (48%)
High-volume disease	213 (50%)	0 (0%)	213 (100%)	69 (46%)	144 (52%)
Gleason grade at diagnosis					
<7	19 (5%)	11 (6%)	8 (4%)	19 (14%)	0 (0%)
3+4	30 (8%)	16 (8%)	14 (7%)	25 (18%)	5 (2%)
4+3	55 (14%)	33 (17%)	22 (12%)	42 (30%)	13 (5%)
8	88 (23%)	44 (23%)	44 (23%)	27 (19%)	61 (25%)
9–10	192 (50%)	90 (46%)	102 (54%)	27 (19%)	165 (68%)
(Missing)	40	17	23	9	31
PSA at diagnosis (ng/mL)	17.2 (6.6–88.8)	12.2 (6.0–42.2)	29.2 (6.9–257.7)	6.7 (4.5–11.0)	46.6 (12.7–245.8)
Sample tissue					
Bone	61 (14%)	38 (18%)	23 (11%)	35 (23%)	26 (9%)
Liver	15 (4%)	0 (0%)	15 (7%)	7 (5%)	8 (3%)
Lung	26 (6%)	0 (0%)	26 (12%)	21 (14%)	5 (2%)
Lymph node	93 (22%)	65 (31%)	28 (13%)	52 (35%)	41 (15%)
Other soft tissue	25 (6%)	3 (1%)	22 (10%)	21 (14%)	4 (1%)
Prostate	204 (48%)	105 (50%)	99 (46%)	13 (9%)	191 (69%)
On continuous ADT	91 (22%)	49 (23%)	42 (20%)	53 (36%)	38 (14%)

Note: Shown are counts (percentages across columns) or median (interquartile range).

Abbreviations: ADT, androgen deprivation therapy; PSA, prostate-specific antigen.

values at cancer diagnosis, disease volume, tissue sample, and continuous treatment with ADT (Table 1). Differences between patients with low-volume and high-volume disease were less pronounced, except for differences in profiled metastatic tissue (Table 1) due to the definition of high-volume disease. Median follow-up for development of castration resistance was 27.2 months and median follow-up for overall survival was 30.5 months.

Clinical phenotypes and prognosis

Of 213 patients with high-volume disease, 139 developed castration resistance, compared with 101 of the 211 patients with low-volume disease (adjusted HR, 1.84; 95% CI, 1.40–2.41; Fig. 1A). Disease volume was even more strongly associated with overall survival, with 77 deaths among patients with high-volume disease compared with 25 deaths among patients with low-volume disease (adjusted HR, 3.71; 95% CI, 2.28–6.02; Fig. 1B). Over 36 months (3 years) of follow up, patients with high-volume disease lived, on average, 4.8 months shorter (95% CI, 3.3–6.3) than patients with low-volume disease.

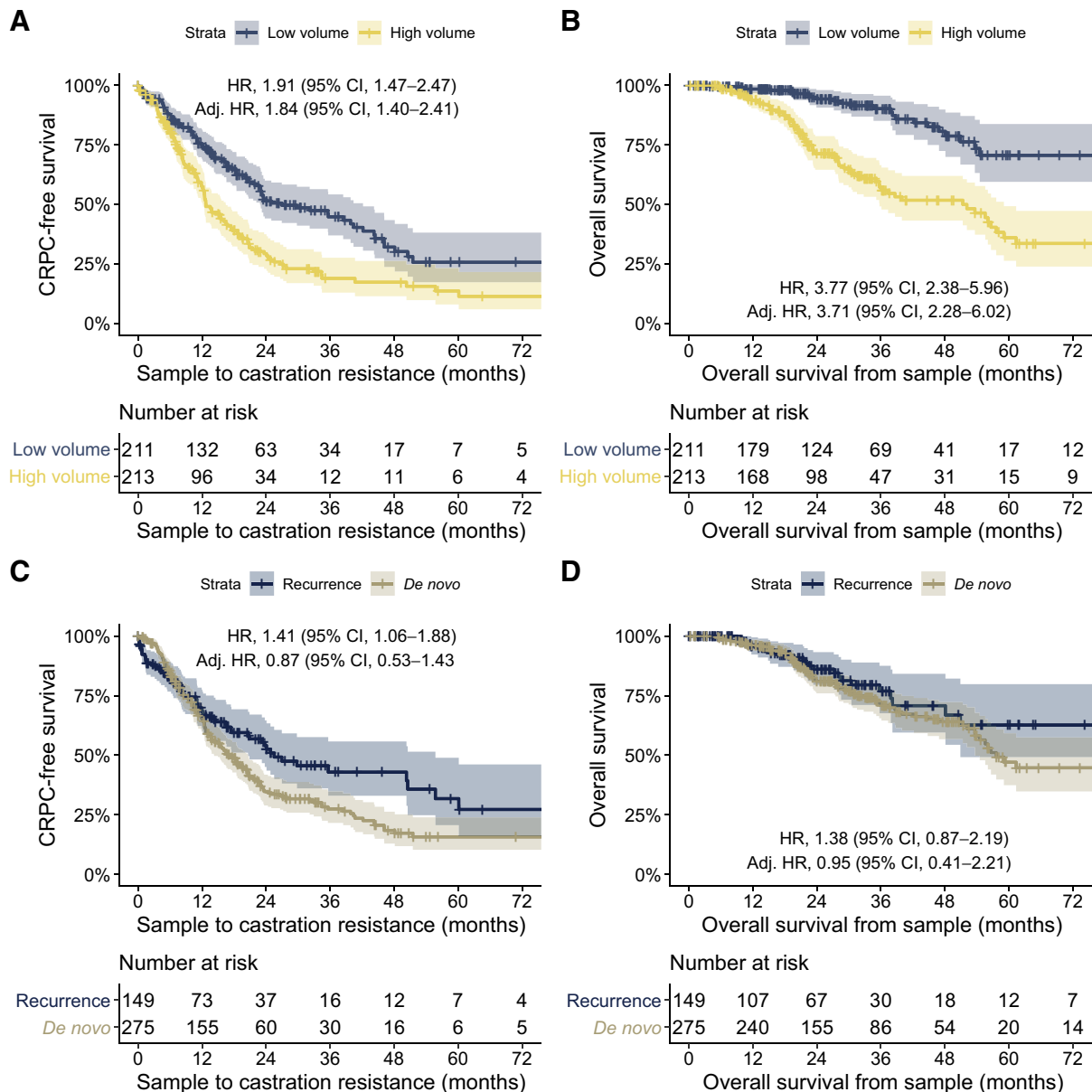
Associations between timing of metastases and prognosis were weaker. Of 275 patients with *de novo* metastatic disease, 175 developed castration resistance, compared with 65 of the 149 patients with metastatic recurrence. Timing of metastases was associated with development of castration resistance when adjusting for age at sampling, disease volume, and time from metastasis to sample (HR, 1.52; 95% CI, 1.11–2.10). However, timing of metastases was not prognostic

once accounting for higher PSA at diagnosis and time since initial diagnosis (HR, 0.87; 95% CI, 0.53–1.43; Fig. 1C). There were 78 deaths among patients with *de novo* metastatic disease compared to 24 deaths among patients with metastatic recurrences (adjusted HR, 0.95; 95% CI, 0.41–2.21; Fig. 1D).

Genomics and clinical phenotypes

The burden of copy number alterations, a consequence of genomic instability and measured as fraction genome altered (median, 32%; interquartile range, 24–48), was higher in high-volume disease than in low-volume disease (adjusted mean difference in percentage points, 4.6; 95% CI, 1.5–7.7; Fig. 2). Fraction genome altered was similar between metastatic-recurrent and *de novo* metastatic disease (adjusted mean difference in percentage points, 1.1; 95% CI, -3.1 to 5.2; Fig. 2). As expected in noncastration-resistant prostate cancer, tumor mutation burden was mostly low (median, 2.6; interquartile range, 1.8–4.4) and similar across clinical phenotypes (Supplementary Table S1). Tumors had a median of three oncogenic alterations (interquartile range, 2–5; Supplementary Fig. S1). A total of 211 tumors (50%) had at least one potentially actionable alteration (Supplementary Fig. S2).

To assess oncogenic alterations in biologically grounded pathways as a potential basis of clinical phenotypes, 144 genes were grouped into 11 pathways (Supplementary Tables S2 and S3). In high-volume disease, for example, alterations in the NOTCH pathway were

**Figure 1.**

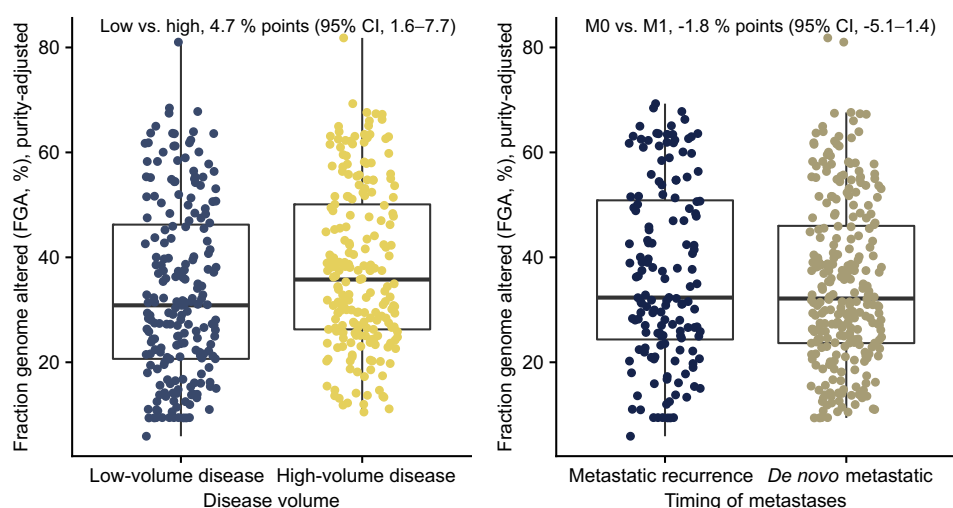
Clinical phenotypes and outcomes. Kaplan-Meier curves by disease volume (low-volume vs. high-volume disease; **A** and **B**) and by timing of metastases (metastatic recurrence vs. *de novo* metastases; **C** and **D**) for time to castration-resistant prostate cancer (CRPC; **A** and **C**) and overall survival (**B** and **D**). Shaded areas denote 95% CIs for survival functions; hazard ratios are from Cox regression models. Adjusted models include timing of metastases (for models of disease volume) or disease volume (for models of timing of metastases) as well as age at sampling, time from diagnosis to sampling, time from metastasis to sampling, and prostate-specific antigen at cancer diagnosis.

4.2 percentage points more common (95% CI, 0.1–8.3; **Fig. 3A**); the next-ranked candidate pathways were the cell cycle and the epigenetic modifiers pathways. Results persisted when adjusting for modest differences in tumor purity or sequencing depth across clinical phenotypes, particularly by timing of metastases (Supplementary Table S1; Supplementary Figs. S3 and S4).

In comparisons by timing of metastatic disease (**Fig. 3B**), *AR* alterations were 8.6 percentage points (95% CI, 3.3–13.8) more frequent in metastatic recurrences than in *de novo* metastatic disease.

However, this association was null once adjusted for continuous ADT treatment at the time of sampling. Alterations in *SPOP* and the cell-cycle and *NOTCH* pathways were less common in *de novo* metastatic disease, but results were imprecise.

There were no single genes differentially altered by volume of disease (Supplementary Fig. S5; Supplementary Table S4). *CDK12* alterations were 6.7 percentage points (95% CI, 3.0–10.4) more common in *de novo* metastatic disease compared with metastatic recurrence (false discovery rate, 0.037; Supplementary Table S5).

**Figure 2.**

Fraction genome altered, the proportion of genes with copy number alterations and a consequence of genomic instability, is compared by clinical phenotypes (with unadjusted mean group difference and 95% CI). Thick lines indicate medians, boxes span interquartile ranges.

Genomic alterations and prognosis

Rates of progression to castration resistance were 1.6-fold to 5-fold higher with alterations in *AR* and *TP53* as well as the cell-cycle and *MYC* pathways and approximately 1.5-fold lower with *SPOP* and *WNT* pathway alterations (Fig. 4A, right). Associations persisted in a multivariable model that also adjusted for alterations in all other pathways (Fig. 4A, right). Over 36 months of follow-up, on average, patients with pathway alterations had 6 to 11 months shorter (*AR*, cell cycle, *MYC*, *TP53*) and 2 to 5 months longer times to castration resistance (*WNT*, *SPOP*; Fig. 4A, left; Supplementary Table S6).

Overall survival rates (Fig. 4B, right) differed 2-fold to 4-fold according to alterations in *AR*, *SPOP* (inverse), and the cell cycle and *WNT* (inverse) pathways. Over 36 months of follow-up, differences in average survival were between 1 to 4 months (Fig. 4B, left).

A potential association between *TP53* alterations and overall survival was attenuated in a multivariable model, as were associations of *NOTCH* pathway alterations with castration resistance; estimates for overall survival were imprecise. Notably, associations of alterations in DNA repair, *PI3K*, *RAS/RAF/MAPK*, and epigenetic modifiers pathways with castration resistance and overall survival were null, particularly in multivariable models (Fig. 4). In a *post hoc* sensitivity analysis, assessing the most commonly altered genes of each pathway (Supplementary Fig. S6), results were generally consistent. For *BRCA2* alterations, results were compatible with higher rates of castration resistance (adjusted HR, 1.60; 95% CI, 0.98–2.63); associations with overall survival could not be ruled out (adjusted HR, 1.08; 95% CI, 0.50–2.33). *PTEN* alterations were associated with a worse prognosis only if assessed in isolation; in multivariable models, there were no strong associations with castration resistance (adjusted HR, 1.27; 95% CI, 0.94–1.73) and overall survival (adjusted HR, 0.95; 95% CI, 0.58–1.56).

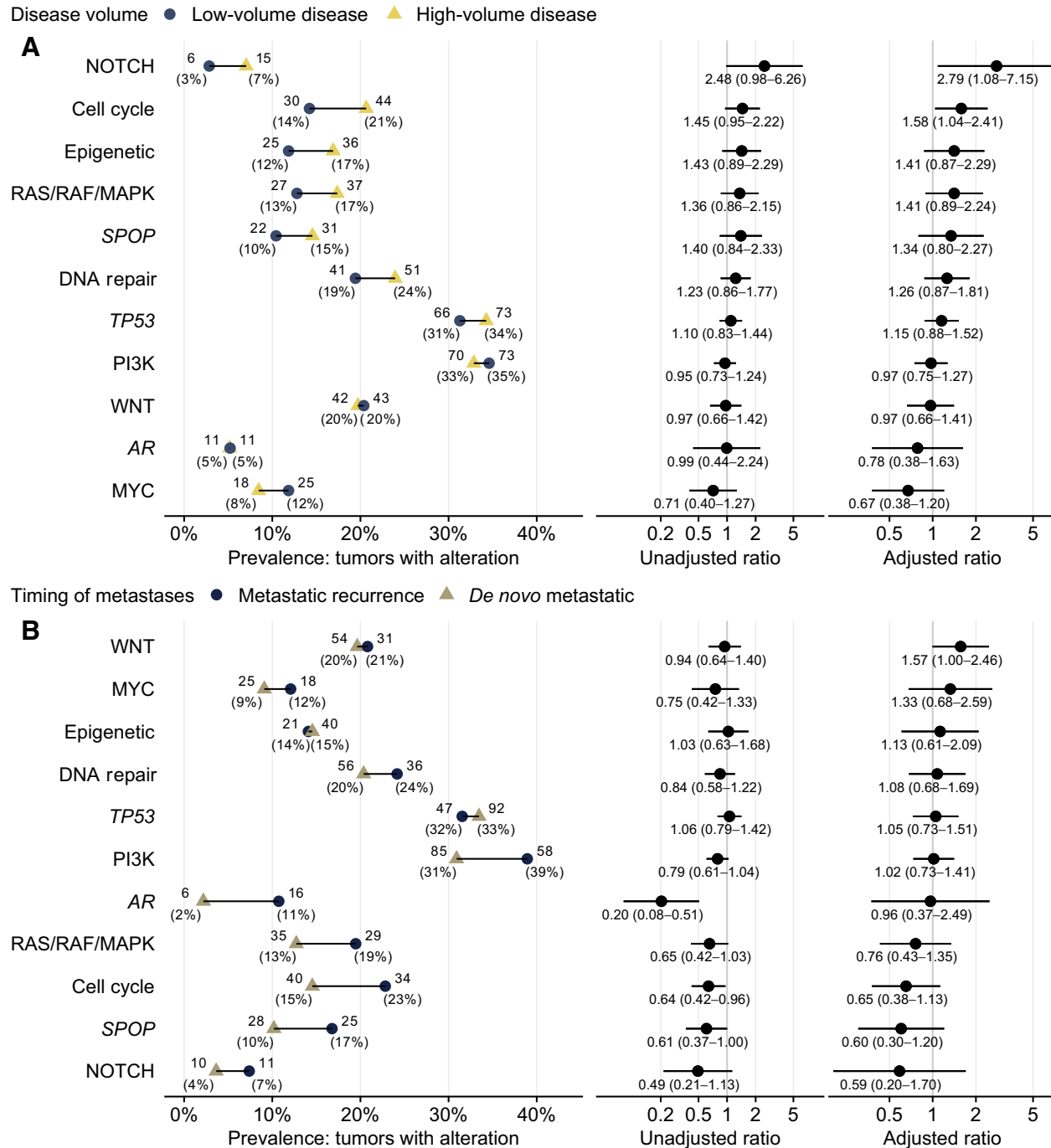
Discussion

In this large clinical-grade sequencing study of paired tumor–blood samples from patients with metastatic castration-sensitive prostate cancer, we identified genomic characteristics of high-volume disease and genomic characteristics of tumors with inferior prognosis. Tumors from high-volume disease had a higher fraction genome altered indicative of genomic instability, more oncogenic alterations in the *NOTCH*, cell cycle, and potentially epigenetic modifiers pathways, and

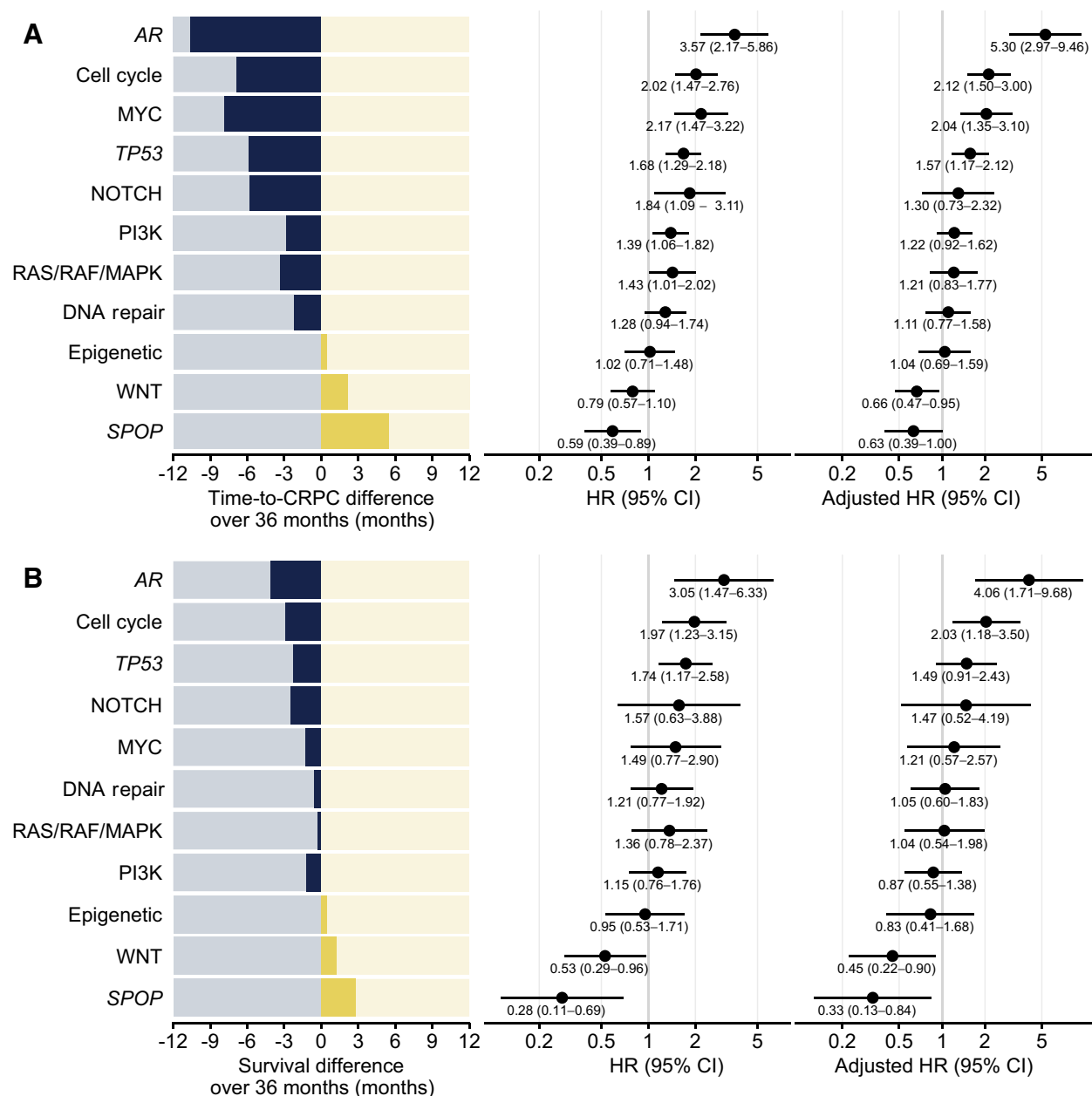
an inferior prognosis compared with low-volume disease. We were unable to detect unequivocal differences between *de novo* metastatic disease and recurrent metastatic disease in both clinical outcomes and genomics, except for more frequent *CDK12* alterations in *de novo* metastatic disease. Importantly, we demonstrate that specific oncogenic signaling pathways are associated with two major clinical endpoints in metastatic castration-sensitive prostate cancer, castration resistance, and overall survival. Alterations in *AR*, *TP53*, and the cell-cycle and *MYC* pathways occurred in tumors with worse prognosis, while tumors with *SPOP* and *WNT* pathway alterations had better prognosis.

Genomic “landscape” studies of tumor DNA profiling in prostate cancer generally excluded metastatic castration-sensitive tumors, focusing on localized disease (25–30) or metastatic castration-resistant disease (13, 31–37). Exceptions are two smaller studies (14, 15) and a study that included archival castration-naïve tumors only from patients who developed castration-resistant disease (38). We connected genomics with clinical phenotypes and clinical outcomes in castration-sensitive disease. Differences between high-volume and low-volume disease in genomic instability, a poor prognostic factor at least in early prostate cancer (39, 40), may explain some of the differences in prognosis by disease volume, yet they are currently difficult to address therapeutically. Our focus on well-defined oncogenic signaling pathways (19) suggested some potentially better targets for the high-volume disease, a subgroup that has long been recognized to have inferior prognosis (22). Oncogenic alterations in single genes of the *NOTCH* pathway, such as *SPEN* (34), were individually uncommon, yet our analysis suggests this pathway as one potential driver of high-volume disease. *NOTCH* alterations have been suggested as a determinant of resistance to docetaxel (41), a therapy that some clinicians recommend just for patients with high-volume disease. Overall, it is critical that differences in pathway alteration prevalence be validated in future tumor sequencing studies. More precise estimates are particularly needed for additional candidates, such as the epigenetic modifiers pathway, *SPOP*, and *CDK12*, which emerged in a gene-level discovery effort. Future studies, which may need to be an order of magnitude larger, should also assess how genomic alterations differ by specific metastatic site, a distinction that a “volume” definition obscures.

De novo metastatic disease was associated with modestly inferior outcomes in unadjusted analyses compared with metastatic recurrences. Surprisingly however, once we accounted for systematic differences

**Figure 3.**

Alterations in genomic pathways by clinical phenotype (**A**, disease volume; **B**, timing of metastases). The left panels show absolute prevalences of pathway alterations, including counts of samples with alterations (above symbols) and prevalence (below symbols). The right panels show prevalence ratios between clinical phenotypes (with 95% CIs). Higher ratios indicate that pathways had more frequent oncogenic alterations in high-volume disease (**A**) or in *de novo* metastatic disease (**B**). Pathways are ordered according to adjusted prevalence ratios. Adjusted models include timing of metastases (for models of disease volume) or disease volume (for models of timing of metastases), type of sample (prostate in the setting of metastatic disease vs. metastasis), age at sampling, time from metastasis to sample, whether the sample was obtained while on continuous androgen deprivation therapy, and sequencing depth.

**Figure 4.**

Genomic pathways and time to castration resistance (**A**) and overall survival (**B**). The left panels show absolute differences in mean time to castration resistance or survival over a 36-month period associated with an oncogenic pathway alteration. The two right panels show hazard ratios (95% CI) from Cox proportional hazards models. Adjusted models include all genomic pathways mutually, disease volume, timing of metastases, age at sampling, PSA at cancer diagnosis, type of sample (prostate vs. metastasis), and fraction genome altered.

between patients who were diagnosed with metastatic prostate cancer *de novo* and those who experienced metastatic recurrence of initially nonmetastatic disease, we were unable to detect prognostic differences. The relatively small number of metastatic recurrences made results imprecise, and systematic differences between patient groups may have obscured true differences in genomics. Nonetheless, our data suggest that the timing of metastasis is likely not a strong prognostic factor in metastatic castration-sensitive disease, and differential treatment just based on this clinical feature may not be needed.

Genomic alterations that characterized tumors with poor prognosis did not appear to be created equal. AR alterations, while uncommon in the metastatic castration-sensitive setting, were the strongest predictor of development of castration resistance and death. Relatively strong associations between cell cycle and MYC pathways, as well as SPOP and TP53 alterations, with prognosis in this disease state are concordant with observations in metastatic castration-resistant prostate cancer (13). However, WNT pathway alterations were associated with better prognosis, including longer overall survival when accounting for

clinical factors and other genomic alterations. This observation is in contrast to two smaller studies in metastatic castration-resistant prostate cancer (37, 42), where WNT pathway alterations, including *CTNNB1*, were associated with worse prognosis.

DNA repair and PI3K pathway alterations had weak to modest associations with development of castration resistance when assessed in isolation. Neither pathway remained associated with clinical outcomes once accounting for other genomic features and clinical factors. It is possible that specific alterations summarized in the DNA repair pathway could be drivers of poor prognosis, such as *BRCA2*. For the PI3K pathway and *PTEN* alterations, our data suggest no strong associations with disease progression and overall survival in this disease state, consistent with results in castration-resistant disease (13).

This study shares its main limitation with all other tumor sequencing studies published on prostate cancer to date (13–15, 25–38) in that it was hospital-based, relying on patients seen at an academic referral center. It is widely appreciated that such study populations are not representative of patients nationally or globally (affecting generalizability). Perhaps more importantly, such a restriction may also introduce selection bias (affecting validity) that is difficult to address analytically, particularly since only a subset of patients undergoes tumor biopsies. An important next step will be to nest studies of the tumor genome in ongoing population-based cohort studies with tumor biorepositories that can address these issues. With evolving sequencing technologies, future studies will also explore the cancer genome more broadly, complementary to our approach that concentrated on deep-coverage sequencing of known cancer susceptibility genes with curated functional annotation.

Taken together, our study takes the first step towards a genomic classification of metastatic castration-sensitive prostate cancer. In describing some of the genomic alterations that occurred more frequently in high-volume disease, we highlight how specific oncogenic signaling pathways may be a potentially targetable biologic underpinning of this poor-prognosis subtype. Our results on prognostically relevant genomic pathways highlight potential drivers of disease progression that may help prioritization in developing targeted therapies and that could be targeted in studies of intensified upfront therapy.

Disclosure of Potential Conflicts of Interest

H.I. Scher is an employee/paid consultant for Ambry Genetics Corp/Konika Minota, Inc., Pfizer, WCG Oncology, reports receiving commercial research grants to the institution from Epic Science, Illumina, Janssen, Menarini Silicon Biosystems, ThermoFisher, and is an advisory board member/unpaid consultant for Amgen,

Bayer, ESSA Pharma, Janssen Research & Development, Janssen Biotech, Menarini Silicon Biosystems, and Sanofi Aventis. M.J. Morris is an advisory board member/unpaid consultant for Bayer, Blue Earth, Advanced Accelerator Applications, Tokai, ORIC, and Johnson and Johnson. D.B. Solit is an employee/paid consultant for Pfizer, Loxo Oncology, Lilly Oncology, QED Therapeutics, Vivideon Therapeutics, and Illumina. P.W. Kantoff reports paid consulting positions with Bavarian Nordic, SEER, DRGT, Progenity, and OncoCellMDX, ownership interest in Context, DRGT, Placon, and SEER, advisory board relationships with Merck, Roche, Context, and Tarveda, and other remuneration from Poling Law Firm. W. Abida is an employee/paid consultant for Clovis Oncology, Janssen Pharmaceutica, MORE Health, ORIC Pharmaceuticals, and reports receiving commercial research grants from AstraZeneca, Zenith Epigenetics, Clovis Oncology, and GlaxoSmithKline. No potential conflicts of interest were disclosed by the other authors.

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Acknowledgments

This work was funded in part by a National Cancer Institute Cancer Center Support Grant (P30CA008748) and Prostate Cancer SPOR (P50CA092629), the Department of Defense (Early Investigator Research Award W81XWH-18-1-0330, to K.H. Stopsack; Physician Research Award W81XWH-17-1-0124, to W. Abida), and the Marie-Josée and Henry R. Kravis Center for Molecular Oncology. K.H. Stopsack, N. Schultz, and W. Abida are Prostate Cancer Foundation Young Investigators.

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Received January 16, 2020; revised March 10, 2020; accepted March 23, 2020; published first March 27, 2020.

References

- VanderWeele DJ, Antonarakis ES, Carducci MA, Dreicer R, Fizazi K, Gillissen S, et al. Metastatic hormone-sensitive prostate cancer: clinical decision making in a rapidly evolving landscape of life-prolonging therapy. *J Clin Oncol* 2019;37:2961–7.
- Kyriakopoulos CE, Chen YH, Carducci MA, Liu G, Jarrard DF, Hahn NM, et al. Chemohormonal therapy in metastatic hormone-sensitive prostate cancer: long-term survival analysis of the randomized phase III E3805 CHAARTED trial. *J Clin Oncol* 2018;36:1080–7.
- Fizazi K, Tran N, Fein L, Matsubara N, Rodriguez-Antolin A, Alekseev BY, et al. Abiraterone plus prednisone in metastatic, castration-sensitive prostate cancer. *N Engl J Med* 2017;377:352–60.
- Gravis G, Boher JM, Joly F, Soulie M, Albiges L, Priou F, et al. Androgen deprivation therapy (ADT) plus docetaxel versus ADT alone in metastatic non castrate prostate cancer: impact of metastatic burden and long-term survival analysis of the randomized phase 3 GETUG-AFU15 trial. *Eur Urol* 2016;70:256–62.
- James ND, de Bono JS, Spears MR, Clarke NW, Mason MD, Dearnaley DP, et al. Abiraterone for prostate cancer not previously treated with hormone therapy. *N Engl J Med* 2017;377:338–51.
- Davis ID, Martin AJ, Stockler MR, Begbie S, Chi KN, Chowdhury S, et al. Enzalutamide with standard first-line therapy in metastatic prostate cancer. *N Engl J Med* 2019;381:121–31.
- Chi KN, Agarwal N, Bjartell A, Chung BH, Pereira de Santana Gomes AJ, Given R, et al. Apalutamide for metastatic, castration-sensitive prostate cancer. *N Engl J Med* 2019;381:13–24.
- James ND, Sydes MR, Clarke NW, Mason MD, Dearnaley DP, Spears MR, et al. Addition of docetaxel, zoledronic acid, or both to first-line long-term hormone therapy in prostate cancer (STAMPEDE): survival results from an adaptive, multi-arm, multistage, platform randomised controlled trial. *Lancet* 2016;387:1163–77.
- Gillissen S, Attard G, Beer TM, Beltran H, Bossi A, Bristow R, et al. Management of patients with advanced prostate cancer: the report of the advanced prostate cancer consensus conference APCCC 2017. *Eur Urol* 2018;73:178–211.

10. Gravis G, Boher JM, Chen YH, Liu G, Fizazi K, Carducci MA, et al. Burden of metastatic castrate naive prostate cancer patients, to identify men more likely to benefit from early docetaxel: further analyses of CHAARTED and GETUG-AFU15 studies. *Eur Urol* 2018;73:847–55.
11. Clarke NW, Ali A, Ingleby FC, Hoyle A, Amos CL, Attard G, et al. Addition of docetaxel to hormonal therapy in low- and high-burden metastatic hormone sensitive prostate cancer: long-term survival results from the STAMPEDE trial. *Ann Oncol* 2019;30:1992–2003.
12. Finianos A, Gupta K, Clark B, Simmens SJ, Aragon-Ching JB. Characterization of differences between prostate cancer patients presenting with *de novo* versus primary progressive metastatic disease. *Clin Genitourin Cancer* 2018;16:85–9.
13. Abida W, Cyrta J, Heller G, Prandi D, Armenia J, Coleman I, et al. Genomic correlates of clinical outcome in advanced prostate cancer. *Proc Natl Acad Sci U S A* 2019;116:11428–36.
14. Hamid AA, Gray KP, Shaw G, MacConaill LE, Evan C, Bernard B, et al. Compound genomic alterations of TP53, PTEN, and RB1 tumor suppressors in localized and metastatic prostate cancer. *Eur Urol* 2019;76:89–97.
15. Abida W, Armenia J, Gopalan A, Brennan R, Walsh M, Barron D, et al. Prospective genomic profiling of prostate cancer across disease states reveals germline and somatic alterations that may affect clinical decision making. *JCO Precis Oncol* 2017;2017.
16. Cheng DT, Mitchell TN, Zehir A, Shah RH, Benayed R, Syed A, et al. Memorial sloan kettering-integrated mutation profiling of actionable cancer targets (MSK-IMPACT): a hybridization capture-based next-generation sequencing clinical assay for solid tumor molecular oncology. *J Mol Diagn* 2015;17:251–64.
17. Shen R, Seshan VE. FACETS: allele-specific copy number and clonal heterogeneity analysis tool for high-throughput DNA sequencing. *Nucleic Acids Res* 2016;44:e131.
18. Chakravarty D, Gao J, Phillips SM, Kundra R, Zhang H, Wang J, et al. OncoKB: a precision oncology knowledge base. *JCO Precis Oncol* 2017;2017.
19. Sanchez-Vega F, Mina M, Armenia J, Chatila WK, Luna A, La KC, et al. Oncogenic signaling pathways in the cancer genome atlas. *Cell* 2018;173:321–37 e10.
20. Knijnenburg TA, Wang L, Zimmermann MT, Chambwe N, Gao GF, Cherniack AD, et al. Genomic and molecular landscape of DNA damage repair deficiency across the cancer genome atlas. *Cell Rep* 2018;23:239–54 e6.
21. Spiegelman D, Hertzmark E. Easy SAS calculations for risk or prevalence ratios and differences. *Am J Epidemiol* 2005;162:199–200.
22. Crawford ED, Eisenberger MA, McLeod DG, Spaulding JT, Benson R, Dorr FA, et al. A controlled trial of leuprolide with and without flutamide in prostatic carcinoma. *N Engl J Med* 1989;321:419–24.
23. Francini E, Gray KP, Xie W, Shaw GK, Valenca L, Bernard B, et al. Time of metastatic disease presentation and volume of disease are prognostic for metastatic hormone sensitive prostate cancer (mHSPC). *Prostate* 2018;78:889–95.
24. Trinquart L, Bill-Axelson A, Rider JR. Restricted mean survival times to improve communication of evidence from cancer randomized trials and observational studies. *Eur Urol* 2019;76:137–9.
25. Cancer Genome Atlas Research Network. The molecular taxonomy of primary prostate cancer. *Cell* 2015;163:1011–25.
26. Barbieri CE, Baca SC, Lawrence MS, Demicheli F, Blattner M, Theurillat JP, et al. Exome sequencing identifies recurrent SPOP, FOXA1 and MED12 mutations in prostate cancer. *Nat Genet* 2012;44:685–9.
27. Beltran H, Wyatt AW, Chedgy EC, Donoghue A, Annala M, Warner EW, et al. Impact of therapy on genomics and transcriptomics in high-risk prostate cancer treated with neoadjuvant docetaxel and androgen deprivation therapy. *Clin Cancer Res* 2017;23:6802–11.
28. Fraser M, Sabelnykova VY, Yamaguchi TN, Heisler LE, Livingstone J, Huang V, et al. Genomic hallmarks of localized, non-indolent prostate cancer. *Nature* 2017;541:359–64.
29. Wedge DC, Gundem G, Mitchell T, Woodcock DJ, Martincorena I, Ghorri M, et al. Sequencing of prostate cancers identifies new cancer genes, routes of progression and drug targets. *Nat Genet* 2018;50:682–92.
30. Gerhauser C, Favero F, Risch T, Simon R, Feuerbach L, Assenov Y, et al. Molecular evolution of early-onset prostate cancer identifies molecular risk markers and clinical trajectories. *Cancer Cell* 2018;34:996–1011 e8.
31. Robinson D, Van Allen EM, Wu YM, Schultz N, Lonigro RJ, Mosquera JM, et al. Integrative clinical genomics of advanced prostate cancer. *Cell* 2015;161:1215–28.
32. Kumar A, Coleman I, Morrissey C, Zhang X, True LD, Gulati R, et al. Substantial interindividual and limited intraindividual genomic diversity among tumors from men with metastatic prostate cancer. *Nat Med* 2016;22:369–78.
33. Grasso CS, Wu YM, Robinson DR, Cao X, Dhanasekaran SM, Khan AP, et al. The mutational landscape of lethal castration-resistant prostate cancer. *Nature* 2012;487:239–43.
34. Armenia J, Wankowicz SAM, Liu D, Gao J, Kundra R, Reznik E, et al. The long tail of oncogenic drivers in prostate cancer. *Nat Genet* 2018;50:645–51.
35. Beltran H, Prandi D, Mosquera JM, Benelli M, Puca L, Cyrta J, et al. Divergent clonal evolution of castration-resistant neuroendocrine prostate cancer. *Nat Med* 2016;22:298–305.
36. Quigley DA, Dang HX, Zhao SG, Lloyd P, Aggarwal R, Alumkal JJ, et al. Genomic hallmarks and structural variation in metastatic prostate cancer. *Cell* 2018;174:758–69 e9.
37. Chen WS, Aggarwal R, Zhang L, Zhao SG, Thomas GV, Beer TM, et al. Genomic drivers of poor prognosis and enzalutamide resistance in metastatic castration-resistant prostate cancer. *Eur Urol* 2019;76:562–71.
38. Mateo J, Seed G, Bertan C, Rescigno P, Dolling D, Figueiredo I, et al. Genomics of lethal prostate cancer at diagnosis and castration resistance. *J Clin Invest* 2020;130:1743–51.
39. Hieronymus H, Murali R, Tin A, Yadav K, Abida W, Moller H, et al. Tumor copy number alteration burden is a pan-cancer prognostic factor associated with recurrence and death. *Elife* 2018;7.
40. Stopsack KH, Whittaker CA, Gerke TA, Loda M, Kantoff PW, Mucci LA, et al. Aneuploidy drives lethal progression in prostate cancer. *Proc Natl Acad Sci U S A* 2019;116:11390–5.
41. Domingo-Domenech J, Vidal SJ, Rodriguez-Bravo V, Castillo-Martin M, Quinn SA, Rodriguez-Barrueco R, et al. Suppression of acquired docetaxel resistance in prostate cancer through depletion of notch- and hedgehog-dependent tumor-initiating cells. *Cancer Cell* 2012;22:373–88.
42. Wang L, Dehm SM, Hillman DW, Sicotte H, Tan W, Gormley M, et al. A prospective genome-wide study of prostate cancer metastases reveals association of wnt pathway activation and increased cell cycle proliferation with primary resistance to abiraterone acetate-prednisone. *Ann Oncol* 2018;29:352–60.



Non-BRCA DNA Damage Repair Gene Alterations and Response to the PARP Inhibitor Rucaparib in Metastatic Castration-Resistant Prostate Cancer: Analysis From the Phase II TRITON2 Study

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ABSTRACT

Purpose: Genomic alterations in DNA damage repair (DDR) genes other than *BRCA* may confer synthetic lethality with PARP inhibition in metastatic castration-resistant prostate cancer (mCRPC). To test this hypothesis, the phase II TRITON2 study of rucaparib included patients with mCRPC and deleterious non-*BRCA* DDR gene alterations.

Patients and Methods: TRITON2 enrolled patients who had progressed on one or two lines of next-generation androgen receptor-directed therapy and one taxane-based chemotherapy for mCRPC. Key endpoints were investigator-assessed radiographic response per modified RECIST/PCWG3 and PSA response ($\geq 50\%$ decrease from baseline).

Results: TRITON2 enrolled 78 patients with a non-*BRCA* DDR gene alteration [*ATM* ($n = 49$), *CDK12* ($n = 15$), *CHEK2* ($n = 12$), and other DDR genes ($n = 14$)]. Among patients evaluable for

each endpoint, radiographic and PSA responses were observed in a limited number of patients with an alteration in *ATM* [2/19 (10.5%) and 2/49 (4.1%), respectively], *CDK12* [0/10 (0%) and 1/15 (6.7%), respectively], or *CHEK2* [1/9 (11.1%) and 2/12 (16.7%), respectively], including no radiographic or PSA responses in 11 patients with confirmed biallelic *ATM* loss or 11 patients with *ATM* germline mutations. Responses were observed in patients with alterations in the DDR genes *PALB2*, *FANCA*, *BRIPI1*, and *RAD51B*.

Conclusions: In this prospective, genomics-driven study of rucaparib in mCRPC, we found limited radiographic/PSA responses to PARP inhibition in men with alterations in *ATM*, *CDK12*, or *CHEK2*. However, patients with alterations in other DDR-associated genes (e.g., *PALB2*) may benefit from PARP inhibition.

See related commentary by Sokolova et al., p. 2439

Introduction

PARP inhibitors are approved for use in *BRCA1* and *BRCA2* (*BRCA*)-mutant breast and ovarian cancers (1, 2), and have also shown evidence of activity in patients with *BRCA*-mutant metastatic

castration-resistant prostate cancer (mCRPC), based on a well-described synthetically lethal effect (3). Confirmed radiographic and PSA responses were observed in 25 (43.9%) of 57 and 51 (52.0%) of 98 patients with a deleterious *BRCA* alteration, respectively, in the phase

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (<http://clincancerres.aacrjournals.org/>).

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Clin Cancer Res 2020;26:2487-96

doi: 10.1158/1078-0432.CCR-20-0394

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Translational Relevance

Deleterious alterations in *BRCA1*, *BRCA2*, and other DNA damage repair genes are thought to sensitize tumor cells to PARP inhibition. In this analysis of patients with metastatic castration-resistant prostate cancer from TRITON2, loss of function alterations in *ATM*, *CDK12*, or *CHEK2* alone did not significantly confer synthetic lethality with the PARP inhibitor rucaparib, as measured by objective radiographic and PSA responses. Responses were observed, however, in a number of patients with alterations in *FANCA*, *PALB2*, *BRIP1*, or *RAD51B*, genes associated with homologous recombination repair; these promising results warrant further studies. This analysis expands current understanding of predictive genomic biomarkers for treatment with a PARP inhibitor in patients with prostate cancer, but also in patients with other malignancies, including breast, pancreatic, and ovarian cancer, where these biomarkers are also being explored.

II TRITON2 study (CO-338-052; NCT02952534) evaluating the PARP inhibitor rucaparib in men who progressed on an androgen receptor (AR)-directed therapy and chemotherapy (4). Olaparib and niraparib also have reported activity in patients with *BRCA*-mutant mCRPC (5–8). Rucaparib, olaparib, and niraparib have received breakthrough therapy designations from the FDA for the treatment of patients with *BRCA*-mutant mCRPC (9–11).

Beyond *BRCA*, deleterious alterations in other genes may also lead to DNA damage repair (DDR) deficiency and are hypothesized to render tumor cells sensitive to PARP inhibition. For example, *ATM* and *CHEK2* are sensors of DNA damage, *CDK12* is a positive regulator of *BRCA* genes, and *PALB2* and *FANCA* interact with *BRCA1* and/or *BRCA2* during DNA repair (12–16). Clinical responses, defined variably as a radiographic response, or a decline in PSA or circulating tumor cell count, have been reported in patients with mCRPC with alterations in *ATM*, *CHEK2*, *FANCA*, *PALB2*, or other non-*BRCA* DDR genes in studies evaluating olaparib [TOPARP-A (NCT01682772), TOPARP-B (NCT01682772)], and niraparib [GALAHAD (NCT02854436)], and patients with alterations in these genes were included in a randomized study comparing olaparib to physician's choice of abiraterone or enzalutamide [PROfound (NCT02987543); refs. 5, 6, 8, 17]. Notably, the co-occurrence of alterations in different non-*BRCA* DDR genes, as seen in the TOPARP and PROfound studies (5, 6, 18), makes it difficult to attribute PARP inhibitor sensitivity to a given gene.

The available data provide a compelling rationale to explore PARP inhibition in men with mCRPC and a DDR gene alteration, and to define the set of DDR gene deficiencies that confer a synthetically lethal effect with PARP inhibitors. The TRITON2 study is investigating the PARP inhibitor rucaparib in patients with mCRPC associated with alterations in 15 DDR genes. Here, we report an *ad hoc* analysis of patients with deleterious alterations in non-*BRCA* DDR genes, including *ATM*, *CDK12*, *CHEK2*, *FANCA*, and *PALB2*.

Patients and Methods

Study design

TRITON2 is an open-label, phase II study evaluating the safety and efficacy of rucaparib in men with mCRPC associated with DDR deficiency. This trial is being conducted at 144 centers in 12 countries. Eligible patients at least 18 years old had histologically or cytologically confirmed adenocarcinoma or poorly differentiated carcinoma of the

prostate that was metastatic and that had progressed on second-generation, AR-directed therapy (e.g., abiraterone acetate, enzalutamide, or apalutamide) for prostate cancer and one prior line of taxane-based chemotherapy for mCRPC. Disease progression on prior therapy was based on any of the following criteria: rise in PSA (minimum of two consecutive rising levels, with an interval of 1 week or more between each determination; the most recent screening measurement must have been greater than or equal to 2 ng/mL); transaxial imaging with new or progressive soft tissue masses on CT or MRI as defined by RECIST version 1.1; or radionuclide bone scan with at least two new metastatic lesions. Patients were surgically or medically castrated, with serum testosterone levels of 50 ng/dL or less.

Patients with and without measurable visceral or nodal disease per RECIST criteria were eligible for the study; patients without measurable disease were required to have PSA levels of greater than 2 ng/mL on the most recent measurement. Patients had an Eastern Cooperative Oncology Group Performance Status of 0 or 1 and adequate organ function. Patients who had prior treatment with any PARP inhibitor, mitoxantrone, cyclophosphamide, or any platinum-based chemotherapy were excluded.

The study was approved by national or local institutional review boards and was carried out in accordance with the Declaration of Helsinki and Good Clinical Practice Guidelines of the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use. Patients provided written informed consent before participation.

Procedures

Patients were screened for the presence of a deleterious somatic or germline alteration in *BRCA1*, *BRCA2*, *ATM*, *BARD1*, *BRIP1*, *CDK12*, *CHEK2*, *FANCA*, *NBN*, *PALB2*, *RAD51*, *RAD51B*, *RAD51C*, *RAD51D*, or *RAD54L* through central genomic testing of plasma or tumor tissue (archival or contemporaneous), or through local testing. Central testing was performed by Foundation Medicine, Inc (19, 20). To classify alterations as somatic or germline, whole-blood samples were tested by Color Genomics using an assay validated to detect germline alterations (21, 22).

Patients received oral rucaparib 600 mg twice daily until confirmed radiographic disease progression as assessed by investigator on the basis of modified RECIST and/or Prostate Cancer Clinical Trials Working Group 3 (PCWG3) criteria, unequivocal clinical disease progression, death, or other reason for discontinuation. Dose reductions were permitted if a patient had a grade 3 or greater or a persistent grade 2 adverse event (AE).

Tumor assessments (CT/MRI and bone scans) were performed every 8 weeks for 24 weeks, then every 12 weeks, and at treatment discontinuation. PSA was evaluated at baseline and every 4 weeks thereafter.

After discontinuation, all patients had a 28-day follow-up visit, and subsequent treatment and survival information was collected every 12 weeks from the last dose of rucaparib until death, loss to follow-up, or withdrawal of consent from study. For patients who discontinued for a reason other than disease progression, tumor assessments continued as above.

Outcomes

The proportion of patients achieving a confirmed complete or partial response per modified RECIST/PCWG3 criteria as assessed by the investigator is reported for patients with measurable disease at baseline (hereafter, "evaluable patients"). The proportion of patients with a confirmed PSA response (50% or greater decrease from baseline

confirmed by a second consecutive measurement at least 3 weeks later) is reported for patients with or without measurable disease at baseline. Additional outcomes reported here include clinical benefit rate [proportion of patients without radiographic progression per RECIST/PCWG3 criteria who were receiving treatment at a given time point (e.g., at 6 or 12 months)] and time to PSA progression.

The tumor responses were assessed by investigators per modified RECIST/PCWG3 criteria. Modified RECIST allows up to 10 target lesions, with a maximum of five per site, not including prostatic bed or bone lesions; MRI is allowed. For PSA response ($\geq 50\%$ decrease from baseline confirmed by a second consecutive measurement at least 3 weeks later), measurements were performed by local laboratories. Early rises in PSA (before 12 weeks following the first dose of study drug) were not considered in determining PSA progression.

The clinical benefit rate was calculated as the number of patients without radiographic progression who were on study through the given time interval divided by the number of patients who had the given amount of follow-up time. Time to PSA progression was defined as the time from the first dose of rucaparib to the date that a 25% or greater increase and absolute increase of at least 2 ng/mL above the nadir (or baseline if there was no PSA decline after baseline) in PSA was measured, plus 1 day.

Safety was assessed by monitoring AEs and vital signs, laboratory testing, and physical examination.

Statistical analysis

For this *ad hoc* analysis, data are reported for patients with a non-BRCA DDR gene alteration enrolled in TRITON2 as of January 4, 2019, using a visit cutoff date of April 29, 2019; therefore, patients in this analysis had a minimum of 16 weeks of follow-up, allowing for at least two radiographic tumor assessments and for confirmation of response. The safety population included all patients who received at least one dose of study treatment.

Time to PSA progression was summarized using Kaplan–Meier methodology. Statistical analyses were performed using SAS version 9.4 (SAS Institute).

Results

Patients and genomic characteristics

Between February 15, 2017, and January 4, 2019, 78 patients were enrolled on the basis of a non-BRCA DDR gene alteration identified during screening. Patients were allowed to submit multiple tissue and plasma samples for genomic testing. The majority of patients had

results from one genomic test [44 (56.4%) patients], with the remainder having results from two [31 (39.7%) patients] or three [3 (3.8%) patients; Supplementary Table]. Patients were grouped by gene alteration (*ATM*, *CDK12*, *CHEK2*, and other DDR gene), and patients with co-occurring alterations were included in multiple gene groups. For example, a patient with co-occurring *CDK12* and *CHEK2* alterations was included in both the *CDK12* and *CHEK2* groups.

Including cases of co-occurring alterations identified prior to enrollment or in samples tested after enrollment, 49 patients had an *ATM* alteration, 15 patients had a *CDK12* alteration, and 12 patients had a *CHEK2* alteration (Fig. 1; Supplementary Table). Among 14 patients with other DDR gene alterations, 4 had a *FANCA* alteration, 4 had an *NBN* alteration, 2 had a *BRIP1* alteration, 2 had a *PALB2* alteration, and 1 each had a *RAD51*, *RAD51B*, or *RAD54L* alteration (Fig. 1). Among 49 patients with an *ATM* alteration, 11 (22.4%) had a germline alteration; the most frequent type of *ATM* alteration was a frameshift mutation (36.1% of alterations; Supplementary Table and Supplementary Figs. S1 and S2). Zygosity could be determined for 17 (34.7%) patients with an *ATM* alteration, 11 of whom had a biallelic alteration and 6 had a monoallelic alteration. Among the 15 patients with *CDK12* alterations, 7 (47%) had two distinct *CDK12* alterations presumed to result in biallelic inactivation, and an additional 2 (13%) had LOH of the other allele. We could not confirm that these alterations were somatic due to the absence of *CDK12* from our germline panel, but *CDK12* alterations have not been reported to occur in the germline (16). Of the 12 patients with *CHEK2* alterations, 4 had germline alterations and 8 somatic, and biallelic inactivation was identified in three of four evaluable cases, two of which were germline (Fig. 2; Supplementary Table).

Patients with an *ATM* alteration generally had lower Gleason scores at diagnosis and less measurable disease at study entry than those in the other groups (Table 1). At the visit cutoff date, patients still receiving treatment included 13 (26.5%) with an *ATM* alteration, 6 (50.0%) with a *CHEK2* alteration, and 7 (50.0%) with another DDR gene alteration.

Efficacy outcomes

ATM cohort

Among 19 evaluable patients with an *ATM* alteration, 2 (10.5%) had confirmed partial radiographic responses, including 1 with a co-occurring *CHEK2* alteration (Table 2; Fig. 2A). Both patients had ongoing responses (1.8+ and 2.0+ months from the date of radiographic response) at the time of the visit cutoff (Fig. 3A). A confirmed PSA response was observed in 2 (4.1%) of 49 overall patients with an

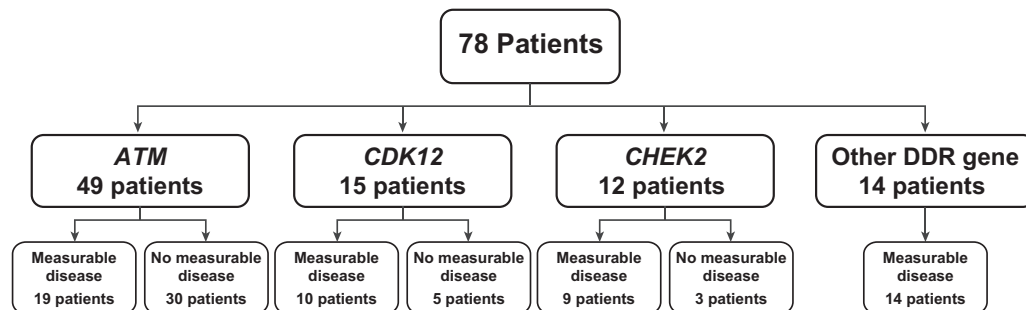


Figure 1.

Overview of analysis groups for patients from TRITON2 with non-BRCA DDR gene alterations. Groups are not mutually exclusive and contain patients with co-occurring DDR gene alterations (see Supplementary Table). The “other DDR gene” group includes patients with an alteration in *FANCA* ($n = 4$), *NBN* ($n = 4$), *BRIP1* ($n = 2$), *PALB2* ($n = 2$), *RAD51* ($n = 1$), *RAD51B* ($n = 1$), and/or *RAD54L* ($n = 1$). Abbreviation: DDR, DNA damage repair.

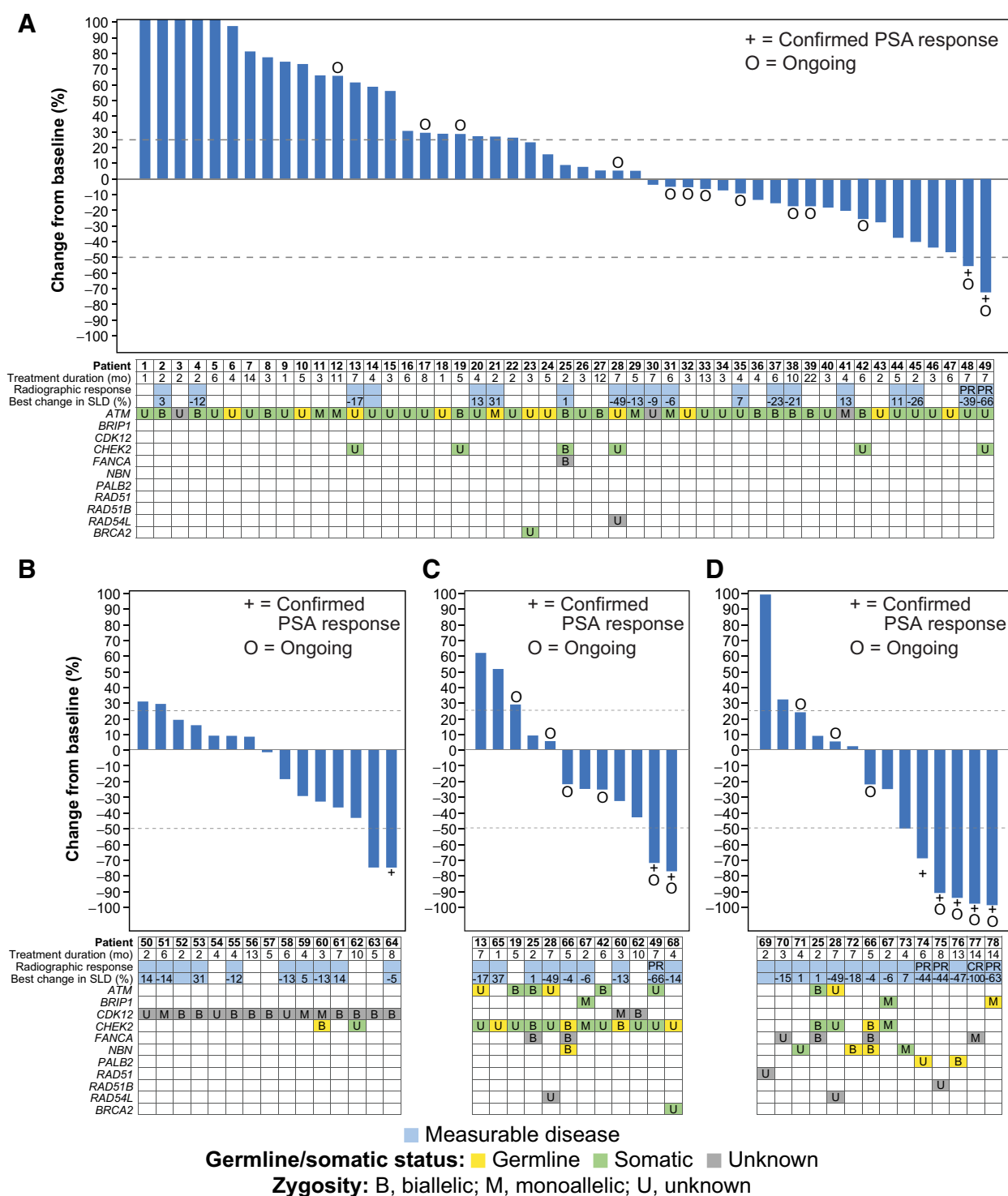


Figure 2.

Best change from baseline in PSA in patients with an *ATM* alteration (A), *CDK12* alteration (B), *CHEK2* alteration (C), or other DDR gene alteration (D). PSA increases for patients 1–5 were 319%, 142%, 126%, 109%, and 106%; bars were capped at 100% for visual clarity. Patients 55, 56, 57, 61, 62, 63, and 64 had two distinct *CDK12* alterations identified through tissue and/or plasma testing and were considered to have biallelic loss. Abbreviations: CR, complete response; mo, months; PR, partial response; SLD, sum of the longest diameter.

Table 1. Baseline patient demographics and disease characteristics.

	DDR gene group			
	ATM (n = 49)	CDK12 (n = 15)	CHEK2 (n = 12)	Other ^a (n = 14)
Age, median (IQR), years	73.0 (68.0–77.0)	64.0 (56.0–72.0)	71.5 (64.5–75.0)	66.5 (61.0–72.0)
Race, n (%)				
White	35 (71.4)	6 (40.0)	6 (50.0)	11 (78.6)
Black or African American	3 (6.1)	2 (13.3)	1 (8.3)	0
Unknown	11 (22.4)	7 (46.7)	5 (41.7)	3 (21.4)
ECOG PS, n (%)				
0	21 (42.9)	7 (46.7)	5 (41.7)	2 (14.3)
1	27 (55.1)	8 (53.3)	7 (58.3)	11 (78.6)
≥2	1 (2.0)	0	0	1 (7.1)
Baseline PSA, median (IQR), ng/mL	108.7 (19.7–286.8)	72.7 (37.2–615.0)	119.9 (33.2–256.5)	98.8 (33.2–255.8)
Baseline alkaline phosphatase, median (IQR), U/L	102.0 (72.0–198.0)	107.0 (78.0–172.0)	88.5 (66.5–164.5)	84.5 (72.0–126.0)
Baseline albumin, median (IQR), g/L	38.6 (36.0–41.0)	39.0 (35.0–44.0)	37.0 (34.5–40.0)	38.5 (35.0–42.0)
Baseline lactate dehydrogenase, median (IQR), ukat/L	3.9 (3.2–6.3)	4.4 (2.8–6.7)	4.2 (3.6–7.3)	4.4 (3.3–7.2)
Gleason score ≥ 8 at diagnosis, n (%)	20 (40.8)	14 (93.3)	6 (50.0)	8 (57.1)
No. prior CRPC therapies, median (IQR) ^b	2 (2–3)	3 (2–4)	3 (2–4)	2 (2–3)
Prior therapies, n (%) ^c				
Abiraterone	34 (69.4)	11 (73.3)	11 (91.7)	12 (85.7)
Enzalutamide	36 (73.5)	14 (93.3)	9 (75.0)	7 (50.0)
Abiraterone and enzalutamide	23 (46.9)	10 (66.7)	8 (66.7)	5 (35.7)
Docetaxel	46 (93.9)	13 (86.7)	12 (100)	13 (92.9)
Cabazitaxel	4 (8.2)	2 (13.3)	0	2 (14.3)
Sipuleucel-T	6 (12.2)	1 (6.7)	1 (8.3)	0
Radium-223	11 (22.4)	2 (13.3)	3 (25.0)	1 (7.1)
Measurable disease status and type (per investigator), n (%)				
Measurable disease	19 (38.8)	10 (66.7)	9 (75.0)	14 (100)
Only measurable nodal disease	9 (47.4)	6 (60.0)	8 (88.9)	9 (64.3)
Measurable visceral ± nodal disease	10 (52.6)	4 (40.0)	1 (11.1)	5 (35.7)
Nonmeasurable disease	30 (61.2)	5 (33.3)	3 (25.0)	0
Bone-only disease	25 (83.3)	2 (40.0)	1 (33.3)	0
Other nonmeasurable disease	5 (16.7)	3 (60.0)	2 (66.7)	0

Note: Visit cutoff date: April 29, 2019.

Abbreviations: CRPC, castration-resistant prostate cancer; DDR, DNA damage repair; ECOG PS, Eastern Cooperative Oncology Group Performance Status; IQR, interquartile range.

^aIncludes patients with an alteration in *FANCA* (n = 4), *NBN* (n = 4), *BRIPI* (n = 2), *PALB2* (n = 2), *RAD51* (n = 1), *RAD51B* (n = 1), and/or *RAD54L* (n = 1).

^bDoes not include luteinizing hormone-releasing hormone analogues, first-generation antiandrogens, hormones, corticosteroids, bone-targeted agents, hematopoietic growth factors, or docetaxel given for hormone-sensitive disease.

^cCategories are not mutually exclusive.

ATM alteration (**Table 2**; Supplementary Fig. S3A), occurring in the same 2 patients who achieved a radiographic response. The 6- and 12-month clinical benefit rate for patients with an *ATM* alteration with or without measurable disease was 28.6% (12/42 patients) and 16.7% (3/18 patients; **Table 2**).

The 2 patients who had radiographic and PSA responses had somatic *ATM* alterations detected through cell-free DNA (cfDNA) screening, so the possibility of homozygous loss of another DDR gene (e.g., *BRCA2* or *PALB2*) cannot be ruled out. Archival tissue was not available for these patients to assess *ATM* alteration zygosity. However, none of the 11 patients with known biallelic alterations in *ATM* or 11 patients with germline alterations in *ATM* had a radiographic or PSA response.

CDK12 cohort

No confirmed radiographic responses were observed among the 10 evaluable patients with a *CDK12* alteration (**Table 2**). Among 15 overall patients with a *CDK12* alteration, 1 (6.7%) had a confirmed PSA response lasting 1.8 months (**Table 2**; **Fig. 3B**; Supplementary Fig. S3B). The 6- and 12-month clinical benefit rate in the *CDK12* cohort was 20.0% (3/15 patients) and 7.1% (1/14 patients; **Table 2**).

The patient with a PSA response had two distinct *CDK12* alterations that are presumed to be biallelic and were identified in both cfDNA and tissue (Supplementary Table; Supplementary Fig. S2B).

CHEK2 cohort

Among 9 evaluable patients with a *CHEK2* alteration, 1 (11.1%) with a co-occurring *ATM* alteration had a confirmed partial radiographic response, as described previously (**Table 2**). A confirmed PSA response was observed in 2 (16.7%) of 12 overall patients with a *CHEK2* alteration, 1 of whom was the patient with a radiographic response. Both were still on study as of the visit cutoff date and had ongoing PSA responses (3.7+ and 0.9+ months; **Fig. 3C**; Supplementary Fig. S3C). The 6-month clinical benefit rate for patients with a *CHEK2* alteration was 37.5% (3/8 patients); no patients with a *CHEK2* alteration were still receiving treatment at 12 months (**Table 2**).

CHEK2 alterations for both patients with PSA responses were identified through cfDNA screening; one was somatic and the other was germline. The PSA responder with a germline *CHEK2* alteration (patient 68) was found to have a somatic *BRCA2* alteration through tissue analysis conducted after study enrollment (Supplementary Table).

Table 2. Response by DDR gene alteration.

	By DDR gene group			
	<i>ATM</i> (n = 49)	<i>CDK12</i> (n = 15)	<i>CHEK2</i> (n = 12)	Other ^a (n = 14)
Confirmed investigator-assessed objective response ^b	2/19 (10.5) (1.3–33.1)	0/10 (0) (0.0–30.8)	1/9 (11.1) (0.3–48.2)	4/14 (28.6) (8.4–58.1)
Complete response	0/19 (0.0)	0/10 (0)	0/9 (0)	1/14 (7.1)
Partial response	2/19 (10.5)	0/10 (0)	1/9 (11.1)	3/14 (21.4)
Stable disease	9/19 (47.4)	6/10 (60.0)	6/9 (66.7)	8/14 (57.1)
Progressive disease	7/19 (36.8)	3/10 (30.0)	2/9 (22.2)	1/14 (7.1)
Not evaluable	1/19 (5.3)	1/10 (10.0)	0/9 (0)	1/14 (7.1)
6-month clinical benefit rate ^c	12/42 (28.6) (15.7–44.6)	3/15 (20.0) (4.3–48.1)	3/8 (37.5) (8.5–75.5)	6/11 (54.5) (23.4–83.3)
12-month clinical benefit rate ^d	3/18 (16.7) (3.6–41.4)	1/14 (7.1) (0.2–33.9)	0/5 (0) (0.0–52.2)	3/8 (37.5) (8.5–75.5)
Confirmed PSA response ^e	2/49 (4.1) (0.5–14.0)	1/15 (6.7) (0.2–31.9)	2/12 (16.7) (2.1–48.4)	5/14 (35.7) (12.8–64.9)
Median time to PSA progression, mo (95% CI)	3.1 (2.8–4.6)	3.2 (2.8–4.6)	7.4 (2.8–7.4)	11.1 (3.0–NR)

Note: Visit cutoff date: April 29, 2019. Data are n/N (%) (95% CI) unless stated otherwise.

Abbreviations: CI, confidence interval; DDR, DNA damage repair; NR, not reached; PCWG3, Prostate Cancer Clinical Trials Working Group 3; RECIST, Response Evaluation Criteria In Solid Tumors version 1.1.

^aIncludes patients with an alteration in *FANCA* (n = 4), *NBN* (n = 4), *BRIP1* (n = 2), *PALB2* (n = 2), *RAD51* (n = 1), *RAD51B* (n = 1), and/or *RAD54L* (n = 1).

^bPer modified RECIST/PCWG3 criteria; includes patients who had measurable disease at baseline per the investigator and ≥16 weeks of follow-up.

^cProportion of patients without radiographic progression per RECIST/PCWG3 criteria who were ongoing with treatment at 6 months.

^dProportion of patients without radiographic progression per RECIST/PCWG3 criteria who were ongoing with treatment at 12 months.

^eDefined as ≥50% reduction in PSA from baseline; includes patients who had ≥16 weeks of follow-up.

Other DDR gene cohort

Of the 14 evaluable patients with other DDR gene alterations, 4 (28.6%) had a confirmed radiographic response and 5 (35.7%) had a PSA response (Table 2; Fig. 3D). Both patients with a *PALB2* alteration had PSA responses, and 1 patient also had a partial radiographic response that was ongoing for 3.9+ months as of the visit cutoff; the other patient had a 47% reduction in tumor volume but had not undergone a follow-up scan for confirmation of the response as of the visit cutoff. Of 4 patients with a *FANCA* alteration, 1 patient with a monoallelic truncating alteration had complete radiographic and PSA responses that were ongoing at the time of the visit cutoff. One of 2 patients with a *BRIP1* alteration had a partial radiographic and a PSA response, both ongoing. The patient with a *RAD51B* alteration had a partial radiographic and a PSA response, both ongoing.

Safety

The safety population included 78 patients with a non-*BRCA* DDR gene alteration who received at least one dose of rucaparib. At the visit cutoff date, the median (range) treatment duration was 4.3 (0.7–22.1) months.

A treatment-emergent AE (TEAE) of any grade occurred in 76 (97.4%) patients, the most frequent (reported in at least 20% of patients) were asthenia or fatigue, nausea, decreased appetite, anemia, constipation, vomiting, and diarrhea (Table 3). Among all 39 (50.0%) patients with grade 3 or higher TEAEs, anemia, asthenia or fatigue, and thrombocytopenia were the most frequent (Table 3). No TEAEs of myelodysplastic syndrome or acute myeloid leukemia were reported.

Treatment interruption due to a TEAE occurred in 34 (43.6%) patients, most commonly due to asthenia or fatigue [10 (12.8%) patients] and anemia [8 (10.3%)]. Dose reduction due to a TEAE occurred in 21 (26.9%) patients, most commonly due to asthenia or fatigue [6 (7.7%) patients], anemia [3 (3.8%)], and thrombocytopenia [3 (3.8%)].

Four (5.1%) patients discontinued because of a TEAE (excluding disease progression), 1 each due to asthenia or fatigue, decreased appetite, hematuria, and postoperative respiratory failure. One (1.5%) death due to intestinal ischemia was reported and was considered unrelated to rucaparib by the investigator.

Discussion

PARP inhibitors have shown dramatic responses in heavily pre-treated mCRPC patients with *BRCA* gene alterations; however, the utility of alterations in non-*BRCA* DDR genes as biomarkers for PARP inhibitor sensitivity is not well established. This report describes 78 patients with mCRPC and prospectively identified non-*BRCA* DDR gene alterations treated with the PARP inhibitor rucaparib. The safety profile of rucaparib in these patients was consistent with that in patients with ovarian cancer and other solid tumors (23, 24). A limited number of radiographic and PSA responses was observed in patients with *ATM*, *CDK12*, or *CHEK2* gene alterations, whereas responses were observed in patients with alterations in other DDR genes less frequently altered in mCRPC such as *PALB2*, *BRIP1*, *FANCA*, and *RAD51B*. These results suggest there are non-*BRCA* biomarkers associated with PARP inhibitor activity in mCRPC that warrant further investigation.

Radiographic and PSA responses in the *ATM* cohort of this study were observed in 10.5% and 4.1% of patients evaluable for each endpoint, respectively. The *ATM* cohort included patients with frame-shift mutations, truncating mutations, homozygous deletions, and rearrangements (Supplementary Figs. S1 and S2A) expected to result in complete loss of function of that allele, whereas few patients (11.5%) had missense alterations predicted to be oncogenic. The *ATM* cohort also included patients with confirmed germline *ATM* alterations and patients with biallelic loss of *ATM* (Fig. 2A). Despite the various types and locations of *ATM* alterations observed, there was no clear

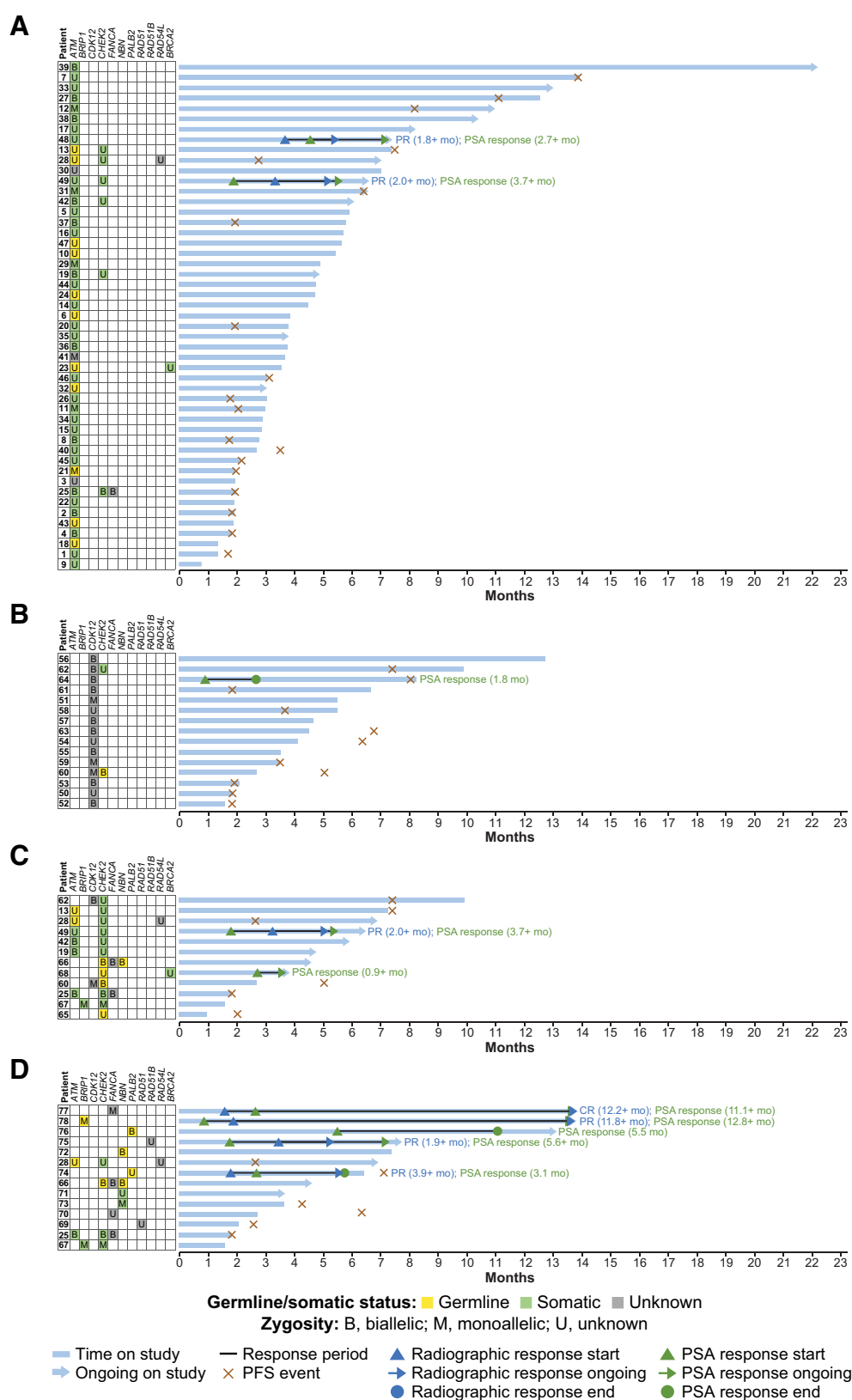


Figure 3.

Treatment duration in patients with an *ATM* alteration (**A**), *CDK12* alteration (**B**), *CHEK2* alteration (**C**), or other DDR gene alteration (**D**). Patients 55, 56, 57, 61, 62, 63, and 64 had two distinct *CDK12* alterations identified through tissue and/or plasma testing and were considered to have biallelic loss. Abbreviations: CR, complete response; DDR, DNA damage repair; mCRPC, metastatic castration-resistant prostate cancer; mo, months; PR, partial response.

Table 3. Most common (10% or more of patients) TEAEs of any grade in the safety population.

	Overall (n = 78)	
	Any grade	Grade ≥ 3
Asthenia or fatigue	41 (52.6)	7 (9.0)
Nausea	35 (44.9)	1 (1.3)
Decreased appetite	27 (34.6)	1 (1.3)
Anemia ^a	24 (30.8)	12 (15.4)
Constipation	18 (23.1)	0
Vomiting	18 (23.1)	1 (1.3)
Diarrhea	17 (21.8)	1 (1.3)
ALT or AST increased	14 (17.9)	2 (2.6)
Back pain	14 (17.9)	2 (2.6)
Edema, peripheral	14 (17.9)	0
Dizziness	13 (16.7)	0
Weight decreased	13 (16.7)	0
Blood creatinine increased	12 (15.4)	0
Dysgeusia	10 (12.8)	0
Thrombocytopenia ^b	10 (12.8)	4 (5.1)
Arthralgia	9 (11.5)	1 (1.3)
Cough	8 (10.3)	0
Hematuria	8 (10.3)	1 (1.3)
Pain in extremity	8 (10.3)	0

Note: Visit cutoff date: April 29, 2019. Data are n (%).

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; TEAE, treatment-emergent adverse event.

^aIncludes preferred terms of anemia and decreased hemoglobin.

^bIncludes preferred terms of thrombocytopenia and decreased platelets.

association between response and the characteristics of the genomic alterations. These results suggest that ATM deficiency alone is not sufficient to result in synthetic lethality with PARP inhibition in mCRPC. In the TOPARP-A study, which did not enroll patients based on biomarker selection, although PSA and/or circulating tumor cell responses were observed in 4 patients with an ATM alteration, none of these patients had a radiographic response. In TOPARP-B, RECIST and PSA responses were observed in 8.3% and 5.3% of patients with an ATM alteration (6). Similarly, among 23 men with mCRPC who received olaparib, no PSA responses were observed in 6 patients with an ATM alteration, compared with 13 (76.4%) of 17 patients with a BRCA alteration (25). In PROfound, median radiographic progression-free survival (PFS) was similar in patients with an ATM alteration who received olaparib and those who received enzalutamide or abiraterone (8). In our study, although only a limited number of patients with an ATM alteration had a radiographic or PSA response, most patients had a best response of stable disease. These data, as well as the 6- and 12-month clinical benefit rates, suggest that rucaparib may provide, at best, disease stabilization in patients with an ATM alteration, although it should be noted that TRITON2 was not designed to assess this outcome. Detailed results from randomized studies such as PROfound or the phase III TRITON3 study of rucaparib (NCT02975934) are needed to more fully evaluate the effect of PARP inhibition in this patient subgroup while controlling for a potential prognostic effect of ATM alterations. The apparent lower sensitivity to PARP inhibitors of tumors harboring an ATM alteration may be due to ATM's role as a sensor of DNA damage rather than a mediator of homologous recombination repair (15).

Most patients with CDK12 alterations enrolled in TRITON2 had a best response of stable disease and only 1 patient had a PSA

response; as a result, enrollment of patients with a CDK12 alteration into TRITON2 was halted per protocol. CDK12 plays a role in the elongation phase of transcription, and studies suggest that inactivation of CDK12 can reduce DDR gene expression and sensitize tumor cells to PARP inhibition (26–28). However, a recent study reported that tumors with biallelic inactivation of CDK12 have distinct genomic and transcriptional profiles compared with DDR-deficient tumors (16). In TOPARP-B, no radiographic or PSA responses were observed in the 20 patients with a CDK12 alteration (6). Similarly, in a recent retrospective study, none of 11 men with CDK12-altered advanced prostate cancer had a radiographic or PSA response to PARP inhibitor treatment (29). In PROfound, median radiographic PFS in patients with a CDK12 alteration was longer with olaparib than with enzalutamide or abiraterone, although the statistical significance of this finding is unclear (8). Together, the genomics and clinical data suggest that CDK12 deficiency does not reliably predict response to PARP inhibitors.

We identified one confirmed radiographic response in the CHEK2 cohort, and 2 patients had a PSA response. However, CHEK2 alterations alone do not appear sufficient to render tumor cells responsive to PARP inhibitor treatment as co-occurring alterations in other DDR genes were often observed in TRITON2 patients with a CHEK2 alteration. In particular, both patients with CHEK2 alterations that responded were enrolled from plasma testing, and subsequent tissue testing in 1 patient revealed the presence of a somatic BRCA2 alteration.

Among patients with alterations in other DDR genes, patients with alterations in PALB2, FANCA, BRIP1, and RAD51B had both confirmed radiographic and PSA responses. The duration of response was up to a year in some of these patients, highlighting the durability of the responses. These data are consistent with reports of PARP inhibitor activity in patients with mCRPC (5, 6, 30, 31). For example, radiographic and/or PSA responses were observed in 57% of patients (4/7) with PALB2 alterations in TOPARP-B (6). In addition, reversion mutations in PALB2 have been detected in a patient with mCRPC who progressed on olaparib treatment, providing strong evidence that the original mutation sensitized the tumor to PARP inhibitor treatment (31). Although it is challenging to draw conclusions because of the small sample size, these data strongly support continued examination of patients with alterations in this subset of DDR genes to determine whether the alterations are robustly associated with PARP inhibitor sensitivity.

In this article, we describe a large population of patients with mCRPC who were prospectively enrolled on the basis of a deleterious gene alteration in a non-BRCA DDR gene, enabling analysis of the impact of PARP inhibitor treatment on these relatively infrequent gene alterations. In addition, many patients had genomic test results from two or three assays, helping to overcome some of the limitations inherent to both tissue and plasma profiling and allowing for a more comprehensive, real-world evaluation of genomic alterations. Furthermore, we present results grouped by presence of at least one alteration in a given DDR gene to enable better assessment of the predictive utility of a specific DDR gene alteration.

One limitation of this study is the small sample size for the CDK12, CHEK2, and other non-BRCA DDR gene cohorts. In addition, tissue and plasma test results were not available for all patients. Thus, a complete evaluation of co-occurring alterations cannot be performed, and tumor zygosity could be determined only for a subset of patients with central tissue test results. Loss of heterozygosity may occur more frequently with certain gene alterations than others, calling into question whether a lack of response is related to alteration zygosity

or whether loss of the gene is not truly synthetically lethal with PARP inhibition. Although biallelic DDR gene alterations were identified among these cohorts without apparent association with response, overall the number of biallelic alterations observed for each gene was small. Further research to add to these findings with greater numbers is warranted.

Although PARP inhibitor treatment has demonstrated radiographic and PSA responses in patients with *BRCA* alterations (4–8), the data presented here offer compelling evidence that response to PARP inhibitors is limited in men with mCRPC harboring an *ATM*, *CDK12*, or *CHEK2* alteration. These data provide an optimistic outlook for treatment of prostate cancer with other DDR gene alterations, such as *PALB2*. Further work is needed to determine whether PARP inhibitors provide disease stabilization in patients with an *ATM* or *CDK12* alteration or whether there are other genomic characteristics for patients who achieve a response, and to better define the subset of other DDR gene alterations that confer sensitivity to PARP inhibitors in the setting of mCRPC.

Disclosure of Potential Conflicts of Interest

W. Abida is an employee/paid consultant for Clovis Oncology, Janssen, ORIC Pharmaceuticals, and MORE Health. A. Patnaik is an employee/paid consultant for and reports receiving commercial research grants from Clovis. B. Sautois is an employee/paid consultant for Clovis Oncology, Astellas, Janssen, and Sanofi, and reports receiving other commercial research support from Janssen. A.H. Bryce reports receiving speakers bureau honoraria from Astellas and OncoLive. R. McDermott is an employee/paid consultant for Bayer, Pfizer, and Janssen, and reports receiving other commercial research support from Bristol-Myers Squibb, Clovis, Amgen, Merck, and Bayer. J. Rowe reports receiving speakers bureau honoraria from Dendreon. J. Zhang is an employee/paid consultant for Seattle Genetics, Clovis Oncology, Bayer, Janssen, and Dendreon, and reports receiving speakers bureau honoraria from Sanofi and Merck. J.M. Piulats is an employee/paid consultant for Roche, reports receiving commercial research grants from Janssen, Bristol-Myers Squibb, Merck Sharp & Dhome (MSD), Pfizer, and AstraZeneca, and speakers bureau honoraria from Bristol-Myers Squibb, Janssen, Astellas, VCN Biotech, BeiGene, Merck Sharp & Dhome (MSD), and AstraZeneca. K. Fizazi is an advisory board member/unpaid consultant for Clovis, Orion, AstraZeneca, Bayer, CureVac, Janssen, Sanofi, Astellas, Amgen, and ESSA. A.S. Merseburger reports receiving other commercial research support from Clovis Oncology, speakers bureau honoraria from Janssen, Astellas, Bayer, GlaxoSmithKline, Ipsen, Novartis, Pfizer, TEVA, and Wyeth, and is an advisory board member/unpaid consultant for Astellas, Bayer, GlaxoSmithKline, Hexal, and Sanofi Aventis. C.S. Higano is an employee/paid consultant for Pfizer, Janssen, Bayer, Blue Earth Diagnostics, Clovis, Ferring, Carrick Therapeutics, Merck, Hironaka, Myriad, Tolmar, and Astellas. L.E. Krieger is an employee/paid consultant for and is an advisory board member/unpaid consultant for Clovis. F.Y. Feng is an employee/paid consultant for Clovis, Janssen, Sanofi, Astellas, Bayer, and Genentech, and is an advisory board member/unpaid consultant for PFS Genomics. A.D. Simmons, M. Dowson, and S.P. Watkins are employees/paid consultants for and hold ownership interest (including patents) in Clovis. A. Loehr, D. Despain,

F. Green, and T. Golsorkhi are employees/paid consultants for Clovis. S. Chowdhury reports receiving speakers bureau honoraria from Clovis Oncology, Astellas, Janssen, Beigene, and Pfizer, holds ownership interest (including patents) in CurveLife, and is an advisory board member/unpaid consultant for Bayer, Beigene, Janssen, and Novartis. No potential conflicts of interest were disclosed by the other authors.

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Acknowledgments

TRITON2 was designed by the funder and the first/last authors (W. Abida and S. Chowdhury). This article was written by the authors, with medical writing and copy editing support paid for by the funder. Data were collected by the investigators, analyzed by the funder, and interpreted by all authors. All authors had full access to all trial data and had final responsibility for the decision to submit for publication. Medical writing and editorial support funded by Clovis Oncology were provided by Nathan Yardley and Frederique H. Evans of Ashfield Healthcare Communications. W. Abida is funded by National Cancer Institute (NCI) Cancer Center Support Grant P30 CA 008748, NCI Prostate Specialized Program of Research Excellence (SPORE) grant P50 CA092629-16; Department of Defense Prostate Cancer Research Program grant W81XWH-17-1-0124, and a Prostate Cancer Foundation Young Investigator Award.

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Received January 30, 2020; revised February 12, 2020; accepted February 18, 2020; published first February 21, 2020.

References

- Cook SA, Tinker AV. PARP inhibitors and the evolving landscape of ovarian cancer management: a review. *BioDrugs* 2019;33:255–73.
- Keung MYT, Wu Y, Vadgama JV. PARP inhibitors as a therapeutic agent for homologous recombination deficiency in breast cancers. *J Clin Med* 2019;8: E435.
- Lord CJ, Ashworth A. PARP inhibitors: synthetic lethality in the clinic. *Science* 2017;355:1152.
- Abida W, Campbell D, Patnaik A, Sautois B, Shapiro J, Vogelzang NJ, et al. Preliminary results from the TRITON2 study of rucaparib in patients (pts) with DNA damage repair (DDR)-deficient metastatic castration-resistant prostate cancer (mCRPC): updated analyses. *Ann Oncol* 2019;30 (suppl 5):abst 846PD.
- Mateo J, Carreira S, Sandhu S, Miranda S, Mossop H, Perez-Lopez R, et al. DNA-repair defects and olaparib in metastatic prostate cancer. *N Engl J Med* 2015;373: 1697–708.
- Mateo J, Porta N, Bianchini D, McGovern U, Elliott T, Jones R, et al. Olaparib in patients with metastatic castration-resistant prostate cancer with DNA repair gene aberrations (TOPAR-B): a multicentre, open-label, randomised, phase 2 trial. *Lancet Oncol* 2020;21:162–74.
- Smith MR, Sandhu SK, Kelly WK, Scher HI, Efsthathiou E, Lara P, et al. Phase II study of niraparib in patients with metastatic castration-resistant prostate cancer (mCRPC) and biallelic DNA-repair gene defects (DRD): preliminary results of GALAHAD. *J Clin Oncol* 2019;37 (suppl 7): abst 202.
- Hussain M, Mateo J, Fizazi K, Saad F, Shore ND, Sandhu S, et al. PROfound: phase III study of olaparib versus enzalutamide or abiraterone for metastatic castration-resistant prostate cancer (mCRPC) with homologous recombination repair (HRR) gene alterations. *Ann Oncol* 2019;30 (suppl 5):abst LBA12.

9. Clovis Oncology, Inc. Clovis Oncology receives breakthrough therapy designation for Rubraca® (rucaparib) for treatment of BRCA1/2-mutated metastatic castration resistant prostate cancer (mCRPC). Available from: <https://ir.clovisoncology.com/investors-and-news/news-releases/press-release-details/2018/Clovis-Oncology-Receives-Breakthrough-Therapy-Designation-for-Rubraca-rucaparib-for-Treatment-of-BRCA12-Mutated-Metastatic-Castration-Resistant-Prostate-Cancer-mCRPC/default.aspx>; 2018.
10. AstraZeneca PLC. Lynparza™ (olaparib) granted breakthrough therapy designation by US FDA for treatment of BRCA1/2 or ATM gene mutated metastatic castration resistant prostate cancer. Available from: <https://www.astrazeneca.com/media-centre/press-releases/2016/Lynparza-Olaparib-granted-Breakthrough-Therapy-Designation-by-US-FDA-for-treatment-of-BRCA1-2-or-ATM-gene-mutated-metastatic-Castration-Resistant-Prostate-Cancer-28012016.html#>; 2016.
11. Janssen Pharmaceuticals, Inc. Janssen announces U.S. FDA breakthrough therapy designation granted for niraparib for the treatment of metastatic castration-resistant prostate cancer. Available from: https://www.janssen.com/us/sites/www_janssen_com_usa/files/janssen_announces_us_fda_breakthrough_therapy_designation_granted_for_niraparib.pdf; 2019.
12. Zhang F, Fan Q, Ren K, Andreassen PR. PALB2 functionally connects the breast cancer susceptibility proteins BRCA1 and BRCA2. *Mol Cancer Res* 2009;7:1110–8.
13. Folias A, Matkovic M, Bruun D, Reid S, Hejna J, Grompe M, et al. BRCA1 interacts directly with the Fanconi anemia protein FANCA. *Hum Mol Genet* 2002;11:2591–7.
14. Zannini L, Delia D, Buscemi G. CHK2 kinase in the DNA damage response and beyond. *J Mol Cell Biol* 2014;6:442–57.
15. Weigelt B, Bi R, Kumar R, Blecua P, Mandelker DL, Geyer FC, et al. The landscape of somatic genetic alterations in breast cancers from ATM germline mutation carriers. *J Natl Cancer Inst* 2018;110:1030–4.
16. Wu YM, Cieslik M, Lonigro RJ, Vats P, Reimers MA, Cao X, et al. Inactivation of CDK12 delineates a distinct immunogenic class of advanced prostate cancer. *Cell* 2018;173:1770–82.
17. Smith MR, Sandhu SK, Kelly WK, Scher HI, Efsthathiou E, Lara PN, et al. Pre-specified interim analysis of GALAHAD: a phase II study of niraparib in patients (pts) with metastatic castration-resistant prostate cancer (mCRPC) and biallelic DNA-repair gene defects (DRD). *Ann Oncol* 2019; 30 (suppl 5):abst LBA50.
18. de Bono JS, Fizazi K, Saad F, Shore N, Sandhu SK, Mehra N, et al. Central, prospective detection of homologous recombination repair gene mutations (HRRm) in tumour tissue from >4000 men with metastatic castration-resistant prostate cancer (mCRPC) screened for the PROfound study. *Ann Oncol* 2019;30 (suppl 5):abst 847PD.
19. Clark TA, Chung JH, Kennedy M, Hughes JD, Chennagiri N, Lieber DS, et al. Analytical validation of a hybrid capture-based next-generation sequencing clinical assay for genomic profiling of cell-free circulating tumor DNA. *J Mol Diagn* 2018;20:686–702.
20. Frampton GM, Fichtenholtz A, Otto GA, Wang K, Downing SR, He J, et al. Development and validation of a clinical cancer genomic profiling test based on massively parallel DNA sequencing. *Nat Biotechnol* 2013;31:1023–31.
21. Crawford B, Adams SB, Sittler T, van den Akker J, Chan S, Leitner O, et al. Multi-gene panel testing for hereditary cancer predisposition in unsolved high-risk breast and ovarian cancer patients. *Breast Cancer Res Treat* 2017; 163:383–90.
22. Neben CL, Zimmer AD, Stedden W, van den Akker J, O'Connor R, Chan RC, et al. Multi-gene panel testing of 23,179 individuals for hereditary cancer risk identifies pathogenic variant carriers missed by current genetic testing guidelines. *J Mol Diagn* 2019;21:646–57.
23. Clovis Oncology, Inc. Rubraca (rucaparib) tablets [prescribing information]. Boulder, CO: Clovis Oncology, Inc.; 2018.
24. Clovis Oncology Ireland Ltd. Rubraca (rucaparib) tablets [summary of product characteristics]. Swords, Ireland: Clovis Oncology Ireland Ltd.; 2019.
25. Marshall CH, Sokolova AO, McNatty AL, Cheng HH, Eisenberger MA, Bryce AH, et al. Differential response to olaparib treatment among men with metastatic castration-resistant prostate cancer harboring BRCA1 or BRCA2 versus ATM mutations. *Eur Urol* 2019;76:452–8.
26. Joshi PM, Sutor SL, Huntoon CJ, Karnitz LM. Ovarian cancer-associated mutations disable catalytic activity of CDK12, a kinase that promotes homologous recombination repair and resistance to cisplatin and poly(ADP-ribose) polymerase inhibitors. 2014;289:9247–53.
27. Ekumi KM, Paculova H, Lenasi T, Pospichalova V, Böskén CA, Rybarikova J, et al. Ovarian carcinoma CDK12 mutations misregulate expression of DNA repair genes via deficient formation and function of the Cdk12/CycK complex. *Nucleic Acids Res* 2015;43:2575–89.
28. Bajrami I, Frankum JR, Konde A, Miller RE, Rehman FL, Brough R, et al. Genome-wide profiling of genetic synthetic lethality identifies CDK12 as a novel determinant of PARP1/2 inhibitor sensitivity. *Cancer Res* 2014;74: 287–97.
29. Antonarakis ES, Velho PI, Agarwal N, Santos VS, Maughan BL, Pili R, et al. CDK12-altered prostate cancer: clinical features and therapeutic outcomes to standard systemic therapies, PARP inhibitors, and PD1 inhibitors. *Ann Oncol* 2019;30 (suppl 5):abst 845PD.
30. Horak P, Weischenfeldt J, von Amsberg G, Beyer B, Schütte A, Uhrig S, et al. Response to olaparib in a PALB2 germline mutated prostate cancer and genetic events associated with resistance. *Cold Spring Harbor Mol Case Stud* 2019;5:pii a003657.
31. Goodall J, Mateo J, Yuan W, Mossop H, Porta N, Miranda S, et al. Circulating cell-free DNA to guide prostate cancer treatment with PARP inhibition. *Cancer Discov* 2017;7:1006–17.

Platinum-Based Chemotherapy in Metastatic Prostate Cancer With DNA Repair Gene Alterations

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PURPOSE Alterations in DNA damage repair (DDR) genes occur in up to 25% of patients with metastatic castration-resistant prostate cancer (mCRPC) and may sensitize to platinum chemotherapy. We aimed to evaluate the efficacy of platinum-based chemotherapy in DDR-mutant (DDRmut) mCRPC.

METHODS We assessed response to platinum chemotherapy based on DDR gene alteration status in men with mCRPC who underwent tumor and germline genomic profiling. Patients with deleterious alterations in a gene panel that included *BRCA2*, *BRCA1*, *ATM*, *PALB2*, *FANCA*, and *CDK12* were considered DDRmut.

RESULTS A total of 109 patients with mCRPC received platinum-based chemotherapy between October 2013 and July 2018. Sixty-four of 109 patients were taxane refractory and poly (ADP-ribose) polymerase inhibitor (PARPi) naïve. Within this subset, DDRmut was found in 16/64 patients (25%) and was associated with an increased likelihood of achieving a prostate-specific antigen (PSA) decline of 50% or more from baseline (PSA50; odds ratio, 7.0; 95% CI, 1.9 to 29.2). Time on platinum chemotherapy tended to be longer in the DDRmut group (median, 3.0 v 1.6 months; hazard ratio, 0.55, 95% CI, 0.29 to 1.24). No difference in survival was detected. Of 8 patients with DDRmut disease who received platinum-based therapy after a PARPi, 3/7 evaluable patients had radiographic partial response or stable disease, and 2/7 had a PSA50 response. None of 4 patients with *ATM* mutations had platinum responses regardless of prior PARPi exposure.

CONCLUSION Patients with DDRmut disease had better response to platinum-based chemotherapy, suggesting that DDR status warrants prospective validation as a potential biomarker for patient selection. Responses to platinum chemotherapy were observed in *BRCA*-altered prostate cancer after PARPi progression. Additional studies are needed to determine the predictive role of individual genes on platinum sensitivity in the context of other clinical and genomic factors.

JCO Precis Oncol 4:355-366. © 2020 by American Society of Clinical Oncology

INTRODUCTION

Platinum-based chemotherapy has been shown to confer palliative benefit, objective responses, and longer progression-free survival in phase II studies of metastatic castration-resistant prostate cancer (mCRPC), although improved overall survival (OS) has not been demonstrated.¹⁻⁴ Specific patient subpopulations may derive more meaningful benefit, including patients with aggressive variants of prostate cancer⁵ or patients with genomic defects in DNA damage repair (DDR) pathways.^{6,7}

Up to 25% of men with mCRPC harbor tumor somatic or germline alterations in DDR.⁸⁻¹¹ Deleterious genomic alterations in these genes, including *BRCA2*, *BRCA1*, *ATM*, *PALB2*, and *FANCA*, are associated with deficiency in DNA damage sensing or repair and may sensitize tumors to platinum chemotherapy^{6,7,12} or to poly (ADP-ribose) polymerase (PARP) inhibitors

(PARPi).^{13,14} Several studies are ongoing to confirm the role of *BRCA* alterations in predicting response to PARPi and to explore the role of less frequently altered genes in this context (ClinicalTrials.gov identifiers: [NCT02952534](#), [NCT02975934](#), [NCT02987543](#), and [NCT02854436](#)).

We leveraged a prospective, institution-wide, tumor somatic and germline molecular profiling initiative to examine the association between somatic and germline mutations in DDR and response to platinum chemotherapy. We also asked whether patients with DDR gene alterations could respond to platinum therapy after progression on a PARPi.

METHODS

Study Design and Patients

We searched the Memorial Sloan Kettering Cancer Center (MSKCC) clinical database to identify patients with prostate cancer who underwent tumor and

ASSOCIATED CONTENT

Appendix

Author affiliations and support information (if applicable) appear at the end of this article.

Accepted on February 28, 2020 and published at ascopubs.org/journal/po on April 16, 2020; DOI <https://doi.org/10.1200/P0.19.00346>

CONTEXT

Key Objective

Do genomic alterations in DNA damage repair (DDR) genes sensitize to platinum chemotherapy in metastatic castration-resistant prostate cancer (mCRPC)?

Knowledge Generated

In a single-center dataset of patients with mCRPC who received platinum therapy and underwent somatic and germline DNA profiling, we found that DDR gene alterations were associated with better response to platinum-based chemotherapy after taxane therapy. Responses were observed among patients with *BRCA* mutations after poly (ADP-ribose) polymerase (PARP) inhibitor progression, although no responses were observed among 4 patients with *ATM* mutations, regardless of previous exposure to PARP inhibitors.

Relevance

Genomic DDR gene deficiency is associated with response to platinum chemotherapy in mCRPC and, in conjunction with clinical factors, may be useful for patient selection.

germline genomic sequencing and received platinum-based chemotherapy between October 1, 2013, and July 30, 2018, as part of the prospective Memorial Sloan Kettering Integrated Molecular Profiling of Actionable Cancer Targets (MSK-IMPACT) initiative (ClinicalTrials.gov identifier: [NCT01775072](#)). Eligible patients had histologically confirmed mCRPC and received at least 1 cycle of carboplatin or cisplatin as monotherapy or in combination with a taxane or etoposide. We excluded patients with pure nonadenocarcinoma histology (eg, pure small-cell carcinoma); patients who received platinum chemotherapy for non-mCRPC, DDR mutant (DDRmut) disease with microsatellite instability (MSI)-high prostate cancer; and patients whose profiled tumor was acquired more than 90 days after receiving platinum chemotherapy (Fig 1). Chart review was performed to extract clinical and pathologic data. MSKCC Institutional Review Board approval was obtained prior to any study-related procedures.

Tumor Sequencing and DDR Gene Status

Tumor sequencing was performed using the MSK-IMPACT clinical sequencing assay, a hybridization capture-based, next-generation sequencing platform.^{15,16} Sixty-six percent of patients also consented to matched germline analysis.¹⁷ Patients were defined as DDRmut if they harbored a deleterious somatic alteration¹⁸ or pathogenic germline alteration¹⁷ in a gene associated with DNA repair pathway as previously described.¹⁰ *CHEK2*,¹⁹ *NBN*,²⁰ *RAD50*,²¹ *RAD51*,²² and *RAD51C*²³ were added to this panel based on a literature search showing these genes are also implicated in homologous recombination/DNA damage recognition and repair. Therefore, our DDR panel consisted of a total of 13 genes: *ATM*, *ATR*, *BRCA1*, *BRCA2*, *CDK12*, *CHEK2*, *FANCA*, *MRE11*, *NBN*, *PALB2*, *RAD50*, *RAD51*, and *RAD51C*. Tumors with deleterious alterations in at least 1 of these genes, predicted to result in loss of function of at least 1 allele per OncoKB annotation,¹⁷ were considered DDRmut. Tumors harboring no alterations or only variants

of unknown significance (per OncoKB)¹⁸ in these genes were categorized as DDR wild type (DDRwt). Patients with known MSI-high prostate cancer were excluded from analysis if they had a mutation in a DDR gene because of the high likelihood that these mutations represented passenger alterations in patients whose tumors exhibited a high tumor mutation burden.²⁴ Zygosity for patients with DDRmut was determined using FACETS.²⁵ Biallelic loss was defined as loss of wild-type alleles through mutation, deletion, or chromosomal rearrangement, or a combination of these events, including a deleterious alteration with loss of heterozygosity. Two distinct deleterious alterations in a single tumor were assumed to result in biallelic loss. Monoallelic loss indicates a deleterious alteration with retention of the wild-type allele.

Outcomes and Statistics

The association between DDR mutations and a 50% prostate-specific antigen (PSA) decline from baseline while receiving platinum therapy (PSA50) response was evaluated using a logistic regression model. Patients were considered evaluable for PSA50 response if they had a baseline PSA (ie, PSA within 3 weeks prior to chemotherapy start) of at least 2.0 ng/mL and at least 1 PSA value beginning 30 days after the start of platinum-based chemotherapy. The relationship between DDR mutations and time on treatment (ToT), defined as the time from start of platinum-based chemotherapy to the last day of treatment or OS from start of platinum-based chemotherapy was evaluated using the Cox proportional hazards model. All outcomes were adjusted for pretreatment PSA, Gleason score, and the presence of visceral metastasis. Radiologic responses were determined by RECIST 1.1, as assessed by an experienced radiologist (A.W.).

RESULTS

Cohort Characteristics

A total of 140 patients with prostate cancer were identified as having received platinum-based chemotherapy, either

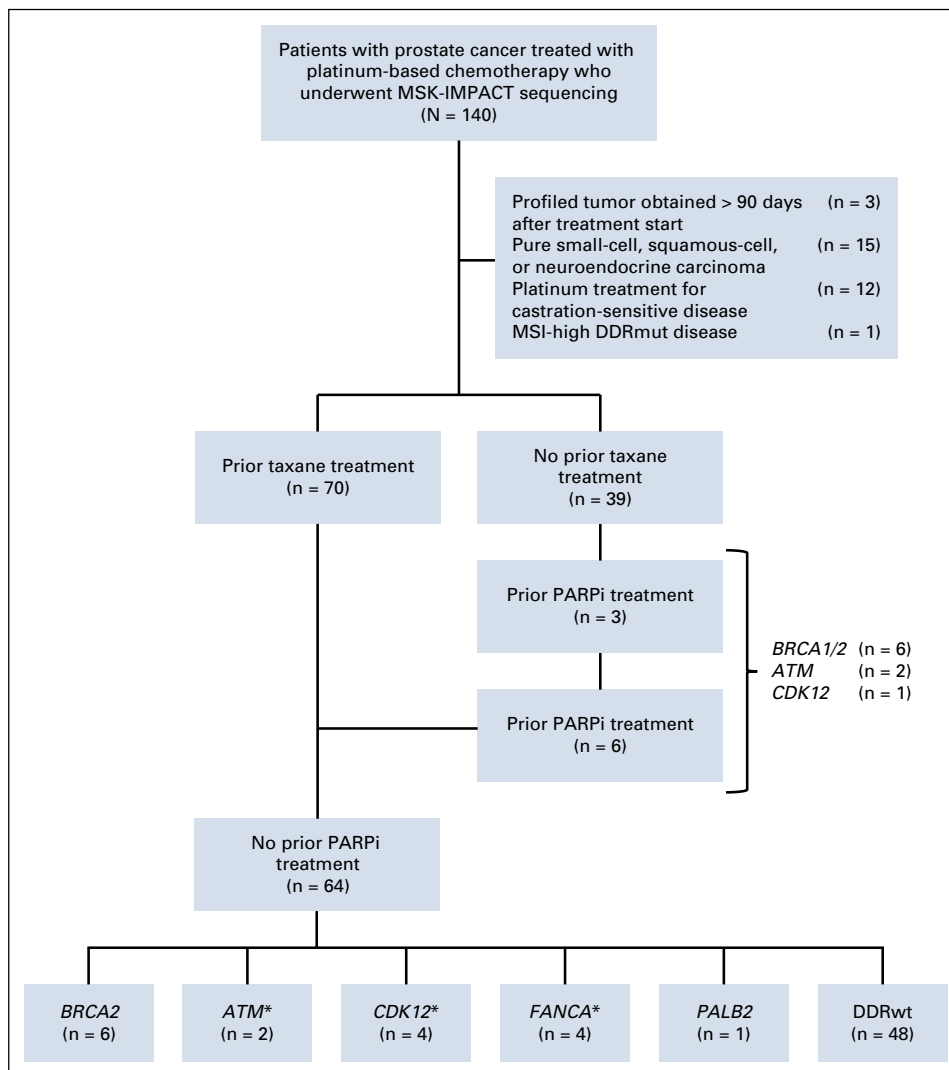


FIG 1. Description of patients with prostate cancer treated with platinum. A total of 140 patients with prostate cancer who underwent tumor genomic profiling received platinum chemotherapy. Thirty-nine patients were excluded from downstream analysis for the reasons listed. A total of 64 patients with metastatic castration-resistant prostate cancer received platinum-based chemotherapy after a taxane and were poly (ADP-ribose) polymerase inhibitor (PARPi) naïve. Six patients with *BRCA* and 2 with *ATM* alterations received platinum chemotherapy after progression on a PARPi. (*) One patient had concurrent *ATM* and *RAD51* alterations, and another had concurrent *FANCA* and *CDK12* alterations. DDRmut, DNA damage repair mutant; DDRwt, DNA damage repair wild type; MSI, microsatellite instability; MSK-IMPACT, Memorial Sloan-Kettering Integrated Molecular Profiling of Actionable Cancer Targets.

as monotherapy or in combination, and having undergone genomic profiling. Of these, 31/140 (22%) were excluded from analysis because of pure nonadenocarcinoma histology (eg, small cell), platinum therapy given for non-castration-resistant disease, tumor profiling performed on a sample acquired after platinum therapy, or MSI-high status (Fig 1). Of the remaining 109 patients, we initially focused on 64 patients who were PARPi naïve and taxane refractory prior to starting platinum-based chemotherapy, where response was less likely to be attributed to the platinum-combination agent if it was a taxane (Fig 1).

Of these 64 patients, 16 (25%) were DDRmut and 48 (75%) were DDRwt, in line with frequencies identified in larger datasets.^{8,9} The most frequently altered DDR gene was *BRCA2* in 6 patients (9% of total and 37% of the DDRmut population), with deleterious alterations also observed in *ATM*, *FANCA*, *CDK12*, *PALB2*, and *RAD51* (Fig 1). One patient had concurrent germline *ATM* and somatic *RAD51* alterations, and another patient had concurrent *CDK12* and *FANCA* alterations. Patient clinical characteristics are summarized in Table 1. Median age and the proportion of patients with neuroendocrine features on

histopathologic reviews were similar in the DDRmut and DDRwt groups. Ninety-four percent of patients in both groups had prior treatment with a next-generation androgen receptor targeted agent (enzalutamide or abiraterone acetate). Visceral metastases at baseline were more common in the DDRwt (22/64; 46%) than the DDRmut group (4/16; 25%). The DDRmut and DDRwt groups were balanced for concomitant treatment with a taxane (11/16 [69%] v 30/64 [63%], respectively). Details of the specific mutations identified in the DDRmut group are shown in Table 2.

Response to Platinum-Based Chemotherapy for DDRmut Versus DDRwt mCRPC

We assessed the proportion of PSA50 in the 64 patients who received platinum-based chemotherapy after receiving a taxane. Of these, 56 patients (DDRmut, n = 16; DDRwt, n = 40) were evaluable for a PSA50 response. In total, 13/56 evaluable patients (23%) achieved a PSA50 response (Fig 2A; Table 3). A PSA50 response was more likely in DDRmut (8/16; 50%) compared with DDRwt (5/40;

13%) patients (unadjusted odds ratio [OR], 7.0; 95% CI, 1.9 to 29.2; $P = .005$; adjusted OR, 8.0; 95% CI, 1.9 to 39.9; $P = .006$). Notably, 4/6 patients with *BRCA2* mutations (67%) achieved a PSA50 response (unadjusted OR, 9.1; 95% CI, 1.5 to 73.6; $P = .019$; adjusted OR, 9.5; 95% CI, 1.5 to 82.9; $P = .022$; compared with DDRwt), consistent with the reported sensitivity of BRCA-deficient tumors of several lineages to platinum-based chemotherapy.^{6,7,26,27} Other DDR gene alterations in the PSA50 responder group included *PALB2*, *FANCA*, and *CDK12*. We found no clear association of other genomic characteristics, including alterations in *TP53* and *RB1*, with PSA50 response (Fig 2A). Of the 8 patients with DDRwt disease who were not evaluable for PSA50 response, 4 had a baseline PSA < 2.0 ng/mL, and 4 had no PSA measurement after the start of platinum-based chemotherapy. All 8 patients received a limited duration of platinum chemotherapy (≤ 2.1 months), with the exception of 1 patient who received treatment for 8.6 months at the time of the data freeze, with ongoing clinical benefit. This patient had a baseline PSA < 2.0 ng/mL and no evidence of

TABLE 1. Baseline Characteristics of Patients With Taxane-Exposed, PARP Inhibitor–Naïve Metastatic Castration-Resistant Prostate Cancer Treated With Platinum-Based Chemotherapy

Characteristic	Entire Cohort (N = 64)	DDRmut (n = 16)	DDRwt (n = 48)
Age at diagnosis, years			
Median (interquartile range)	68 (63-74)	68 (63-73)	67 (63-74)
Gleason score			
6-7	18 (28)	3 (19)	15 (31)
≥ 8	43 (67)	13 (81)	30 (63)
Not evaluable	3 (5)	0 (0)	3 (6)
Baseline PSA, ng/mL			
Median (interquartile range)	121 (32-337)	119 (45-634)	121 (22-308)
Histologic neuroendocrine features in genomically profiled tissue	3 (5)	1 (6)	3 (6)
Sites of metastasis			
Bone	56 (88)	16 (100)	42 (88)
Lymph node	53 (83)	15 (94)	38 (79)
Visceral	26 (40)	4 (25)	22 (46)
Lung	17 (27)	2 (13)	12 (25)
Liver	14 (22)	3 (19)	24 (29)
Prior treatment exposure			
Taxane	64 (100)	16 (100)	49 (100)
Abiraterone/enzalutamide	60 (94)	15 (94)	45 (94)
PARP inhibitor	0 (0)	0 (0)	0 (0)
Concomitant treatment			
Taxane	41 (64)	11 (69)	30 (63)
Etoposide	2 (3)	0 (0)	2 (4)

NOTE. Data are No. (%) unless otherwise indicated.

Abbreviations: DDRmut, DNA damage repair mutant; DDRwt, DNA damage repair–wild type; PARP, poly (ADP-ribose) polymerase; PSA, prostate-specific antigen.

TABLE 2. Genomic Alterations of Patients With DDRmut Disease and PSA50 Response

PARP Inhibitor–Naïve Patients					
Patient	Gene	Setting	Genomic Alteration	Zygosity	PSA50 Response
P-0005806	<i>ATM</i>	Germline	Q852*	Biallelic	No
	<i>RAD51</i>	Somatic	HOMDEL	Biallelic	
P-0034393	<i>ATM</i>	Somatic	E2236*	Biallelic	No
P-0008098	<i>BRCA2</i>	Somatic	HOMDEL	Biallelic	Yes
P-0014408	<i>BRCA2</i>	Somatic	HOMDEL	Biallelic	Yes
P-0006749	<i>BRCA2</i>	Somatic	X228_splice	Biallelic	Yes
P-0012132	<i>BRCA2</i>	Somatic	HOMDEL	Biallelic	Yes
P-0023961	<i>BRCA2</i>	Somatic	HOMDEL	Biallelic	No
P-0000441	<i>BRCA2</i>	Germline	Q699Sfs*31	Biallelic	No
		Somatic	L2362Cfs*5		
P-0002796	<i>CDK12</i>	Somatic	L447Vfs*15	Biallelic	Yes
P-0006231	<i>CDK12</i>	Somatic	L122Tfs*4	Biallelic	No
P-0019332	<i>CDK12</i>	Somatic	P934Kfs*12	Biallelic	No
P-0005374	<i>FANCA</i>	Somatic	HOMDEL	Biallelic	Yes
	<i>CDK12</i>	Somatic	P792Tfs*27	Monoallelic	
P-0009056	<i>FANCA</i>	Somatic	HOMDEL	Biallelic	Yes
P-0001698	<i>FANCA</i>	Somatic	HOMDEL	Biallelic	No
P-0000683	<i>FANCA</i>	Somatic	HOMDEL	Biallelic	No
P-0015284	<i>PALB2</i>	Somatic	T284IFS*4	Undefined	Yes
Post–PARP Inhibitor Patients					
Patient	Gene	Setting	Genomic Alteration	Zygosity	PSA50 Response
P-0008315	<i>ATM</i>	Somatic	D639Ifs*10, X24_splice	Biallelic	No
P-0011465	<i>ATM</i>	Germline	Y959*	Biallelic	No
P-0000377	<i>BRCA1</i>	Germline	Q1756Pfs*74	Biallelic	N/A
P-0016296	<i>BRCA2</i>	Somatic	E2846*	Monoallelic	No
P-0000541	<i>BRCA2</i>	Germline	S1982Rfs*22	Monoallelic	Yes
P-0001092	<i>BRCA2</i>	Germline	P3039P	Undefined	No
P-0017524	<i>BRCA2</i>	Somatic	P655Qfs*5	Biallelic	No
P-0004489	<i>BRCA2</i>	Somatic	HOMDEL	Biallelic	Yes

Abbreviations: DDRmut, DNA damage repair mutant; HOMDEL, homozygous deletion; N/A, nonevaluable; PARP, poly (ADP-ribose) polymerase; PSA50, 50% prostate-specific antigen decline from baseline.

visceral metastasis or histologic neuroendocrine differentiation. We also evaluated ToT as a surrogate of clinical benefit in the overall population of 64 patients (Fig 2B). Median ToT for the DDRwt group was 1.6 months, compared with 3.0 months for DDRmut (hazard ratio [HR], 0.55; 95% CI, 0.29 to 1.04; $P = .064$) and 3.9 months for patients with *BRCA2* mutations (HR, 0.63; 95% CI, 0.26 to 1.52; $P = .300$). OS from the start of platinum-based therapy was not significantly different in the DDRmut and DDRwt groups (HR, 0.79; 95% CI, 0.41 to 1.52; $P = .480$; Fig 2C).

Platinum-Based Chemotherapy After PARPi Treatment

We evaluated response to platinum chemotherapy in patients with DDRmut disease who received platinum

chemotherapy after experiencing progression on a PARPi. We focused on the 8 patients with an alteration in either *BRCA2* ($n = 5$), *BRCA1* ($n = 1$), or *ATM* ($n = 2$), given the previously reported sensitivity of these tumors to PARP inhibition,¹³ with US Food and Drug Administration (FDA) breakthrough therapy designation having been granted for olaparib for *BRCA1/2* and *ATM*-mutated mCRPC. Baseline clinical characteristics are summarized in Appendix Table A1. Median time on the prior PARPi for all 8 patients was 4.6 months (range, 1.0–13.1 months). Best radiographic response on the prior PARPi was partial response (PR) in 2/8, stable disease (SD) in 3/8, and progression of disease in 3/8, although all ultimately experienced progression (Fig 3A).

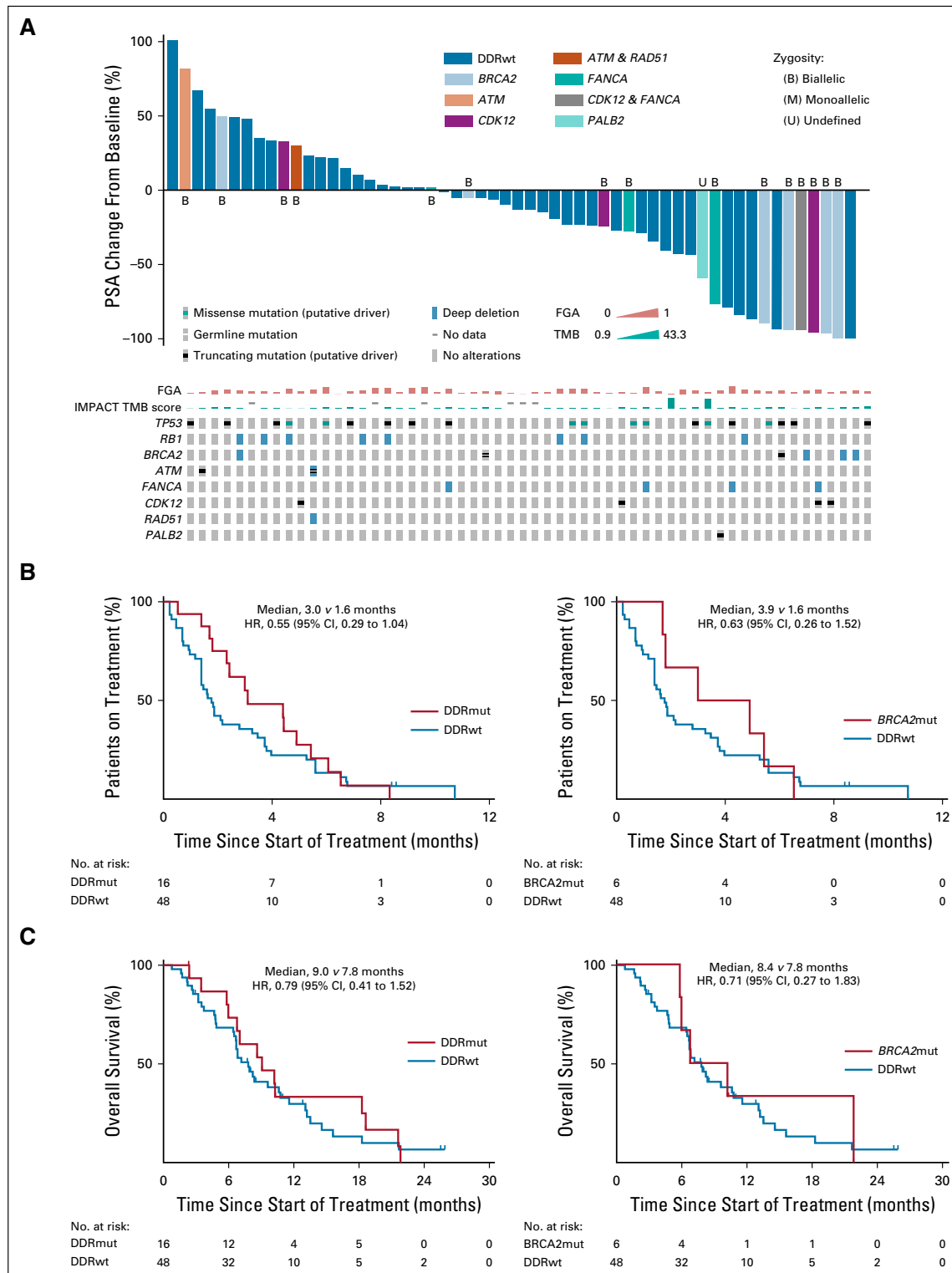


FIG 2. Response to platinum-based chemotherapy in patients with DNA damage repair mutant (DDRmut) and DNA damage repair–wild type (DDRwt) disease. A total of 64 patients received platinum-based chemotherapy after a taxane. (A) Waterfall plot showing best prostate-specific antigen (PSA) change from baseline for patients with DDRmut and DDRwt disease among 56 patients evaluable for PSA response (top panel). The oncoprint (bottom panel) shows details of the types of alterations in DNA damage repair (DDR) genes, as well as alterations in *TP53*, *RB1*, tumor mutation burden (TMB; in mutations per megabase), and fraction of the genome altered (FGA). Zygosity status for the relevant DDR genes is indicated. (B) Time on treatment and (C) overall survival with platinum chemotherapy for DDRmut (left) and the *BRCA2*-mutated (*BRCA2*mut) subset (right) compared with patients with DDRwt disease. HR, hazard ratio.

TABLE 3. PSA50 Response Rate by DDR Gene Status

Response	DDRmut (n = 16)	BRCA2mut (n = 6)	DDRwt (n = 40)
PSA50 responses, No. (%)	8 (50)	4 (67)	5 (13)
Odds ratio (95% CI) ^a	8.0 (1.9 to 39.9)	9.5 (1.5 to 82.9)	1.0 (reference)

Abbreviations: DDR, DNA damage repair; DDRmut, DNA damage repair mutant; DDRwt, DNA damage repair–wild type; PSA50, 50% prostate-specific antigen decline from baseline.

^aAdjusted for pretreatment prostate-specific antigen, Gleason score, and the presence of visceral metastasis.

The median time on platinum-based chemotherapy for these 8 patients after progression on PARPi was 2.1 months (range, 1.8 to 15.6 months). Six of 7 PSA-evaluable patients (86%) had a decline in PSA from baseline on platinum therapy, 2 of whom achieved a PSA50 response (Fig 3A). Two of 7 evaluable patients, both with *BRCA2* mutations, achieved SD, with PSA declines of 61% and 79% (Fig 3B). Both of these patients received platinum chemotherapy as monotherapy when their PSA responses could not be attributed to another agent. One patient with a *BRCA1* germline mutation and nonmeasurable PSA achieved a radiographic PR on carboplatin plus docetaxel after progression on a PARPi (Fig 3C). However, this patient was taxane naïve at baseline; therefore, his response could not be definitely attributed to the platinum agent. Of note, none of the 4 patients with deleterious mutations in *ATM*, regardless of prior PARPi or taxane exposure (Fig 1), had a PSA or radiographic response to platinum-based chemotherapy (Figs 2A and 3A; Table 2).

DISCUSSION

In this study, we retrospectively assessed response to platinum-based chemotherapy in patients with mCRPC who underwent clinical tumor and germline genomic sequencing at a tertiary referral center. We found that PSA responses occurred more frequently in patients who harbored genomic alterations in DDR genes. There was a trend toward longer ToT in the DDR-mutant group, but we could not detect a difference in OS. Importantly, we limited the analysis to patients who received platinum-based chemotherapy after progression on a taxane, where response was more likely attributable to the platinum agent, because many patients with mCRPC receive platinum chemotherapy in combination with a taxane.^{2,28}

Our findings are consistent with prior reports of improved response of *BRCA*-altered tumors to platinum-based chemotherapy^{6,7,12} and identify responses in tumors with non-*BRCA* DDR gene alterations, including *PALB2*, *FANCA*, and *CDK12*,^{8,9} suggesting that a broader DDR gene panel encompassing nearly 25% of patients with mCRPC could be used to identify patients who are more likely to derive benefit from platinum chemotherapy, either administered alone or concurrently with a taxane. The limited sample size of our study likely made it difficult to reliably detect differences in ToT and OS. Importantly, other clinical disease subsets, sometimes described as

“aggressive variants” of prostate cancer, including those with low PSA expression, visceral metastasis, or histologic neuroendocrine differentiation, may also derive particular benefit from platinum chemotherapy,⁵ and the presence of a genomic alteration in a DDR gene is only 1 variable that may aid in patient selection for this type of therapy.

We also examined responses to platinum chemotherapy after progression on a PARPi for patients with *BRCA* and *ATM* mutations. The PARPi olaparib and rucaparib were recently granted FDA breakthrough therapy designation for *BRCA*-mutated mCRPC, based on phase II studies showing a high rate of objective responses in this setting.^{14,29} However, it remains unknown whether tumors that acquire resistance to PARPi³⁰ can still respond to other DNA damage-targeting agents, including platinum chemotherapy. We found that 3/ 8 patients with DDR mutations (37%) derived some clinical benefit from platinum-based chemotherapy after progression on a PARPi, with 1 patient achieving a radiographic PR, although outcomes in this advanced patient population were generally poor.

Of note, our study included 4 patients with deleterious alterations in *ATM* who received platinum-based chemotherapy either before or after receiving a PARPi. None of these patients achieved a PSA50 response, and all experienced rapid disease progression. This finding is from a limited sample size and will need to be confirmed in larger studies, but it reinforces the need for novel therapeutic approaches for the approximately 4% of patients with mCRPC who harbor deleterious alterations in *ATM*.³¹

In summary, our study suggests that a subset of patients with DDR gene alterations detected by tumor or germline sequencing may derive benefit from platinum-based chemotherapy, including patients with *BRCA* mutations who have progressed after treatment with a PARPi, a group with particular clinical relevance. Our study differs from prior studies in that it represents a single-institution experience using a single panel sequencing assay that includes DDR genes beyond *BRCA* and includes both somatic and germline alterations in DDR genes. We recognize that our study is limited by its retrospective nature, the incorporation of distinct DDR genes with varying functions into a single panel, and sample size; thus, our findings will need to be validated in larger prospective

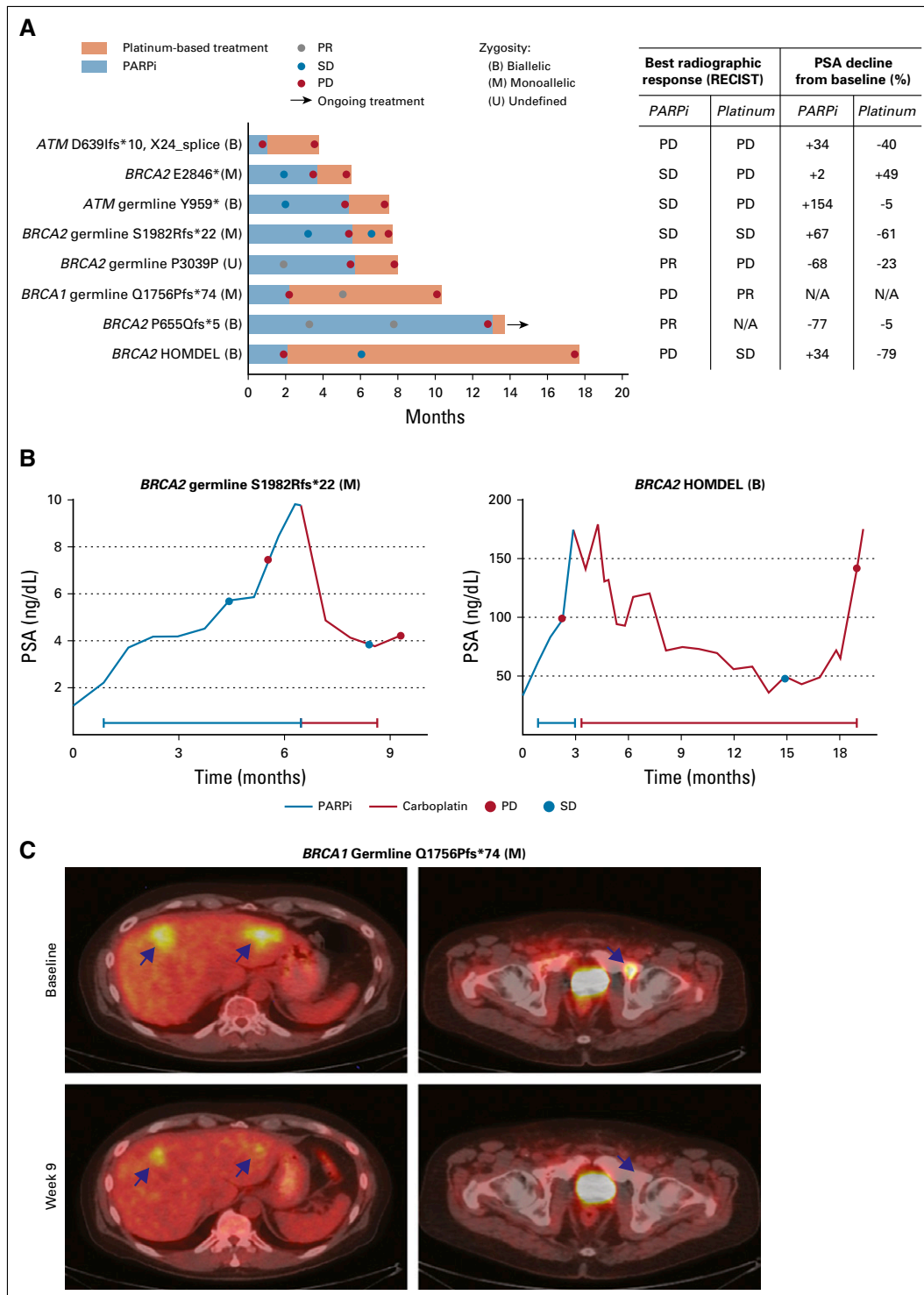


FIG 3. Platinum-based chemotherapy after poly (ADP-ribose) polymerase inhibitor (PARPi) progression. (A) Time on treatment on PARPi (blue) and subsequent platinum-based chemotherapy (orange), which did not necessarily occur immediately after PARPi therapy. Best radiographic and prostate-specific antigen (PSA) responses are summarized in the table. Zygosity status for the DNA damage repair gene is indicated. Three of 7 evaluable patients had RECIST 1.1 stable disease (SD) or partial response (PR) on platinum-based chemotherapy. (B) Six of 7 evaluable patients had PSA decrease on platinum therapy, with 2 *BRCA2*-altered patients achieving a 50% prostate-specific antigen decline from baseline response. (C) One patient with a *BRCA1* germline mutation had a RECIST PR on platinum chemotherapy after progression on a PARPi, with representative ^{18}F -labeled fluorodeoxyglucose–positron emission tomography/computed tomography images. This patient was taxane naïve and received carboplatin with docetaxel; therefore, his response cannot be definitely attributed to the platinum agent alone. HOMDEL, homozygous deletion; N/A, not evaluable; PD, progression of disease; PR, partial response; SD, stable disease.

studies, which are currently ongoing. We also recognize that other clinical factors linked to aggressive variants of prostate cancer are associated with response to platinum

chemotherapy and that a combination of genomic and clinical characteristics may ultimately aid in patient selection.

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PRIOR PRESENTATION

Presented in part at the ASCO 2019 Annual Meeting, Chicago, IL, May 31 to June 4, 2019.

SUPPORT

Supported by Young Investigator Awards from the Prostate Cancer Foundation (W.A., K.H.S.); Department of Defense Prostate Cancer Research Program Physician Research Award W81XWH-17-1-0124 (W.A.) and Early Investigator Research Award W81XWH-18-1-0330 (K.H.S.); National Cancer Institute (NCI) cancer center support Grant No. P30CA008748; NCI prostate cancer Specialized Programs of Research Excellence (SPORE) Grant No. P50CA092629; and Marie-Josée and Henry R. Kravis Center for Molecular Oncology.

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Financial support: David B. Solit, Wassim Abida

Administrative support: Ethan Barnett, David B. Solit, Wassim Abida

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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Open Payments is a public database containing information reported by companies about payments made to US-licensed physicians ([Open Payments](http://OpenPayments.org)).

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Patents, Royalties, Other Intellectual Property: Gene expression profile associated with prostate cancer

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Patents, Royalties, Other Intellectual Property: Method for predicting the risk of prostate cancer morbidity and mortality, predicting and treating prostate cancer, methods for predicting likelihood of responding to treatment, chromosome copy number gain as a biomarker of urothelial carcinoma lethality, drug combinations to treat cancer, somatic ERCC2 mutations correlate with cisplatin sensitivity in muscle-invasive urothelial carcinoma (patent), Up-to-Date royalties, Wolters Kluwer Royalties, methods and kits for determining sensitivity to cancer treatment, composition and methods for screening and diagnosis of prostate cancer.

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No other potential conflicts of interest were reported.

REFERENCES

1. Sternberg CN, Petrylak DP, Sartor O, et al: Multinational, double-blind, phase III study of prednisone and either satraplatin or placebo in patients with castrate-refractory prostate cancer progressing after prior chemotherapy: The SPARC trial. *J Clin Oncol* 27:5431-5438, 2009
2. Ross RW, Beer TM, Jacobus S, et al: A phase 2 study of carboplatin plus docetaxel in men with metastatic hormone-refractory prostate cancer who are refractory to docetaxel. *Cancer* 112:521-526, 2008
3. Hager S, Ackermann CJ, Joerger M, et al: Anti-tumour activity of platinum compounds in advanced prostate cancer—a systematic literature review. *Ann Oncol* 27:975-984, 2016
4. Corn PG, Heath EI, Zurita A, et al: Cabazitaxel plus carboplatin for the treatment of men with metastatic castration-resistant prostate cancers: A randomised, open-label, phase 1-2 trial. *Lancet Oncol* 20:1432-1443, 2019
5. Aparicio AM, Harzstark AL, Corn PG, et al: Platinum-based chemotherapy for variant castrate-resistant prostate cancer. *Clin Cancer Res* 19:3621-3630, 2013
6. Cheng HH, Pritchard CC, Boyd T, et al: Biallelic inactivation of BRCA2 in platinum-sensitive metastatic castration-resistant prostate cancer. *Eur Urol* 69:992-995, 2016
7. Pomerantz MM, Spisák S, Jia L, et al: The association between germline BRCA2 variants and sensitivity to platinum-based chemotherapy among men with metastatic prostate cancer. *Cancer* 123:3532-3539, 2017
8. Robinson D, Van Allen EM, Wu Y-M, et al: Integrative clinical genomics of advanced prostate cancer. *Cell* 162:454, 2015
9. Abida W, Armenia J, Gopalan A, et al: Prospective genomic profiling of prostate cancer across disease states reveals germline and somatic alterations that may affect clinical decision making. *JCO Precis Oncol* 10.1200/PO.17.00029
10. Armenia J, Wankowicz SAM, Liu D, et al: The long tail of oncogenic drivers in prostate cancer. *Nat Genet* 50:645-651, 2018 [Erratum *Nat Gen* 51:1194, 2019]
11. Pritchard CC, Mateo J, Walsh MF, et al: Inherited DNA-repair gene mutations in men with metastatic prostate cancer. *N Engl J Med* 375:443-453, 2016
12. Zafeiriou Z, Bianchini D, Chandler R, et al: Genomic analysis of three metastatic prostate cancer patients with exceptional responses to carboplatin indicating different types of DNA repair deficiency. *Eur Urol* 75:184-192, 2019
13. Mateo J, Carreira S, Sandhu S, et al: DNA-repair defects and olaparib in metastatic prostate cancer. *N Engl J Med* 373:1697-1708, 2015
14. Abida W, Bryce AH, Vogelzang NJ, et al: Preliminary results from TRITON2: A phase II study of rucaparib in patients (pts) with metastatic castration-resistant prostate cancer (mCRPC) associated with homologous recombination repair (HRR) gene alterations. *Ann Oncol* 29, 2018 (suppl 8; abstr 793PD)
15. Cheng DT, Mitchell TN, Zehir A, et al: Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT): A hybridization capture-based next-generation sequencing clinical assay for solid tumor molecular oncology. *J Mol Diagn* 17:251-264, 2015
16. Zehir A, Benayed R, Shah RH, et al: Mutational landscape of metastatic cancer revealed from prospective clinical sequencing of 10,000 patients. *Nat Med* 23:703-713, 2017 [Erratum: *Nat Med* 23:1004, 2017]
17. Mandelker D, Zhang L, Kemel Y, et al: Mutation detection in patients with advanced cancer by universal sequencing of cancer-related genes in tumor and normal DNA vs guideline-based germline testing. *JAMA* 318:825-835, 2017
18. Chakravarty D, Gao J, Phillips SM, et al: OncoKB: A precision oncology knowledge base. *JCO Precis Oncol* 10.1200/PO.17.00011
19. Zannini L, Delia D, Buscemi G: CHK2 kinase in the DNA damage response and beyond. *J Mol Cell Biol* 6:442-457, 2014
20. Varon R, Vissinga C, Platzer M, et al: Nibrin, a novel DNA double-strand break repair protein, is mutated in Nijmegen breakage syndrome. *Cell* 93:467-476, 1998
21. Hopfner KP, Karcher A, Shin DS, et al: Structural biology of Rad50 ATPase: ATP-driven conformational control in DNA double-strand break repair and the ABC-ATPase superfamily. *Cell* 101:789-800, 2000
22. Bhattacharya S, Srinivasan K, Abdisalaam S, et al: RAD51 interconnects between DNA replication, DNA repair and immunity. *Nucleic Acids Res* 45:4590-4605, 2017
23. Somyajit K, Subramanya S, Nagaraju G: Distinct roles of FANCO/RAD51C protein in DNA damage signaling and repair: Implications for Fanconi anemia and breast cancer susceptibility. *J Biol Chem* 287:3366-3380, 2012 [Erratum: *J Biol Chem* 294:13198, 2019]
24. Abida W, Cheng ML, Armenia J, et al: Analysis of the prevalence of microsatellite instability in prostate cancer and response to immune checkpoint blockade. *JAMA Oncol* 5:471-478, 2019
25. Shen R, Seshan VE: FACETS: Allele-specific copy number and clonal heterogeneity analysis tool for high-throughput DNA sequencing. *Nucleic Acids Res* 44:e131, 2016

26. Kang J, D'Andrea AD, Kozono D: A DNA repair pathway-focused score for prediction of outcomes in ovarian cancer treated with platinum-based chemotherapy. *J Natl Cancer Inst* 104:670-681, 2012
27. Telli ML, Timms KM, Reid J, et al: Homologous recombination deficiency (HRD) score predicts response to platinum-containing neoadjuvant chemotherapy in patients with triple-negative breast cancer. *Clin Cancer Res* 22:3764-3773, 2016
28. Regan MM, O'Donnell EK, Kelly WK, et al: Efficacy of carboplatin-taxane combinations in the management of castration-resistant prostate cancer: A pooled analysis of seven prospective clinical trials. *Ann Oncol* 21:312-318, 2010
29. Mateo J, Porta N, McGovern UB, et al: TOPARP-B: A phase II randomized trial of the poly(ADP)-ribose polymerase (PARP) inhibitor olaparib for metastatic castration resistant prostate cancers (mCRPC) with DNA damage repair (DDR) alterations. *J Clin Oncol* 37, 2019 (suppl; abstr 5005)
30. Lin KK, Harrell MI, Oza AM, et al: *BRCA* reversion mutations in circulating tumor DNA predict primary and acquired resistance to the PARP inhibitor rucaparib in high-grade ovarian carcinoma. *Cancer Discov* 9:210-219, 2019
31. Abida W, Campbell D, Patnaik A, et al: Non-BRCA DNA damage repair gene alterations and response to the PARP inhibitor rucaparib in metastatic castration-resistant prostate cancer: Analysis from the phase 2 TRITON2 study. *Clin Cancer Res* [10.1158/1078-0432.CCR-20-0394](https://doi.org/10.1158/1078-0432.CCR-20-0394) [epub ahead of print on February 21, 2020]



APPENDIX

TABLE A1. Baseline Clinical Characteristics of Patients With PARP Inhibitor–Refractory Metastatic Castration-Resistant Prostate Cancer Treated With Platinum-Based Chemotherapy

Characteristic	n = 8
Age at diagnosis (years)	
Median (interquartile range)	62 (57-66)
Baseline PSA (ng/mL)	
Median (interquartile range)	127.2 (6.7-1206)
Histologic neuroendocrine features in genomically profiled tissue	0 (0)
Visceral metastasis	3 (38)
Prior treatment exposure	
Taxane	5 (63)
Abiraterone	7 (88)
Enzalutamide	6 (75)
Concomitant treatment	
Taxane	4 (50)
Etoposide	1 (13)

NOTE. Data are No. (%) unless otherwise specified.

Abbreviation: PARP, poly (ADP-ribose) polymerase; PSA, prostate-specific antigen.



Differences in Prostate Cancer Genomes by Self-reported Race: Contributions of Genetic Ancestry, Modifiable Cancer Risk Factors, and Clinical Factors

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ABSTRACT

Purpose: Black men die from prostate cancer twice as often as White men, a disparity likely due to inherited genetics, modifiable cancer risk factors, and healthcare access. It is incompletely understood how and why tumor genomes differ by self-reported race and genetic ancestry.

Experimental Design: Among 2,069 men with prostate cancer (1,841 self-reported White, 63 Asian, 165 Black) with access to clinical-grade sequencing at the same cancer center, prevalence of tumor and germline alterations was assessed in cancer driver genes reported to have different alteration prevalence by race.

Results: Clinical characteristics such as prostate-specific antigen and age at diagnosis as well as cancer stage at sample procurement differed by self-reported race. However, most genomic differences persisted when adjusting for clinical characteristics. Tumors from Black men harbored fewer *PTEN*

mutations and more *AR* alterations than those from White men. Tumors from Asian men had more *FOXA1* mutations and more *ZFHX3* alterations than White men. Despite fewer *TP53* mutations, tumors from Black men had more aneuploidy, particularly chromosome arm 8q gains, an adverse prognostic factor. Genetic ancestry was associated with similar tumor alterations as self-reported race, but also with modifiable cancer risk factors. Community-level average income was associated with chr8q gains after adjusting for race and ancestry.

Conclusions: Tumor genomics differed by race even after accounting for clinical characteristics. Equalizing access to care may not fully eliminate such differences. Therapies for alterations more common in racial minorities are needed. Tumor genomic differences should not be assumed to be entirely due to germline genetics.

Introduction

Each year in the United States, there are 38 deaths from prostate cancer per 100,000 Black men, compared with 18 per 100,000 White men (1), a disparity that is greater than for any other cancer. It is critical to understand the contributions of the potential underlying causes, including differences in inherited (germline) genetics, modifiable risk factors, and healthcare access. Prostate

cancer is highly heritable (2), and germline risk loci for prostate cancer differ by ancestry (3). In addition, the burden of modifiable risk factors for prostate cancer–related death, such as smoking (4), is higher among Black men (5). Racial and ethnic groups have unequal healthcare access to cancer screening, diagnostics, and treatment. Consequently, prostate cancer among Black men may be diagnosed and treated at later stages with shorter survival after diagnosis.

Another potential contributor to racial differences is underlying differences in molecular tumor features that may indicate different cancer etiologies as well as higher or lower sensitivity of the tumor to targeted therapies. Prostate tumors differ by race in key somatic DNA alterations (6–10). For example, in the largest study to date (8), metastatic prostate tumors from Black men, compared with those from White men, were reported to have more DNA mutations in the androgen receptor *AR*, in *BRAF*, in DNA repair genes, and in genes considered “actionable,” and fewer *TP53* alterations than tumors from Asian men. These differences suggest that for some therapies, treatment benefits may be different by race. However, previous genomic studies had limited or no data on differences in healthcare access or cancer risk factors, and they typically relied on either race or genetic ancestry.

A first question is whether differences in tumor genomics are a consequence of disparities in healthcare access. For example, if Black men had tumor sequencing later in their disease course than White men, finding more *AR* alterations in their tumors could merely reflect barriers to healthcare access. Conversely, if genomic differences

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Clin Cancer Res 2022;28:318–26

doi: 10.1158/1078-0432.CCR-21-2577

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Translational Relevance

Prostate cancer has the greatest racial disparities in death rates in the United States of all cancers. As one contributor, tumor genomes have been suggested to differ by self-reported race. This study demonstrates that the same differences in tumor genomics as by self-reported race are observed if comparing men by genetic ancestry. There were notable differences in clinical factors by self-reported race among the men who underwent tumor-normal sequencing, but differences in tumor genomics persisted after adjusting for clinical characteristics. Tumors from Black men had more poor-prognosis chr8q gains, which beyond race and ancestry were also associated with a crude, area-level measure of socioeconomic status. These results underscore that therapies for genomic alterations more common in racial minorities are needed. Differences in tumor genomes should neither be assumed to result solely from access to care nor solely from germline genetics.

persisted once consequences of healthcare access were accounted for, then race may need to be considered for precision prevention and treatment selection (11, 12).

A second question is the interpretation of “race” and to what extent genetic ancestry is associated with genomic tumor differences associated with self-reported race. Race is a self-ascribed or socially-ascribed identity, whereas genetic ancestry describes components of an individual’s genetic origin (13). Yet both concepts are highly correlated and must be carefully disentangled.

Finally, a third question is to what extent differences in tumor genomics are driven by germline genetics or could be reduced by acting on racial disparities in cancer risk factors. We addressed these questions in one of the largest, clinical-grade sequencing cohorts of prostate cancer.

Materials and Methods

Study population

This study included men with histologically confirmed prostate cancer who had access to care at a comprehensive cancer center and who reported race on intake forms (Supplementary Fig. S1). Patients gave written informed consent for this institutional review board–approved study that was conducted in accordance with the U.S. Common Rule. Freely available definitions of data elements for a clinical-genomic research database, their validation, and the analysis pipeline were described elsewhere (14). Area-level median household income per 5-digit ZIP code was from the U.S. Census 2015–2019 American Community Survey.

Genomics

Tumor profiling used archival or fresh formalin-fixed, paraffin-embedded tumor samples. Germline DNA from peripheral blood was only analyzed with specific consent. An FDA-cleared targeted hybridization capture-based gene panel, MSK-IMPACT, including 341 to 468 genes depending on its version, was used to capture single-nucleotide variants, small insertions and deletions, copy-number alterations, and structural rearrangements (15).

TMPRSS2 and *ERG* alterations, more common in men of European ancestry (16), were not included in analyses given the suboptimal sensitivity of DNA panel sequencing for gene fusions like *TMPRSS2:ERG* and the analytical focus on absolute differences. Oncogenic

or likely oncogenic function was annotated using OncoKB 2.8 (September 17, 2020; ref. 17).

For copy-number gains and deletions, absolute log₂ copy-number deviations >0.2 were required. The fraction of copy number–altered genome was defined as the proportion of the genome with such a copy-number change relative to the size of the genome with copy number profiled. Estimates were corrected for pathologist-assessed tumor cellularity; there were no differences in tumor cellularity by race (Supplementary Fig. S7). CNTools v1.46, copy number v1.30, and, for calling arm-level events, ASCETS (18) with breadth of coverage >0.5 were used.

To infer genetic ancestry, we used the 1000 Genomes Project (1KGP) with potentially admixed individuals excluded as reference. We selected 5,072 bi-allelic autosomal single-nucleotide polymorphism (SNP) markers from 1KGP with minor allele frequency >1% and within MSK-IMPACT468 bait intervals. For each patient, we genotyped these markers on the matched normal BAM files using GATK v4.0 Pileup, merged with 1KGP reference data and pruned with PLINK v1.9 (parameters –indep-pairwise 500kb 50 0.2) to keep only those markers that were in linkage equilibrium. We then performed supervised ADMIXTURE v1.3 (19) to estimate proportions of African (AFR), European (EUR), East Asian (EAS), NAM (Native American; not included in analyses), and South Asian (SAS) ancestries.

Statistical analyses

Prevalence differences for genomic alterations by race (categorical) or ancestry (each ancestry modeled as a continuous variable) were estimated using marginal standardization of logistic regression, with bias-corrected accelerated (BC_a) 95% confidence intervals (CI) from parametric bootstrapping with 10,000 repeats (20). Regression models were used for differences in age at diagnosis and time to sequencing (quantile regression for the median), counts of actionable alterations (Poisson model), arithmetic mean ratios of fraction genome altered (generalized linear model with Gaussian distribution and log link), prevalence differences in chr8q gain by continuously modeled income (binomial model with identity link), and survival from sequencing to death or last contact (Cox regression with separate baseline hazards by disease extent at sequencing).

Data availability

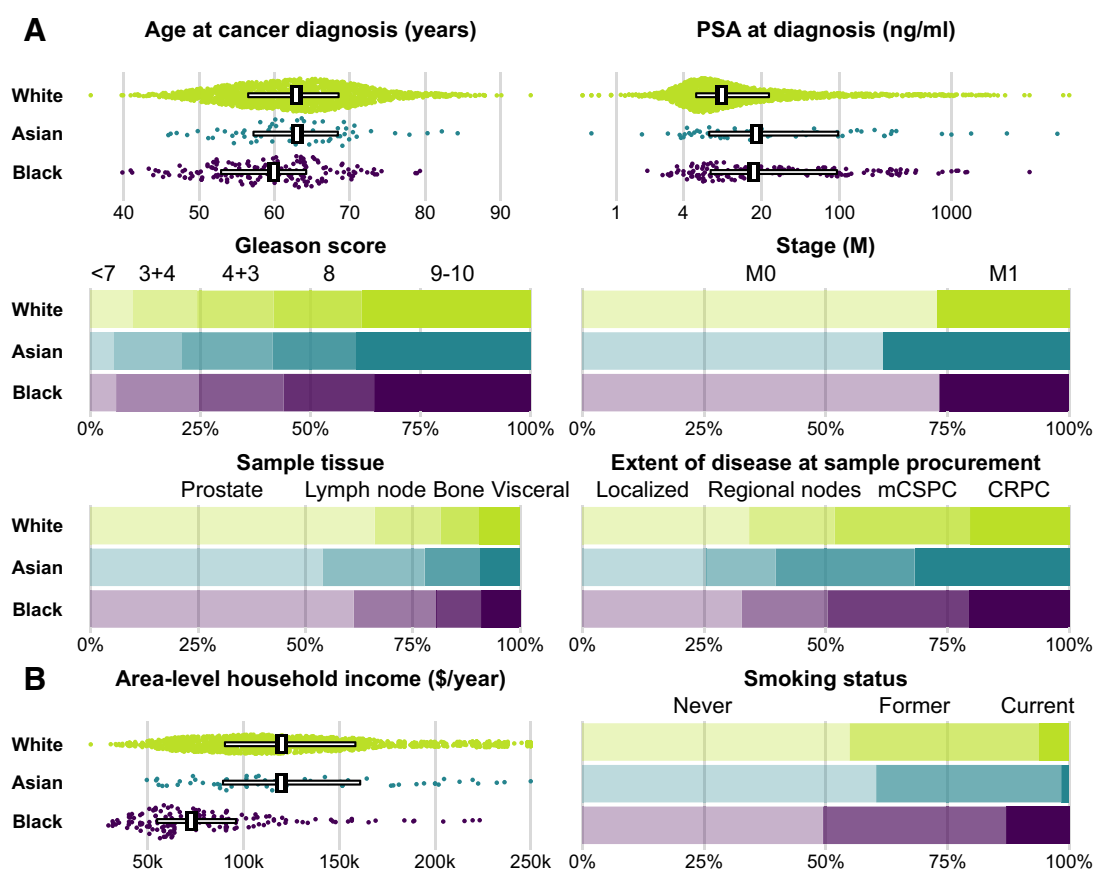
Clinical and genomic data from this study are available via cBioPortal (https://www.cbioportal.org/study/summary?id=prad_msk_stopsack_2021).

Results

Clinical characteristics and cancer risk factors

We first compared clinical features of 2,069 men with prostate cancer by self-reported race including 63 Asian men, 165 Black men, and 1,841 White men (Fig. 1A; Table 1). Despite being 3.0 years younger at diagnosis (95% CI, 0.6 to 5.4), Black men had median serum prostate-specific antigen (PSA) levels at diagnosis of 17 ng/mL, higher than White men (9 ng/mL; difference, 8.2 ng/mL; 95% CI, 0.1 to 16.3). Asian men had a more advanced cancer stage at sample procurement (32% castration-resistant) than White men (19%; difference, 13 percentage points; 95% CI, 1 to 24). Time to tumor sequencing was similar by race in our study (Table 1).

These differences in clinical cancer features prompted us to investigate two examples of potential upstream factors (Fig. 1B).

**Figure 1.**

Clinical factors (A) and cancer risk factors (B) by self-reported race. For age, PSA, and area-level household income, individual data points are shown, with horizontal bars covering the 25th to 75th percentile and vertical bars indicating the median. For other characteristics, bars indicate proportions. CRPC, castration-resistant prostate cancer.

Socioeconomic status has profound influences on cancer risk and survival (21) through cancer risk factors and healthcare use and access. Black men lived in ZIP code areas with an average household income 36% lower than that of White men (95% CI, 30% to 42%). There were no noticeable differences between Asian and White men. Twice as many Black men (13%) as White men (6%) were current smokers at the time of cancer diagnosis (difference, 7 percentage points; 95% CI, 2 to 12), a strong risk factor for prostate cancer-related death (4).

Tumor genomics by self-reported race before and after accounting for clinical features

We quantified alteration prevalence in oncogenes and tumor suppressors in tumor DNA (Fig. 2; Supplementary Fig. S2). Our focus was on genes that putatively differed by race in previous studies (7–10). Unlike previous studies, we considered all types of DNA alterations (mutations, copy-number alterations, and fusions) but limited analyses to those classified as oncogenic or likely oncogenic based on a curated precision oncology database (17). We first compared expected tumor genomics by self-reported race because genetic ancestry is typically unknown in clinical practice until after a sequencing test is performed.

Alterations in the androgen receptor gene (AR) occurred in 19% of tumors from Asian men and 18% among Black men, as compared with

12% among White men. Potential differences in AR alteration prevalence between tumors from Asian and White men were completely attenuated (prevalence difference, 0 percentage points; 95% CI, –5 to 6) after adjusting for clinical features (age, disease extent, PSA, stage, sample type) and thus accounting for the more advanced disease state when samples had been procured from Asian men. In contrast, AR alterations were slightly more common in tumors from Black men than White men even after adjusting for clinical features (difference, 5 percentage points; 95% CI, 1 to 8).

Other differences in tumor genomics by race persisted when adjusting for clinical features (Fig. 2), suggesting that the observed differences in tumor genomics were not driven exclusively by differences in disease extent. The strongest differences were in *FOXA1*, *ZFH3*, *TP53*, and *PTEN*, with a smaller difference in *MYC* (copy-number gains) and *BRAF* alterations (Fig. 2).

In *FOXA1*, 27% of tumors from Asian men had alterations compared to 13% in White men (adjusted difference, 15 percentage points; 95% CI, 6 to 26), a difference driven by class-1 *FOXA1* mutations in the forkhead domain (22), which occurred in 21% of tumors from Asian men compared with 8% from White men (Supplementary Fig. S3A). *ZFH3* alterations, both mutations and copy-number alterations, were enriched in tumors from Asian men (17%) compared with White men (7%; adjusted difference, 8 percentage points; 95% CI, 1 to 17; Supplementary Fig. S2).

Table 1. Characteristics of men and tumors with MSK-IMPACT sequencing^a.

	Overall	By self-reported race		
		White	Asian	Black
N	2,069	1,841	63	165
Age at diagnosis (years)	63 (56–68)	63 (56–68)	63 (57–68)	60 (53–64)
Age at sample (years)	66 (59–72)	66 (59–72)	66 (60–70)	62 (56–69)
Diagnosis to sample (months)	3 (0–43)	3 (0–42)	4 (0–29)	3 (0–50)
Diagnosis to sequencing (months)	26 (6–83)	27 (6–83)	18 (7–60)	24 (6–84)
Smoking status				
Never	1,099 (55%)	981 (55%)	38 (60%)	80 (49%)
Former	783 (39%)	698 (39%)	24 (38%)	61 (38%)
Current	132 (7%)	110 (6%)	1 (2%)	21 (13%)
Unknown	55	52	0	3
Area-level household income (\$/year)	115,844 (84,643–156,558)	119,884 (90,293–158,420)	119,780 (89,288–160,935)	72,568 (54,886–96,086)
Unknown	40	39	0	1
PSA at diagnosis (ng/mL)	9.2 (5.3–26.8)	8.8 (5.2–23.3)	17.9 (6.8–97.0)	17.0 (7.0–94.5)
Unknown	116	104	2	10
Gleason score				
<7	171 (9%)	159 (9%)	3 (5%)	9 (6%)
3+4	287 (15%)	249 (15%)	9 (16%)	29 (19%)
4+3	336 (18%)	294 (17%)	12 (21%)	30 (19%)
8	381 (20%)	338 (20%)	11 (19%)	32 (21%)
9–10	726 (38%)	648 (38%)	23 (40%)	55 (35%)
Unknown	168	153	5	10
Stage (M)				
M0	1,501 (73%)	1,341 (73%)	39 (62%)	121 (73%)
M1	568 (27%)	500 (27%)	24 (38%)	44 (27%)
Sample tissue				
Prostate	1,352 (65%)	1,217 (66%)	34 (54%)	101 (61%)
Lymph node	331 (16%)	284 (15%)	15 (24%)	32 (19%)
Bone	191 (9%)	166 (9%)	8 (13%)	17 (10%)
Visceral	125 (6%)	110 (6%)	3 (5%)	12 (7%)
Other soft tissue	70 (3%)	64 (3%)	3 (5%)	3 (2%)
Extent of disease at sample				
Localized	700 (34%)	630 (34%)	16 (25%)	54 (33%)
Regional nodes	362 (17%)	324 (18%)	9 (14%)	29 (18%)
Metastatic hormone-sensitive	578 (28%)	512 (28%)	18 (29%)	48 (29%)
Nonmetastatic castration-resistant	17 (1%)	16 (1%)	0 (0%)	1 (1%)
Metastatic castration-resistant	405 (20%)	353 (19%)	20 (32%)	32 (19%)
Metastatic, variant histology	7 (0%)	6 (0%)	0 (0%)	1 (1%)
Tumor cellularity by pathologist (%)	40 (20–50)	40 (20–50)	40 (20–50)	40 (20–50)
Unknown	11	9	1	1
Mean sequencing coverage (X)	611 (461–754)	612 (461–753)	648 (539–793)	581 (453–737)
Consent for germline analyses	1,781 (86%)	1,583 (86%)	54 (86%)	144 (87%)

^aValues are count (percent) or median (quartile 1–quartile 3).

TP53 alterations were less common in tumors from Black men (19%) than White men (30%; adjusted difference, –10 percentage points; 95% CI, –3 to –17). This difference was likely driven by fewer *TP53* missense mutations among tumors from Black men (11%) than White men (18%; adjusted difference, –4 percentage points; 95% CI, –9 to 1; Supplementary Fig. S3B). Differences were not explained by frameshift or truncating mutations or single hotspots, such as the most common mutation at amino acid R273 (prevalence among White men, 2%).

Alterations in the tumor suppressor *PTEN* were less common in tumors from Asian and Black men (14%) compared with White men (20%; adjusted difference Black vs. White, –7 percentage points; 95% CI, –12 to –1), particularly due to fewer *PTEN* mutations (8% in Black

vs. 3% in White; adjusted difference, –4 percentage points; 95% CI, –7 to 0; Supplementary Fig. S3C).

In contrast to a previous study (8), we did not observe differences in the prevalence of DNA repair alterations (Fig. 2) or other potentially actionable alterations, nor did we find the (low) prevalence of microsatellite instability and the tumor mutation burden to differ by race (Supplementary Table S1). When combining gene-level alterations in oncogenic signaling pathways (23), differences in PI3K, RAS/RAF/MAPK, and MYC signaling pathways (Supplementary Fig. S4) were consistent with the observed differences in *PTEN*, *BRAF*, and *MYC*, respectively. In addition to these confirmatory analyses, we conducted an exploratory analysis of frequently altered genes (Supplementary Fig. S5), without additional candidates.

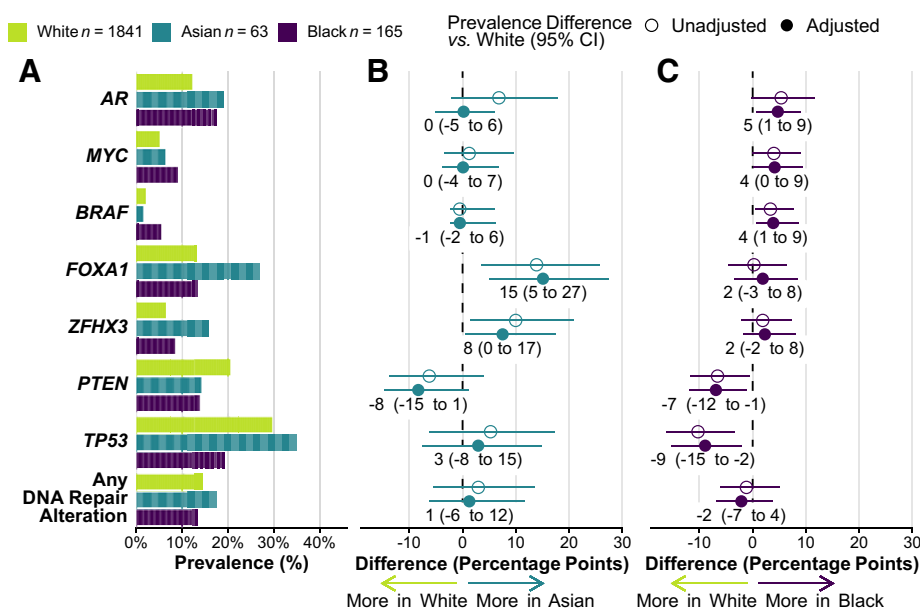


Figure 2.

Self-reported race and tumor alterations. **A**, Prevalence of oncogenic alterations by self-reported race. Prevalence is a proportion; each “brick” represents one patient with an alteration (not visible for White men because of the high number). **B**, Differences in alteration prevalence, comparing Asian men versus White men. **C**, Differences in alteration prevalence, comparing Black men versus White men. **B** and **C** show absolute differences in percentage points. Adjusted models include age at sample (linear), disease extent (localized, regional nodes, metastatic hormone-sensitive, or castration-resistant), PSA levels at diagnosis (log_e-transformed, mean-imputed), stage at diagnosis (*de novo* metastatic versus nonmetastatic), and sample type (prostate, lymph node, bone, or visceral). Individual genes are ordered by prevalence difference in Black men vs. White men. Only 1,924 samples (93%) were profiled for *ZFH3* due to its inclusion in a later panel version. For comparability with ref. 8, DNA repair alterations encompass *ERCC5*, *MRE11*, *TP53BP1*, *POLE*, *RAD21*, *MSH2*, *MSH6*, *BRCA1*, *BRCA2*, *ATR*, and *ATM*.

Tumor genomics and cancer risk factors by genetic ancestry

SNPs captured by the sequencing panels allowed us to infer genetic ancestry, which was strongly associated with but, as expected, not perfectly predictive of self-reported race (Fig. 3A). We then assessed how higher proportions of specific genetic ancestries were associated with tumor alterations that differed by self-reported race. We qualitatively observed the same associations between genetic ancestry and tumor alterations as between self-reported race and tumor alterations (Fig. 3B). For example, *TP53* alteration prevalence was –13 percentage points lower (95% CI, –19 to –5) in tumors from men with 100% AFR genetic ancestry markers than with 0% AFR ancestry.

We then probed to what extent ancestry can be interpreted causally as the influence of germline genetics or whether ancestry was also associated with nongenetic factors. We again encountered differences in smoking prevalence and in community-wide median household income, a unidimensional and imprecise measure of socioeconomic status, when comparing men by genetic ancestry (Fig. 3C). Among men with 100% AFR ancestry, 15% were predicted to live in a community with a median household income of at least \$100,000 annually, compared with 68% among men with 0% AFR ancestry (difference, 52 percentage points; 95% CI, 46 to 58).

We assessed to what extent differences in tumor alteration prevalence could directly result from alterations present in the germline, which were included in all analyses. Consent was given by 1,781 (86%) men for germline genetic testing for alterations in genes associated with heritable cancer risk, without differences in consent by self-reported race. While germline alterations were included in all analyses,

none of the genes with prevalence differences by race and ancestry had more than a single germline mutation among the 1,781 patients (Supplementary Table S2).

TP53 alterations and genomic instability

Tumor aneuploidy is strongly tied to *TP53* alterations and genomic instability (24). Despite fewer *TP53* alterations, tumors from Black men did not have a lower proportion of the tumor genome affected by copy-number alterations (Fig. 4A). When adjusting for *TP53* alteration status, tumors from Black men, but not Asian men, had slightly more copy-number alterations than those from White men.

Patterns of copy-number alterations appeared generally similar by race, except for chromosome arm 8q gains (Fig. 4B; Supplementary Fig. S6). Forty-nine percent of tumors from Black men and 47% of tumors from Asian men had chr8q gains, as compared with 37% of tumors from White men. Adjusting for clinical factors attenuated the difference in chr8q gain prevalence for Asian men (difference to White, 5 percentage points; 95% CI, –6 to 17) but not for Black men (difference to White, 11 percentage points; 95% CI, 4 to 18; Fig. 4C).

As expected, chr8q gains were an adverse prognostic factor. Over a median of 30 months, 441 deaths occurred among 1,690 White men with follow-up data after sequencing, 15 among 58 Asian men (precluding survival analyses), and 45 among 156 Black men. These data were not indicative of a generally worse prognosis among Black men in our study population, but we cannot rule out a difference (HR compared with White men, 1.15; 95% CI, 0.85 to 1.57). Tumors with chr8q gain, compared with tumors without chr8q gain but the same

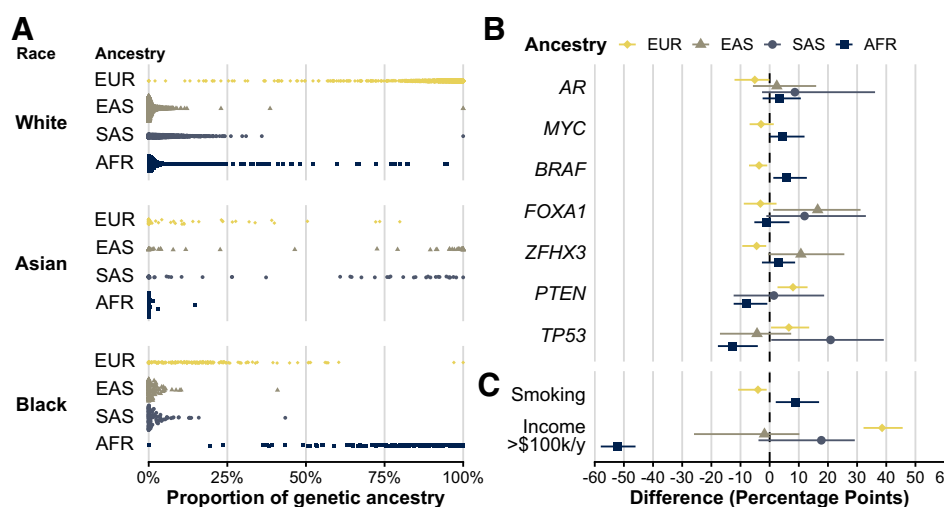


Figure 3.

Genetic ancestry and tumor alterations. **A**, Self-reported race (left-most) and proportion of inferred genetic ancestry (x-axis), by genetic superpopulation (EUR, EAS, SAS, AFR). **B** and **C**, Genetic ancestry and prevalence differences in tumors alterations (**B**) and cancer risk factors (**C**; current smoking vs. others; annual area-level household income >\$100,000 vs. others). Each genetic ancestry was modeled continuously, and estimates are expressed as percentage-point differences in the outcome (with 95% CIs) for an extreme comparison of men with 100% of the respective genetic ancestry to those with 0%. No estimates for a specific ancestry are shown if <3 tumor alterations (or current smokers) were observed when categorizing men by predominant genetic ancestry.

disease extent at sequencing, had a worse prognosis both among White men (HR, 1.61; 95% CI, 1.33 to 1.96) and among Black men (HR, 2.00; 95% CI, 1.00 to 4.01).

The difference in chr8q gain prevalence by race was mirrored by genetic ancestry. Tumors from men with 100% AFR ancestry had a 13 percentage-point higher prevalence of chr8q gains compared with 0% AFR ancestry (95% CI, 4 to 23). Yet even after adjusting for both self-reported race and genetic ancestry on an individual level, area-level median household income was associated with chr8q gain (Fig. 4D). For each \$50,000/year increase, men of the same self-reported race and genetic ancestry were 2 percentage points less likely to have tumors with chr8q gain (95% CI, -4 to 0). Conversely, adjusting for community-level income reduced the above-noted difference in chr8q gains from 13 to 10 percentage points (95% CI, 0 to 20).

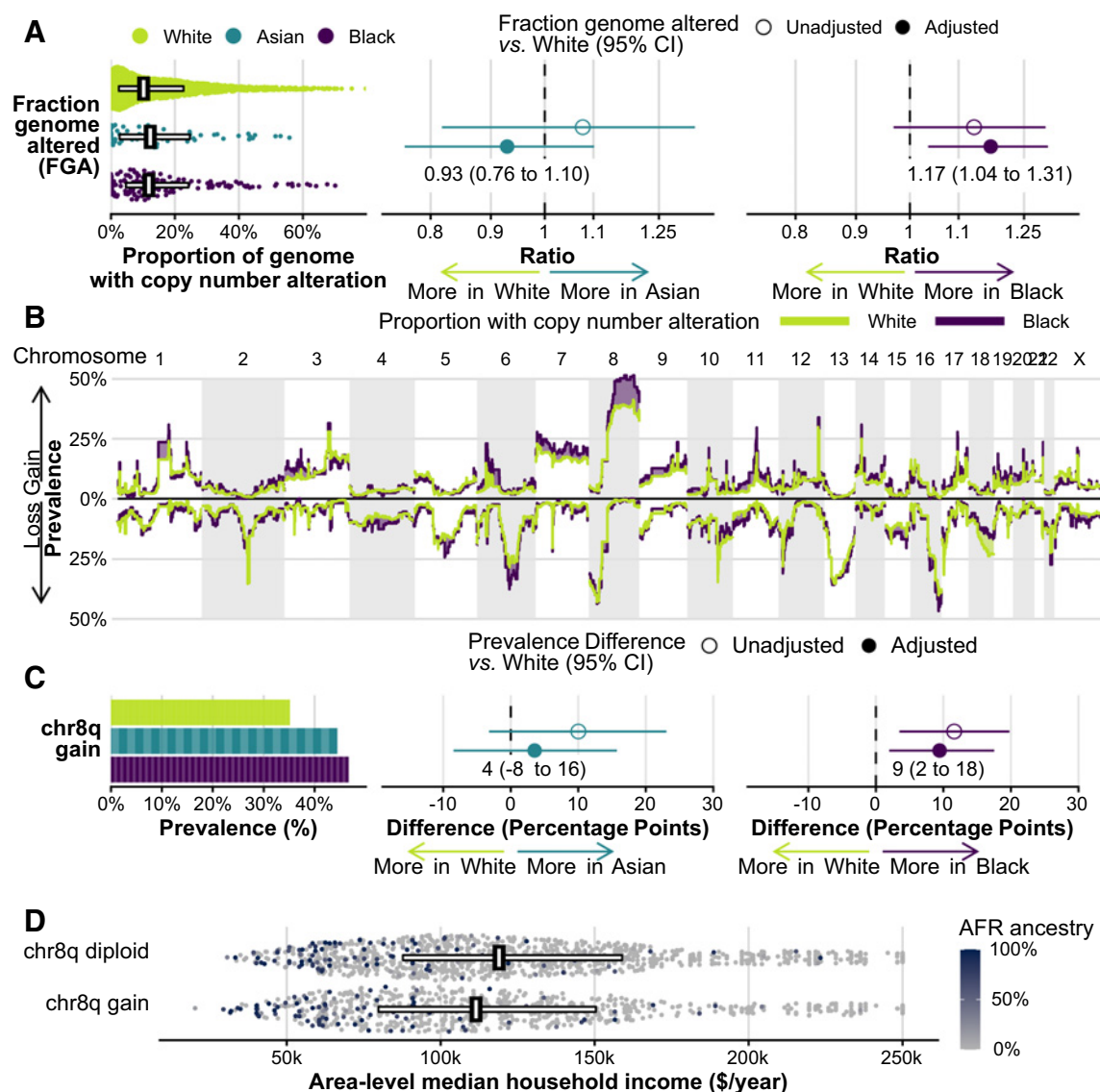
Discussion

Even within a group of men that ultimately had access to healthcare and tumor sequencing at the same cancer center, clinical factors and cancer risk factors differed noticeably by self-reported race and ancestry. These features are an essential part of the perspective on differences in tumor genomics. Tumor genomics still differed between men with similar prostate cancer clinical features but different self-reported race or genetic ancestry, in particular, prevalence of *AR* and *BRAF* alterations, class-1 *FOXA1* mutations, *ZFH3* alterations, mutations of *PTEN*, missense mutations in *TP53*, and gains of chromosome arm 8q. Despite fewer *TP53* alterations, tumors from Black men and those with greater AFR ancestry had more aneuploidy at chr8q, which may contribute to their aggressiveness. Our results suggest that self-reported race carries some information on what tumor genomic profiles to expect in clinic before a sequencing test is obtained. They also highlight the need for targeted treatments for genomic alterations more common in non-White men.

Our findings on differences in alteration prevalence of commonly altered genes in prostate cancer are generally consistent with previous studies (8–10, 25). Some men in this study were included in a previous study (8) through Project GENIE (26). Our data are thus not an independent validation that alteration prevalence differs. Instead, the importance of our findings is the demonstration that most differences persist when restricting to oncogenic driver alterations, when accounting for different clinical features between men with different race, and when considering genetic ancestry.

The higher prevalence of *AR* and *BRAF* alterations in tumors from Black men is directly relevant in light of currently available targeted therapies (8). *FOXA1*, in which class-1 mutations in the DNA-binding forkhead domain were more common among tumors from Asian men, has been described functionally (22) but is not yet therapeutically actionable. In contrast to the class-1 *FOXA1* mutations that occur early during carcinogenesis (22), a previously noted higher prevalence of (generally late-occurring) *AR* alterations in tumors from Asian men (8) was explained by more advanced disease among Asian men and thus should not influence clinical decision making. A lower prevalence of *PTEN* alterations in non-White men is relevant for PI3K inhibitors, which have reached phase III testing in prostate cancer (27).

Ancestry differences in aneuploidy, albeit in varying directions, have been observed in other cancer types (28). In our study, tumors with fewer *TP53* alterations in Black men, specifically missense mutations, would be expected to have fewer chromosome arm losses and gains, given the strong association between *TP53* alterations and aneuploidy burden (24). Surprisingly, tumors from Black men and those with higher AFR ancestry were no less aneuploid and instead harbored more chr8q gains, which are a poor prognostic indicator in primary prostate cancer (29) as well as in Black and White men in our study. chr8q gains explain differences in *MYC* alterations. Therapeutic targets on chr8q beyond *MYC* might be particularly relevant for Black men.

**Figure 4.**

Tumor genomic instability. **A**, Fraction genome altered, the proportion of the genome affected by copy-number alterations, by self-reported race. **B**, Segmental copy-number profiles for White men and Black men, showing the proportion of tumors with copy-number gains (upwards) or losses (downwards). Purple-colored areas indicate areas where copy-number alterations are more common among tumors from Black men. **C**, Chromosome arm 8q gain by self-reported race. In **A** and **C**, the left panels show absolute values; one “brick” is one patient. The middle panel, compares tumors from Asian men with tumors from White men; the right panel compares tumors from Black men with tumors from White men, giving the relative fraction genome altered (ratio, with 95% CI, in **A**) and the absolute difference in chr8q gain prevalence (with 95% CI, in **C**) from unadjusted comparisons and from adjusted comparisons that include the clinical factors as in **Fig. 2** plus *TP53* alteration status. **D**, Annual area-level household income, by chr8q status. One point is one individual; color indicates the proportion of AFR ancestry markers; horizontal bars cover the 25th to 75th percentile and vertical bars indicate the median.

Many germline risk loci for prostate cancer are located at chr8q24, and some differ by ancestry (30). In our study, genetic ancestry had similar associations with chr8q gain and the other somatic alterations as did self-reported race. Germline genetics are clearly a contributor to tumor genomics. However, chr8q gains were also associated with a crude measure of socioeconomic status of neighborhoods, which differed strongly between Black and White men and by genetic ancestry. This crude, ecological measure does not allow pinpointing causes, which may include limited education opportunities and social services, neighborhood violence, environmental pollution, food inse-

curity, and many others (12), in addition to individual-level risk factors. Yet that modifiable risk factors have a strong impact is evidenced by prostate cancer rates among Asian-American men, which are lower than among Whites but substantially higher than for men of Asian ancestry living in Asia (31)—without changes in germline genetics upon immigration to the U.S. Our findings illustrate that studies on genetic ancestry as a potential cause of differences in tumor genomics need to consider confounding by nongenetic factors.

The underrepresentation of Asian and Black men in our study partially reflects disparities in access to healthcare. Increasing

representation of racial and ethnic minorities will be important to allow for assessing differences by Hispanic ethnicity, between EAS and SAS ancestries, and by ancestry within each of the ancestry super-populations, particularly between Northern, Eastern, and Western African ancestry that have been subsumed under AFR (11, 32). Other facets of tumor genomics, and a richer set of cancer risk factors, need to be assessed. Even more important are population-based, rather than hospital-based study designs, without which we cannot know the denominator of men at risk of developing cancers with certain genomic features—a prerequisite for causal attributions and thus interventions that address causes, rather than just correlates of racial differences.

Taken together, tumor genomes differed by self-reported race even after accounting for differences in clinical features between men of different race. Cautiously considering race and tumor genomics jointly has potential to contribute to reducing the significant disparities in prostate cancer death rates that exist in the United States today (13). Differences in tumor genomics by race and ancestry should not be assumed to be completely genetically determined and thus immutable in the future.

Authors' Disclosures

S.M. McBride reports grants from Janssen, AstraZeneca, and Roche outside the submitted work. M.J. Morris reports personal fees from Oric Pharmaceuticals, Curium, Athenex, Exelixis, and AstraZeneca outside the submitted work. D.E. Rathkopf reports uncompensated consulting or advisory roles for Genentech/Roche, Janssen Oncology, AstraZeneca, and Myovant, as well as institutional research funding from AstraZeneca, BMS/Celgene, Ferring, Genentech/Roche, Janssen Oncology, Medivation, Millennium, Novartis, Taiho Pharmaceutical, Takeda, TRACON Pharma, and Phosphatin. D.C. Danila reports research support from US Department of Defense, American Society of Clinical Oncology, Prostate Cancer Foundation, Stand Up 2 Cancer, Janssen Research & Development, Astellas, Medivation, Agensys, Genentech, and CreaTV, as well as consultancy for Angle LLT, Axiom LLT, Janssen Research & Development, AstraZeneca, Clovis, Astellas, Medivation, Pfizer, and Agensys. K.A. Autio reports grants from AstraZeneca, Pfizer, Amgen, and Parker Institute for Cancer Immunotherapy outside the submitted work. H.I. Scher reports grants from NCI during the conduct of the study. H.I. Scher also reports personal fees from Asterias Biotherapeutics, Bayer, Ambray Genetics Corp/Konica Minolta Inc, Pfizer Inc, Sun Pharmaceuticals Industries Inc, WCG Oncology, and Elsevier Ltd; grants from Epic Sciences, Illumina Inc, Janssen, Menarini Silicon Biosystems, and Thermo Fisher; and non-financial support from Amgen, ESSA Pharma, Janssen, Menarini Silicon Biosystems, Amgen, Asterias Biotherapeutics, Bayer, ESSA Pharma Inc, Phosphatin, Pfizer Inc, and WCG Oncology outside the submitted work. L.A. Mucci reports grants from AstraZeneca and Janssen, as well as personal fees from Bayer outside the submitted work. D.B. Solit reports personal fees from Pfizer, Loxo/Lilly Oncology, Vividion Therapeutics, Scorpion Therapeutics, FORE Therapeutics, and BridgeBio outside the submitted work. Y. Chen reports

grants from NCI during the conduct of the study, as well as other support from Oric Pharmaceuticals outside the submitted work. M.F. Berger reports personal fees from Eli Lilly and personal fees from PetDx outside the submitted work. W. Abida reports personal fees and other support from Clovis Oncology and ORIC Pharmaceuticals; personal fees from Janssen, Daiichi Sankyo, OncLive, Roche, Medscape, and Aptitude Health; other support from AstraZeneca, Zenith Epigenetics, and Epizyme outside the submitted work. No disclosures were reported by the other authors.

Authors' Contributions

K.H. Stopsack: Conceptualization, data curation, software, formal analysis, funding acquisition, visualization, writing—original draft, writing—review and editing. **S. Nandakumar:** Data curation, investigation, visualization, writing—review and editing. **K. Arora:** Data curation, formal analysis, methodology, writing—review and editing. **B. Nguyen:** Data curation, investigation, writing—review and editing. **S.E. Vasselmann:** Data curation, writing—review and editing. **B. Nweji:** Data curation, writing—review and editing. **S.M. McBride:** Resources, funding acquisition, writing—review and editing. **M.J. Morris:** Resources, writing—review and editing. **D.E. Rathkopf:** Resources, writing—review and editing. **S.F. Slovin:** Resources, writing—review and editing. **D.C. Danila:** Resources, writing—review and editing. **K.A. Autio:** Resources, writing—review and editing. **H.I. Scher:** Resources, funding acquisition, writing—review and editing. **L.A. Mucci:** Investigation, writing—review and editing. **D.B. Solit:** Resources, funding acquisition, writing—review and editing. **M. Gönen:** Validation, writing—review and editing. **Y. Chen:** Resources, writing—review and editing. **M.F. Berger:** Methodology, writing—review and editing. **N. Schultz:** Resources, supervision, investigation, writing—review and editing. **W. Abida:** Resources, data curation, supervision, funding acquisition, writing—review and editing. **P.W. Kantoff:** Resources, supervision, funding acquisition, writing—review and editing.

Acknowledgments

This work was funded in part by the NCI (1P01CA228696, to P.W. Kantoff; P30CA008748, Cancer Center Support grant; P50CA092629, Prostate Cancer SPORE), the Department of Defense (Early Investigator Research Award W81XWH-18-1-0330, to K.H. Stopsack; Physician Research Award W81XWH-17-1-0124, to W. Abida), and the Marie-Josée and Henry R. Kravis Center for Molecular Oncology. K.H. Stopsack, S.M. McBride, D.E. Rathkopf, L.A. Mucci, N. Schultz, and W. Abida are Prostate Cancer Foundation Young Investigators. We are grateful for contributions of the Molecular Diagnostics Service and Marc Ladanyi, MD, to the MSK-IMPACT platform.

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Note

Supplementary data for this article are available at Clinical Cancer Research Online (<http://clincancerres.aacrjournals.org/>).

Received July 15, 2021; revised August 23, 2021; accepted October 12, 2021; published first October 19, 2021.

References

- Howlader N, Noone AM, Krapcho M, Miller D, Brest A, Yu M, et al. SEER cancer statistics review, 1975–2017. National Cancer Institute. [cited 2020 Feb 8]. Available from: https://seer.cancer.gov/csr/1975_2017.
- Hjelmberg JB, Scheike T, Holst K, Skythe A, Penney KL, Graff RE, et al. The heritability of prostate cancer in the Nordic Twin Study of Cancer. *Cancer Epidemiol Biomarkers Prev* 2014;23:2303–10.
- Conti DV, Darst BF, Moss LC, Saunders EJ, Sheng X, Chou A, et al. Trans-ancestry genome-wide association meta-analysis of prostate cancer identifies new susceptibility loci and informs genetic risk prediction. *Nat Genet* 2021;53:65–75.
- Kenfield SA, Stampfer MJ, Chan JM, Giovannucci E. Smoking and prostate cancer survival and recurrence. *JAMA* 2011;305:2548–55.
- Cornelius ME, Wang TW, Jamal A, Loretan CG, Neff LJ. Tobacco product use among adults - United States, 2019. *MMWR Morb Mortal Wkly Rep* 2020;69:1736–42.
- Awasthi S, Berglund A, Abraham-Miranda J, Rounbehler RJ, Kensler K, Serna A, et al. Comparative genomics reveals distinct immune-oncologic pathways in African American men with prostate cancer. *Clin Cancer Res* 2021;27:320–9.
- Li J, Xu C, Lee HJ, Ren S, Zi X, Zhang Z, et al. A genomic and epigenomic atlas of prostate cancer in Asian populations. *Nature* 2020;580:93–9.
- Mahal BA, Alshalalfa M, Kensler KH, Chowdhury-Paulino I, Kantoff P, Mucci LA, et al. Racial differences in genomic profiling of prostate cancer. *N Engl J Med* 2020;383:1083–5.
- Yuan J, Kensler KH, Hu Z, Zhang Y, Zhang T, Jiang J, et al. Integrative comparison of the genomic and transcriptomic landscape between prostate cancer patients of predominantly African or European genetic ancestry. *PLoS Genet* 2020;16:e1008641.

10. Koga Y, Song H, Chalmers ZR, Newberg J, Kim E, Carrot-Zhang J, et al. Genomic profiling of prostate cancers from men with African and European ancestry. *Clin Cancer Res* 2020;26:4651–60.
11. Davis MB. Genomics and cancer disparities: the justice and power of inclusion. *Cancer Discov* 2021;11:805–9.
12. Rebbeck TR. Prostate cancer disparities by race and ethnicity: from nucleotide to neighborhood. *Cold Spring Harb Perspect Med* 2018;8:a030387.
13. Borrell LN, Elhawary JR, Fuentes-Afflick E, Witonsky J, Bhakta N, Wu AHB, et al. Race and genetic ancestry in medicine - a time for reckoning with racism. *N Engl J Med* 2021;384:474–80.
14. Keegan NM, Vasselmann SE, Barnett ES, Nweji B, Carbone EA, Blum A, et al. Clinical annotations for prostate cancer research: defining data elements, creating a reproducible analytical pipeline, and assessing data quality. *medRxiv* 2021. doi: <https://doi.org/10.1101/2021.09.20.21263842>.
15. Cheng DT, Mitchell TN, Zehir A, Shah RH, Benayed R, Syed A, et al. Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT): a hybridization capture-based next-generation sequencing clinical assay for solid tumor molecular oncology. *J Mol Diagn* 2015;17:251–64.
16. Pettersson A, Graff RE, Bauer SR, Pitt MJ, Lis RT, Stack EC, et al. The TMPRSS2:ERG rearrangement, ERG expression, and prostate cancer outcomes: a cohort study and meta-analysis. *Cancer Epidemiol Biomarkers Prev* 2012;21:1497–509.
17. Chakravarty D, Gao J, Phillips SM, Kundra R, Zhang H, Wang J, et al. OncoKB: a precision oncology knowledge base. *JCO Precis Oncol* 2017;2017:PO.17.00011.
18. Spurr LF, Touat M, Taylor AM, Dubuc AM, Shih J, Meredith DM, et al. Quantification of aneuploidy in targeted sequencing data using ASCETS. *Bioinformatics* 2021;37:2461–3.
19. Alexander DH, Novembre J, Lange K. Fast model-based estimation of ancestry in unrelated individuals. *Genome Res* 2009;19:1655–64.
20. Stopsack KH. risks: Estimating risk ratios and risk differences using regression. Available from: <https://stopsack.github.io/risks/>.
21. Ward E, Jemal A, Cokkinides V, Singh GK, Cardinez C, Ghafoor A, et al. Cancer disparities by race/ethnicity and socioeconomic status. *CA Cancer J Clin* 2004;54:78–93.
22. Parolia A, Cieslik M, Chu SC, Xiao L, Ouchi T, Zhang Y, et al. Distinct structural classes of activating FOXA1 alterations in advanced prostate cancer. *Nature* 2019;571:413–8.
23. Sanchez-Vega F, Mina M, Armenia J, Chatila WK, Luna A, La KC, et al. Oncogenic signaling pathways in The Cancer Genome Atlas. *Cell* 2018;173:321–37.
24. Taylor AM, Shih J, Ha G, Gao GF, Zhang X, Berger AC, et al. Genomic and functional approaches to understanding cancer aneuploidy. *Cancer Cell* 2018;33:676–89.
25. Petrovics G, Li H, Stumpel T, Tan SH, Young D, Katta S, et al. A novel genomic alteration of LSAMP associates with aggressive prostate cancer in African American men. *EBioMedicine* 2015;2:1957–64.
26. AACR Project GENIE Consortium. AACR project GENIE: powering precision medicine through an international consortium. *Cancer Discov* 2017;7:818–31.
27. de Bono JS, Bracarda S, Sternberg CN, Chi KN, Olmos D, Sandhu S, et al. LBA4 IPATential150: Phase III study of ipatasertib (ipat) plus abiraterone (abi) vs placebo (pbo) plus abi in metastatic castration-resistant prostate cancer (mCRPC). *Ann Oncol* 2020;31:S1153–S4.
28. Yuan J, Hu Z, Mahal BA, Zhao SD, Kensler KH, Pi J, et al. Integrated analysis of genetic ancestry and genomic alterations across cancers. *Cancer Cell* 2018;34:549–60.
29. Stopsack KH, Whittaker CA, Gerke TA, Loda M, Kantoff PW, Mucci LA, et al. Aneuploidy drives lethal progression in prostate cancer. *Proc Natl Acad Sci U S A* 2019;116:11390–5.
30. Troutman SM, Sissung TM, Cropp CD, Venzon DJ, Spencer SD, Adesunloye BA, et al. Racial disparities in the association between variants on 8q24 and prostate cancer: a systematic review and meta-analysis. *Oncologist* 2012;17:312–20.
31. Yu H, Harris RE, Gao YT, Gao R, Wynder EL. Comparative epidemiology of cancers of the colon, rectum, prostate and breast in Shanghai, China versus the United States. *Int J Epidemiol* 1991;20:76–81.
32. Oni-Orisan A, Mavura Y, Banda Y, Thornton TA, Sebore R. Embracing genetic diversity to improve Black health. *N Engl J Med* 2021;384:1163–7.

Rucaparib in Men With Metastatic Castration-Resistant Prostate Cancer Harboring a *BRCA1* or *BRCA2* Gene Alteration

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PURPOSE *BRCA1* or *BRCA2* (*BRCA*) alterations are common in men with metastatic castration-resistant prostate cancer (mCRPC) and may confer sensitivity to poly(ADP-ribose) polymerase inhibitors. We present results from patients with mCRPC associated with a *BRCA* alteration treated with rucaparib 600 mg twice daily in the phase II TRITON2 study.

METHODS We enrolled patients who progressed after one to two lines of next-generation androgen receptor-directed therapy and one taxane-based chemotherapy for mCRPC. Efficacy and safety populations included patients with a deleterious *BRCA* alteration who received ≥ 1 dose of rucaparib. Key efficacy end points were objective response rate (ORR; per RECIST/Prostate Cancer Clinical Trials Working Group 3 in patients with measurable disease as assessed by blinded, independent radiology review and by investigators) and locally assessed prostate-specific antigen (PSA) response ($\geq 50\%$ decrease from baseline) rate.

RESULTS Efficacy and safety populations included 115 patients with a *BRCA* alteration with or without measurable disease. Confirmed ORRs per independent radiology review and investigator assessment were 43.5% (95% CI, 31.0% to 56.7%; 27 of 62 patients) and 50.8% (95% CI, 38.1% to 63.4%; 33 of 65 patients), respectively. The confirmed PSA response rate was 54.8% (95% CI, 45.2% to 64.1%; 63 of 115 patients). ORRs were similar for patients with a germline or somatic *BRCA* alteration and for patients with a *BRCA1* or *BRCA2* alteration, while a higher PSA response rate was observed in patients with a *BRCA2* alteration. The most frequent grade ≥ 3 treatment-emergent adverse event was anemia (25.2%; 29 of 115 patients).

CONCLUSION Rucaparib has antitumor activity in patients with mCRPC and a deleterious *BRCA* alteration, but with a manageable safety profile consistent with that reported in other solid tumor types.

J Clin Oncol 38:3763-3772. © 2020 by American Society of Clinical Oncology

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ASSOCIATED CONTENT

See accompanying editorial on page 3735

Data Supplement Protocol

Author affiliations and support information (if applicable) appear at the end of this article.

Accepted on July 8, 2020 and published at ascopubs.org/journal/jco on August 14, 2020; DOI <https://doi.org/10.1200/JCO.20.01035>

Written on behalf of the TRITON2 investigators.

INTRODUCTION

Therapies such as androgen receptor (AR)-directed therapy and taxane chemotherapy have led to improved outcomes for men with metastatic castration-resistant prostate cancer (mCRPC).¹⁻⁴ However, patients will eventually progress, and subsequent treatment options are limited, highlighting the need for additional effective therapies.

Approximately 12% of men with mCRPC harbor a deleterious *BRCA1* or *BRCA2* (*BRCA*) alteration (*BRCA1*, 2%; *BRCA2*, 10%).⁵ Men with a germline *BRCA* alteration have an increased risk for prostate cancer and more commonly have nodal involvement and/or distant metastases.^{6,7} Poly(ADP-ribose) polymerase (PARP) inhibitors can induce cytotoxicity via synthetic lethality in tumor

cells that are deficient in homologous recombination-directed DNA damage repair (DDR), including those carrying loss-of-function alterations in *BRCA* genes.⁸⁻¹⁰

The phase II TRITON2 study is evaluating the PARP inhibitor rucaparib for the treatment of men with mCRPC associated with a deleterious alteration in *BRCA* or other DDR gene who have progressed after next-generation AR-directed therapy and a taxane-based chemotherapy. Here, we present efficacy and safety data from TRITON2 for patients with mCRPC with a *BRCA* alteration.

METHODS

TRITON2 (ClinicalTrials.gov identifier: [NCT02952534](https://clinicaltrials.gov/ct2/show/study/NCT02952534)) is a fully enrolled, ongoing, international, open-label,

CONTEXT

Key Objective

We evaluated rucaparib as treatment for men with metastatic castration-resistant prostate cancer (mCRPC) associated with a *BRCA* gene alteration who had received prior taxane and androgen receptor–directed therapy.

Knowledge Generated

A substantial proportion of patients achieved a confirmed radiographic response with rucaparib treatment in both blinded, central independent radiology review– and investigator-assessed analyses. Furthermore, we provide evidence of radiographic and prostate-specific antigen responses across subgroups based on baseline characteristics (eg, number of prior lines of therapy) and genomic characteristics (eg, gene, zygosity, and alteration types).

Relevance

Men with mCRPC and a *BRCA* alteration who receive a poly(ADP-ribose) polymerase inhibitor in this setting achieve higher objective and prostate-specific antigen response rates than those observed with previously approved therapies in an unselected population. These data illustrate the potential benefit of rucaparib in patients with mCRPC associated with a *BRCA* alteration.

phase II study evaluating rucaparib in patients with mCRPC associated with DDR deficiency. Men aged ≥ 18 years with histologically or cytologically confirmed mCRPC, Eastern Cooperative Oncology Group performance status of 0 or 1, and adequate organ function were enrolled. Eligible patients had a deleterious germline or somatic alteration in *BRCA1*, *BRCA2*, or another prespecified DDR gene that may confer sensitivity to PARP inhibition, as well as disease progression after one to two lines of next-generation AR-directed therapy for prostate cancer and one prior taxane-based chemotherapy for castration-resistant disease. Patients were required to receive a concomitant gonadotropin-releasing hormone analog or to have had prior bilateral orchiectomy. Patients who were previously treated with a PARP inhibitor, mitoxantrone, cyclophosphamide, or platinum-based chemotherapy or with an active secondary malignancy were excluded. Patients were enrolled irrespective of measurable disease status (Data Supplement). Full eligibility criteria are described in the protocol (Data Supplement).

Patients received a starting dose of 600 mg oral rucaparib twice daily. Dose reductions, in decrements of 100 mg, were permitted for grade ≥ 3 or persistent grade 2 treatment-emergent adverse events (TEAEs).

The study was approved by national or local institutional review boards and performed in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines of the International Council for Harmonisation. Patients provided written informed consent before participation.

Patient Populations

Efficacy and safety populations included patients with a deleterious *BRCA* alteration identified before enrollment who received ≥ 1 dose of rucaparib 600 mg. Patients with a non-*BRCA* DDR gene alteration (without a deleterious

BRCA alteration) were not part of this analysis (data reported previously).¹¹

Efficacy was assessed in the overall efficacy population (all evaluable patients regardless of measurable disease status), independent radiology review (IRR)–evaluable population (patients who had measurable disease at baseline per blinded, central IRR assessment), and investigator–evaluable population (patients who had measurable disease at baseline per investigator assessment).

Efficacy and safety analyses included all patients meeting the above criteria enrolled by May 8, 2019. The visit cutoff date for safety analyses was September 13, 2019. The visit cutoff date for efficacy analyses was December 23, 2019, to allow for more complete assessment (≥ 32 weeks of follow up) of the efficacy end points.

Analysis Outcomes

The primary end point was objective response rate (ORR) by blinded IRR per modified RECIST v1.1 and Prostate Cancer Clinical Trials Working Group 3 (PCWG3) criteria (IRR-evaluable population), supported by confirmed ORR by investigator assessment (investigator-evaluable population).

Secondary end points included duration of response (DOR) for radiographic response, rate of confirmed locally assessed prostate-specific antigen (PSA) response ($\geq 50\%$ decrease from baseline confirmed by a consecutive measurement ≥ 3 weeks later), time to PSA progression, radiographic progression-free survival (rPFS), overall survival (OS), and safety.

Exploratory subgroup analyses of confirmed ORR and confirmed PSA response rate were performed based on baseline disease characteristics (number of prior lines of therapy, measurable disease status, presence of hepatic metastases, and age) and genomic characteristics (*BRCA1*

or *BRCA2*, germline or somatic alteration, zygosity, and alteration types).

Safety was assessed by monitoring TEAEs, vital signs, laboratory testing, and physical examination. Dose intensity was calculated as the actual dose received divided by the first dose.

Procedures

Patients were screened for the presence of a deleterious somatic or germline alteration in *BRCA1*, *BRCA2*, or other DDR gene through central genomic testing of plasma or tumor tissue (archival or contemporaneous), or through local testing. Central testing was performed by Foundation Medicine.^{12,13} Germline testing was performed by Color Genomics.^{14,15} Additional details are provided in the Data Supplement.

Patients received rucaparib until radiographic disease progression (soft tissue or bone lesion) assessed by investigator per modified RECIST/PCWG3 criteria, unequivocal clinical progression, unacceptable toxicity or inability to tolerate additional treatment, loss to follow up, or withdrawal of consent. Tumor assessments by computed tomography or magnetic resonance imaging and bone scans were performed during screening, every 8 weeks for 24 weeks, then every 12 weeks thereafter. PSA assessments were conducted every 4 weeks.

TEAEs were monitored from the first dose of rucaparib until 28 days after the last dose. TEAEs were coded using Medical Dictionary for Drug Regulatory Activities v20.1¹⁶ and graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events v4.03.¹⁷ Additional details are provided in the Data Supplement.

Statistical Analyses

A Simon 2-stage design was used as a futility rule. Assuming a total sample size of 83 patients in the IRR-evaluable population, characteristics of the design included a null hypothesis of ORR = 20%, type I error rate of 5% (one sided), and 90% power when the true response rate is 35%. However, the final number of patients was to be defined based on regulatory considerations. Confirmed ORR and PSA responses were summarized descriptively with frequencies and 95% CIs (Clopper-Pearson).

DOR, time to PSA progression, and rPFS were summarized using Kaplan-Meier methodology. DOR was defined as the time from the date of the first confirmed response to the date progression was first documented plus 1 day. Time to PSA progression was defined as the time from the first rucaparib dose to the date of an increase $\geq 25\%$ and absolute increase of ≥ 2 ng/mL above the nadir in PSA plus 1 day. PSA increases must have been confirmed by a consecutive assessment conducted ≥ 3 weeks later; early rises (< 12 weeks) were not considered in determining PSA progression.¹⁸ rPFS was defined as the time from first rucaparib dose to the date of first objective

evidence of radiographic progression or death due to any cause, whichever occurred first.

Best change from baseline in the sum of the diameter of target lesions and PSA were summarized graphically. Statistical analyses were performed using SAS v9.4 (SAS Institute, Cary, NC).

RESULTS

Efficacy

The overall efficacy population included 115 patients who received ≥ 1 dose of rucaparib and had a deleterious *BRCA* alteration (*BRCA1* [n = 13], *BRCA2* [n = 102], germline [n = 44], and somatic [n = 71]; Data Supplement). Baseline patient demographics and disease characteristics are shown in Table 1 and the Data Supplement. At baseline, 62 patients had measurable disease per blinded IRR assessment (IRR-evaluable population) and 65 patients had measurable disease per investigator assessment (investigator-evaluable population); 57 patients had measurable disease by both IRR and investigator. Genomic characteristics (eg, germline/somatic status, zygosity, and alteration type) and co-occurring alterations are shown in the Data Supplement.

Median treatment duration for the overall efficacy population was 8.1 months (range, 0.5-30.3 months), and the median follow up was 17.1 months (range, 7.6-31.5 months). As of December 23, 2019, 29 patients (25.2%) remained on treatment.

The confirmed ORR for the IRR-evaluable population was 43.5% (95% CI, 31.0% to 56.7%; 27 of 62), and the confirmed ORR for the investigator-evaluable population was 50.8% (95% CI, 38.1% to 63.4%; 33 of 65; Table 2). Most patients had a best response of stable disease or better (88.7% in the IRR-evaluable population and 89.2% in the investigator-evaluable population). Eight patients had a confirmed complete response in soft-tissue disease per blinded IRR and/or investigator assessment. Among these patients, one had hepatic metastases, one had a non-nodal pelvic mass, and 6 had nodal-only disease at baseline per IRR; one had a *BRCA1* alteration and seven had a *BRCA2* alteration. Among responders in the IRR-evaluable population, 19 of 27 patients (70.4%) demonstrated a response by the first tumor assessment (week 8; Data Supplement). Median DOR in the IRR-evaluable population was not reached (NR; 95% CI, 6.4 months to NR; Data Supplement) and was 6.4 months in the investigator-evaluable population (95% CI, 5.5 to 11.7 months; Data Supplement). Within the IRR-evaluable population, 15 of 27 patients with a confirmed objective response had a DOR ≥ 6 months; three patients with ongoing responses were followed for < 6 months from the onset of response. In the IRR- and investigator-evaluable populations, 64.5% and 63.1% of patients demonstrated

TABLE 1. Baseline Patient and Disease Characteristics in TRITON2 Patients With a *BRCA* Alteration

Characteristic	Value (N = 115)
Median age, years (range)	72.0 (50-88)
Race, No. (%)	
White	85 (73.9)
Black or African American	7 (6.1)
Other	2 (1.7)
Unknown	21 (18.3)
ECOG PS, No. (%)	
0	37 (32.2)
1	76 (66.1)
≥ 2	2 (1.7)
Median baseline PSA, ng/mL (range)	61.1 (0.0-4,782.0)
Median baseline ALP, U/L (range)	98.0 (34.0-5,490.0)
Median baseline albumin, g/L (range)	39.0 (24.0-49.0)
Median baseline LDH, U/L (range)	240 (84-2,058)
Gleason score ≥ 8 at diagnosis, No. (%)	77 (67.0)
Median No. of prior CRPC therapies (range) ^a	2 (1-8)
Prior therapies, No. (%) ^b	
Next-generation AR-directed therapy	
Abiraterone acetate	74 (64.3)
Enzalutamide	82 (71.3)
Apalutamide	3 (2.6)
Darolutamide	0
≥ 2 AR-directed therapies	42 (36.5)
Docetaxel (castration sensitive)	17 (14.8)
Docetaxel (castration resistant)	108 (93.9)
Cabazitaxel	8 (7.0)
Sipuleucel-T	12 (10.4)
Radium-223	14 (12.2)
Measurable disease (per blinded IRR), No. (%) ^c	
Measurable disease	62 (53.9)
Only measurable nodal disease	41 (66.1)
Measurable visceral ± nodal disease	21 (33.9)
Nonmeasurable disease	53 (46.1)
Bone-only disease	36 (67.9)
Other nonmeasurable disease	17 (32.1)
Site of metastases (per blinded IRR), No. (%)	
Hepatic	14 (12.2)
Lung	14 (12.2)
≥ 10 bone lesions (per blinded IRR), No. (%)	54 (47.0)

NOTE. Visit cutoff date: December 23, 2019.

Abbreviations: AR, androgen receptor; CRPC, castration-resistant prostate cancer; ECOG PS, Eastern Cooperative Oncology Group Performance Status; LDH, lactate dehydrogenase; IRR, independent radiology review; PSA, prostate-specific antigen.

^aDoes not include luteinizing hormone–releasing hormone analogs, first-generation antiandrogens, hormones, corticosteroids, bone-targeted agents, hematopoietic growth factors, or docetaxel administered for castration-sensitive disease (per protocol, docetaxel in this setting was not counted toward the number of prior metastatic CRPC therapies for study eligibility).

^bCategories are not mutually exclusive.

^cBased on modified RECIST/Prostate Cancer Clinical Trials Working Group 3 criteria.

a ≥ 30% reduction in target lesion size from baseline (Fig 1A and Data Supplement).

In the overall efficacy population, 63 of 115 patients had a confirmed PSA response (54.8%; 95% CI, 45.2% to 64.1%; Table 2), and median time to PSA response was 1.9 months (95% CI, 1.3 to 1.9 months). Median time to PSA progression was 6.5 months (95% CI, 5.9 to 7.8 months; Data Supplement). The majority of patients (60.0%) demonstrated a single best PSA reduction ≥ 50% from baseline (Fig 1B).

Although ORRs were similar across subgroups based on baseline clinical or genomic characteristics, some differences were observed with PSA response rates (Fig 2 and Data Supplement). PSA responses were observed among patients with *BRCA1* (15.4%; 2 of 13 patients) or mono-allelic alterations (11.1%; 1 of 9 patients), albeit at a lower rate than the overall population; however, the number of patients in these subgroups was low. Conversely, a higher proportion of patients with biallelic alterations (75.0%; 27 of 36 patients) and/or homozygous loss (81.0%; 17 of 21 patients) experienced a PSA response compared with the overall population.

Median rPFS was 9.0 months (95% CI, 8.3 to 13.5 months) per blinded IRR assessment (Fig 3) and 8.5 months (95% CI, 8.1 to 11.2 months) per investigator assessment (Data Supplement). Although OS data were not yet mature at the time of the analysis (41% of events reported), the Kaplan-Meier estimate of 12-month OS was 73.0% (95% CI, 62.9% to 80.7%).

Safety

The safety population included 115 patients who received one or more dose of rucaparib. As of the visit cutoff, median treatment duration was 6.5 months (range, 0.5-26.7 months), and median follow up was 13.7 months (range, 4.2-28.2 months). Mean dose intensity was 0.88 (standard deviation, 0.15).

A TEAE of any grade occurred in 114 patients (99.1%), and a grade ≥ 3 TEAE was reported in 70 patients (60.9%; Table 3 and Data Supplement). The most frequent TEAEs (any grade) were asthenia/fatigue (61.7%), nausea (52.2%), and anemia/decreased hemoglobin (43.5%). The most frequent grade ≥ 3 TEAE was anemia/decreased hemoglobin (25.2%). Overall, 32 patients (27.8%) received ≥ 1 transfusion of packed RBCs. Other TEAEs of interest are presented in the Data Supplement.

ALT/AST elevations (any grade) were observed in 38 patients (33.0%)—the majority were grade 1 or 2. These typically occurred within the first 4 weeks of rucaparib treatment, normalized over time with continued treatment, and were not associated with abnormal increases in bilirubin or other criteria for drug-induced hepatotoxicity (Data Supplement). Increases in creatinine—predominantly grade 1 or 2—were also observed with the initiation of

rucaparib and typically stabilized by the third week (Data Supplement) without being accompanied by a change in blood urea nitrogen. Decreased phosphate was observed in 75 (67.6%) of 111 patients (Data Supplement); however, most patients had low baseline phosphate and/or were receiving concomitant medications associated with decreased phosphate (eg, antacids or bone-targeting agents).

Treatment interruption due to a TEAE occurred in 65 patients (56.5%), most commonly because of anemia/decreased hemoglobin (21.7%), thrombocytopenia/decreased platelets (13.9%), and asthenia/fatigue (9.6%). Dose reduction due to a TEAE occurred in 47 patients (40.9%), most commonly due to anemia/decreased hemoglobin (13.0%), asthenia/fatigue (9.6%), and thrombocytopenia/decreased platelets (7.0%). Overall, 73 patients (63.5%) had either a treatment interruption or dose reduction due to a TEAE.

Nine patients (7.8%) discontinued because of a TEAE, one patient each due to acute respiratory distress syndrome; ALT/AST increased; anemia; balance disorder; cardiac failure; decreased appetite, fatigue, and weight decreased; leukopenia and neutropenia; pneumonia; and prolonged QT. There were three deaths as a result of TEAEs, including one each from pneumonia and prolonged QT, both considered unrelated to rucaparib, and one from acute respiratory distress syndrome, considered related to rucaparib by the investigator (additional details are in the Data Supplement).

DISCUSSION

Rucaparib treatment demonstrated significant clinical activity in men with mCRPC associated with a *BRCA* alteration, resulting in meaningful radiographic and PSA responses, including complete responses in soft-tissue disease, with a manageable safety and tolerability profile. Based on these results, rucaparib was granted accelerated approval by the US Food and Drug Administration for the treatment of men

with a deleterious *BRCA* mutation (germline and/or somatic) associated mCRPC who have been treated with AR-directed therapy and a taxane-based chemotherapy.¹⁹

Baseline characteristics in the efficacy population were as expected for a population of patients with mCRPC receiving third-line or later treatment. More than one third of patients were treated with ≥ 2 next-generation AR-directed therapies.

For men with mCRPC with disease progression after next-generation AR-directed therapy and taxane-based chemotherapy, response to previously approved therapies has been historically poor, with confirmed ORRs ranging from 8%-15% and PSA response ($\geq 50\%$ decrease) rates ranging from 8%-39%.²⁰⁻²² Of note, these data come from studies of men with mCRPC who were not selected based on a potential predictive biomarker (eg, *BRCA* alteration).

Data from TRITON2 illustrate the importance of genomic screening to identify men who may benefit from treatment with a PARP inhibitor.^{2,4,23} TRITON2 patients with mCRPC associated with a *BRCA* alteration who received rucaparib had substantially higher RECIST and PSA response rates than those typically observed with other treatments in an unselected population, including responses in clinically relevant subgroups. For example, although the numbers are small, the confirmed ORR of 46.2% (6 of 13) among patients with hepatic metastases (Fig 2) is potentially clinically important as hepatic metastases are known to be an indicator of poor prognosis in mCRPC.²⁴

RECIST and PSA responses were observed in patients with *BRCA2* and *BRCA1* alterations, germline and somatic alterations, and all categories of alteration zygosity. Although the rates of confirmed PSA response were higher in *BRCA2* versus *BRCA1* and biallelic versus monoallelic subgroups, the numbers of patients in the *BRCA1* and monoallelic subgroups were small. Due to the limited number of tissue

TABLE 2. Rate of Response to Rucaparib Treatment

Response	Investigator-Evaluable Population (n = 65)	IRR-Evaluable Population (n = 62)
Confirmed ORR, No. (%; 95% CI) ^a	33 (50.8; 38.1 to 63.4)	27 (43.5; 31.0 to 56.7)
Complete response	4 (6.2)	7 (11.3)
Partial response	29 (44.6)	20 (32.3)
Stable disease	25 (38.5)	28 (45.2)
Progressive disease	6 (9.2)	6 (9.7)
Not evaluable	1 (1.5)	1 (1.6)
Overall Efficacy Population (n = 115)		
Confirmed PSA response rate, No. (%; 95% CI)	63 (54.8; 45.2 to 64.1)	

NOTE. Data presented as No. (%) unless otherwise indicated. Visit cutoff date: December 23, 2019.

Abbreviations: IRR, independent radiology review; ORR, objective response rate; PSA, prostate-specific antigen.

^aPer modified RECIST/Prostate Cancer Clinical Trials Working Group 3 criteria.

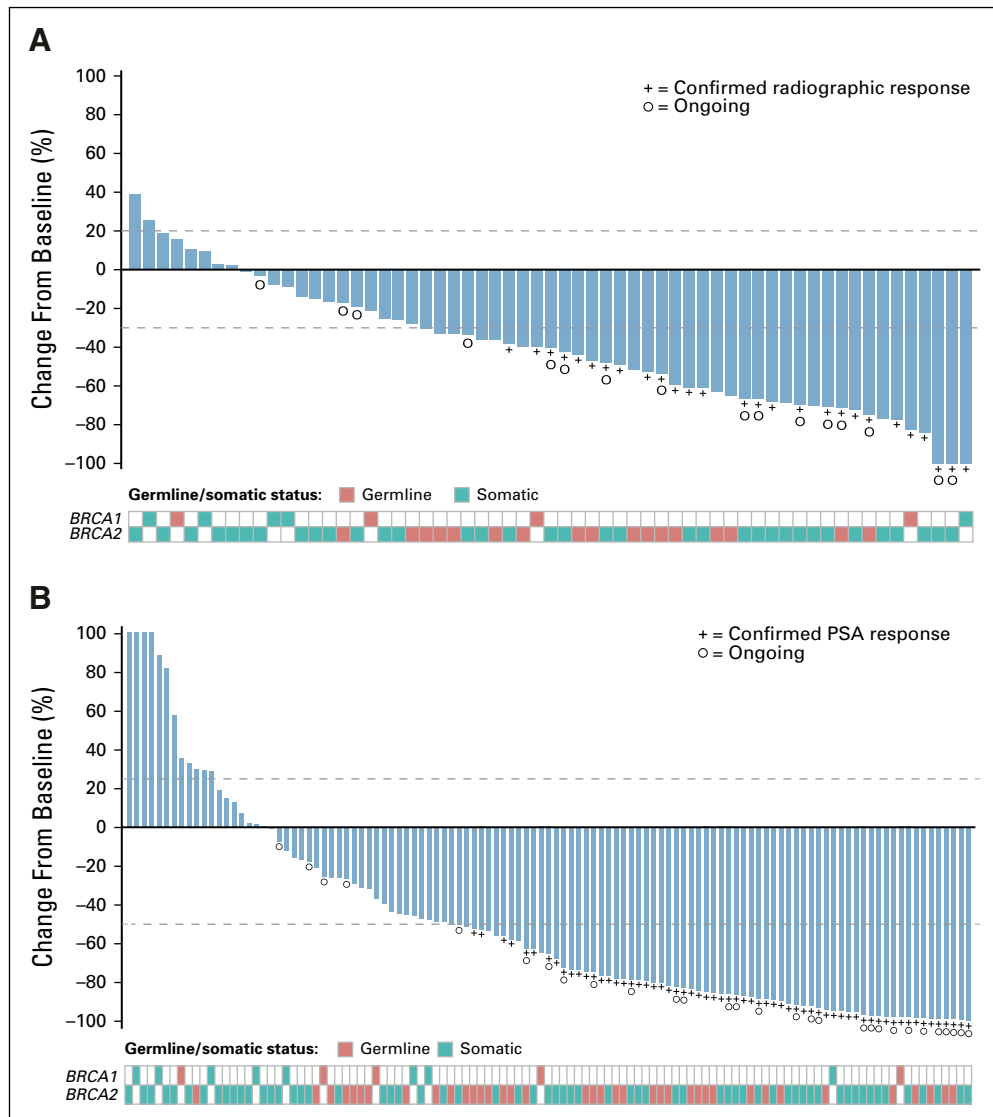


FIG 1. Best change from baseline in (A) sum of target lesion(s) in the independent radiology review–evaluable population and in (B) prostate-specific antigen (PSA) in the overall efficacy population. Visit cutoff date: December 23, 2019. In (A), the upper dotted line indicates the threshold for progressive disease, a 20% increase in the sum of the longest diameter of the target lesions, whereas the lower dotted line indicates the threshold for partial response, a 30% decrease in the sum of the longest diameter of the target lesions. In (B), the upper dotted line indicates the threshold for PSA progression, a 25% increase from baseline (accompanied by an absolute increase of ≥ 2 ng/mL above the nadir), whereas the lower dotted line indicates the threshold for PSA response, a 50% decrease from baseline. Bars were capped at 100% for visual clarity. PSA increases for the 4 leftmost patients were 689%, 231%, 183%, and 133%. In both panels, patients with 0% change from baseline are shown as 0.5% for visual clarity.

samples received (66 of 115 patients [57.4%]), alteration zygosity could only be determined in 45 patients (39.1%) based on the availability of sufficiently high-quality next-generation sequencing data from tissue for copy-number analysis. Importantly, despite the relatively small numbers, confirmed PSA responses were observed in all molecular and clinical subgroups examined.

Our results are consistent with those from other studies demonstrating the clinical activity of PARP inhibitors

(olaparib, niraparib, and talazoparib) in patients with mCRPC and a *BRCA* alteration who received prior AR-directed therapy.²⁵⁻²⁹ For example, in the PROfound study, patients with a *BRCA* alteration and prior AR-directed therapy showed improved rPFS with olaparib versus abiraterone acetate or enzalutamide (median, 9.8 v 3.0 months; hazard ratio, 0.22; 95% CI, 0.15 to 0.32).²⁹ Although there are important differences in the study designs for these trials (eg, differences in the method of determining

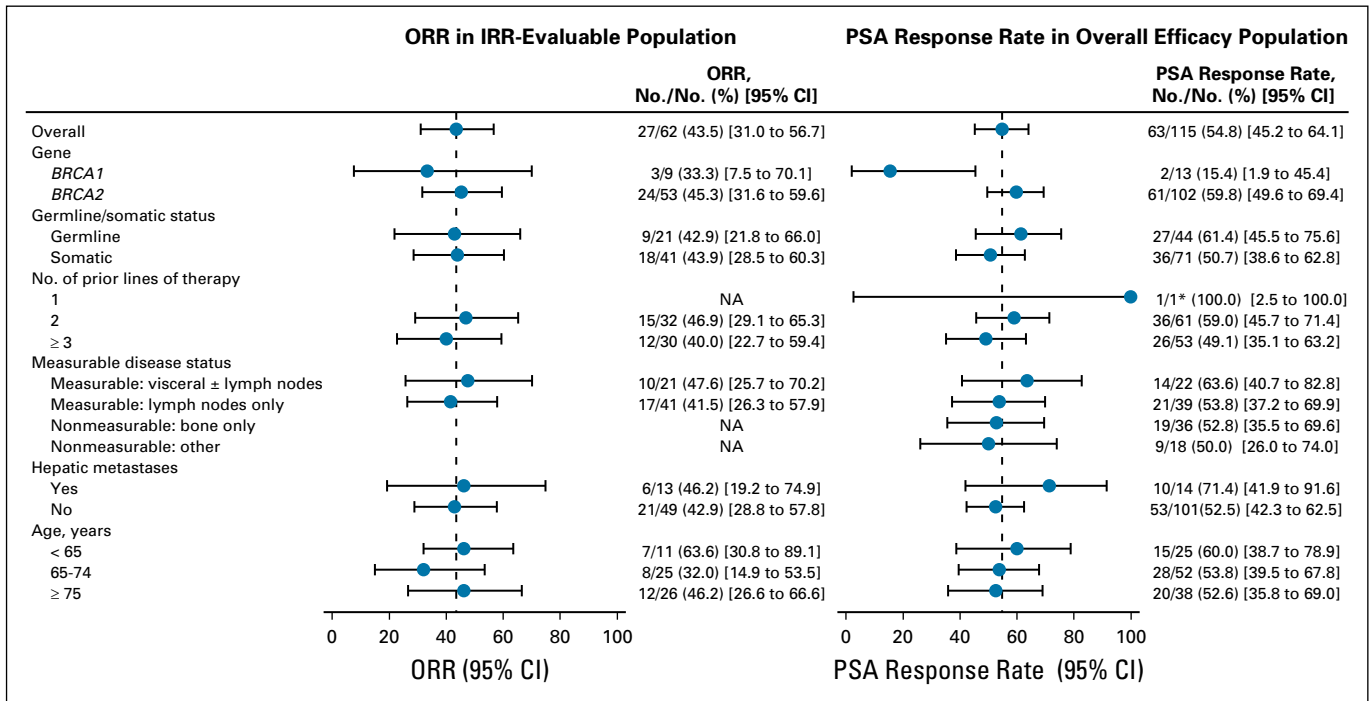


FIG 2. Subgroup analysis of objective response rate (ORR) in independent radiology review (IRR)-evaluable population and prostate-specific antigen (PSA) response rate in overall efficacy population by baseline characteristics. Visit cutoff date: December 23, 2019. The vertical dotted line corresponds to the overall ORR or PSA response. (*)One patient received taxane in the hormone-sensitive setting only, which per protocol was not counted as a line of therapy for eligibility; not receiving taxane for castration-resistant prostate cancer was considered a protocol deviation. NA, not applicable.

genomic alteration, type of alterations eligible for enrollment, assessment of response by RECIST, PSA decrease, or a composite that includes changes in circulating tumor cell count), these studies reinforce the potential benefit of PARP inhibitors in patients with mCRPC associated with a *BRCA* alteration.

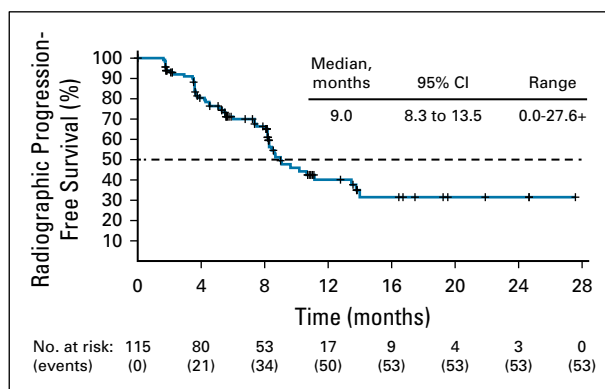


FIG 3. Radiographic progression-free survival by blinded independent radiology review assessment. Visit cutoff date: December 23, 2019. Progression was assessed per modified RECIST/PWCG3 criteria. Details on reasons for censoring are provided in the Data Supplement. IRR, independent radiology review; PCWG3, Prostate Cancer Clinical Trials Working Group 3; RECIST, Response Evaluation Criteria In Solid Tumors version 1.1.

The safety profile of rucaparib in patients with mCRPC was consistent with that observed in prior studies conducted in patients with ovarian cancer and other solid tumor types,^{19,30,31} as well as in studies of men with mCRPC who received other PARP inhibitors, with asthenia/fatigue, GI adverse effects, and myelosuppression among the most common TEAEs reported.^{25-27,29} Similar to studies of rucaparib in ovarian cancer, elevations in ALT, AST, and creatinine were commonly reported; however, these laboratory abnormalities were not associated with liver or kidney toxicity. Elevated creatinine has been observed with multiple PARP inhibitors and is thought to be due to inhibition of renal transporters (eg, MATE-1, MATE2-K, OCT2) rather than a direct impact on renal function.^{19,32-35} Although there have been reports of fatal pneumonitis with other PARP inhibitors,³⁴ interstitial lung disease has not been identified as a potential risk from rucaparib treatment when evaluated across studies in multiple tumor types; the majority of cases had an alternative etiology, and most resolved with continued rucaparib treatment or after dose interruption with negative rechallenge.

A strength of our analysis is the inclusion of both IRR- and investigator-assessed end points; a substantial proportion of patients achieved a confirmed radiographic response with rucaparib treatment via both assessment methods. Discordance in assessment by independent reviewers and investigators may be influenced by variations in lesion

TABLE 3. Most Commonly Reported TEAEs (N = 115)

Individual TEAE (preferred terms) Occurring in ≥ 15% of Patients	Any Grade	Grade ≥ 3
Asthenia/fatigue	71 (61.7)	10 (8.7)
Nausea	60 (52.2)	3 (2.6)
Anemia/decreased hemoglobin	50 (43.5)	29 (25.2)
ALT/AST increased	38 (33.0)	6 (5.2)
Decreased appetite	32 (27.8)	2 (1.7)
Constipation	31 (27.0)	1 (0.9)
Thrombocytopenia/decreased platelets	29 (25.2)	11 (9.6)
Vomiting	25 (21.7)	1 (0.9)
Diarrhea	23 (20.0)	0
Dizziness	21 (18.3)	0
Blood creatinine increased	18 (15.7)	1 (0.9)

NOTE. Data presented as No. (%). Visit cutoff date: September 13, 2019. TEAEs were graded according to National Cancer Institute–Common Terminology Criteria for Adverse Events version 4.03. There were no TEAEs of myelodysplastic syndrome or acute myeloid leukemia reported. Abbreviation: TEAE, treatment-emergent adverse event.

selection or by historical/clinical information available to investigators but not the blinded IRR.³⁶ One limitation of our analysis was that the DOR and rPFS results for the IRR-evaluable population were more heavily impacted by censoring. Per the protocol, radiographic assessments were to be continued until disease progression was observed by the investigator. If the investigator reported disease progression on a scan but the blinded IRR did not, the patient became censored in the IRR analysis. Additional limitations include the lack of a control arm and the immaturity of OS data. The phase III TRITON3 study (ClinicalTrials.gov identifier: [NCT02975934](https://clinicaltrials.gov/ct2/show/study?term=NCT02975934)) is ongoing to define the clinical benefit (eg, rPFS and OS) of rucaparib in an earlier disease setting among patients with

mCRPC associated with a *BRCA* or *ATM* alteration who have progressed after one next-generation AR-directed therapy and who have not received taxane-based chemotherapy in the mCRPC setting. Rucaparib is being compared with physician's choice of next-generation AR-directed therapy or docetaxel and will provide additional evidence of the effects of rucaparib treatment in men with mCRPC.

Altogether, results from the TRITON2 study demonstrate that rucaparib has meaningful antitumor activity and a manageable safety profile in patients with mCRPC, as well as a deleterious germline or somatic *BRCA* alteration, and support the use of rucaparib in this patient population.

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CLINICAL TRIAL

NCT02952534

SUPPORT

Funded by Clovis Oncology; supported in part by the National Cancer Institute (NCI) Cancer Center Support Grant No. P30-CA008748, NCI Prostate Specialized Program of Research Excellence (SPORE) Grant No. P50-CA092629-16, Department of Defense Prostate Cancer Research Program Grant No. W81XWH-17-1-0124, and a Prostate Cancer Foundation Young Investigator Award (W.A.); and supported in part by a Prostate Cancer Foundation Challenge Award and NCI Prostate SPORE Grant No. P50-CA180995 (A.P.).

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST AND DATA AVAILABILITY STATEMENT

Disclosures provided by the authors and data availability statement (if applicable) are available with this article at DOI <https://doi.org/10.1200/JCO.20.01035>.

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ACKNOWLEDGMENT

The authors thank Cheryl Chun and Vivian Chen of Clovis Oncology for assistance in manuscript preparation. Medical writing and editorial support funded by Clovis Oncology were provided by Nathan Yardley and Frederique H. Evans of Ashfield Healthcare Communications.

REFERENCES

1. National Comprehensive Cancer Network: NCCN Clinical Practice Guidelines in Oncology: Prostate cancer (version 2.2020). https://www.nccn.org/professionals/physician_gls/pdf/prostate.pdf
2. Parker C, Gillessen S, Heidenreich A, et al: Cancer of the prostate: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 26: v69-v77, 2015
3. European Association of Urology: Guidelines: Prostate cancer. <http://uroweb.org/guideline/prostate-cancer/>
4. Gillessen S, Attard G, Beer TM, et al: Management of patients with advanced prostate cancer: Report of the Advanced Prostate Cancer Consensus Conference 2019. *Eur Urol* 77:508-547, 2020
5. Abida W, Armenia J, Gopalan A, et al: Prospective genomic profiling of prostate cancer across disease states reveals germline and somatic alterations that may affect clinical decision making. *JCO Precis Oncol* 2017:1-16, 2017
6. Castro E, Goh C, Olmos D, et al: Germline BRCA mutations are associated with higher risk of nodal involvement, distant metastasis, and poor survival outcomes in prostate cancer. *J Clin Oncol* 31:1748-1757, 2013
7. Bancroft EK, Page EC, Castro E, et al: Targeted prostate cancer screening in BRCA1 and BRCA2 mutation carriers: Results from the initial screening round of the IMPACT study. *Eur Urol* 66:489-499, 2014 [Erratum: *Eur Urol* 67:e126, 2015]
8. Drew Y, Mulligan EA, Vong WT, et al: Therapeutic potential of poly(ADP-ribose) polymerase inhibitor AG014699 in human cancers with mutated or methylated BRCA1 or BRCA2. *J Natl Cancer Inst* 103:334-346, 2011
9. Nguyen M, Simmons AD, Harding TC: Preclinical assessment of the PARP inhibitor rucaparib in homologous recombination deficient prostate cancer models. *Cancer Res* 77, 2017 (abstr 2476)
10. Robillard L, Nguyen M, Harding TC, et al: In vitro and in vivo assessment of the mechanism of action of the PARP inhibitor rucaparib. *Cancer Res* 77, 2017 (abstr 2475)
11. Abida W, Campbell D, Patnaik A, et al: Non-BRCA DNA damage repair gene alterations and response to the PARP inhibitor rucaparib in metastatic castration-resistant prostate cancer: Analysis from the phase 2 TRITON2 study. *Clin Cancer Res* 26:2487-2496, 2020
12. Clark TA, Chung JH, Kennedy M, et al: Analytical validation of a hybrid capture-based next-generation sequencing clinical assay for genomic profiling of cell-free circulating tumor DNA. *J Mol Diagn* 20:686-702, 2018
13. Frampton GM, Fichtenholtz A, Otto GA, et al: Development and validation of a clinical cancer genomic profiling test based on massively parallel DNA sequencing. *Nat Biotechnol* 31:1023-1031, 2013
14. Crawford B, Adams SB, Sittler T, et al: Multi-gene panel testing for hereditary cancer predisposition in unsolved high-risk breast and ovarian cancer patients. *Breast Cancer Res Treat* 163:383-390, 2017
15. Neben CL, Zimmer AD, Stedden W, et al: Multi-gene panel testing of 23,179 individuals for hereditary cancer risk identifies pathogenic variant carriers missed by current genetic testing guidelines. *J Mol Diagn* 21:646-657, 2019
16. Brown EG, Wood L, Wood S: The medical dictionary for regulatory activities (MedDRA). *Drug Saf* 20:109-117, 1999
17. National Cancer Institute: NCI term browser: CTCAE. https://ncit.nci.nih.gov/ncitbrowser/pages/multiple_search.jsf?nav_type=terminologies
18. Scher HI, Morris MJ, Stadler WM, et al: Trial design and objectives for castration-resistant prostate cancer: Updated recommendations from the Prostate Cancer Clinical Trials Working Group 3. *J Clin Oncol* 34:1402-1418, 2016
19. Clovis Oncology: Rubraca (rucaparib) tablets [prescribing information]. Boulder, CO, Clovis Oncology, 2020

20. Lortot 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* 24:1807-1812, 2013
21. Pezaro CJ, Omlin AG, Altavilla A, et al: Activity of cabazitaxel in castration-resistant prostate cancer progressing after docetaxel and next-generation endocrine agents. *Eur Urol* 66:459-465, 2014
22. Al Nakouzi N, Le Moulec S, Albigès L, et al: Cabazitaxel remains active in patients progressing after docetaxel followed by novel androgen receptor pathway targeted therapies. *Eur Urol* 68:228-235, 2015
23. Nuhn P, De Bono JS, Fizazi K, et al: Update on systemic prostate cancer therapies: Management of metastatic castration-resistant prostate cancer in the era of precision oncology. *Eur Urol* 75:88-99, 2019
24. Halabi S, Lin C-Y, Kelly WK, et al: Updated prognostic model for predicting overall survival in first-line chemotherapy for patients with metastatic castration-resistant prostate cancer. *J Clin Oncol* 32:671-677, 2014
25. Mateo J, Carreira S, Sandhu S, et al: DNA-repair defects and olaparib in metastatic prostate cancer. *N Engl J Med* 373:1697-1708, 2015
26. Mateo J, Porta N, Bianchini D, et al: Olaparib in patients with metastatic castration-resistant prostate cancer with DNA repair gene aberrations (TOPARP-B): A multicentre, open-label, randomised, phase 2 trial. *Lancet Oncol* 21:162-174, 2020
27. Smith MR, Sandhu SK, Kelly WK, et al: Pre-specified interim analysis of GALAHAD: A phase II study of niraparib in patients (pts) with metastatic castration-resistant prostate cancer (mCRPC) and biallelic DNA-repair gene defects (DRD). *Ann Oncol* 30:v851-v934, 2019 (abstr 3405)
28. De Bono JS, Mehra N, Hígano CS, et al: TALAPRO-1: A phase II study of talazoparib (TALA) in men with DNA damage repair mutations (DDRMut) and metastatic castration-resistant prostate cancer (mCRPC)—First interim analysis (IA). *J Clin Oncol* 38, 2020 (suppl 6; abstr 119)
29. de Bono J, Mateo J, Fizazi K, et al: Olaparib for metastatic castration-resistant prostate cancer. *N Engl J Med* 382:2091-2102, 2020
30. Clovis Oncology: Rubraca (rucaparib) tablets [summary of product characteristics]. Swords, Ireland, Clovis Oncology, 2019
31. Kristeleit R, Shapiro GI, Burris HA, et al: A phase I-II study of the oral PARP inhibitor rucaparib in patients with germline BRCA1/2-mutated ovarian carcinoma or other solid tumors. *Clin Cancer Res* 23:4095-4106, 2017
32. McCormick A, Swaisland H: In vitro assessment of the roles of drug transporters in the disposition and drug-drug interaction potential of olaparib. *Xenobiotica* 47:903-915, 2017
33. Kikuchi R, Lao Y, Bow DA, et al: Prediction of clinical drug-drug interactions of veliparib (ABT-888) with human renal transporters (OAT1, OAT3, OCT2, MATE1, and MATE2K). *J Pharm Sci* 102:4426-4432, 2013
34. AstraZeneca: Lynparza (olaparib) tablets [prescribing information]. Wilmington, DE, AstraZeneca, 2020
35. Zibetti Dal Molin G, Westin SN, Msaouel P, et al: Discrepancy in calculated and measured glomerular filtration rates in patients treated with PARP inhibitors. *Int J Gynecol Cancer* 30:89-93, 2020
36. Tang PA, Pond GR, Chen EX: Influence of an independent review committee on assessment of response rate and progression-free survival in phase III clinical trials. *Ann Oncol* 21:19-26, 2010

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Rucaparib in Men With Metastatic Castration-Resistant Prostate Cancer Harboring a *BRCA1* or *BRCA2* Gene Alteration

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No other potential conflicts of interest were reported.