Original Study

# A Panel-Based Mutational Signature of Mismatch Repair Deficiency is Associated With Durable Response to Pembrolizumab in Metastatic Castration-Resistant Prostate Cancer

Daniel Boiarsky,[1] Doga C. Gulhan,[2] Hunter Savignano,[3] Gitanjali Lakshminarayanan,[3] Heather M. McClure,[3] Rebecca Silver,[3] Michelle S. Hirsch,[4] Lynette M. Sholl,[4] Atish D. Choudhury,[3] Guruprasad Ananda,[5] Peter J. Park,[2] Alok K. Tewari,[3] Jacob E. Berchuck[3]

## Abstract

**Immune checkpoint inhibitors (ICIs) have limited efficacy in prostate cancer (PCa) and better biomarkers are needed to predict responses to ICIs. In this study, we found that SigMA detects additional cases of mismatch repair deficiency as compared to microsatellite testing in PCa and identifies patients likely to experience durable response to pembrolizumab.**

**Introduction/Background:** Immune checkpoint inhibitors (ICIs) have limited efficacy in prostate cancer (PCa). Better biomarkers are needed to predict responses to ICIs. We sought to demonstrate that a panel-based mutational signature identifies mismatch repair (MMR) deficient (MMRd) PCa and is a biomarker of response to pembrolizumab. **Patients and Methods:** Clinico-genomic data was obtained for 2664 patients with PCa sequenced at Dana-Farber Cancer Institute (DFCI) and Memorial Sloan Kettering (MSK). Clinical outcomes were collected for patients with metastatic castration-resistant PCa (mCRPC) treated with pembrolizumab at DFCI. SigMA was used to characterize tumors as MMRd or MMR proficient (MMRp). The concordance between MMRd with microsatellite instability (MSI-H) was assessed. Radiographic progression-free survival (rPFS) and overall survival (OS) were collected for patients treated with pembrolizumab. Event-time distributions were estimated using Kaplan-Meier methodology. **Results:** Across both cohorts, 100% (DFCI: 12/12; MSK: 43/43) of MSI-H tumors were MMRd. However, 14% (2/14) and 9.1% (6/66) of MMRd tumors in the DFCI and MSK cohorts respectively were microsatellite stable (MSS), and 26% (17/66) were MSI-indeterminate in the MSK cohort. Among patients treated with pembrolizumab, those with MMRd ($n = 5$) versus MMRp ($n = 14$) mCRPC experienced markedly improved rPFS (HR = 0.088, 95% CI: 0.011-0.70; $P = .0064$) and OS (HR = 0.11, 95% CI: 0.014-0.80; $P = .010$) from start of treatment. Four patients with MMRd experienced remissions of >= 2.5 years. **Conclusion:** SigMA detects additional cases of MMRd as compared to MSI testing in PCa and identifies patients likely to experience durable response to pembrolizumab.

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## Introduction

Immune checkpoint inhibitors (ICIs) have dramatically improved overall survival (OS) in many advanced solid tumors, but have limited efficacy in mCRPC.[1-5] Due to the low prevalence (3%-5%) of mismatch repair (MMR) deficiency (MMRd) in prostate cancer (PCa),[6-8] only 6 patients with MMRd or microsatellite unstable (MSI-H) PCa were included in the clinical trial that led to the tumor-agnostic FDA approval of pembrolizumab.[9] Subsequent retrospective studies have confirmed the clinical activity

[1]Department of Medicine, Tufts Medical Center, Boston, MA
[2]Department of Biomedical Informatics, Harvard Medical School, Boston, MA
[3]Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA
[4]Department of Pathology, Brigham and Women's Hospital, Boston, MA
[5]Department of Data Science, Dana-Farber Cancer Institute, Boston, MA

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Address for correspondence: Jacob E. Berchuck, Department of Medical Oncology, Dana-Farber Cancer Institute, 450 Brookline Ave, Boston, MA, 02215
E-mail contact: jacob_berchuck@dfci.harvard.edu

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of pembrolizumab in MMRd/MSI-H mCRPC, though data are limited to small numbers of patients and short follow-up.[7,10–13]

Current methods for detecting MMRd have limitations. Immunohistochemical (IHC) assessment for MMR protein loss and polymerase chain reaction (PCR) or next-generation sequencing (NGS) assessment of MSI-H are commonly used to identify MMRd, but fail to identify a subset of patients who benefit from ICI.[14-18] The NCCN and the European Society for Medical Oncology recommend using NGS to identify MSI-H in PCa.[19,20] However, different algorithms for predicting MSI-H are used in tumor-normal and tumor-only sequencing, precluding the use of a standardized algorithm across NGS platforms.[21] These limitations highlight the need for a reliable computational approach that can be applied across NGS platforms to identify patients likely to benefit from ICIs.

Mutational signature analysis has been shown to be a promising approach to detect DNA repair deficiencies[22-27] and could aid in identifying patients likely to benefit from ICIs. However, commercially available assays for tumor genomic profiling rely on targeted gene panel sequencing, precluding use of most mutational signature tools due to insufficient genomic coverage.[23] SigMA is a novel computational tool that can be used to detect signatures from panel data[25] that integrates MSI as well as single-base substitution (SBS) mutational signatures to detect MMRd.[28] We therefore hypothesized that SigMA would detect additional MMRd cases not detected by MSI testing. Furthermore, SigMA can be applied to both tumor-normal and tumor-only panel sequencing and is therefore a promising tool to identify a uniform biomarker for ICIs across NGS platforms.

In this study, we demonstrate that in 2 large independent cohorts comprising more than 2600 patients, SigMA accurately identifies MSI-H tumors as MMRd, and also identifies additional MMRd cases in the absence of MSI-H. Further, in a cohort of mCRPC patients with long-term follow-up (median 17 months), SigMA-predicted MMRd strongly associated with a high response rate and durable responses to Pembrolizumab.

## Patients and Methods

### *Patient Cohorts*

Two clinico-genomic cohorts of patients with prostate adenocarcinoma were identified. The "DFCI" cohort comprised adult patients with prostate adenocarcinoma treated at Dana-Farber Cancer Institute (DFCI) who underwent tumor targeted panel gene sequencing with OncoPanel Version (V3) from October 10, 2016 to February 25, 2022.[29] For patients with more than 1 sample, the first biopsied sample was selected (Supplemental Figure 1A). For the "MSK" cohort, adult patients with prostate adenocarcinoma treated at Memorial-Sloan Kettering (MSK) Cancer Center and who underwent molecular profiling by MSK-IMPACT, identified from the previously published MSK-MET cohort, were included.[30] One sample was selected per patient as previously described (Supplemental Figure 1B).[30] Clinical, mutation and copy number data were downloaded from https://doi.org/10.5281/zenodo.5801902 on November 7, 2022.

### *MMR Annotations*

Across the DFCI and MSK cohorts, we uniformly employed SigMA[25] to identify tumors with MMRd mutational signatures and accompanying enrichment of indels at microsatellite regions. SigMA uses a multiclass gradient boosting classifier to categorize samples into MMRd or MMRpThe SigMA classifier underwent training on simulated targeted gene panels using features including the likelihood of MMRd SBS signatures and the total number of small indels overlapping with microsatellite regions. Simulated panel data used in the training set were generated by subsampling The Cancer Genome Atlas (TCGA) whole-exome sequencing data. The classification was validated with respect to MMR gene IHC results in an orthogonal cohort of colorectal and endometrial cancers sequenced with MSK-IMPACT panels. The code can be accessed at https://github.com/parklab/SigMA. The related publication is under review.

### *Clinical Outcomes*

Patients in the DFCI cohort treated with pembrolizumab for mCRPC were identified from pharmacy records. Clinical data was collected blinded to patients' genomic status. Clinical outcomes including best PSA response, PSA progression-free survival (PSA-PFS), radiographic progression-free survival (rPFS) and OS[31] were collected per PCWG3.[31]

### *Statistical Analysis*

Differences between 2 groups were compared using Fisher's exact test and Mann-Whitney test for categorical and continuous variables respectively, and differences between more than 2 groups were compared using chi-squared test for categorical variables. Event-time distributions were estimated using Kaplan-Meier methodology. All significance tests are 2-tailed and confidence intervals are at the 95% level, with significance predefined to be at $< 0.05$.

## Results

### *SigMA-Predicted MMRd Associates With Deleterious Alterations in MMR Genes*

We identified a total of 2664 patients with prostate adenocarcinoma comprising 492 and 2171 patients in the DFCI and MSK cohorts, respectively. We first sought to establish the correlation between SigMA-predicted MMRd and the presence of deleterious alterations in the 4 primary MMR genes (*MSH2, MSH6, PMS2, MLH1)*. The prevalence of deleterious MMR gene alterations was similar across the DFCI and MSK cohorts for *MSH2* (2.6% and 1.4%), *MSH6* (2.2% and 1.4%), *PMS2* (1.6% and 0.4%), and *MLH1* (1.0% and 0.3%) (Figure 1A). The higher rate of PVs in the DFCI cohort is likely a result of paired tumor-normal sequencing and filtering of germline variants in the MSK cohort. Three percent of patients weref identified as MMRd in both the DFCI and MSK cohorts, which is similar to the frequency of MMRd/MSI-H reported in previous studies (Figure 1B).[6-8] Clinical characteristics were similar between patients with MMRd and MMRp tumors (Supplemental Tables 1 and 2). MMRd tumors were highly enriched for deleterious alterations in MMR genes (Figure 1C). Across both cohorts, 100% of tumors harboring 2CL in *MSH2* or *MSH6* were MMRd compared to 0.25% of tumors without a deleterious MMR



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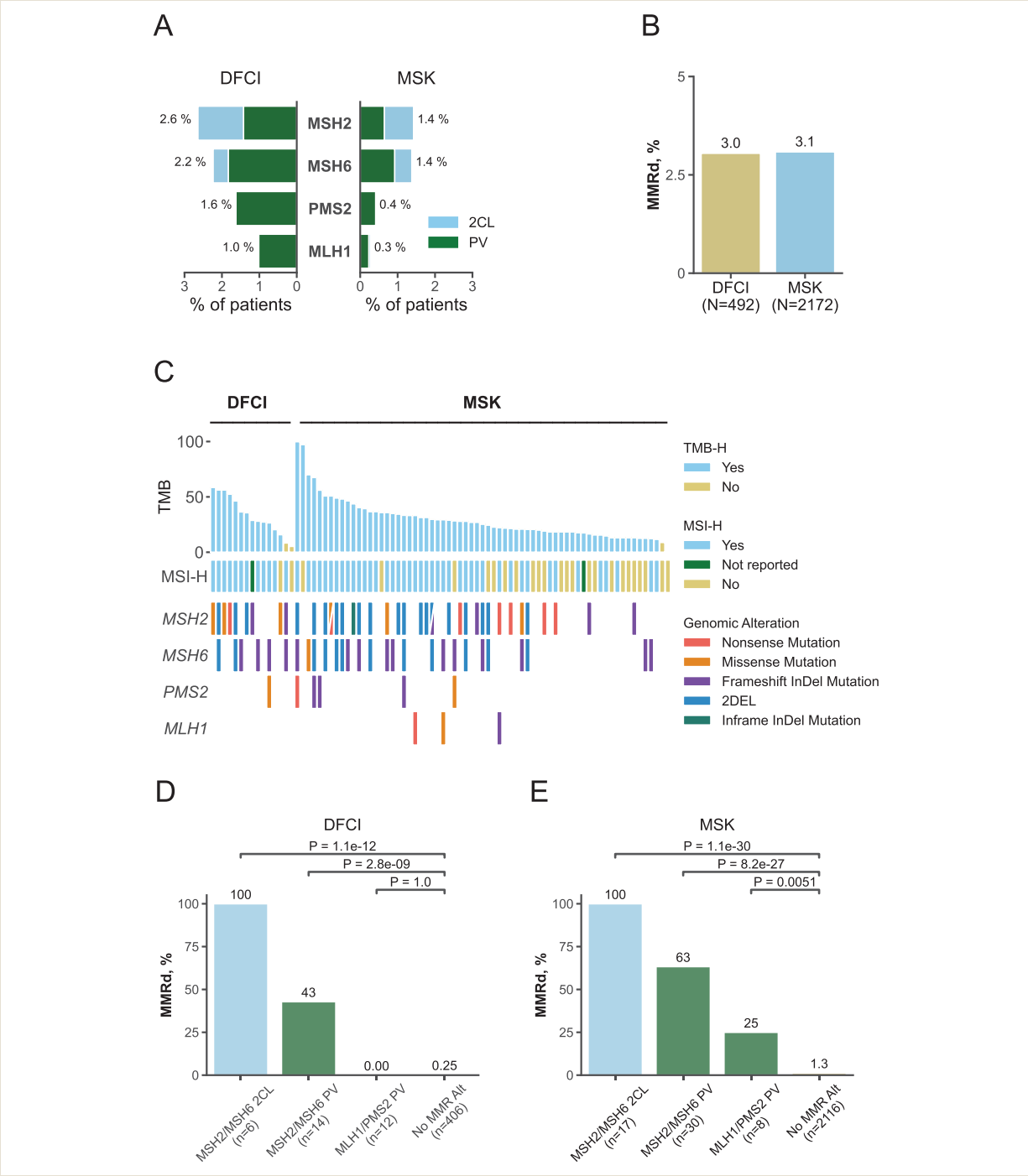
**Figure 1** Association of SigMA-predicted MMRd with MMR gene alterations. (A) Barplots showing the proportion of patients with 2CL and PVs in MMR genes in the DFCI and MSK cohorts. (B) Barplot showing the proportion of MMRd tumors in the DFCI and MSK cohorts. (C) Comutation plot showing the distribution of MMR gene alterations among MMRd tumors in the DFCI and MSK cohorts. Association of 2CL and PVs in *MSH2* or *MSH6* and *PMS2* or *MLH1* with MMRd in the (D) DFCI and (E) MSK cohorts. Abbreviations: 2CL = 2-copy loss; DFCI = Dana-Farber Cancer Institute;MMR = mismatch repair deficient; MMRd = SigMA-predicted mismatch repair deficient; MSI-H = microsatellite instability-high; MSK = Memorial Sloan Kettering; PV = pathogenic variant.



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gene alteration in the DFCI cohort ($P$ = 1.1e-12, Odds Ratio [OR] = infinity) and 1.3% in the MSK cohort ($P$ = 1.1e-30, OR = infinity) (Figure 1D and E). Likewise, a significantly higher proportion of tumors with a PV in *MSH2* or *MSH6* were MMRd compared to those lacking a deleterious MMR gene alteration in both cohorts (DFCI: 43%, $P$ = 2.8e-09, OR = 304; MSK: 63%, $P$ = 8.2e-27, OR = 141) (Figure 1D and E). Notably, PVs in *MLH1* or *PMS2* were only associated with a significantly higher likelihood of being MMRd compared to tumors without a deleterious MMR gene alteration in the MSK cohort (DFCI: 0% vs. 0.25%, $P$ = 1.0, OR = 0.0; MSK: 25% vs. 1.3%, $P$ = .0051, OR = 25) (Figure 1D and E). Of the MMRd tumors in the MSK cohort without a deleterious alteration in 1 of the 4 primary MMR genes, 18% (5/28) harbored a PV in either *PMS1* or *MSH3*. Only 1 tumor across the 2 cohorts harbored *MLH1* or *PMS2* 2CL, a sample with *MLH1* 2CL that was not MMRd. These data suggest that SigMA-predicted MMRd strongly associates with deleterious alterations in MMR genes with the most robust association for *MSH2* or *MSH6* 2CL, followed by *MSH2* or *MSH6* PVs, and a weaker association for *MLH1* or *PMS2* alterations.

### *SigMA-predicted MMRd identifies additional cases of MMRd as compared to MSI*

We next assessed the concordance between SigMA and MSI testing to identify MMRd tumors. Given that SigMA utilizes indels at microhomologies reflecting MSI status as well as SBS signatures, we hypothesized that SigMA could identify additional MMRd tumors in the absence of microsatellite instability. Across both cohorts, MSI status was reported for the majority of cases (DFCI: 80%; MSK: 88%). Among tumors for which MSI status was reported, 100% (DFCI: 12/12; MSK: 43/43) of MSI-H tumors were identified as MMRd. However, 14% (2/14) and 9.1% (6/66) of MMRd tumors in the DFCI and MSK cohorts respectively were MSS (Figure 2A and B). Among tumors with indeterminate MSI status (MSI-indeterminate) in the MSK cohort, a high proportion were MMRd (56%; 17/30) (Figure 2C). Only 2 tumors were MSI-indeterminate in the DFCI cohort and neither were MMRd. The difference in the number of MSI-indeterminate cases across the 2 cohorts is attributable to the different methods in classifying tumors as MSI-indeterminate. Whereas tumors in the MSK cohort are classified as MSI-indeterminate by the MSI calling algorithm,[32,33] those in the DFCI cohort are classified as MSI-indeterminate by a pathologist in the presence of limited sequencing evidence for MMR and other genomic and clinical characteristics suggesting microsatellite instability.[33] Finally, we sought to assess whether MMRd/MSS tumors displayed MMR protein loss on immunohistochemistry staining. Clinical immunohistochemistry staining was available for 1 MMRd/MSS tumor in the DFCI cohort, which showed MSH6 protein loss despite lacking a genomic alteration in MSH6 (Figure 2D) Thus, SigMA reliably identified MSI-H tumors as MMRd, and also identified additional MMRd tumors that were not identified by MSI analysis.

Finally, we sought to verify that SigMA identified MMRd even in the absence of MSI-H. We compared MSS or MSI-indeterminate MMRd samples to those in the MMRd/MSI-H and MMRp/MSS categories in the MSK cohort. We found that they displayed a higher number of SBSs and indels overlapping with microsatellite regions (Supplemental Figure 2A), but their values were lower than MMRd/MSI-H tumors. These samples also displayed well-characterized MMRd signatures (Figure 2E), with subtle differences such as a higher contribution from SBS15 in MMRd/MSI-indeterminate compared to MMRd/MSI-H tumors. The same conclusion held when focusing on MSI-indeterminate samples without MMR alterations, suggesting that even tumors without MMR alterations share the characteristics of MMRd tumors (Supplemental Figure 2A and C). MMRd/MSS samples also displayed MMRd signatures but with a lower contribution of these specific signatures to the overall mutational burden. (Supplemental Figure 2B and C). Consistently, SigMA-predicted MMRp tumors did not exhibit MMRd mutational signatures (Figure 2E). The additional tumors identified as MMRd by SigMA displayed characteristic MMRd signatures and were hypermutated, suggesting that despite not qualifying as MSI-H, they are likely to be MMRd.

### *SigMA-Predicted MMRd Associates With Durable Response to Pembrolizumab in mCRPC*

We identified 19 patients with mCRPC in the DFCI cohort treated with pembrolizumab, of which 5 had MMRd tumors and 14 had MMRp tumors (Table 1). Of the MMRp patients, 5 were treated off-label following standard-of-care treatments and 9 were treated on a clinical trial of pembrolizumab and radium-223 (NCT03093428).[34] Among patients treated with pembrolizumab, most self-identified as white (95%), 42% had *de novo* metastatic disease, the median age at starting Pembrolizumab was 72, the median lines of prior therapy for mCRPC was 3, and the median PSA prior to starting pembrolizumab was 35 ng/ml. Median follow-up from the time of pembrolizumab start was 17 months (range 4-67) for all patients, 36 months (range 12-67) for the MMRd patients, and 17 months (range 4-51) for the MMRp patients. The only significant difference in clinical characteristics between patients with MMRd versus MMRp tumors was older age at the time of pembrolizumab start in the MMRd patients (median years 77 vs. 68, $P$ = .025) (Table 1). There was a trend towards lower PSA prior to starting pembrolizumab in the MMRd versus MMRp patients (median PSA 4.2 vs. 55, $P$ = .055) and fewer lines of prior therapy (median 1 vs. 3.5, $P$ = .11). Four of the 5 MMRd tumors harbored a deleterious alteration in an MMR gene and 1 did not (Figure 3A). Additionally, 4 of the 5 MMRd tumors were MSI-H and 1 did not have MSI status reported. Amongst the 14 MMRp tumors, only 1 harbored a deleterious MMR alteration (PV in *PMS2*) and all were MSS (Figure 3A).

We next evaluated the association of SigMA-predicted MMR status with clinical outcomes in patients with mCRPC treated with pembrolizumab. A significantly higher proportion of patients with MMRd than MMRp tumors achieved a PSA decline of at least 50% (PSA50) following treatment with pembrolizumab (80% vs. 0%, $P$ = .0013) (Figure 3B). Similarly, patients with MMRd tumors experienced dramatically improved PSA-PFS (median 55 vs. 2.9 months; HR = 0.079, 95% CI: 0.0099-0.60; $P$ = .0032), rPFS (median 54 vs. 3.7 months; HR = 0.088, 95% CI: 0.011-0.70; $P$ = .0064), and OS (median not reached vs. 3.7 months;



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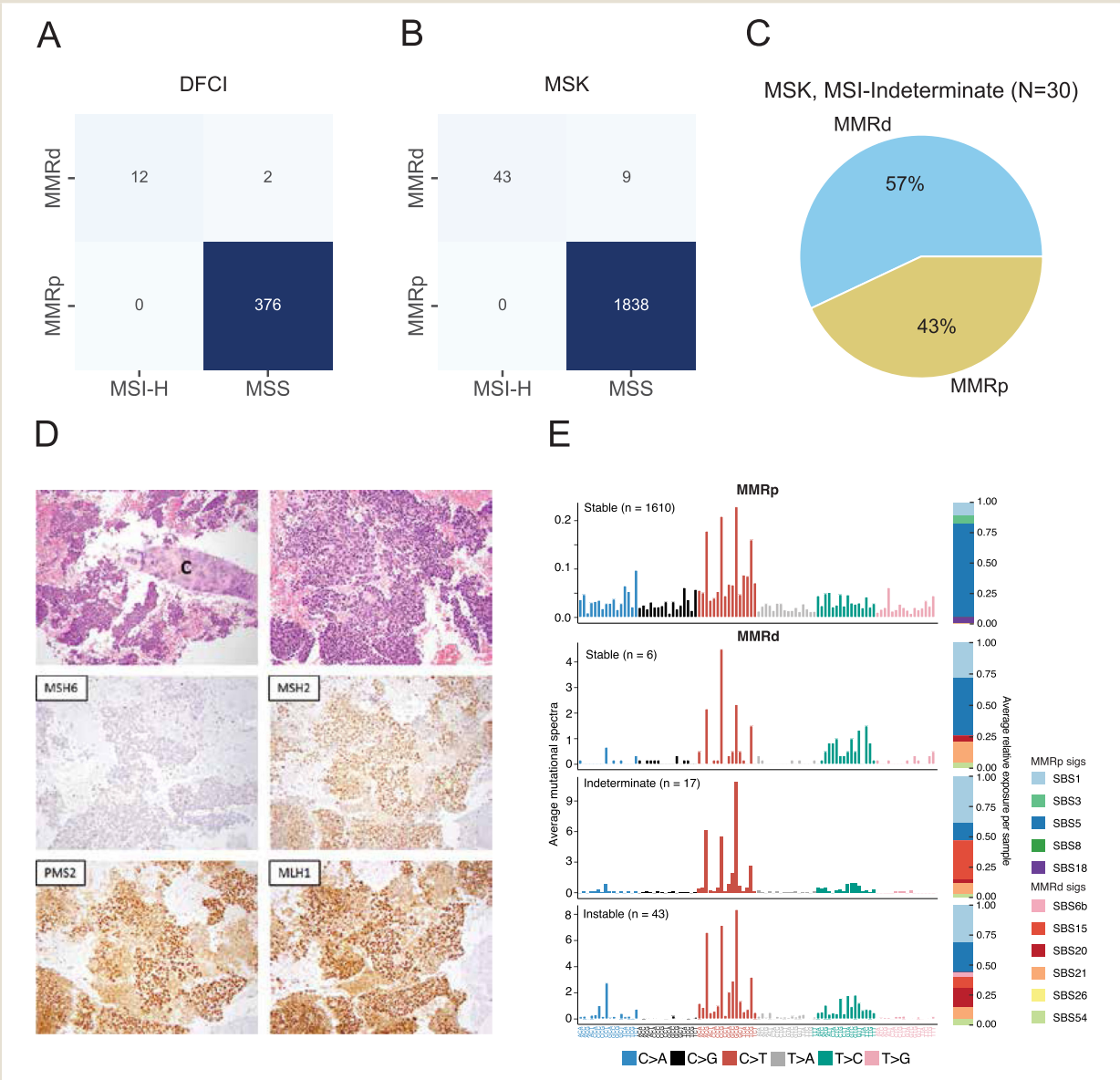
**Figure 2** Concordance of SigMA-predicted MMRd and MSI-H. Contingency tables showing the concordance of MMRd vs MMRp with MSI-H vs MSS in the (A) DFCI and (B) MSK cohorts. (C) Piechart showing the proportion of MSI-indeterminate tumors which were MMRd in the MSK cohort. (D) Pathology slides with hematoxylin and eosin staining (first row) and immunohistochemical staining for MMR proteins (second and third rows) from a MMRd/MSS tumor in the DFCI cohort. Immunohistochemical staining for MSH2, PMS2, and MLH1 demonstrates intact nuclear staining whereas staining for MSH6 demonstrates loss of nuclear staining in tumor cells but intact nuclear staining in inflammatory cells (negative control). (E) Average mutational spectra of tumors by MMR and MSI status. Abbreviations: C = cartilage; DFCI = Dana-Farber Cancer Institute; MMR = mismatch repair; MMRd = mismatch repair deficient; MMRp = mismatch repair proficient; MSI = microsatellite instability; MSI-H = microsatellite instability-high; MSK = Memorial Sloan Kettering; MSS = microsatellite stable.




HR = 0.11, 95% CI: 0.014-0.80; $P$ = .010) from start of pembrolizumab treatment (Figure 3C-E). These long-term follow-up data demonstrate that SigMA-predicted MMR status discriminates between patients with mCRPC likely and unlikely to derive durable clinical benefit from treatment with pembrolizumab.

Four patients with MMRd mCRPC experienced an exceptional response to pembrolizumab with remissions lasting for more than 2.5 years, 3 of which were ongoing at the time of study cut-off (Figure 4A and B). The patient with the longest rPFS of 54 months harbored 2CL in *MSH2* and received pembrolizumab as first-



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**Table 1 Characteristics of Patients Treated With Pembrolizumab**

| | All | MMRd | MMRp | *P* |
|---|---|---|---|---|
| Patients | 19 | 5 (26) | 14 (74) | |
| Age at pembro txt, median (range) | 72 (65-75) | 77 (72-78) | 68 (64-72) | .025 |
| Prior lines of treatment in mCRPC, median (range) | 3 (1-4) | 1 (1-3) | 3.5 (2.3-4.8) | .10 |
| Race (%) | | | | 1.0 |
| White | 18 (95) | 5 (100) | 13 (93) | |
| Black or African American | 0 (0) | 0 (0) | 0 (0) | |
| Asian | 1 (5.3) | 0 (0) | 1 (7.1) | |
| Other/unknown | 0 (0) | 0 (0) | 0 (0) | |
| Stage at prostate cancer diagnosis (%) | | | | .60 |
| Localized (N0M0) | 11 (58) | 2 (40) | 9 (64) | |
| Locoregional (N1M0) | 0 (0) | 0 (0) | 0 (0) | |
| Metastatic (NxM1) | 8 (42) | 3 (60) | 5 (36) | |
| PSA prior to treatment, median (range) | 35 (10-109) | 4.2 (0.84-31) | 55 (19-350) | .055 |
| Microsatellite status (%) | | | | .0010 |
| Instable | 4 (29) | 4 (100) | 0 (0) | |
| Indeterminate | 0 (0) | 0 (0) | 0 (0) | |
| Stable | 10 (71) | 0 (0) | 10 (100) | |
| Not reported | 5 | 1 | 4 | |
| TMB >= 10 (%) | 5 (26) | 5 (100) | 0 (0) | 8.5e-05 |

Abbreviations: MMRd = SigMA-predicted mismatch repair deficient; MMRp = SigMA-predicted mismatch repair proficient; Pembro = pembrolizumab; PSA = prostate-specific antigen; TMB = tumor mutational burden.

line treatment for mCRPC. Despite discontinuing pembrolizumab after 6 cycles due to ICI-induced hepatitis, he experienced an additional 49 months of rPFS. Of the 3 exceptional responders with ongoing complete responses, 1 harbored an *MSH2* PV and received pembrolizumab as a second-line mCRPC treatment following docetaxel, 1 did not have an MMR gene alteration (though was MSI-H) and received pembrolizumab as second-line mCRPC treatment following Abiraterone, and 1 harbored a deep deletion in *MSH2* and received pembrolizumab as fifth-line treatment. The 1 patient with MMRd mCRPC who experienced rPFS of only 4 months received pembrolizumab as fourth-line treatment (Figure 4A and B).

Finally, we compared outcomes between patients with MMRd mCRPC based on whether or not they were treated with pembrolizumab. We identified 11 patients with MMRd mCRPC, 5 of whom received pembrolizumab and 6 who did not. Patients treated with pembrolizumab experienced significantly longer OS from the time of mCRPC compared to those who never received pembrolizumab (median 62 vs. 33 months, $P = .018$) (Figure 5A).

## Discussion

Pembrolizumab is an FDA-approved, NCCN guideline-recommended therapy that can induce durable responses in a subset of patients with MMRd/MSI-H mCRPC. Reliable biomarkers of MMRd are needed to identify patients likely to benefit from pembrolizumab. In this study, we applied a novel computational tool, SigMA, to detect a mutational signature of MMRd in targeted panel sequencing from 2664 PCa tumors across 2 independent cohorts. We observed that SigMA-predicted MMRd (1) strongly associated with the presence of deleterious genomic alterations in MMR genes, (2) reliably identified MSI-H tumors as determined by next-generation sequencing as MMRd and also identified additional MMRd tumors in the absence of MSI-H, and (3) strongly associated with response to pembrolizumab in patients with mCRPC. Further, we report the longest survival outcomes following pembrolizumab treatment in patients with MMRd mCRPC, with several responses lasting more than 2.5 years, highlighting the potential for durable responses to ICIs in patients with PCa.

Consistent with previous studies,[6–8] SigMA-predicted MMRd was present in 3% of patients in 2 large clinico-genomic PCa cohorts. The similar performance across 4 targeted gene panels with variable genomic coverage, including tumor-normal and tumor-only sequencing panels, from 2 independent cohorts supports the robustness of SigMA to accurately predict MMRd. MMRd was associated with alterations in MMR genes, with the strongest association with *MSH2* or *MSH6* 2CL, followed by *MSH2* or *MSH6* PVs, and a less robust association with *MLH1 or PMS2* PVs. The observation that MMR gene 2CL resulted in a higher likelihood of MMRd than a single PV suggests that biallelic inactivation is necessary to disrupt MMR heterodimers and highlights the need for further investigation of whether MMRd prostate tumors with a single PV undergo epigenetic silencing of the second MMR gene copy or contain a shallow deletion that was not detectable with targeted panel sequencing. This finding has clinical implications in mCRPC where 2CL of homologous recombination repair genes is associated with greater benefit from PARP inhibitor treatment compared to a single PV.[35] To our knowledge, our study reports the first data to suggest variability in MMRd in PCa depending on the



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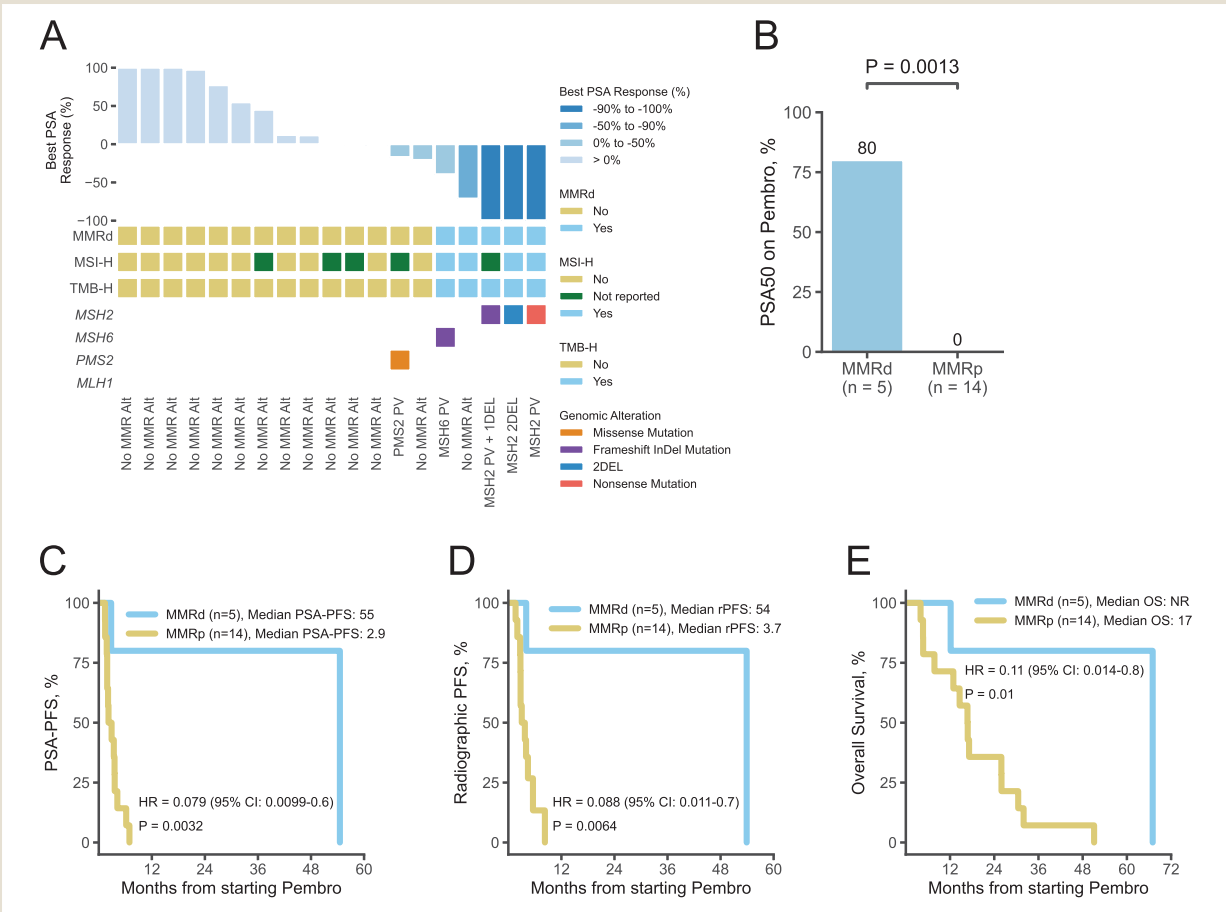
**Figure 3** Clinical outcomes in patients with mCRPC treated with pembrolizumab by SigMA-precited MMR status. (A) Waterfall plot showing best PSA response on pembrolizumab and comutation plot showing the genomic alterations in MMR genes in mCRPC patients treated with pembrolizumab. (B) Barplots comparing the proportion of patients with PSA50 response on pembrolizumab between patients with MMRd and MMRp tumors. Kaplan-meir curves comparing (C) PSA-PFS, (D) radiographic PFS, (E) overall survival between patients with MMRd and MMRp tumors treated with pembrolizumab. Abbreviations: mCRPC = metastatic castration-resistant prostate cancer; MMR = mismatch repair; MMRd = SigMA-predicted mismatch repair deficient; MMRp = SigMA-predicted mismatch repair proficient; PFS = progression-free survival; PSA = prostate specific antigen; PSA50 = decline in prostate specific antigen of at least 50% from baseline.




specific MMR gene altered. However, this is consistent with a previous analysis of 1,057 MSI-H solid tumors, which reported significantly higher tumor mutational burden in patients with *MSH2* or *MSH6* alterations as compared to *MLH1* or *PMS2* alterations.[36] These observations highlight the need for further studies to characterize the association of specific MMR gene alterations with phenotypic MMRd and pembrolizumab response in PCa.

Across 2 large independent PCa cohorts, SigMA classified 100% of MSI-H tumors as MMRd, indicating that SigMA is a reliable tool for identifying MMRd in MSI-H tumors. We also observed that SigMA identified MMRd even in the absence of MSI-H, as 14% and 9.1% of MMRd tumors in the DFCI and MSK cohorts respectively were MSS and 56% of MSI-indeterminate tumors in the MSK cohort were MMRd. Notably, MMRd tumors displayed similar mutational signatures in the presence and absence of MSI-H. IHC staining was available for 1 MMRd/MSS tumor in the DFCI cohort and demonstrated loss of MSH6 protein expression. The absence of microsatellite instability in this tumor could be due to MSH6 deficient tumors having lower levels of homopolymer insertion-deletions and higher levels of characteristic MMRd SBS signatures as compared to those with MSH2 loss.[37] Taken together, these findings suggest that combining MSI testing with mutational signature analysis may improve the detection of MMRd tumors over MSI testing alone. Additionally, a major limitation in the use of MSI-H as a biomarker for pembrolizumab in PCa is the lack of standardization in how MSI testing is implemented across NGS platforms. SigMAcan be applied to both tumor-normal and tumor-only sequencing data, including targeted panels beyond OncoPanel and MSK-IMPACT that are more widely used in the U.S., and could therefore serve as a standardized tool for identi-



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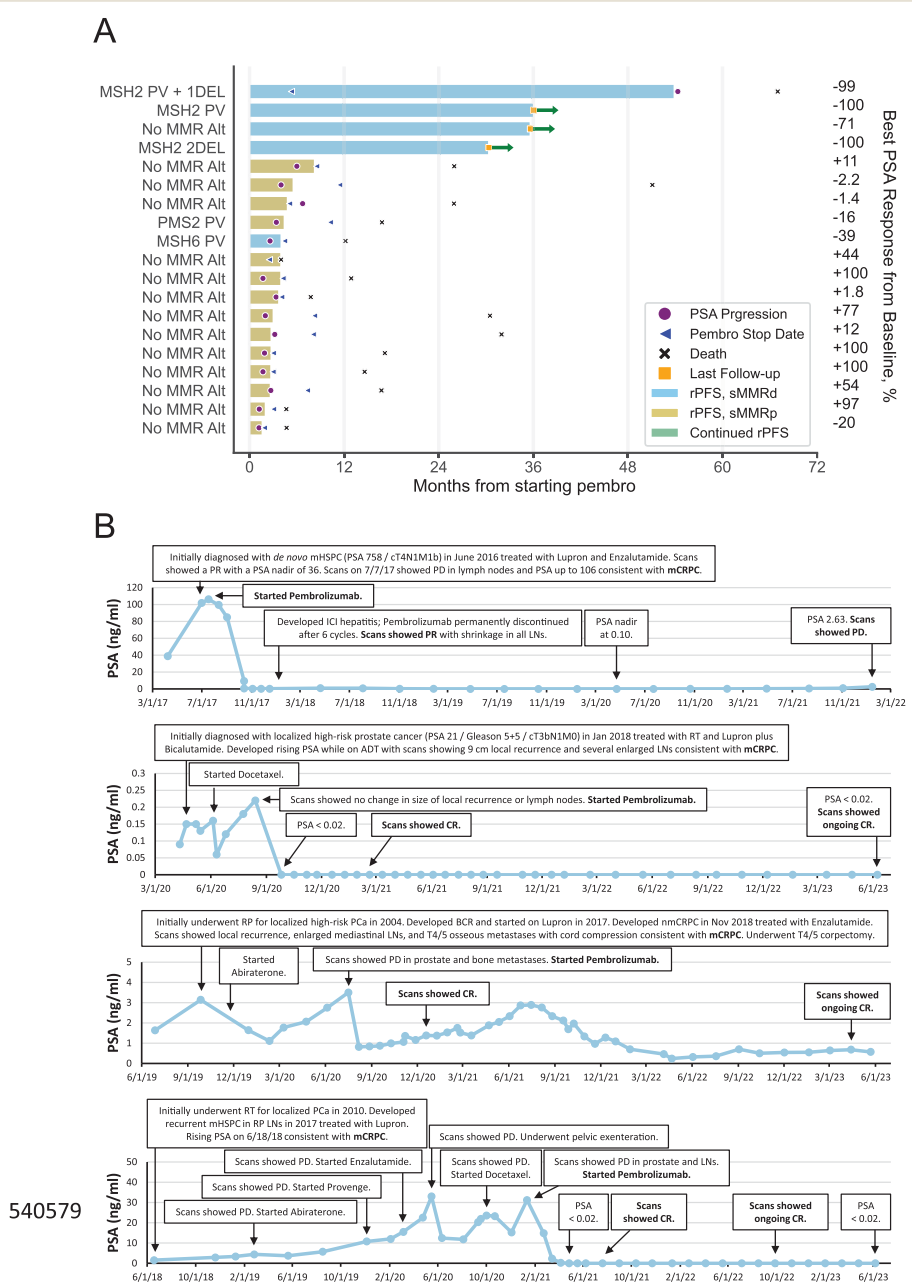
**Figure 4** Clinical histories of exceptional responders to pembrolizumab. (A) Swimmers plot showing rPFS from treatment start in patients treated with pembrolizumab. (B) Scatterplots of PSA over time in 4 exceptional responders to pembrolizumab with clinical annotations. Abbreviations: CR = complete response; ICI = immune-checkpoint inhibitor; LN = lymph node; mCRPC = metastatic castration-resistant prostate cancer; mHSPC = metastatic hormone-sensitive prostate cancer; MMRd = mismatch repair deficient; MMRp = mismatch repair proficient; MSI-H = microsatellite instability-high; PD = progressive disease; pembro = pembrolizumab; PSA = prostate specific antigen; rPFS = radiographic progression-free survival; RT = radiation therapy; T4/T5 = thoracic vertebrae 4 and 5.




fying a biomarker of pembrolizumab response in PCa across NGS platforms.

Among patients with mCRPC treated with pembrolizumab, SigMA-predicted MMRd strongly associated with durable response to pembrolizumab. Of the 5 patients with MMRd mCRPC treated with pembrolizumab, 4 experienced radiographic responses ranging from 30 to 54 months, 3 of whom had an ongoing response at study cut-off. Interestingly, 1 patient with MMRd mCRPC who



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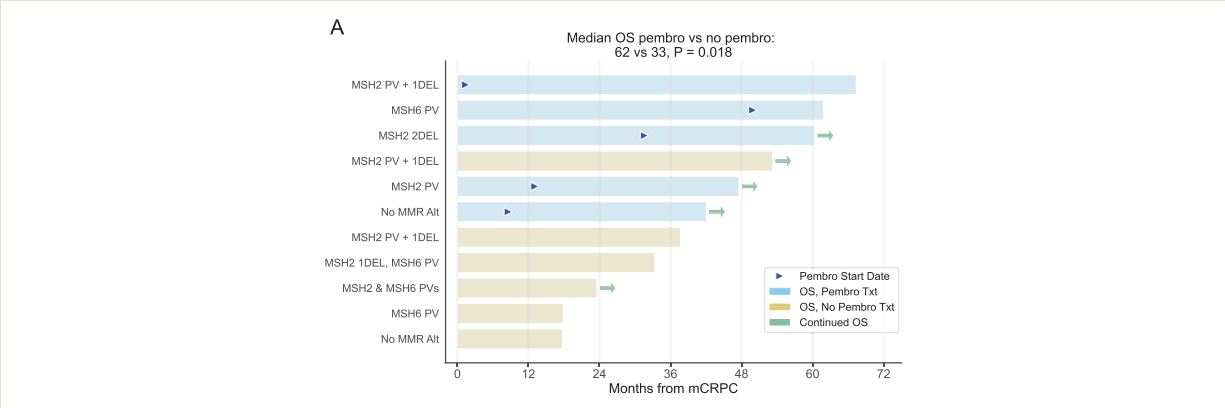
**Figure 5** Clinical outcomes in patients with mismatch repair deficient tumors by treatment with and without pembrolizumab (pembro). Swimmers plot showing overall survival (OS) from the time of diagnosis of metastatic castration-resistant prostate cancer (mCRPC) in patients with mismatch-repair deficient tumors by treatment with and without pembrolizumab.




had ongoing response at study cut-off did not have genomic MMR gene loss, highlighting the need to further investigate the biological mechanisms leading to MMRd. Prior retrospective studies of ICIs in MMRd mCRPC reported ongoing radiographic responses approaching 2 years, however, to our knowledge, herein we report the longest survival outcomes (median follow-up of 3 years) following pembrolizumab treatment in patients with PCa, highlighting the potential for durable responses beyond 2 years to ICIs in patients with mCRPC, as seen in other solid tumors. The observation that patients with MMRd mCRPC who received pembrolizumab experienced significantly longer OS than those who did not suggests that detecting MMRd and treating with pembrolizumab is important to optimize outcomes for patients with advanced PCa. Notably, the longest radiographic response in an MMRp patient was less than 9 months with 13 of 14 MMRp patients experiencing rPFS less than 6 months, consistent with the reported lack of clinical activity for ICIs in patients with mCRCP who lack evidence of MMRd.[2–5]

The clinical data reported in our study has several limitations. First, we do not have IHC data available to assess whether SigMA-predicted MMRd tumors without MSI-H exhibited MMR protein loss. Second, this is a retrospective study, introducing potential for unmeasured bias. Notably, there was a trend towards MMRd patients treated with pembrolizumab receiving fewer lines of prior therapy for mCRPC and having a lower PSA prior to pembrolizumab treatment compared to MMRp patients, both of which could predispose to better outcomes in the MMRd patients. Third, the small number of patients with mCRPC treated with pembrolizumab limits our ability to draw definitive conclusions from these data. For example, the higher PSA50 rate to ICI in MMRd mCRPC in our study (80%) compared to published cohorts (44%-65%) is likely due to variability inherent to analyzing a small number of patients.[7,10-13] Finally, the small sample size of patients treated with pembrolizumab limited our ability to disentangle the predictive value of tumor mutational burden from MSI. Further research is needed to disambiguate the independent association of MMRd and tumor mutational burden on response to ICI in mCRPC.

In summary, we demonstrate that a novel computational tool (SigMA) reliably detects a mutational signature of MMRd in PCa across multiple NGS platforms, and that this biomarker strongly associates with durable response to pembrolizumab in patients with mCRPC. With further validation, SigMA-predicted MMRd could be a valuable clinical biomarker to optimize selection of PCa patients likely to benefit from ICIs. Additionally, we report the longest follow-up to date of MMRd mCRPC patients treated with pembrolizumab, highlighting the possibility of durable responses lasting several years. We are optimistic that ongoing clinical trials evaluating ICIs earlier in the disease course for patients with MMRd PCa, combined with validation of biomarkers that optimally identify patients most likely to benefit from ICI therapy, will improve outcomes for patients with PCa.

### *Clinical Practice Points*

- **What is already known about this topic?** Pembrolizumab can induce durable remissions in many cancers but has limited efficacy in prostate cancer (PCa). Microsatellite instability and mismatch repair deficiency (MMRd) are biomarkers of response to pembrolizumab in prostate cancer, but current methods to assess these biomarkers can fail to identify patients who may benefit from pembrolizumab.
- **What are the new findings?** We identified a panel-based mutational signature of MMRd that detects additional cases as compared to microsatellite instability testing and was associated with remissions of greater than 2.5 years in patients with metastatic-castration resistant prostate cancer (mCRPC) treated with pembrolizumab.
- **How might it impact on clinical practice in the foreseeable future?** SigMA is a promising and novel computational



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tool to identify patients with mCRPC who would benefit from pembrolizumab. Further studies in larger cohorts are needed to validate SigMA as a clinical biomarker of response to pembrolizumab in mCRPC.

## Data availability statement

Data for the DFCI cohort is available to those who request it from the authors. Data for the MSK cohort is publicly available as referenced above.

## CRediT authorship contribution statement

**Daniel Boiarsky:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Formal analysis, Data curation, Conceptualization. **Doga C. Gulhan:** Writing – review & editing, Visualization, Software, Methodology, Formal analysis, Data curation. **Hunter Savignano:** Data curation. **Gitanjali Lakshminarayanan:** Data curation. **Heather M. McClure:** Data curation. **Rebecca Silver:** Data curation. **Michelle S. Hirsch:** Writing – review & editing. **Lynette M. Sholl:** Writing – review & editing. **Atish D. Choudhury:** Writing – review & editing. **Guruprasad Ananda:** Writing – review & editing, Data curation. **Peter J. Park:** Writing – review & editing, Funding acquisition. **Alok K. Tewari:** Writing – review & editing, Formal analysis. **Jacob E. Berchuck:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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## Disclosure

Jacob E. Berchuck certifies that all conflicts of interest including specific financial interests and relationships and affiliates relevant to the subject matter or materials discussed in the manuscripts (eg, employment/affiliation, grants or funding, consultancies, honoraria, stock ownership or options, expert testimony, royalties, or patents filed, received, or pending), are the following: Rebecca Silver has research funding from Bayer; Alok K. Tewari has equity in Moderna Therapeutics and Teladoc, honoraria from UroToday, and is an advisor/consultant to Best Doctors, Inc and B.A.I. Technologies, and receives travel reimbursements from AbbVie; Jacob E. Berchuck is an advisor/consultant to Genome Medical, Oncotect, Precede, TracerDx, Musculo, and JucaBio, has equity in Cityblock Health, Genome Medical, Oncotect, Precede, TracerDx, and Musculo, has received speaker honoraria from Guardant Health, and has an institutional patent on methods to detect neuroendocrine prostate cancer through tissue-informed cell-free DNA methylation analysis; Doga C. Gulhan and Peter J. Park are listed as inventors on a pending patent application for SigMA; All other authors have no financial disclosures to report.

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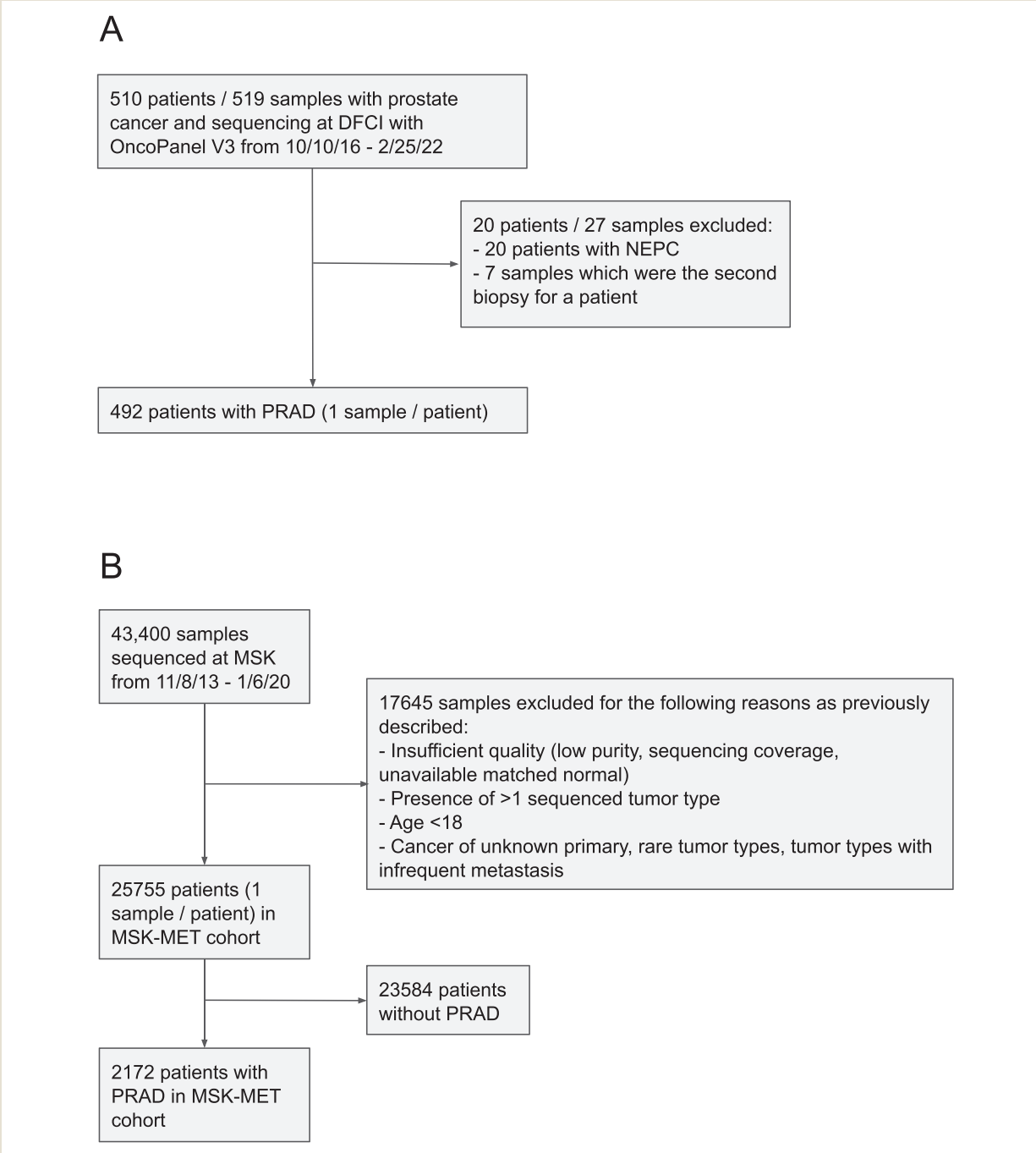
## Appendix

Supplemental Figure 1A, Supplemental Figure 2A, Supplemental Table 1A, Supplemental Table 2A.

**Supplemental Figure S1** Consort diagrams for the (A) DFCI and (B) MSK cohorts. NEPC: neuroendocrine prostate cancer; PRAD: prostate adenocarcinoma; DFCI: Dana-Farber Cancer Institute; MSK: Memorial Sloan Kettering.



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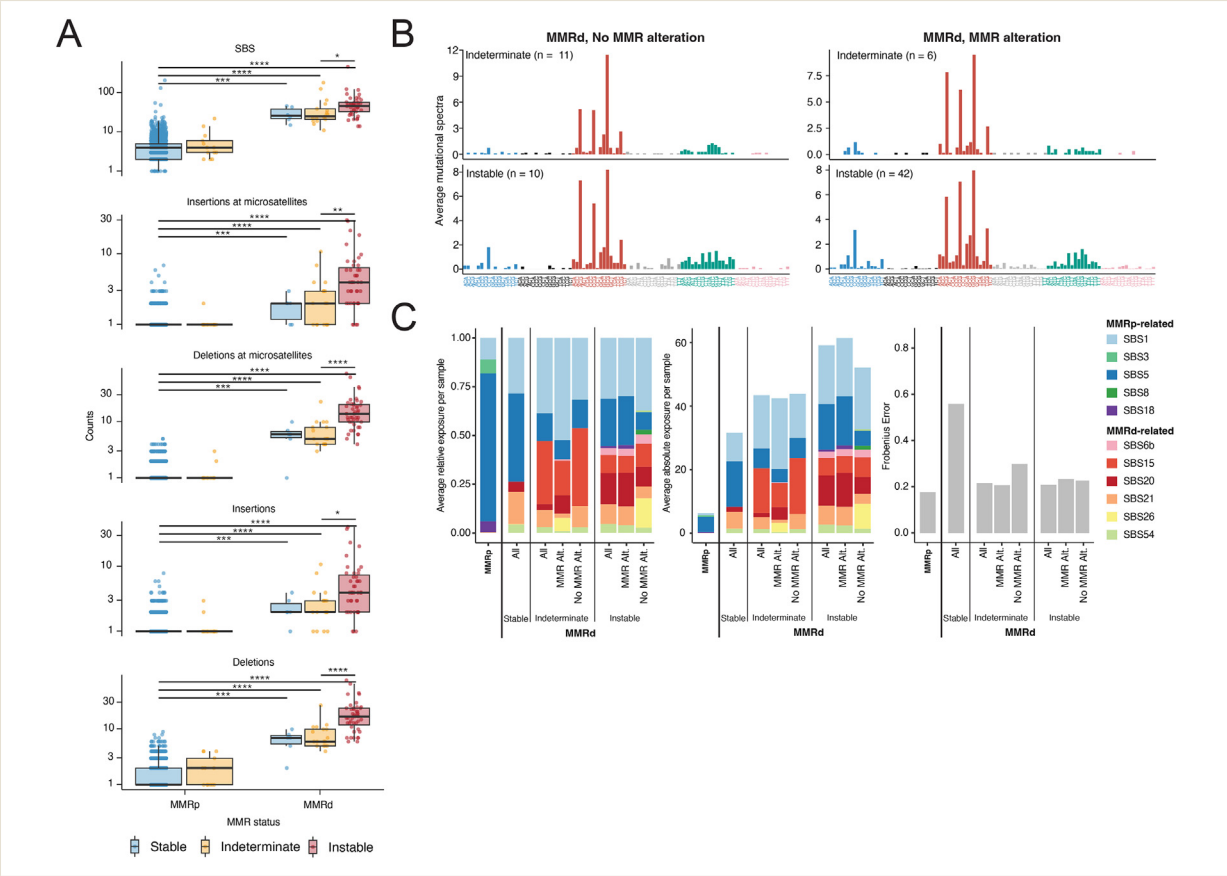
**Supplemental Figure 2** Investigation of characteristics of MMRd samples in the MSK cohort according to their MSI status and presence of MMR gene alterations. (A) From top to bottom, the SBS counts, small insertions and deletions overlapping at microsatellite regions and their total counts independently of the overlap are shown, for subsets of samples classified as MMRd and MMRp further stratified according to their MSI status (MSS, MSI-Indeterminate or MSI-H). (B) The average mutational spectra of MSI-Indeterminate and MSI-H MMRd samples grouped according to presence of MMR Alterations. (C) Relative (Left) and absolute (Middle) exposure of signatures (i.e. contribution of the mutational signature to the overall mutational burden) calculated from average mutational spectra, and Frobenius error (Right) for samples grouped according to MMRd status. The MMRd samples are further grouped according to their MSI status and presence of MMR alterations for MSI-Indeterminate and MSI-H groups. MSS/MMRd group is not stratified further due to lack of sufficient number of samples.



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**Supplemental Table S1** Characteristics of patients in the DFCI cohort. MMRd: SigMA-predicted mismatch repair deficient; MMRp: SigMA-predicted mismatch repair proficient; TMB: tumor mutational burden

| | DFCI Cohort | MMRd | MMRp | *P* |
|---|---|---|---|---|
| **Patients (%)** | 492 (100) | 15 (3.0) | 477 (97) | |
| **Age at diagnosis, median (range)** | 62.8 (58 - 68) | 65 (63- 67) | 63 (58 - 68) | 0.18 |
| **Age at sequencing biopsy, median (range)** | 67 (61 - 723) | 70 (67 - 72) | 67 (61 - 73) | 0.14 |
| **Race (%)** | | | | 0.55 |
| White | 443 (90) | 14 (93) | 429 (90) | |
| Black or African American | 29 (5.9) | 0 (0) | 29 (6.1) | |
| Asian | 7 (1.4) | 0 (0) | 7 (1.5) | |
| Other/unknown | 13 (2.6) | 1 (6.7) | 12 (2.5) | |
| **Stage at prostate cancer diagnosis (%)** | | | | 0.00032 |
| Localized (N0M0) | 295 (60) | 6 (40) | 289 (61) | |
| Locoregional (N1M0) | 149 (30) | 6 (40) | 42 (8.8) | |
| Metastatic (NxM1) | 48 (9.8) | 3 (20) | 146 (31) | |
| **Stage at sequencing biopsy (%)** | | | | 0.86 |
| M0 | 160 (33) | 4 (27) | 156 (33) | |
| M1HSPC | 81 (17) | 3 (20) | 78 (17) | |
| M1CRPC | 246 (50) | 8 (53) | 238 (50) | |
| Not available | 5 | 0 | 5 | |
| **Gleason grade at diagnosis (%)** | | | | 0.064 |
| Low-grade | 22 (5.0) | 2 (14) | 20 (4.7) | |
| Intermediate-grade | 136 (30.6) | 1 (7.1) | 135 (31.4) | |
| High-grade | 286 (64.4) | 11 (79) | 275 (64.0) | |
| Not available | 34 | 1 | 33 | |
| **Metastatic biopsy site (%)** | 273 (56) | 7 (47) | 266 (56) | 0.60 |
| **PSA prior to diagnosis, median (range)** | 10 (5.5 - 36) | 11 (7.2 - 24) | 10 (5.4 - 37) | 0.81 |
| **Treated with pembrolizumab (%)** | 19 (3.9) | 5 (33) | 14 (2.9) | 0.00012 |

**Supplemental Table S2** Characteristics of patients in the MSKCC cohort. MMRd: SigMA-predicted mismatch repair deficient; MMRp: SigMA-predicted mismatch repair proficient; TMB: tumor mutational burden

| | All | MMRd | MMRp | P |
|---|---|---|---|---|
| **Patients (%)** | 2172 (100) | 67 (3.1) | 2105 (96.9) | |
| **Age at sequencing biopsy, median (range)** | 68 (61 - 73) | 68 (59 - 72) | 68 (61 - 73) | 0.58 |
| **Race (%)** | | | | 0.91 |
| White | 1723 (79) | 51 (76) | 1672 (79) | |
| Black or African American | 192 (8.8) | 7 (10) | 185 (8.8) | |
| Asian | 75 (3.5) | 3 (4.5) | 72 (3.4) | |
| Other/Unknown | 182 (8.4) | 6 (9) | 176 (8.4) | |
| **Metastatic biopsy site (%)** | 860 (40) | 33 (49) | 827 (39) | 0.13 |



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