TITLE: A patient-driven clinicogenomic partnership through the Metastatic Prostate Cancer Project

AUTHORS/AFFILIATIONS

Jett Crowdis	1, 2 *
Sara Balch	1, 2 *
Lauren Sterlin	2, 3
Beena S. Thomas	2, 3
Sabrina Y. Camp	1, 2
Michael Dunphy	2, 3
Elana Anastasio	2, 3
Shahrayz Shah	2, 3
Alyssa L. Damon	2, 3
Rafael Ramos	1, 2, 3
Delia M. Sosa	2, 3
Ilan K. Small	2, 3
Brett Tomson	2, 3
Colleen M. Nguyen	2, 3
Mary McGillicuddy	2, 3
Parker S. Chastain	2, 3
Meng Xiao He	1, 2, 4
Alexander T.M. Cheung	1, 2, 5
Stephanie Wankowicz	6, 7
Alok K. Tewari	1
Dewey Kim	1, 2
Saud H. AlDubayan, MD	1, 2, 8
Ayanah Dowdye	2, 3
Benjamin Zola	2, 3
Joel Nowak	9
Jan Manarite	9
Major Idola Henry Gunn, USAF (Ret.)	10
Bryce Olson	11
Eric S. Lander	12, 13, 14
Corrie A. Painter	2, 3
Nikhil Wagle	1, 2, 3
Eliezer M. Van Allen	1, 2, 15 ‡

1. Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA 02115, USA
2. Broad Institute of Harvard and MIT, Cambridge, MA 02142, USA
3. Count Me In, Cambridge, MA 02142, USA
4. Harvard Graduate Program in Biophysics, Boston, MA, 02115, USA
5. NYU Grossman School of Medicine, New York, NY, 10016, USA
6. Biophysics Graduate Program, University of California San Francisco, San Francisco, CA 94143, USA

7. Department of Bioengineering and Therapeutic Sciences, University of California San Francisco, San Francisco, CA 94143, USA
8. College of Medicine, King Saud bin Abdulaziz University for Health Sciences, Riyadh, Saudi Arabia
9. Cancer ABCs, Brooklyn, NY 11201, USA
10. Founder, MomVetWifeWidow.Org
11. Global Strategist, Health and Life Sciences, Intel Corp. Patient advocate, SequenceMe.
12. Broad Institute of Harvard and MIT, Cambridge, MA 02142, USA (on leave)
13. Department of Biology, MIT, Cambridge, MA 02142, USA (on leave)
14. Department of Systems Biology, Harvard Medical School, Boston, MA 02115, USA (on leave).
15. Center for Cancer Genomics, Dana-Farber Cancer Institute, Boston, MA 02115, USA

* These authors contributed equally to this work

‡ Correspondence:
Eliezer M. Van Allen
Dana-Farber Cancer Institute 450 Brookline Ave
Boston, MA 02215
Eliezerm_vanallen@dfci.harvard.edu

ABSTRACT

Molecular profiling studies have enabled numerous discoveries for metastatic prostate cancer (MPC), but they have mostly occurred in academic medical institutions focused on select patient populations. We developed the Metastatic Prostate Cancer Project (MPCproject, mpcproject.org), a patient-partnered initiative to empower MPC patients living anywhere in the U.S. and Canada to participate in molecular research and contribute directly to translational discovery. Here we present clinicogenomic results from our partnership with the first 706 MPCproject participants. We found that a patient-centered and remote research strategy enhanced engagement with patients in rural and medically underserved areas. Furthermore, patient-reported data achieved 90% consistency with abstracted health records for therapies and provided a mechanism for patient-partners to share information about their cancer experience not documented in medical records. Among the molecular profiling data from 333 patient-partners (n = 573 samples), whole exome sequencing of 63 tumor samples obtained from hospitals across the U.S. and Canada and 19 plasma cell-free DNA (cfDNA) samples from blood donated remotely recapitulated known findings in MPC and enabled longitudinal study of prostate cancer evolution. Inexpensive ultra-low coverage whole genome sequencing of 318 cfDNA samples from donated blood revealed clinically relevant genomic changes like *AR* amplification, even in the context of low tumor burden. Collectively, this study illustrates the power of a longitudinal partnership with patients to generate a more representative clinical and molecular understanding of MPC.

Note: To assist our patient-partners and the wider MPC community interpret the results of this study, we have included a glossary of terms in the Supplementary Materials.

INTRODUCTION

23 Prostate cancer is the second most diagnosed cancer in men, with nearly 200,000 men
24 diagnosed in 2020 alone in the U.S.[1] Survival rates for localized disease are high, but the five-
25 year survival rate for the over 300,000 men currently living with metastatic prostate cancer
26 (MPC) is only 31%, representing the third leading cause of death for men[1,2]. Because prostate
27 cancer is largely driven by alterations to DNA, genomic sequencing studies have enabled
28 discoveries of its molecular drivers and new therapeutic targets in both primary and metastatic
29 clinical settings[3–6]. However, obtaining large cohorts of tumor biopsies from MPC patients for
30 molecular study has been challenging. MPC most commonly spreads to bone, and sampling
31 osseous lesions necessitates painful and technically challenging procedures that are not widely
32 accessible or feasible in clinical care. Because prostate cancer can shed cell-free DNA (cfDNA)
33 into the bloodstream, blood biopsies that sample this circulating tumor DNA have proven to be a
34 useful alternative for the study of MPC[7,8].

35 Historically, quaternary care academic medical institutions have had the necessary
36 infrastructure and expertise to lead clinically integrated MPC sequencing studies through clinical
37 trials. However, the resulting clinical and genomic data is often siloed within these institutions,
38 leading many to push for mandatory data sharing[9,10]. These efforts, while critical to
39 democratizing genomic research, do not directly improve access to molecular research programs
40 and do not address underlying ethnic, socioeconomic, and geographic patient disparities in such
41 studies, which threaten to bias findings and eventually care towards select patient populations[11–
42 14]. Commercial sequencing options for prostate cancer are emerging, but such approaches are
43 often proprietary, only available to patients with appropriate insurance, and regularly
44 inaccessible for wider research use[15–17]. Indeed, despite growing interest in clinical and research-

45 based genomic sequencing within the MPC patient community, there are only limited
46 mechanisms for these patients to participate in molecular profiling studies and partner with the
47 research community to accelerate discoveries[18–20].

48 We hypothesized that a patient-partnered framework that empowers MPC patients to
49 share their biological samples, clinical histories, and lived experiences directly with researchers
50 regardless of geographic location or hospital affiliation would lead to new clinicogenomic
51 discoveries and begin to address demographic inequities and data access barriers in molecular
52 studies for this disease. Thus, we established the Metastatic Prostate Cancer Project
53 (MPCproject, mpcproject.org), a research model that leverages patient advocacy and social
54 media to enable MPC patients to participate in genomic research remotely at no personal cost.

RESULTS

55 *Development of a patient-partnered metastatic prostate cancer research model*

56 Working with patients, loved ones, and advocates, we established an MPCproject
57 enrollment process for men living with MPC in the U.S. and Canada (Fig. 1a). The MPCproject
58 outreach model is community-centered and utilizes advocacy partnerships, social media
59 campaigns, and educational initiatives to engage patients (Supplementary Fig. 1). Should they
60 choose to register, patient-partners complete an online survey describing their experience with
61 MPC, followed by signing electronic consent and medical release forms, which allow the
62 MPCproject team to contact their hospitals to request medical records for abstraction and
63 optionally archival tumor tissue for research-grade genomic sequencing (Supplementary Fig. 2).
64 Additionally, enrolled patients can use a mailed kit to donate saliva and/or blood at routine blood
65 draws at no cost, and these samples are sequenced to assess germline DNA and cfDNA,
66 respectively (Supplementary Fig. 3, 4).

67 Our partnership with patients is reciprocal and continuous. Patient-partners and advocates
68 are involved in every step of the project's design and execution—we respond directly to their
69 feedback and keep them informed of our progress and findings (Supplementary Fig. 5). We work
70 with men who choose to continue donating blood to help the research community understand the
71 evolution of metastatic disease, and we regularly release prepublication, deidentified genomic
72 and clinical data in public repositories for research use.

73 ***Partnering with a demographically distinct patient population***

74 To date, the MPCproject has partnered with over 1,000 patients in the U.S. and Canada
75 and has orchestrated three public data releases (Fig. 1b). The analyses presented here are based
76 on the 706 men from the U.S. and Canada who had enrolled (completed consent forms) as of
77 June 1, 2020 (Supplementary Fig. 6).

78 Using patient-reported survey data, we assessed the geographical diversity of our patient-
79 partners. Hailing from 49 U.S. states and 6 Canadian provinces, patient-partners reported
80 receiving care for their prostate cancer at over 1,000 distinct medical institutions, 91% of which
81 were reported by two or fewer patients (Fig. 1c). We found that 55% of patient-partners have
82 never received care at an NCI-designated cancer center, where genomic research is traditionally
83 conducted (Supplementary Table 1). These patient-partners were three times less likely to report
84 participating in a clinical trial, indicating the understudied nature of our cohort and barriers MPC
85 patients face in access to clinical trials (7% vs. 20%, $P = 1 \times 10^{-6}$, Fisher's exact test).

86 Patients in rural and medically underserved areas face unique obstacles and disparities in
87 clinical cancer care[21,22]. To better understand the challenges faced by our patient-partners, we
88 identified the census tracts of patient-reported U.S. home addresses and examined their
89 geographic characteristics (n = 628/706 participants provided U.S. addresses, Methods). We

90 found that 13% of patient-partners live in rural areas defined by the USDA, a proportion
91 consistent with MPC patients in the U.S. generally (11%)[23]. We then examined primary care
92 health physician shortage areas (HPSAs) and medically underserved areas (MUAs) defined by
93 the Health Resources and Services Administration (Methods). We found that 38% of patient-
94 partners live in HPSAs (29%) or MUAs (23%) (Fig. 1d)[24]. These proportions could not be
95 compared with MPC patients in the U.S. due to a lack of published data, but they are
96 significantly enriched compared to the general U.S. population (25% HPSA, 5% MUA, $P = 0.03$
97 and 1×10^{-82} respectively, Fisher's exact test)[25,26]. While living in a rural area was associated
98 with being in a MUA or HPSA, 23% of MPCproject patient-partners live in urban primary care
99 MUAs or HPSAs ($P = 5.7 \times 10^{-13}$, Fisher's exact test).
100 We found that home addresses in rural areas were a median of 160 km farther from
101 institutions where those patients reported receiving treatment, compared to home addresses in
102 urban areas ($P < 10^{-11}$, Mann-Whitney U test) (Methods, Fig. 1e). Although we cannot determine
103 if home addresses changed during treatment, this suggests that patient-partners in rural areas
104 travel significantly farther for cancer care. We did not observe significant differences in baseline
105 clinical factors, therapies received, or likelihood to participate in a clinical trial across patients in
106 rural areas, MUAs, or HPSAs.
107 The combination of the MPCproject's online enrollment and patient-centered outreach
108 through advocacy partnerships enabled the creation of a geographically distinct prostate cancer
109 research program. Despite the project's geographical diversity, however, fewer than 10% of
110 patient-partners self-identify as non-white. While similar to existing studies, this representation
111 remains below the proportion of minority prostate cancer patients generally (20%), a racial

112 imbalance that has spurred new MPCproject initiatives to connect with patients of color
113 (Supplementary Table 2, Discussion)[23].

114 ***Patient-reported data augment medical records to amplify patient stories***

115 Through the patient-reported data, we sought to understand the experiences of those
116 living with MPC. 45% of patient-partners report being diagnosed with *de novo* metastatic
117 disease, with bone (48%) and lymph node (39%) lesions as the most common metastatic sites
118 (Fig. 2a, b). 48% of patient-partners reported a family history of prostate or breast cancer, while
119 24% reported having at least one other cancer diagnosis in their lifetime, 30% of which was a
120 non-skin form of cancer (Fig. 2c, d). The average age at diagnosis was significantly younger than
121 the national average (61 vs. 65 years old, $P < 10^{-39}$, t-test), and 24% of participants were
122 diagnosed with early-onset prostate cancer (≤ 55 years at diagnosis, Supplementary Table 2)[27].

123 We used the MPCproject's comprehensive abstracted medical records taken from
124 medical documentation together with patient-reported data to evaluate the treatments received in
125 this real-world cohort (Methods, Fig. 2e). Patient-partners reported taking an average of 2.8
126 therapies (range 1-13) to treat their prostate cancer. 119 (17%) patient-partners had abstracted
127 medical records at the time of writing, and there was 90% concordance between therapies noted
128 in formal medical records and therapies reported by patients. The overlap was lowest for
129 treatments typically given earlier in the therapeutic timeline (first line androgen deprivation
130 therapy, 83%), supportive care therapies (64%), or treatments abandoned quickly due to side-
131 effects (Fig. 2e). This finding illustrates the value of patient-reported data obtained via surveys
132 for MPC, particularly in the absence of a complete medical record.

133 We also used the patient-reported data to assess how living with prostate cancer has
134 changed the daily lives of our patient-partners. For example, in the survey, we asked participants

135 to list additional medications, alternative medications, or lifestyle changes since their diagnosis
136 of prostate cancer. 56% of patient-partners reported a lifestyle change because of living with
137 their cancer, with the most common being a change in diet or exercise (Fig. 2f). Common
138 nutritional supplements reported include Vitamin D and antioxidant-based supplements, while
139 common non-cancer medications included metformin and statins. Collectively, these results
140 demonstrate the impact of metastatic prostate cancer on patient lifestyles and that patients often
141 pursue supplemental therapies that are not regularly documented in the medical record.

142 ***Whole exome sequencing of a real-world MPC patient cohort***

143 To date, we have completed molecular profiling of 573 samples from 333 patient-
144 partners, including: ultra-low pass whole genome sequencing (ULP-WGS, average depth of
145 0.1x) of cfDNA from 319 donated blood samples; whole exome sequencing (WES) of cfDNA
146 from 47 of those blood samples; WES of 106 tumor samples; and WES of 148 germline samples
147 from donated saliva or blood buffy coat. cfDNA samples underwent WES if ULP-WGS detected
148 a tumor fraction above 0.03 (Methods). In total, 82 exome-sequenced samples (63 tumor and 19
149 cfDNA) from 79 patient-partners enrolled before June 1, 2020 were included in downstream
150 genomic analyses after assessment of sufficient tumor purity ($\geq$10%) and coverage (Methods).

151 Exome sequencing from the tumor and cfDNA samples recapitulated known genomic
152 patterns in metastatic prostate cancer (Fig. 3a). *TP53* and *SPOP* were recurrently altered,
153 consistent with previous studies of both metastatic and primary prostate cancer ($q < 0.1$ via
154 MutSig2CV)[3,4,6]. In primary tumor samples from this cohort, the mutation frequency of *TP53*
155 (30%) was more consistent with metastatic cohorts than those of primary prostate cancer[3,6]. 17
156 (27%) primary tumor samples were from men diagnosed with *de novo* metastatic disease, and
157 samples from these patient-partners were more likely to carry *TP53* mutations ($P = 0.04$, Fisher's

158 exact test). We also observed known patterns of copy number alteration in prostate cancer (Fig.
159 3a). Analysis of gene copy number alterations using GISTIC2.0 revealed recurrent
160 amplifications of *AR* and *FOXA1*, as well as recurrent deletions of *PTEN* ($q < 0.1$)[28]. Whole-
161 genome doubling was present in 5/63 tumor samples and 3/19 cfDNA samples, including in two
162 tumor samples from patient-partners initially diagnosed with localized prostate cancer. In both
163 cases, the patients were diagnosed with metastatic disease within a few months of their initial
164 diagnosis.

165 To understand the mutational processes in this cohort's exome-sequenced samples, we
166 used a mutation-based method (deconstructSigs) to determine the contribution of COSMIC v2.0
167 signatures to each sample[29,30] (Fig. 3b, Methods). We detected the presence of aging-associated
168 clock-like signature 1 in all samples and the presence of signature 3 (associated with homologous
169 recombination deficiency, HRD) and signature 6 (associated with mismatch repair deficiency,
170 MMR) in a subset of samples. These results are consistent with previous studies implicating
171 these signatures in prostate cancer, although they likely overestimate the prevalence of signature
172 6 in tumor samples due to formalin-induced deamination artifacts[31,32]. We found that the
173 presence of signature 3 was enriched in metastasis-associated samples (cfDNA and primary
174 tumors obtained in the metastatic setting) relative to tumor tissue from patients with strictly
175 localized tumors at time of resection ($P < 0.02$, Fisher's exact test). While some samples with
176 signature 3 had alterations in *BRCA1*, *BRCA2*, or another DNA repair gene, this association was
177 not statistically significant, potentially highlighting the presence of HRD-positive tumors without
178 a causative molecular alteration as previously reported in studies of prostate and breast
179 cancer[5,33–36].

180 In 10% of samples (8/82), we observed contributions from COSMIC signatures 2 and 13,
181 which are driven by APOBEC cytidine deaminases and known to operate at a baseline level in
182 prostate cancer[31,37]. APOBEC-driven mutagenesis has been implicated in kataegis—rare,
183 localized hypermutation in specific nucleotide contexts that is associated with genomic
184 instability and increased Gleason score in prostate cancer[38,39]. In a cfDNA sample donated by
185 one patient-partner (patient-partner 0203), we detected eight distinct mutations within a 2 kB
186 window in *KMT2C*, a known driver of prostate cancer (Fig. 3c)[3]. Six of these mutations were in a
187 T(C>T)A nucleotide context, and this sample had a detectable contribution from COSMIC
188 signature 13. We found that two pairs of the mutations, p.S1947F/p.S1954F and
189 p.Q2325*/p.S2337Y, were each present on individual sequencing reads, confirming that these
190 mutations existed within the same cell and strongly implicating *KMT2C* disruption through
191 kataegis (Supplementary Fig. 7). These findings illustrate the ability to detect both frequent and
192 rare clinically relevant molecular events in MPC across diverse contexts using a patient-
193 partnered model.
194 Given the strong heritability of prostate cancer, we also sought to assess inherited
195 germline alterations and their overlap with self-reported family history of cancer[40]. We found
196 that among the 132 patient-partners (19%) with WES of donated saliva or blood buffy coat, 15
197 had pathogenic germline alterations in select genes implicated in prostate cancer heritability (Fig.
198 3d, Supplementary Table 3)[41]. 14% of men that reported a family history of prostate or breast
199 cancer had at least one pathogenic germline alteration, compared to 7% of men that reported no
200 family history, although this difference was not statistically significant ($P = 0.38$, Fisher's exact
201 test). The most mutated gene was *CHEK2* (8 patient-partners), followed by *BRCA2* (4 patient-
202 partners). In three cases, we detected an accompanying somatic loss of a germline-mutated gene

203 (Fig. 3d). These results emphasize the need to further characterize the drivers of germline
204 susceptibility in men with MPC and to expand clinical germline testing beyond *BRCA2* in
205 diverse clinical settings.

206 ***Longitudinal blood biopsies enable study of tumor evolution in a patient-partnered model***

207 Ten patient-partners had WES from both tumor tissue and cfDNA, and three patient-
208 partners had both samples pass quality control metrics. Using the molecular data and abstracted
209 medical records, we sought to explore the evolutionary relationships between these longitudinal
210 samples in the context of patient clinical trajectories. Like most men with MPC, one participant,
211 patient-partner 0495, received a diverse range of treatments between biopsy timepoints (Fig. 4a).
212 After responding to first line anti-androgen therapy (leuprolide + bicalutamide), they took
213 second-generation anti-androgen inhibitors (abiraterone, enzalutamide), as well as experimental
214 radiotherapy and immunotherapy. To explore the relationship between samples, we utilized
215 PhylogicNDT, an algorithm that clusters mutations based on their prevalence in the tumor
216 (cancer cell fraction) into evolutionarily related subclones (Methods)[42]. In the cfDNA sample of
217 patient-partner 0495 but not the primary tumor, we observed two distinct frameshift mutations in
218 *ASXL2*, a gene implicated in castration-resistant metastatic prostate cancer, as well as an
219 amplification of *AR*, a known resistance mechanism to abiraterone and enzalutamide[43,44]. Patient-
220 partner 0093's tumor had clonal mutations in *TP53* and *KMT2D* but harbored an *NF2* mutation
221 solely in the cfDNA sample. Patient-partner 0213's tumor had a *TP53* mutation and APOBEC-
222 associated COSMIC signature 13 detected exclusively in the cfDNA sample.

223 Two of these patient-partners, 0495 and 0093, were initially diagnosed with primary
224 prostate cancer (Gleason score 4 + 3 and 5 + 4, respectively), while patient-partner 0213 was
225 diagnosed with *de novo* metastatic disease. The primary tumor tissues of these participants were

226 obtained at the time of diagnosis and separated from their donated blood samples by a range of
227 years, ranging from 2 to 10 years. Despite these varied disease presentations, clinical trajectories,
228 and biopsy timelines, we observed similar patterns of a "clonal switch" between the primary
229 tumor and cfDNA, wherein different subclones were dominant each sample (Fig. 4b,
230 Supplementary Fig. 8). We did not, however, observe primary tumor-specific copy number
231 alterations, bolstering previous claims that subclonal diversification in MPC via mutations may
232 happen after acquisition of ancestral copy number alterations (Supplementary Fig. 9)[45].
233 Furthermore, we observed primary tumor-specific mutations across all seven other patient-
234 partners with both tumor and cfDNA samples, although their exact clonal structure could not be
235 resolved due to low purity (Supplementary Fig. 10). While we cannot account for the sampling
236 bias of tumor biopsies, these results suggest that such clonal switches may be common in the
237 development of metastatic disease.

238 In two of the three patient-partners with tumor and cfDNA samples that passed quality
239 control, we detected the emergence of an amplification in the androgen receptor (*AR*) between
240 the initial diagnosis and metastatic blood sample that was accurately captured using ULP-WGS
241 of cfDNA (example patient-partner shown in Fig. 4c). This led us to examine *AR* copy number
242 using ULP-WGS of cfDNA samples across the entire cohort, including those that did not have
243 exome sequencing (n = 300 patient-partners, 318 samples, Fig. 4d). We found that patient-
244 partners who reported taking enzalutamide or abiraterone had significantly higher *AR* log copy-
245 ratios across a range of tumor fractions ($P < 0.001$, linear regression). Men who had taken
246 enzalutamide or abiraterone also had significantly higher tumor fractions, likely reflecting a
247 more advanced disease state and subsequent higher tumor burden in blood ($P < 0.001$, Mann-
248 Whitney U test)[46]. We observed that *AR* amplifications are often detectable in ULP-WGS of

249 cfDNA even when the tumor fraction is below 0.03 (Fig. 4e, f). For one patient-partner, the
250 tumor fraction within their donated blood was inferred as undetectable, but we nevertheless
251 observed a clear *AR* amplification (Fig. 4e). This highlights the potential efficacy of cfDNA to
252 reveal clinically relevant changes in MPC, even in cases of very low or undetectable tumor
253 burden. Broadly, these sequencing results illustrate the feasibility of identifying relevant
254 genomic and evolutionary alterations from both archival tumor tissue and donated blood samples
255 irrespective of geographical source site, enabling patient-partners to participate in genomic
256 research at no cost and with little effort.

DISCUSSION

257 Here we describe the MPCproject, a patient-driven framework for partnering with MPC
258 patients in the U.S. and Canada to increase access to genomics research and strengthen our
259 understanding of this disease. The online enrollment process was jointly created with patient-
260 partners to emphasize simplicity, requiring only the completion of basic online consent and
261 survey forms, along with optional mailed saliva and blood kits. To our knowledge, no previous
262 effort in MPC has used patient partnership to integrated demographic, clinical, patient-reported,
263 and genomic data from patients at a national level.

264 To that end, we demonstrated the feasibility of working with over 700 patient-partners,
265 41% of whom live in rural, medically underserved, or health physician shortage areas. We found
266 that patient-partners living in rural areas in this study likely travel significantly farther for their
267 cancer care, which has been shown to independently predict worse outcomes and mortality for
268 cancer patients[47]. Furthermore, a recent study found that incomplete medical records are
269 associated with shorter overall survival for MPC patients, particularly for those with complicated
270 clinical histories or whose care is fragmented between institutions[48]. Our analysis of abstracted

271 medical record data revealed a strong overlap between clinical histories represented in medical
272 records and patient-reported data, even for patient-partners with complex treatment trajectories
273 or who had received treatment at multiple hospitals, supporting the use of patient surveys to
274 improve care in this disease.

275 We also demonstrated that tumor tissue collected from paraffin-embedded archival
276 samples and cfDNA from donated blood samples from across the U.S. and Canada, enriched for
277 samples not obtained from NCI cancer centers, accurately recapitulate known genomic findings
278 in MPC, including somatic alterations, mutational signatures, germline pathogenic variants, and
279 a rare kataegis event. There has been substantial effort in the field to identify molecular features
280 associated with selective response to therapies like PARP inhibition and immunotherapy,
281 including the use of mutational signatures to assess targetable HRD, MMR, and APOBEC
282 deficiencies in cases without a causative molecular alteration[33,49]. Our results strengthen previous
283 findings that such signatures can be detected using cfDNA and, combined with our ability to
284 obtain cfDNA from participants nationwide, demonstrate the scalability of a patient-partnered
285 approach to identify and validate such genomic findings within a 'real world' cohort[50,51].

286 Moreover, we used archival tumor tissue and cfDNA from donated blood to reconstruct
287 tumor phylogenetic profiles, revealing polyclonality between primary and metastatic diagnosis.
288 Despite well-known findings of heterogeneity in both primary and metastatic prostate cancer,
289 there is a paucity of matched primary-metastatic studies, owing mostly to the invasiveness and
290 logistical challenges of longitudinal biopsy studies[31,52]. Our project enables such studies paired
291 with comprehensive clinical histories with minimal patient effort. To that end, we also found
292 clinically relevant *AR* amplifications via low-pass WGS of cfDNA from donated blood, even at
293 very low or undetectable tumor fractions. This result provides additional inexpensive utility to

294 the suggested use of cfDNA tumor fraction as a clinically relevant biomarker in metastatic
295 prostate cancer[50,46]. We are working with patient-partners who continue to donate blood and have
296 been able to collect multiple secondary blood biopsy kits for future longitudinal analysis.
297 Through feedback from patient-partners and advocates, we continue to improve the
298 MPCproject's design and outreach. Despite the geographic diversity of our patient-partners, we
299 recognize that they do not reflect the racial diversity of MPC patients, a critical issue given
300 substantial disparities in both cancer care and genomics research by race and ethnicity[11,53,54]. In
301 light of structural racism and a well-founded mistrust of medical research by patients of color,
302 this unmet disparity demands that we rethink our models of outreach and patient engagement[55].
303 We continue to work with community-based advocacy partners to involve communities of color,
304 and we are building a campaign to amplify Black cancer patient voices and their lived
305 experiences. We are also working to translate enrollment and educational materials into Spanish.
306 In addition, a common request by our patient-partners is to enable return of clinically relevant
307 results to participants and their physicians. While the regulatory hurdles to accomplish this are
308 large, we recognize its importance to our patient-partners and are striving to institute return of
309 results under this project model prospectively.
310 Paired with open-access clinical trials, patient-driven studies hold great promise to
311 achieve equity and accelerate discovery in genomic research[56]. The MPCproject is part of a
312 wider 'Count Me In' patient-partnered initiative (joincountmein.org) that has already yielded
313 new findings in angiosarcoma and has expanded to metastatic breast cancer and osteosarcoma,
314 among others[57–59]. The success of the MPCproject is based entirely on the courage and altruism
315 of the men with whom we partner, who, in the words of one participant, hope that their
316 "participation will help other men… and lead eventually to a cure".

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AUTHOR CONTRIBUTIONS

N.W., C.A.P. and E.M.V.A. conceived and designed the MPCproject with support from E.S.L. J.C., S.B., L.S., and E.M.V.A. designed and prepared the study and interpreted the data. J.C. wrote the manuscript and performed the analyses. S.B. and L.S. led study operations including tumor sample and medical record acquisition, sample sequencing, and patient

338 coordination. L.S., B.S.T., M.D., E.A., S.S., A.L.D., R.R., D.M.S., I.K.S. oversaw medical
339 record abstraction. S.Y.C. provided feedback on various analyses of the study and completed
340 germline variant calling with oversight from S.H.A. S.B., L.S., J.C., B.T., M.D., M.M., and
341 P.S.C. coordinated data releases. M.M., P.S.C., A.D., B.Z. led recent project operations. M.D.
342 supervised early project operations. C.M.N. and E.A. led patient advocacy and outreach efforts.
343 A.T.M.C. and S.W. oversaw early project sequencing analyses. M.X.H. provided feedback of
344 study analyses. A.K.T. provided feedback on medical record abstractions and tissue sample
345 collection. D.K. enabled electronic medical record searching. J.N., J.M., Major I.H.G., B.O.
346 contributed to survey design, project development, assessment of patient criteria, and outreach
347 strategy.

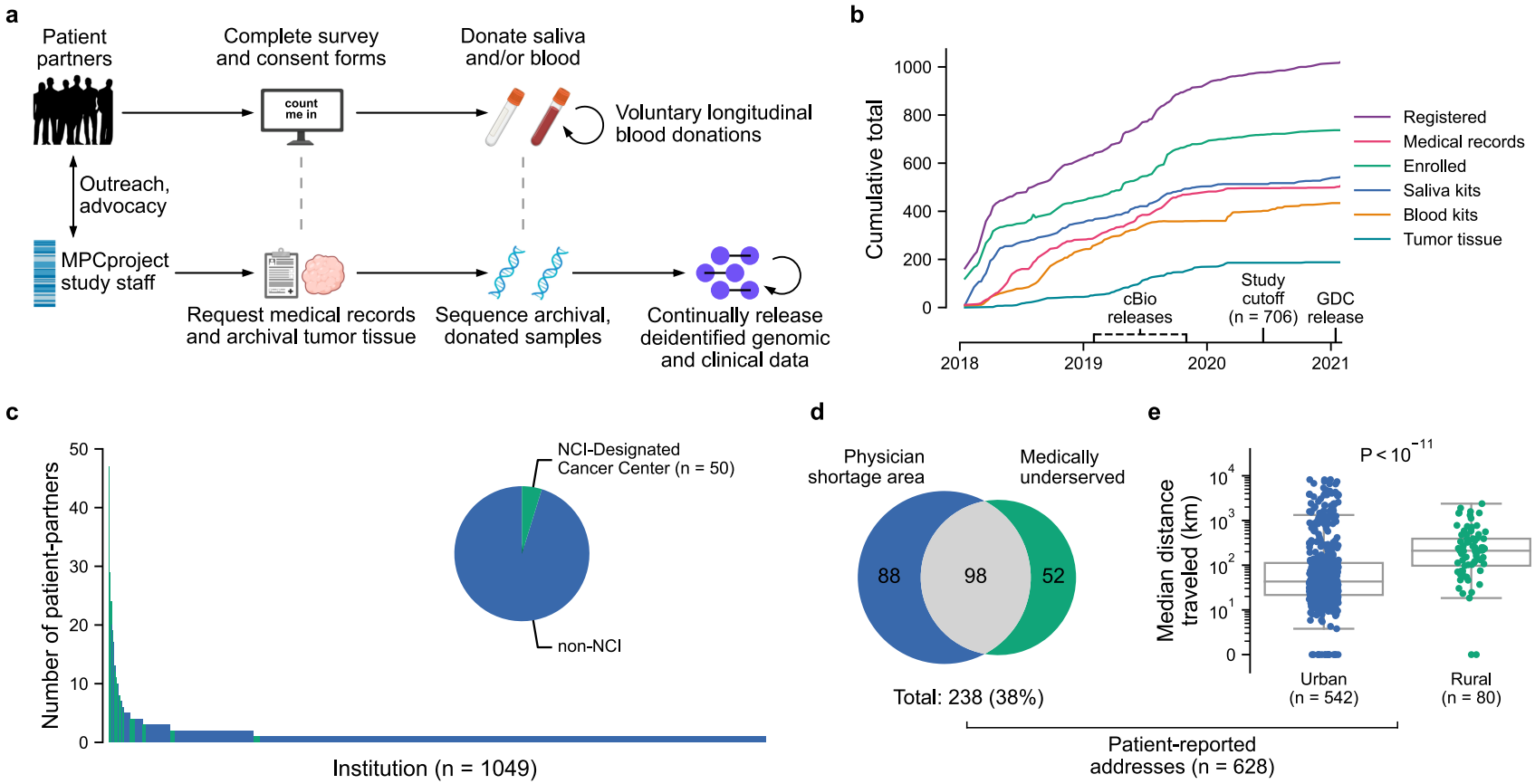
348 **COMPETING INTERESTS**

349 M.X.H. has been a consultant to Amplify Medicines and Ikena Oncology. E.S.L. is
350 currently in the process of divesting any relevant holdings. N.W. reports advisory relationships
351 and consulting with Eli Lilly and Co.; advising and stockholding interest in Relay Therapeutics;
352 and grant support from Puma Biotechnology. E.M.V.A. reports advisory relationships and
353 consulting with Tango Therapeutics, Genome Medical, Invitae, Illumina, Enara Bio, Manifold
354 Bio and Janssen; research support from Novartis and BMS; equity in Tango Therapeutics,
355 Genome Medical, Syapse, Manifold Bio and Enara Bio; and travel reimbursement from Roche
356 and Genentech, outside the submitted work.

357 **DATA AVAILABILITY**

358 Processed, deidentified data is available on cbioportal.org
359 (https://www.cbioportal.org/study/summary?id=prad_mpcproject_2018). Raw sequencing files
360 are available at the Genomic Data Commons (https://portal.gdc.cancer.gov/projects/CMI-MPC).

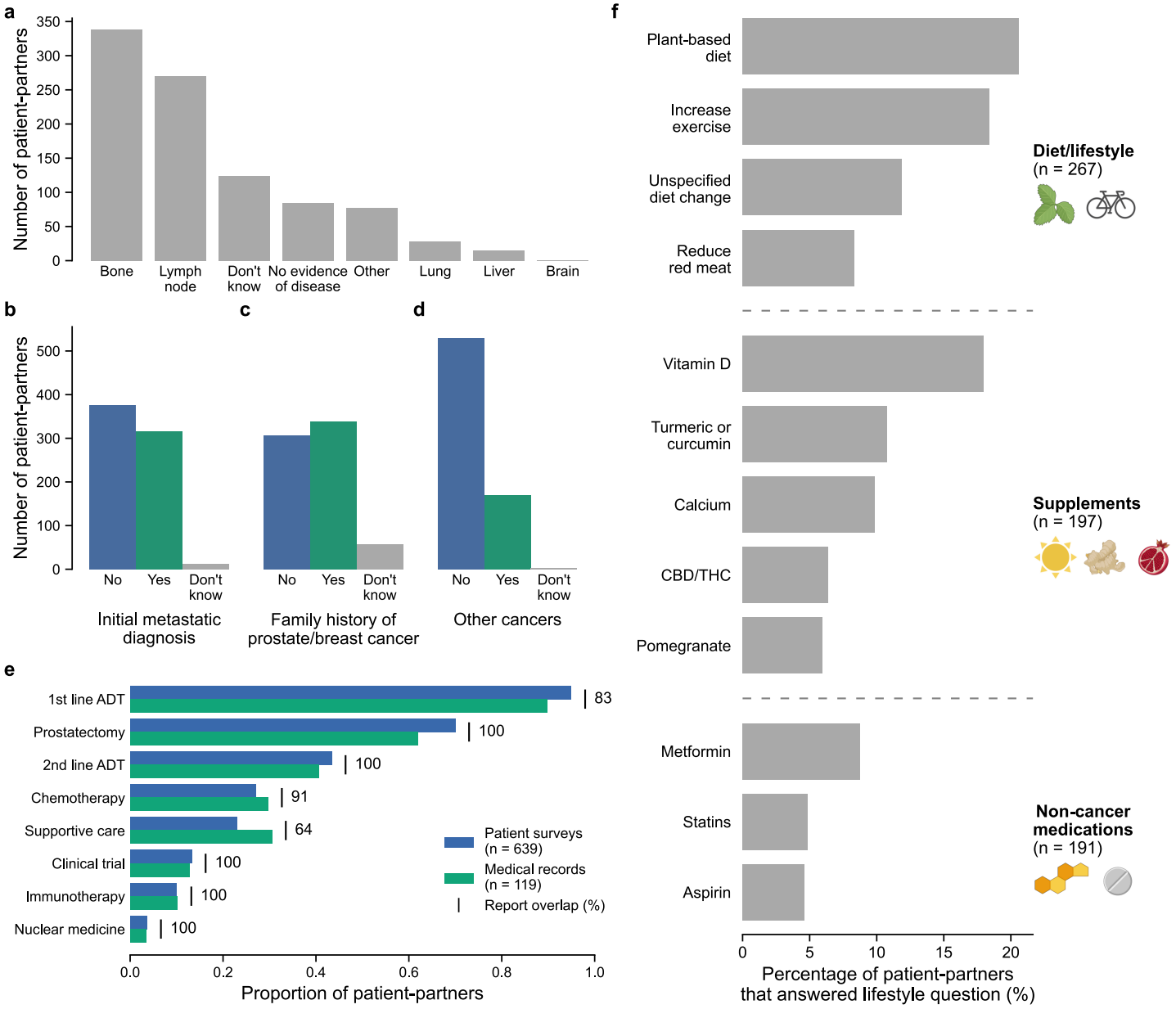
Please note that data is regularly being updated within these repositories and may not currently reflect all data generated from the project to date.


a
Patient partners
Complete survey and consent forms
Donate saliva and/or blood
count me in
Voluntary longitudinal blood donations
Outreach, advocacy
MPCproject study staff
Request medical records and archival tumor tissue
Sequence archival, donated samples
Continually release deidentified genomic and clinical data
b
Cumulative total
1000
800
600
400
200
0
Registered
Medical records
Enrolled
Saliva kits
Blood kits
Tumor tissue
cBio releases
Study cutoff (n = 706)
GDC release
2018
2019
2020
2021
c
Number of patient-partners
50
40
30
20
10
0
Institution (n = 1049)
NCI-Designated Cancer Center (n = 50)
non-NCI
d
Physician shortage area
Medically underserved
88
98
52
Total: 238 (38%)
e
Median distance traveled (km)
P < 10^-11
Urban (n = 542)
Rural (n = 80)
Patient-reported addresses (n = 628)

363

364 **Figure 1. Partnering with diverse patients to enhance our understanding of metastatic**
365 **prostate cancer**
366 **a)** Summary of MPCproject enrollment process. Patients learn about the project primarily
367 through outreach and partnered advocacy groups. If they register, patient-partners complete
368 online intake, consent, and medical release forms, then can opt into donating saliva via a mailed
369 kit and/or blood at routine blood draws at no charge. In parallel, MPCproject staff request
370 medical records and archival tumor samples from patients' medical institutions, then abstract
371 medical information from obtained records and sequence archival tumor tissue and/or donated
372 blood and saliva (Methods). Deidentified clinical, genomic, and patient-reported data are
373 released on a continual, prepublication basis and deposited in public repositories.
374 **b)** Enrollment statistics and timeline for the MPCproject. Depicted are the cumulative number of
375 patients that began the registration process (registered), patients that completed the survey and
376 consent forms (enrolled), patients with at least one medical record received (medical records),
377 and blood kits, saliva kits, and archival tumor tissue received at the Broad Institute for
378 sequencing (blood kits, saliva kits, tumor tissue, respectively). 706 patient-partners enrolled
379 before "Study cutoff", June 1, 2020, and are included in this study's analyses. cBioPortal
380 (cbioportal.org) releases include summary abstracted medical, genomic, and patient-reported
381 data; Genomic Data Commons (GDC) releases include raw sequencing files and demographic
382 data.
383 **c)** Represented medical institutions among patient-partners living in the U.S. and Canada. Shown
384 are the 1049 unique institutions (x-axis) where patient-partners report receiving care for their
385 prostate cancer, with the number of distinct patients at each institution (y-axis). NCI-designated

386 cancer centers are shown in green. Patient-partners that did not complete this survey question (n
387 = 36) and institutions outside the U.S. and Canada (n = 56) are not shown.
388 **d)** Access to medical care among patient-partners living in the U.S. Patient-reported U.S.
389 addresses were overlapped with primary care health physician shortage areas (HPSAs) and
390 medically underserved population/areas obtained from the Health Resources and Services
391 Administration (HRSA.gov). Patient-partners that live in Canada (n = 30), did not provide an
392 address (n = 40), or provided only a P.O. box (n = 8) are not shown.
393 **e)** Patient-partners in rural areas travel farther for clinical care. Using geographic census tract
394 information of self-reported home addresses along with USDA rural-urban continuum codes,
395 patient-partners were categorized as living in urban or rural areas. For each patient-partner, the
396 median Haversine round-trip distance between the zip code of their home address and that of
397 institutions they visited was calculated (Methods). Patient-partners that live in Canada (n = 30),
398 did not provide an address (n = 40), or provided only a P.O. box (n = 8) are not shown. *P*-value
399 calculated via two sided Mann-Whitney U test.


a
Number of patient-partners
350
300
250
200
150
100
50
0
Bone
Lymph node
Don't know
No evidence of disease
Other
Lung
Liver
Brain
b
c
d
Number of patient-partners
500
400
300
200
100
0
No
Yes
Don't know
Initial metastatic diagnosis
Family history of prostate/breast cancer
Other cancers
e
1st line ADT
Prostatectomy
2nd line ADT
Chemotherapy
Supportive care
Clinical trial
Immunotherapy
Nuclear medicine
83
100
100
91
64
100
100
100
Patient surveys (n = 639)
Medical records (n = 119)
Report overlap (%)
0.0
0.2
0.4
0.6
0.8
1.0
Proportion of patient-partners
f
Plant-based diet
Increase exercise
Unspecified diet change
Reduce red meat
Diet/lifestyle (n = 267)
Vitamin D
Turmeric or curcumin
Calcium
CBD/THC
Pomegranate
Supplements (n = 197)
Metformin
Statins
Aspirin
Non-cancer medications (n = 191)
0
5
10
15
20
Percentage of patient-partners that answered lifestyle question (%)

400

401 **Figure 2. Patient voices reveal the landscape of living with metastatic prostate cancer**

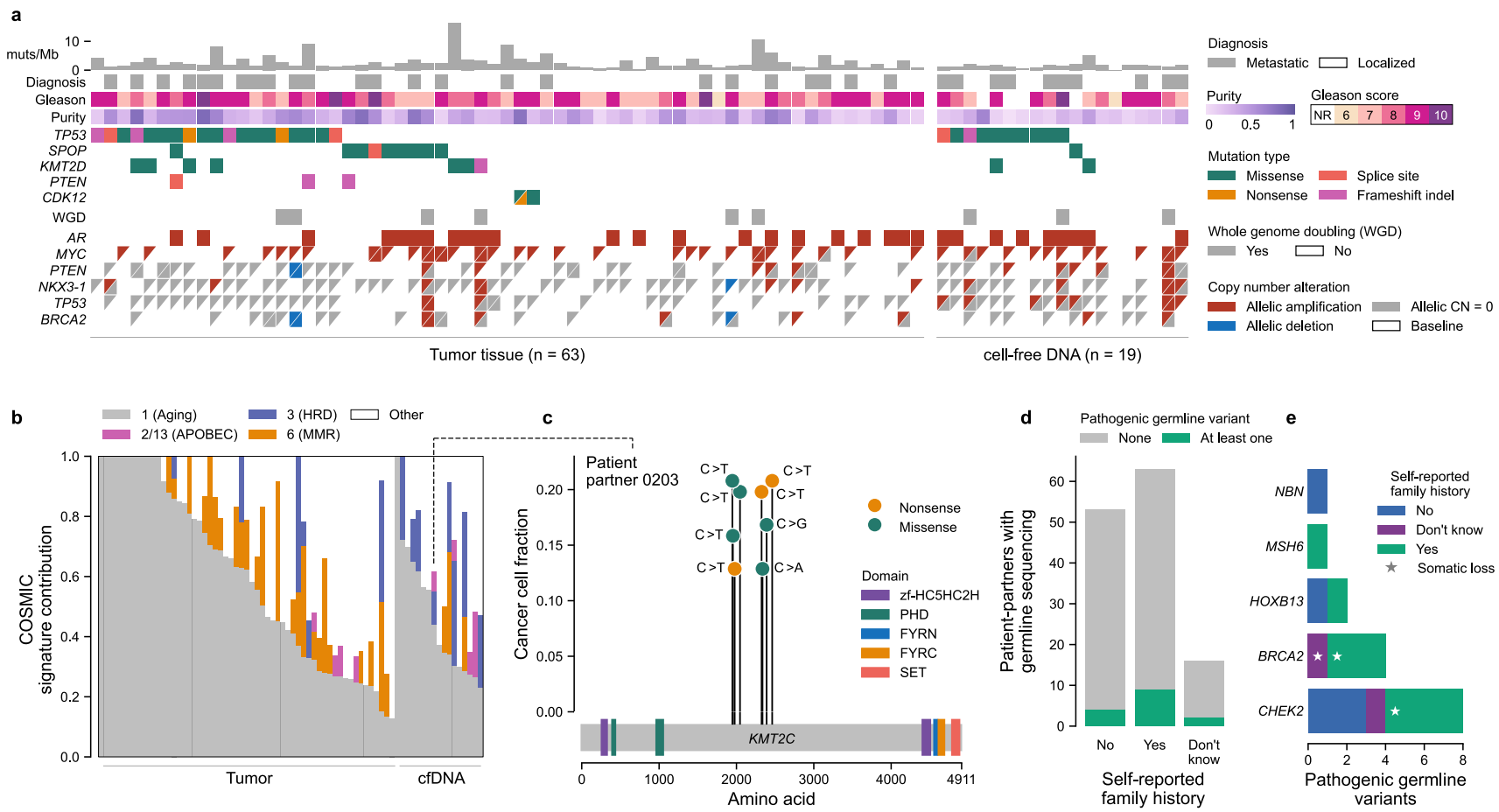
402 **a-d)** Self-reported data of 706 patient-partners related to their prostate cancer. In **a**, patient-
403 partners were asked for the current location of their cancer. Participants were free to choose
404 multiple if their cancer had metastasized to multiple locations. In **b-d**, responses were tabulated
405 from questions asking patient-partners if their initial prostate cancer diagnosis was metastatic
406 (**b**), if they have a family history of prostate/breast cancer (**c)**, or if they have ever had another
407 cancer diagnosis (**d**). Patient-partners who did not complete these questions (n < 5) are not
408 shown.

409 **e)** Self-reported therapies show strong overlap with medical records. Drug categories are shown
410 on the y-axis, with the proportion of patient-partners from each data type (patient surveys and
411 medical records) receiving therapies of that category shown on the x-axis. In the online survey,
412 patient-partners selected therapies they received for their metastatic prostate cancer from a list.
413 639/706 patient-partners reported at least one therapy and are shown. 119 of these participants
414 also had abstracted therapy data from medical records. Report overlap refers to how often
415 patient-partners report receiving a therapy when their medical records show that they have
416 received that therapy, as a percentage. Only drugs available for selection in the patient survey
417 were used in this comparison (Supplementary Table 4).

418 **f)** Landscape of lifestyle changes for patient-partners. Participants were asked to list additional
419 medications, alternative medications, or lifestyle changes since their diagnosis of prostate cancer.
420 Free-text responses were manually abstracted and categorized into diet/lifestyle changes,
421 supplements, and non-cancer medications. The y-axis shows individual instances of diet/lifestyle
422 changes, supplements, or medications. The x-axis shows the percentage of patient-partners with
423 that lifestyle change or taking that supplement/drug out of all patient-partners that responded to

424 the lifestyle question (n = 456). CBD/THC: Cannabidiol/Tetrahydrocannabinol (oils, medical

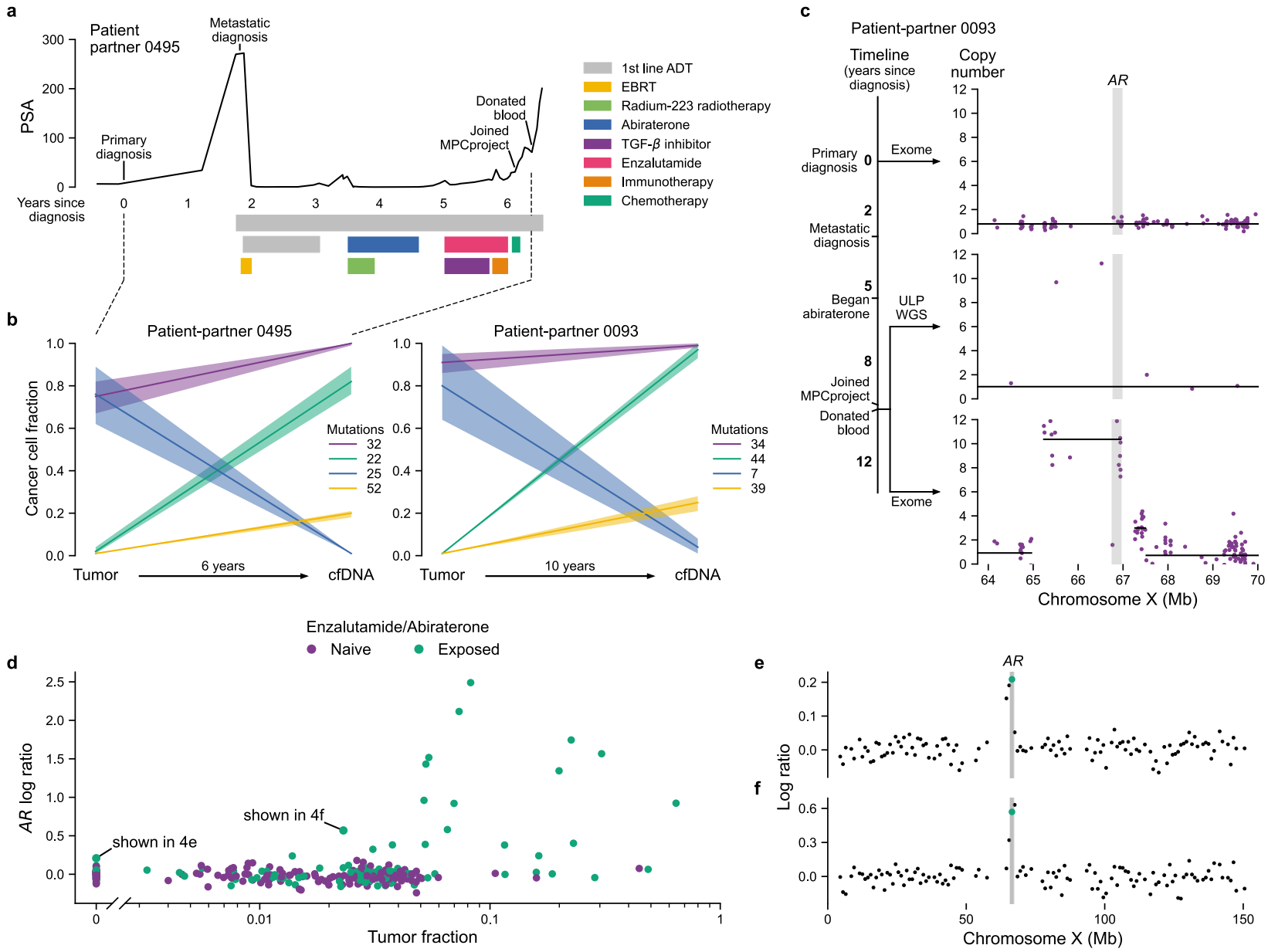
425 marijuana, etc).


a
muts/Mb
Diagnosis
Gleason
Purity
TP53
SPOP
KMT2D
PTEN
CDK12
WGD
AR
MYC
PTEN
NKX3-1
TP53
BRCA2
Tumor tissue (n = 63)
cell-free DNA (n = 19)
Diagnosis
Metastatic
Localized
Purity
Gleason score
Mutation type
Missense
Splice site
Nonsense
Frameshift indel
Whole genome doubling (WGD)
Yes
No
Copy number alteration
Allelic amplification
Allelic CN = 0
Allelic deletion
Baseline
b
1 (Aging)
3 (HRD)
Other
2/13 (APOBEC)
6 (MMR)
COSMIC signature contribution
Tumor
cfDNA
c
Patient partner 0203
Cancer cell fraction
Nonsense
Missense
Domain
zf-HC5HC2H
PHD
FYRN
FYRC
SET
KMT2C
Amino acid
d
Pathogenic germline variant
None
At least one
Patient-partners with germline sequencing
Self-reported family history
e
Self-reported family history
No
Don't know
Yes
Somatic loss
NBN
MSH6
HOXB13
BRCA2
CHEK2
Pathogenic germline variants

426

427 **Figure 3. Donated tumor and cell-free DNA samples obtained through patient partnership**
428 **recapitulate known genomic findings in metastatic prostate cancer**
429 **a)** Genomic and clinical landscape of 82 sequenced samples. Columns represent samples,
430 separated into tumor (prostate, left) and cfDNA (donated blood, right) samples, while rows
431 represent select clinical and genomic features. Gleason scores for tumor samples are taken from
432 the pathology report received with the sample (n = 58) or the patient-partner's medical records (n
433 = 5) if Gleason scores were not provided in the report. Gleason scores for cfDNA were taken
434 from pathology reports in the medical record, with NR representing cases where a Gleason score
435 was not reported in the medical record. Diagnosis refers to whether the initial diagnosis of
436 prostate cancer was localized or metastatic. Multiple mutations in the same gene are represented
437 as triangles. WGD refers to whole genome doubling. Copy number calls are allelic and defined
438 with respect to baseline allelic ploidy (2 for samples with WGD, 1 for those without), with calls
439 for the two alleles indicated by two triangles (except for *AR*, which has only one allele in men
440 and so is shown as a single box). Allelic CN = 0 refers to complete allelic deletions. Allelic
441 deletions that are not complete deletions are possible in samples with WGD. Figure created with
442 CoMut[60].
443 **b)** Mutational signature analysis of sequenced samples. The relative contribution of select
444 COSMIC v2.0 mutational signatures are shown, separated by tumor and cfDNA (donated blood)
445 sample type[30]. APOBEC refers to signatures associated with activity of APOBEC family of
446 cytidine deaminases (signature 2 and 13); MMR to the signature associated with deficient DNA
447 mismatch repair (signature 6); HRD to the signature associated with homologous recombination
448 deficiency (signature 3). Samples with too few mutations for signature analysis (< 50 mutations,
449 n = 5 samples) are not shown.

450 **c)** Instance of localized hypermutation (kataegis) of *KMT2C* in cfDNA from a donated blood
451 sample. The y-axis shows the cancer cell fraction of each mutation while the x-axis shows their
452 amino acid within *KMT2C*. Domains taken from Pfam[61]. The dotted line connects to this
453 sample's mutational signature profile.
454 **d)** Germline pathogenic DNA repair alterations and their overlap with patient reported family
455 history. Pathogenic germline alterations (as annotated by ClinVar) in genes from a select panel
456 of DNA repair genes implicated in prostate cancer were detected in patient-partners with
457 sequenced saliva or blood buffy coat (n = 132) (Methods; Supplementary Table 3)[62]. Survey
458 responses to a question asking about a family history of prostate or breast cancer were tabulated
459 and overlapped with this genomic data. Stars indicate instances where a somatic deletion also
460 affected that gene in a tumor or cfDNA sample from that patient-partner.


a
Patient partner 0495
Metastatic diagnosis
PSA
Primary diagnosis
Donated blood
Joined MPCproject
Years since diagnosis
1st line ADT
EBRT
Radium-223 radiotherapy
Abiraterone
TGF-β inhibitor
Enzalutamide
Immunotherapy
Chemotherapy
b
Patient-partner 0495
Patient-partner 0093
Cancer cell fraction
Mutations
32
22
25
52
34
44
7
39
Tumor
6 years
10 years
cfDNA
c
Patient-partner 0093
Timeline (years since diagnosis)
Copy number
AR
Primary diagnosis
Metastatic diagnosis
Began abiraterone
Joined MPCproject
Donated blood
Exome
ULP WGS
Chromosome X (Mb)
d
Enzalutamide/Abiraterone
Naive
Exposed
AR log ratio
shown in 4f
shown in 4e
Tumor fraction
e
f
Log ratio
Chromosome X (Mb)

462 **Figure 4. cfDNA from donated blood reveals patterns of clonal dynamics and clinically**
463 **relevant genomic changes**

464 **a)** Clinical trajectory of patient-partner 0495. This patient-partner's prostate specific antigen
465 (PSA) trajectory is shown on the y-axis, time in years since initial diagnosis is shown on the x-
466 axis, and bars denote the beginning and end of therapies. EBRT—external beam radiation
467 therapy; 1st line androgen deprivation therapy (ADT)—leuprolide and bicalutamide;
468 immunotherapy—nivolumab; chemotherapy—cisplatin and etoposide.

469 **b)** Tumor evolution from primary tumor to metastatic cfDNA samples. The y-axis shows the
470 cancer cell fraction (CCF) of clonal clusters identified between tumor and cfDNA samples (x-
471 axis). Time between samples shown on the x-axis. Colors indicate how many mutations were
472 identified in each clone, with a 95% confidence interval around the estimated CCF. Purple
473 represents the truncal/ancestral clone. Clusters with CCF < 0.10 across all biopsies are omitted.
474 The clinical trajectory of patient-partner 0495 (left) is shown in **a**, while the trajectory of patient-
475 partner 0093 (right) is shown in **c**.

476 **c)** Emergence of *AR* amplification in patient-partner 0093 induced by anti-androgen therapy. The
477 timeline depicts this patient's clinical trajectory, while the plots show the absolute copy number
478 (y-axis) of the genomic region around *AR* (x-axis, gene body shown in grey). The first plot
479 depicts exome sequencing from the patient's archival tumor tissue; the second and third plots
480 depict ultra-low pass whole-genome sequencing (ULP-WGS) and exome sequencing of cfDNA
481 from the patient's donated blood, respectively. Individual points represent copy number of target
482 regions (exome) or copy number of 1 Mb genomic windows (ULP-WGS). Black lines represent
483 discrete copy number segments.

484 **d – f)** ULP-WGS reveals clinically relevant *AR* amplifications even at low tumor fraction. Tumor
485 fraction of 318 cfDNA samples from donated blood of 300 patient-partners with ULP-WGS
486 sequencing is shown on the x-axis, while the log copy-ratio (logR) of the genomic interval
487 containing *AR* is shown on the y-axis. Points are colored by whether patient-partners self-
488 reported taking enzalutamide or abiraterone. 89 samples are shown with tumor fraction of 0
489 (undetectable), while 229 have nonzero tumor fractions. Two samples, one at a tumor fraction of
490 0 and another at a tumor fraction of 0.023, have chromosome X log copy-ratio profiles shown in
491 **e** and **f**, respectively. The green points represent the values shown in **d,** with the genomic interval
492 containing *AR* highlighted in grey.

METHODS

493 *Statistical computing*

494 Except where otherwise specified, analysis and data visualization were performed with
495 Python 3.8, SciPy v.1.5.2, Matplotlib v.3.3.2, seaborn v.0.11.0 and R v.3.5.1. All statistical tests
496 were two-sided unless otherwise specified. The code used to generate the main figures can be
497 found at https://github.com/vanallenlab/mpcproject-paper.

498 *MPCproject website*

499 The MPCproject utilizes a website (https://mpcproject.org/) to enroll patients through an
500 online consent and release form. The website provides information about the project and
501 advocacy groups that have partnered with the study. The website design, messaging, and
502 workflow were developed with direct input from patient-partners and advocates.

503 *Informed consent*

504 Patients who chose to enroll in this research study are provided informed consent using a
505 web-based consent form approved by the Dana-Farber/Harvard Cancer Center Institutional
506 Review Board (DF/HCC Protocol 15-057B). A link to the electronic informed consent document
507 for formal enrollment in the study (https://mpcproject.org/ConsentAndRelease.pdf) was sent to
508 registrant emails, and upon signing, a copy of the completed form was shared. At minimum,
509 informed consent enabled study staff to request and abstract medical records, send a saliva kit
510 directly to patients, perform sequencing on any returned saliva samples, and release de-identified
511 integrated clinical, genomic, and patient-reported data for research use. Patient-partners had the
512 additional option to consent to study staff obtaining a portion of archived tumor tissue and/or a
513 blood sample for further sequencing analysis.

514 *Patient-reported data*

515 After registering, patient-partners completed a 17-question survey asking them about
516 themselves and their disease (https://mpcproject.org/AboutYouSurvey.pdf). All questions were
517 optional. Information on how question responses were standardized and categorized can be
518 found in the Supplementary Methods.

519 *Acquisition of medical records*

520 Medical records were obtained for patient-partners from the U.S. and Canada who
521 completed the consent and medical release forms. Later in project development, a donated saliva
522 or blood sample was also required. Study staff submitted medical record requests to all
523 institutions and physician offices at which the patient reported receiving clinical care for their
524 prostate cancer. A detailed medical record request form, along with the consent and release
525 forms, were electronically faxed to each facility listed in a patient's release form. Medical
526 records were returned to the project via mail, fax, or secure online portals. If a record request was
527 not fulfilled in six months, study staff called the hospital, and a second request was submitted,
528 with up to three requests made. Patient-partners that communicated with study staff about
529 changes in their treatment could request a medical record update, in which case their current
530 hospital was again contacted for medical records. All medical records were saved in an
531 electronic format to a secure drive at the Broad Institute.

532 *Acquisition of patient samples*

533 All consented patient-partners living in the United States or Canada were mailed saliva
534 kits with appropriate instructions, a sample tube labeled with a unique barcode, and a prepaid
535 return box to send back the saliva sample. Samples were returned to the Broad Institute
536 Genomics Platform, logged, and stored at room temperature (25 °C) until further sequencing.

537 If a consented patient-partner opted into the blood biopsy component of the study, they
538 were sent a blood kit with instructions (https://mpcproject.org/BloodSampleInstructions.pdf,
539 Supplementary Figure 4). Participants could take this kit to their next blood draw and request a
540 courtesy draw by their medical provider; if a courtesy draw was not possible, patients could go to
541 Quest Diagnostics with a complimentary voucher to have their blood drawn. Blood kits were
542 returned free of charge to the Broad Institute Genomics Platform where they were fractionated
543 into plasma and buffy coats and stored at -80°C. If a patient-partner did not provide a saliva
544 sample, buffy coats were used to extract germline DNA for WES. Plasma samples continued to
545 WES if ultra-low pass WGS detected a tumor fraction of circulating tumor DNA greater than
546 0.03. Some patient-partners were selected to provide additional blood samples and were sent a
547 new consent form. If they agreed to submit another blood sample, a new blood kit was shipped.
548 For patient-partners that provided a germline sample and consented to the acquisition of
549 some of their archival tumor tissue, study staff reviewed each patient's medical records and
550 identified available tissue (Supplementary Methods). Patient-partners were screened by the study
551 staff to determine if they had metastatic or advanced prostate cancer based on the definition by
552 our study. If a patient-partner had a sample that met the project's strict requesting criteria, study
553 staff coordinated with that hospital's pathology department to fax a request for one H&E-stained
554 slide as well as either 5-20 5-µm unstained slides or one formalin-fixed paraffin-embedded tissue
555 block. Requests explicitly asked that the pathology department should not exhaust a sample to
556 fulfill the request. Samples were sent to the MPCproject by mail. Tissue samples received as
557 slides were labeled with unique barcode identifiers and submitted for whole exome sequencing.
558 Tissue samples received as blocks were cut into three 30-µm scrolls per block, labeled with
559 unique barcode identifiers, and then submitted for whole exome sequencing.

560 *Medical record abstraction*

561 A data dictionary comprising 60 clinical fields with possible options was curated by
562 trained study staff working with prostate oncologists. Electronic health records were converted to
563 searchable PDF files using the Optical Character Recognition (OCR) engine known as
564 Tesseract[63]. Three study staff abstractors were involved in the abstraction and QC process for
565 each record (Supplementary Methods). If a field had lack of concordance between abstractors or
566 there were outstanding questions, a prostate cancer oncologist reviewed the content. Whenever
567 possible, clinical data was abstracted directly from the records. For information that's not found,
568 it was abstracted as 'NOT FOUND IN RECORD'. In instances where ambiguity or incomplete
569 data was present, inferences were made considering the whole narrative of the medical record.
570 Incomplete dates missing the day or month are abstracted as the first day of the month or first
571 month of the year, respectively. While all medical records will eventually be abstracted, medical
572 records from patient-partners that received molecular sequencing of some form were prioritized
573 for this study, resulting in 125 patient-partners with medical record abstractions, 119 of which
574 had at least one therapy noted. In examining the overlap between patient surveys and medical
575 record therapies, we only considered therapies that were given for metastatic prostate cancer at
576 least one week before the patient enrolled.

577 *Geographic analysis*

578 Using secure Census Bureau geocoding, we identified the census tracts of patient
579 reported home addresses[64]. To identify patient-partners living in rural areas, this information was
580 overlapped with rural-area continuum (RUCA) codes from the United States Department of
581 Agriculture (USDA)[65]. Addresses with a secondary RUCA code greater than 3 were designated
582 as rural. For comparison, the proportion of metastatic prostate cancer patients within each RUCA

583 code from 2004 – 2017 was taken from Surveillance, Epidemiology, and End Results (SEER)
584 using SEER*stat with the following selection table: `{Site and Morphology.Site recode ICD-O-`
585 `3/WHO 2008} = 'Prostate' AND {Stage - Summary/Historic.SEER Combined Summary Stage`
586 `2000 (2004-2017)} != 'In situ', 'Localized only', 'Not applicable',`
587 `'Unknown/unstaged/unspecified/DCO', 'Blank(s)'`[23]. To identify patient-partners living in
588 medical shortage areas, the census tracts of home addresses were overlapped with primary care
589 health physician shortage areas (HPSA) and medically underserved areas (MUA) defined by the
590 Health Resources and Services Administration (HRSA)[25]. Addresses were labelled as existing
591 within a MUA if they were designated as within a medically underserved area or population and
592 as existing within a HPSA if they were designated as within a primary care HPSA. Published
593 geographic datasets of cancer patients (e.g., SEER, NPCR) do not contain census-tract resolved
594 data or summary results of MUA/HPSA status, so for comparison we instead used the total U.S.
595 population living in HPSAs and MUAs, taken from HRSA, divided by the entire U.S. population
596 taken from the U.S. Census[25,26]. To calculate appointment distances, we calculated the round-trip
597 Haversine distances between the zip code of home addresses and the zip code of reported
598 institutions.

599 *Whole exome sequencing analysis*

600 Whole exome sequences were captured using Illumina technology and the sequence data
601 processing and analysis was performed using Picard and FireCloud pipelines on Terra
602 (https://terra.bio/) (Supplementary Methods). The Picard pipeline (http://picard.sourceforge.net)
603 was used to produce a BAM file with aligned reads. This includes alignment to the GRCh37
604 human reference sequence using BWA[66] and estimation and recalibration of base quality score
605 with the Genome Analysis Toolkit (GATK)[67]. Somatic alterations for tumor samples were called

606 using a customized version of the Getz Lab CGA WES Characterization pipeline
607 (https://portal.firecloud.org/#methods/getzlab/CGA_WES_Characterization_Pipeline_v0.1_Dec2
608 018/) developed at the Broad Institute. Briefly, MuTect v1.1.6 algorithm was used to identify
609 somatic mutations[68]. Somatic mutation calls were filtered using a panel of normals (PoN), oxoG
610 filter and an FFPE filter to remove artifacts introduced during the sequencing or formalin
611 fixation process[69]. Small somatic insertions and deletions were detected using the Strelka
612 algorithm[70]. Somatic mutations were annotated using Oncotator[71]. Recurrently altered mutations
613 were identified using MutSig2CV[72]. To define somatic copy ratio profiles, we used GATK
614 CNV[67]. To generate allele-specific copy number profiles and assess tumor purity and ploidy, we
615 used ABSOLUTE and FACETS[73,74]. Final segmentation calls were taken from ABSOLUTE,
616 except for the X chromosome, which was taken from FACETS. We utilized GISTIC2.0 to
617 identify significantly recurrent amplification and deletion peaks[28]. For determining allele-specific
618 copy number alterations, we assessed the absolute allelic copy numbers of the segment
619 containing each gene. Mutation burden was calculated as the total number of mutations (non-
620 synonymous + synonymous) detected for a given sample divided by the length of the total
621 genomic target region captured with appropriate coverage from whole exome sequencing.
622 *Whole exome sequencing quality control*
623 Samples with average coverage below 55x in the tumor sample or below 30x in the
624 normal sample were excluded. Samples with purity < 0.10 from both ABSOLUTE and FACETS
625 were excluded. DeTiN was applied to samples to estimate the amount of tumor contamination in
626 the normal samples; samples with TiN (tumor in normal) > 0.25 were excluded[75]. ContEst was
627 applied to measure the amount of cross-sample contamination in samples; samples with
628 contamination > 0.04 were excluded[76]. The Picard task CrossCheckFingerprints was applied to

629 determine sample mixups; samples with Fingerprints LOD value < 0 were excluded[77]. Samples
630 which passed quality control were submitted to cBioPortal and GDC.

631 *Ultra-low pass whole genome sequencing analysis*

632 ichorCNA was used to assess the tumor fraction in cfDNA samples that completed ultra-
633 low pass whole genome sequencing[50]. The log copy ratio of *AR* was assessed by the log copy
634 ratio of the genomic interval containing *AR.* This value could not consistently be converted to
635 absolute copy number due to the low tumor fractions of many samples.

636 *Mutational signature analysis and kataegis*

637 Mutational processes in our cohort were determined using deconstructSigs with default
638 parameters applying COSMIC v2 signatures as the reference with a maximum number of
639 signatures of 6[29,30]. A signature was assessed as present if the signature contribution was greater
640 than 6%. Because tumor samples were formalin-fixed and paraffin embedded (FFPE), a process
641 known to introduce stranded mutational artifacts in specific nucleotide contexts, we used a filter
642 to remove likely FFPE artifacts according to nucleotide context and strand bias before using
643 deconstructSigs[78]. We also tried to assess the colocalization of the kataegis event with structural
644 variant breakpoints but were limited by targeted sequencing in exomes and low coverage in
645 ULP-WGS. *KMT2C* and its surrounding region were not copy number altered in the sample with
646 kataegis. Kataegis was not identified in any other sample.

647 *Association of DNA-repair alterations and presence of signature 3*

648 Alterations in a select list of genes previously implicated in DNA-repair in prostate
649 cancer were examined (Supplementary Table 3). An alteration was considered if there was a
650 somatic single-copy deletion, double deletion, nonsense mutation, missense mutation, frameshift

651 indel, or splice site mutation. An alteration was also considered if there was a pathogenic
652 germline alteration, denoted by "Pathogenic" in ClinVar[62].
653 *Germline variant discovery*
654 To call short germline single-nucleotide polymorphisms, insertions, and deletions from
655 germline WES data, we used DeepVariant (v0.8.0)[79,80]. Specifically, we used the publicly-
656 released WES model
657 (https://console.cloud.google.com/storage/browser/deepvariant/models/DeepVariant/0.8.0/Deep
658 Variant-inception_v3-0.8.0+data-wes_standard/) to generate single-sample germline variant call
659 files using the human genome reference GRCh37(b37). We filtered variants with bcftools v1.9 to
660 only keep high-quality variants annotated as "PASS" in the "FILTER" column. The high-quality
661 variants were merged into single-sample Variant Call Format (VCF) files using
662 CombineVariants from GATK 3.7 (https://github.com/broadinstitute/gatk/releases). To
663 decompose multiallelic variants and normalize variants, we used the computational package vt
664 v3.13 (https://github.com/atks/vt). Lastly, germline variants were annotated using the VEP v92
665 with the publicly-released GRCh37 cache file (https://github.com/Ensembl/ensembl-vep)[81].
666 Germline variants were denoted as pathogenic if they appeared as "Pathogenic" in ClinVar (Dec
667 2019 version)[62].
668 *Phylogenetic analysis*
669 To compare mutations between distinct samples (tumor and cfDNA) from the same
670 patient, we used a previously described method designed to recover evidence for mutations
671 called in one sample in all other samples derived from the same individual[82]. In brief, the 'force-
672 calling' method uses the strong prior of the mutation being present in at least one sample in the
673 patient to more sensitively detect and recover mutations that might otherwise be missed. A

674 mutation was deemed tumor/cfDNA specific if there were no force-called reads that supported
675 the mutation in the other sample, although this process underestimates the proportion of shared
676 mutations in low purity tumors. The cancer cell fraction (CCF) of mutations were defined using
677 ABSOLUTE, which calculates the CCF based on variant allele frequency, purity, and local
678 allelic copy number[73]. To reconstruct tumor phylogenies, we used PhylogicNDT, which clusters
679 mutations into subclones across multiple samples based on their underlying similar CCFs[42].

680 *Data releases*

681 The MPCproject releases de-identified clinical, patient-reported and research-grade
682 genomic data into public repositories, such as cBioPortal
683 (https://www.cbioportal.org/study/summary?id=prad_mpcproject_2018) and the Genomic Data
684 Commons (https://portal.gdc.cancer.gov/projects/CMI-MPC), at regular intervals and pre-
685 publication. Data is processed and formatted as required by each repository's guidelines. All
686 patient identifiers are stripped prior to data deposition to protect patient privacy. On the
687 MPCproject data release webpage (https://mpcproject.org/data-release), patients can access
688 project data, additional information about the data, list of common terms used in research,
689 methods used to generate the data, and an email address for any additional data-related questions.

REFERENCES

690 1. Siegel, R. L., Miller, K. D. & Jemal, A. Cancer statistics, 2020. *CA. Cancer J. Clin.* **70**, 7–30
691 (2020).
692 2. Litwin, M. S. & Tan, H.-J. The Diagnosis and Treatment of Prostate Cancer: A Review.
693 *JAMA* **317**, 2532–2542 (2017).
694 3. Cancer Genome Atlas Research Network. The Molecular Taxonomy of Primary Prostate
695 Cancer. *Cell* **163**, 1011–1025 (2015).
696 4. Armenia, J. *et al.* The long tail of oncogenic drivers in prostate cancer. *Nat. Genet.* **50**, 645–
697 651 (2018).
698 5. de Bono, J. *et al.* Olaparib for Metastatic Castration-Resistant Prostate Cancer. *N. Engl. J.*
699 *Med.* **382**, 2091–2102 (2020).
700 6. Abida, W. *et al.* Genomic correlates of clinical outcome in advanced prostate cancer. *Proc.*
701 *Natl. Acad. Sci.* **116**, 11428 (2019).
702 7. Annala, M. *et al.* Circulating Tumor DNA Genomics Correlate with Resistance to Abiraterone
703 and Enzalutamide in Prostate Cancer. *Cancer Discov.* **8**, 444 (2018).
704 8. Sonpavde, G. *et al.* Circulating tumor DNA alterations in patients with metastatic castration-
705 resistant prostate cancer. *Cancer* **125**, 1459–1469 (2019).
706 9. Siu, L. L. *et al.* Facilitating a culture of responsible and effective sharing of cancer genome
707 data. *Nat. Med.* **22**, 464–471 (2016).
708 10. Joly, Y., Dove, E. S., Knoppers, B. M., Bobrow, M. & Chalmers, D. Data sharing in the
709 post-genomic world: the experience of the International Cancer Genome Consortium (ICGC)
710 Data Access Compliance Office (DACO). *PLoS Comput. Biol.* **8**, e1002549–e1002549
711 (2012).

712 11. Spratt, D. E. *et al.* Racial/Ethnic Disparities in Genomic Sequencing. *JAMA Oncol.* **2**,
713 1070–1074 (2016).
714 12. Feyman, Y., Provenzano, F. & David, F. S. Disparities in Clinical Trial Access Across
715 US Urban Areas. *JAMA Netw. Open* **3**, e200172–e200172 (2020).
716 13. Huey, R. W., Hawk, E. & Offodile, A. C. Mind the Gap: Precision Oncology and Its
717 Potential to Widen Disparities. *J. Oncol. Pract.* **15**, 301–304 (2019).
718 14. Mamun, A. *et al.* Diversity in the Era of Precision Medicine - From Bench to Bedside
719 Implementation. *Ethn. Dis.* **29**, 517–524 (2019).
720 15. Messner, D. A. *et al.* Barriers to clinical adoption of next generation sequencing:
721 Perspectives of a policy Delphi panel. *Appl. Transl. Genomics* **10**, 19–24 (2016).
722 16. American Cancer Society Cancer Action Network. Payer Coverage Policies of Tumor
723 Biomarker Testing. (2020).
724 17. Chakradhar, S. Tumor sequencing takes off, but insurance reimbursement lags. *Nat. Med.*
725 **20**, 1220–1221 (2014).
726 18. McGuire, A. L. *et al.* To share or not to share: a randomized trial of consent for data
727 sharing in genome research. *Genet. Med. Off. J. Am. Coll. Med. Genet.* **13**, 948–955 (2011).
728 19. Husedzinovic, A., Ose, D., Schickhardt, C., Fröhling, S. & Winkler, E. C. Stakeholders'
729 perspectives on biobank-based genomic research: systematic review of the literature. *Eur. J.*
730 *Hum. Genet. EJHG* **23**, 1607–1614 (2015).
731 20. Sequence Me - Demand Genomic & DNA Testing for Cancer Treatments.
732 https://sequenceme.org/.

733 21. Henley, J. *et al.* Invasive Cancer Incidence, 2004–2013, and Deaths, 2006–2015, in
734 Nonmetropolitan and Metropolitan Counties — United States. *MMWR Surveill Summ 2017*
735 **66**, 1–13 (2017).
736 22. Olaku, O. O. & Taylor, E. A. Cancer in the Medically Underserved Population. *Prim.*
737 *Care* **44**, 87–97 (2017).
738 23. *Surveillance Research Program, National Cancer Institute SEER*Stat software*.
739 24. Economic Research Service - U.S. Department of Agriculture. Rural-Urban Continuum
740 Codes - Documentation. (2013).
741 25. Shortage Areas - Health Resources and Services Administration.
742 26. Population Clock. https://www.census.gov/popclock/.
743 27. Rawla, P. Epidemiology of Prostate Cancer. *World J. Oncol.* **10**, 63–89 (2019).
744 28. Mermel, C. H. *et al.* GISTIC2.0 facilitates sensitive and confident localization of the
745 targets of focal somatic copy-number alteration in human cancers. *Genome Biol.* **12**, R41
746 (2011).
747 29. Rosenthal, R., McGranahan, N., Herrero, J., Taylor, B. S. & Swanton, C.
748 deconstructSigs: delineating mutational processes in single tumors distinguishes DNA repair
749 deficiencies and patterns of carcinoma evolution. *Genome Biol.* **17**, 31 (2016).
750 30. Tate, J. G. *et al.* COSMIC: the Catalogue Of Somatic Mutations In Cancer. *Nucleic Acids*
751 *Res.* **47**, D941–D947 (2019).
752 31. Gerhauser, C. *et al.* Molecular Evolution of Early-Onset Prostate Cancer Identifies
753 Molecular Risk Markers and Clinical Trajectories. *Cancer Cell* **34**, 996-1011.e8 (2018).
754 32. Alexandrov, L. B. *et al.* Signatures of mutational processes in human cancer. *Nature* **500**,
755 415–421 (2013).

756 33. Mateo, J. *et al.* DNA-Repair Defects and Olaparib in Metastatic Prostate Cancer. *N. Engl.*
757 *J. Med.* **373**, 1697–1708 (2015).
758 34. Pritchard, C. C. *et al.* Inherited DNA-Repair Gene Mutations in Men with Metastatic
759 Prostate Cancer. *N. Engl. J. Med.* **375**, 443–453 (2016).
760 35. Polak, P. *et al.* A mutational signature reveals alterations underlying deficient
761 homologous recombination repair in breast cancer. *Nat. Genet.* **49**, 1476–1486 (2017).
762 36. Sztupinszki, Z. *et al.* Detection of Molecular Signatures of Homologous Recombination
763 Deficiency in Prostate Cancer with or without BRCA1/2 Mutations. *Clin. Cancer Res.* **26**,
764 2673 (2020).
765 37. Swanton, C., McGranahan, N., Starrett, G. J. & Harris, R. S. APOBEC Enzymes:
766 Mutagenic Fuel for Cancer Evolution and Heterogeneity. *Cancer Discov.* **5**, 704–712 (2015).
767 38. Nik-Zainal, S. *et al.* Mutational Processes Molding the Genomes of 21 Breast Cancers.
768 *Cell* **149**, 979–993 (2012).
769 39. Fraser, M. *et al.* Genomic hallmarks of localized, non-indolent prostate cancer. *Nature*
770 **541**, 359–364 (2017).
771 40. Mucci, L. A. *et al.* Familial Risk and Heritability of Cancer Among Twins in Nordic
772 Countries. *JAMA* **315**, 68–76 (2016).
773 41. AlDubayan, S. Considerations of multigene test findings among men with prostate cancer
774 - knowns and unknowns. *Can. J. Urol.* **26**, 14–16 (2019).
775 42. Leshchiner, I. *et al.* Comprehensive analysis of tumour initiation, spatial and temporal
776 progression under multiple lines of treatment. *bioRxiv* 508127 (2018) doi:10.1101/508127.
777 43. Grasso, C. S. *et al.* The mutational landscape of lethal castration-resistant prostate cancer.
778 *Nature* **487**, 239–243 (2012).

779 44. Tucci, M. *et al.* Enzalutamide-resistant castration-resistant prostate cancer: challenges
780 and solutions. *OncoTargets Ther.* **11**, 7353–7368 (2018).
781 45. Espiritu, S. M. G. *et al.* The Evolutionary Landscape of Localized Prostate Cancers
782 Drives Clinical Aggression. *Cell* **173**, 1003-1013.e15 (2018).
783 46. Choudhury, A. D. *et al.* Tumor fraction in cell-free DNA as a biomarker in prostate
784 cancer. *JCI Insight* **3**, e122109 (2018).
785 47. Ambroggi, M., Biasini, C., Del Giovane, C., Fornari, F. & Cavanna, L. Distance as a
786 Barrier to Cancer Diagnosis and Treatment: Review of the Literature. *The oncologist* **20**,
787 1378–1385 (2015).
788 48. Yang, D. X. *et al.* Prevalence of Missing Data in the National Cancer Database and
789 Association With Overall Survival. *JAMA Netw. Open* **4**, e211793–e211793 (2021).
790 49. Abida, W. *et al.* Non-BRCA DNA Damage Repair Gene Alterations and Response to the
791 PARP Inhibitor Rucaparib in Metastatic Castration-Resistant Prostate Cancer: Analysis From
792 the Phase II TRITON2 Study. *Clin. Cancer Res.* **26**, 2487 (2020).
793 50. Adalsteinsson, V. A. *et al.* Scalable whole-exome sequencing of cell-free DNA reveals
794 high concordance with metastatic tumors. *Nat. Commun.* **8**, 1324 (2017).
795 51. Ritch, E. *et al.* Identification of Hypermutation and Defective Mismatch Repair in ctDNA
796 from Metastatic Prostate Cancer. *Clin. Cancer Res.* **26**, 1114 (2020).
797 52. Gundem, G. *et al.* The evolutionary history of lethal metastatic prostate cancer. *Nature*
798 **520**, 353–357 (2015).
799 53. Ward, E. *et al.* Cancer Disparities by Race/Ethnicity and Socioeconomic Status. *CA.*
800 *Cancer J. Clin.* **54**, 78–93 (2004).

801 54. Rebbeck, T. R. Prostate Cancer Disparities by Race and Ethnicity: From Nucleotide to
802 Neighborhood. *Cold Spring Harb. Perspect. Med.* **8**, a030387 (2018).
803 55. Bailey, Z. D. *et al.* Structural racism and health inequities in the USA: evidence and
804 interventions. *The Lancet* **389**, 1453–1463 (2017).
805 56. McKay, R. R. *et al.* Tackling Diversity in Prostate Cancer Clinical Trials: A Report From
806 the Diversity Working Group of the IRONMAN Registry. *JCO Glob. Oncol.* 495–505 (2021)
807 doi:10.1200/GO.20.00571.
808 57. Painter, C. A. *et al.* The Angiosarcoma Project: enabling genomic and clinical discoveries
809 in a rare cancer through patient-partnered research. *Nat. Med.* **26**, 181–187 (2020).
810 58. Count Me In. *Count Me In* https://joincountmein.org/.
811 59. Wagle, N. *et al.* The Metastatic Breast Cancer Project: A national direct-to-patient
812 initiative to accelerate genomics research. *J. Clin. Oncol.* **34**, LBA1519–LBA1519 (2016).
813 60. Crowdis, J., He, M. X., Reardon, B. & Van Allen, E. M. CoMut: visualizing integrated
814 molecular information with comutation plots. *Bioinforma. Oxf. Engl.* **36**, 4348–4349 (2020).
815 61. Finn, R. D. *et al.* Pfam: the protein families database. *Nucleic Acids Res.* **42**, D222–D230
816 (2014).
817 62. Landrum, M. J. *et al.* ClinVar: improving access to variant interpretations and supporting
818 evidence. *Nucleic Acids Res.* **46**, D1062–D1067 (2018).
819 63. *Tesseract OCR*.
820 64. Geocoder - U.S. Census Bureau. https://geocoding.geo.census.gov/.
821 65. USDA ERS - Documentation. https://www.ers.usda.gov/data-products/rural-urban-
822 commuting-area-codes/documentation/.

823 66. Li, H. & Durbin, R. Fast and accurate short read alignment with Burrows–Wheeler
824 transform. *Bioinformatics* **25**, 1754–1760 (2009).
825 67. McKenna, A. *et al.* The Genome Analysis Toolkit: a MapReduce framework for
826 analyzing next-generation DNA sequencing data. *Genome Res.* **20**, 1297–1303 (2010).
827 68. Cibulskis, K. *et al.* Sensitive detection of somatic point mutations in impure and
828 heterogeneous cancer samples. *Nat. Biotechnol.* **31**, 213–219 (2013).
829 69. FilterByOrientationBias – GATK. https://gatk.broadinstitute.org/hc/en-
830 us/articles/360037060232-FilterByOrientationBias-EXPERIMENTAL-.
831 70. Saunders, C. T. *et al.* Strelka: accurate somatic small-variant calling from sequenced
832 tumor–normal sample pairs. *Bioinformatics* **28**, 1811–1817 (2012).
833 71. Ramos, A. H. *et al.* Oncotator: cancer variant annotation tool. *Hum. Mutat.* **36**, E2423–
834 E2429 (2015).
835 72. Lawrence, M. S. *et al.* Discovery and saturation analysis of cancer genes across 21
836 tumour types. *Nature* **505**, 495–501 (2014).
837 73. Carter, S. L. *et al.* Absolute quantification of somatic DNA alterations in human cancer.
838 *Nat. Biotechnol.* **30**, 413–421 (2012).
839 74. Shen, R. & Seshan, V. E. FACETS: allele-specific copy number and clonal heterogeneity
840 analysis tool for high-throughput DNA sequencing. *Nucleic Acids Res.* **44**, e131–e131 (2016).
841 75. Taylor-Weiner, A. *et al.* DeTiN: overcoming tumor-in-normal contamination. *Nat.*
842 *Methods* **15**, 531–534 (2018).
843 76. Cibulskis, K. *et al.* ContEst: estimating cross-contamination of human samples in next-
844 generation sequencing data. *Bioinformatics* **27**, 2601–2602 (2011).

845 77. CrosscheckFingerprints (Picard) – GATK. https://gatk.broadinstitute.org/hc/en-
846 us/articles/360037594711-CrosscheckFingerprints-Picard-.
847 78. Prentice, L. M. *et al.* Formalin fixation increases deamination mutation signature but
848 should not lead to false positive mutations in clinical practice. *PloS One* **13**, e0196434–
849 e0196434 (2018).
850 79. Poplin, R. *et al.* A universal SNP and small-indel variant caller using deep neural
851 networks. *Nat. Biotechnol.* **36**, 983–987 (2018).
852 80. AlDubayan, S. H. *et al.* Detection of Pathogenic Variants With Germline Genetic Testing
853 Using Deep Learning vs Standard Methods in Patients With Prostate Cancer and Melanoma.
854 *JAMA* **324**, 1957–1969 (2020).
855 81. McLaren, W. *et al.* The Ensembl Variant Effect Predictor. *Genome Biol.* **17**, 122 (2016).
856 82. Stachler, M. D. *et al.* Paired exome analysis of Barrett's esophagus and adenocarcinoma.
857 *Nat. Genet.* **47**, 1047–1055 (2015).
858