

Genomics of lethal prostate cancer at diagnosis and castration-resistance

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Genomics of primary prostate cancer differs from that of metastatic castration-resistant prostate cancer (mCRPC). We studied genomic aberrations in primary prostate cancer biopsies from patients who developed mCRPC, also studying matching, same patient, diagnostic and mCRPC biopsies following treatment. We profiled 470 treatment-naïve, prostate cancer diagnostic biopsies and for 61 cases, mCRPC biopsies using targeted and low-pass whole genome sequencing ($n = 52$). Descriptive statistics were used to summarize mutation and copy number profile. Prevalence was compared using Fisher's exact test. Survival correlations were studied using log-rank test. *TP53* (27%) and *PTEN* (12%) and DDR gene defects (*BRCA2* 7%; *CDK12* 5%; *ATM* 4%) were commonly detected. *TP53*, *BRCA2*, and *CDK12* mutations were significantly commoner than described in the TCGA cohort. Patients with *RB1* loss in the primary tumour had a worse prognosis. Among 61 men with matched hormone-naïve and mCRPC biopsies, differences were identified in *AR*, *TP53*, *RB1*, and PI3K/AKT mutational status between same-patient samples. In conclusion, the genomics of diagnostic prostatic biopsies acquired from men who develop mCRPC differs to that of the primary prostatic cancers. *RB1/TP53/AR* aberrations are enriched in later stages, but the prevalence of DDR defects in diagnostic samples is similar to mCRPC.

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1 Title: **Genomics of lethal prostate cancer at diagnosis and castration-resistance**

2

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48

49 **Conflicts of Interest:**

50 JM has served as a consultant for AstraZeneca, Roche, Janssen, Clovis and Amgen. TE

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60

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62

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64 resistance.

65 **ABSTRACT**

66 Genomics of primary prostate cancer differs from that of metastatic castration-resistant
67 prostate cancer (mCRPC). We studied genomic aberrations in primary prostate cancer
68 biopsies from patients who developed mCRPC, also studying matching, same patient,
69 diagnostic and mCRPC biopsies following treatment.

70 We profiled 470 treatment-naïve, prostate cancer diagnostic biopsies and for 61 cases,
71 mCRPC biopsies using targeted and low-pass whole genome sequencing (n=52).

72 Descriptive statistics were used to summarize mutation and copy number profile.

73 Prevalence was compared using Fisher's exact test. Survival correlations were studied
74 using log-rank test.

75 *TP53* (27%) and *PTEN* (12%) and DDR gene defects (*BRCA2* 7%; *CDK12* 5%; *ATM*
76 4%) were commonly detected. *TP53*, *BRCA2* and *CDK12* mutations were significantly
77 commoner than described in the TCGA cohort. Patients with *RBI* loss in the primary
78 tumour had a worse prognosis. Among 61 men with matched hormone-naïve and
79 mCRPC biopsies, differences were identified in *AR*, *TP53*, *RBI* and PI3K/AKT
80 mutational status between same-patient samples.

81 In conclusion, the genomics of diagnostic prostatic biopsies acquired from men who
82 develop mCRPC differs to that of the non-lethal primary prostatic cancers.

83 RB1/TP53/AR aberrations are enriched in later stages, but the prevalence of DDR
84 defects in diagnostic samples is similar to mCRPC.

85 INTRODUCTION

86 Inter-patient genomic heterogeneity in prostate cancer is well-recognized (1) . However,
87 molecular stratification of prostate cancer to guide treatment selection based on
88 predictive genomic biomarkers remains an unmet clinical need. Recent genomic studies
89 have elucidated this inter-patient heterogeneity, identifying multiple potentially
90 actionable alterations which are now being evaluated in clinical trials. These studies
91 have also described differences in the genomic landscape of the different clinical states
92 of the disease (localized vs metastatic)(1, 2). Alterations in the *AR* gene (mutations,
93 amplifications and structural variants) are increased the prevalence in mCRPC, and
94 associated with the development of castration-resistance, as well as resistance to
95 abiraterone acetate and enzalutamide (3, 4). Moreover, loss-of-function events in *TP53*,
96 *RBI*, *PTEN* and DNA damage repair (DDR) genes are more common in mCRPC
97 compared to non-metastatic, prostate cancer cohorts. It remains unclear whether these
98 differences are the result of evolutionary processes in response to therapy exposure, or
99 whether these reflect different disease sub-types with differing outcomes.

100

101 An ultimate aim of understanding the genomic landscape of cancer is the
102 implementation of more precise therapeutic strategies, but metastatic biopsy acquisition
103 is a key obstacle for implementing genomic stratification in clinical practice. Liquid
104 biopsies can partially overcome this limitation, but these assays are not yet validated to
105 replace tumour biopsy testing, at least for prostate cancer(5, 6). Understanding if
106 primary tumour biopsies can be used for molecular stratification to guide the treatment
107 of advanced mCRPC years later remains a key question.

108

109 This study aims to describe the genomic profile of primary tumour biopsies from lethal
110 prostate cancers, either presenting as metastatic hormone treatment-naïve prostate
111 cancers, or locoregional tumours that later evolve to metastatic disease; we
112 hypothesized that these primary tumours would be enriched for alterations previously
113 associated with mCRPC, and would be different to those primary prostate tumours that
114 do not recur. Additionally, we assessed a cohort of same-patient, matched, treatment-
115 naïve and mCRPC biopsies to determine if these genomic defects change during
116 treatment with tumour evolution.

117 **RESULTS**

118 *Patient and sample disposition*

119 Between March 2015 and December 2017, 652 primary tumor samples from consenting
120 patients were received; 87 cases (13%) were discarded due to either low DNA yield or
121 excessive DNA degradation. Hence, targeted NGS was successfully performed on 565
122 prostate cancer diagnostic biopsies. Fifty-four cases were excluded due to either: 1) the
123 biopsy not being collected prior to ADT; or 2) diagnosis being based on a metastatic
124 biopsy (Supplementary Figures 1 and 2 in the Appendix). Next generation sequencing
125 of 511 samples was analysed; of those, 41 (8%) cases did not meet quality control
126 criteria for copy-number calling (7) and were discarded, so the final analysis evaluated
127 470 cases. Two cohorts were defined for the planned analyses based on disease extent at
128 the time of original diagnosis: Cohort 1 was composed of 175 cases with locoregional
129 prostate cancer at diagnosis (69.5% confined to the prostate, 30.5% with pelvic nodal
130 extension); Cohort 2 included 292 primary tumours from patients with metastatic
131 disease at diagnosis. The clinical records of 3 subjects were unobtainable (Table 1).

132

133 *Genomic profile of lethal primary prostate tumours*

134 Recurrent aberrations in genes and pathways related to lethal prostate cancer were
135 identified, the commonest being mutations and homozygous loss of *TP53*, (27%)
136 (Figure 1 and Appendix). Deleterious mutations and/or homozygous deletions in genes
137 involved in DNA damage repair pathways were identified in 23% of primary tumours.
138 *BRCA2* was the DDR gene most commonly altered (7%). Alterations in mismatch repair
139 genes were detected in 11/470 (2%) cases.

140

141 Activating mutations in *PIK3CA* and *AKT1* were detected in 5%, with *PTEN* loss-of-
142 function mutations or deep deletions in 12%. Deep deletions of *RBI* were uncommon in
143 the primary tumours (5%), although shallow deletions in *RBI* were frequent. Genes in
144 the WNT pathway (loss of *APC* or activating mutations in *CTNNB1*) were altered in 7%
145 of cases (8, 9). *SPOP* mutations were identified in 7% cases(10, 11).

146

147 Surprisingly, low-allele frequency *AR* T878A or R630Q mutations (always with low
148 MAF, ranging 0.06 to 0.18) were detected in 1% of treatment-naïve samples(12).

149

150 Our Cohort 1 of primary tumours, without detectable metastases at diagnosis, was
151 enriched for alterations in *TP53* (25 vs 8%; $p<0.001$), *BRCA2* (8 vs 3%; $p=0.015$) and
152 *CDK12* (6 vs 2%; $p=0.04$) when compared with the TCGA series (Table 2). Conversely,
153 *SPOP* mutations were less common in our population than in the better prognosis
154 TCGA series (3% vs 11%; $p=0.001$). No relevant differences in prevalence of other
155 mutations were observed when comparing Cohort 1 and Cohort 2. After adjusting for
156 Gleason score, *CDK12* mutations were enriched in Gleason 8 or higher cases (1/105
157 cases in Gleason 6-7 vs 21/353 in Gleason ≥ 8) (Appendix)

158

159 *Clinical outcome based on primary tumour genomics.*

160 Median time to ADT progression and start of first mCRPC therapy was 1.17 years
161 (95%CI: 1.08-1.26 years) among the subset (n=210) of patients with clinical data
162 available. Median overall survival from first evidence of metastatic disease was 4.28
163 years (95%CI: 3.72-4.84 years).

164

165 None of the gene alterations were associated with a significantly different time to ADT
166 progression; patients with germline or somatic *BRCA2* alterations had the lowest
167 median time to ADT progression among the subgroups but the differences were not
168 significant (median 0.92 years, 95%CI 0.5-1.17, p=0.39). (Table 3)

169

170 Patients with *RBI* alterations in the primary tumour had a significantly shorter overall
171 survival (median OS from metastatic disease 2.32 years, 95%CI 1.82-3.84; p=0.006).
172 (Table 3 and Appendix)

173

174 *Changes when assessing clinically actionable genomic alterations in patient-matched*
175 *treatment-naïve and castration-resistant.*

176 We pursued NGS of mCRPC biopsies acquired from 61 patients participating in this
177 study to further investigate if certain gene aberrations were detected more often in
178 biopsies after progression on ADT and subsequent lines of therapy. Overall, we
179 performed targeted NGS on 61 mCRPC biopsies (using the same panel as for the
180 primary treatment-naïve samples) and copy-number profiles for both primary and
181 mCRPC samples were compared using low-pass WGS in 52 cases with sufficient DNA
182 in both samples. Copy number estimation was adjusted for ploidy, and tumour purity,
183 since mCRPC biopsies overall had higher tumour content than the primary prostate
184 biopsies (Appendix).

185

186 The median time between the two same-patient biopsies was 45.2 months (range 12 to
187 211 months). All mCRPC samples were obtained after progression on ADT, and in
188 50/61 (82%) cases after progression on at least 2 further lines of therapy for mCRPC

189 (80% after at least one taxane and 90% after abiraterone acetate and/or enzalutamide)
190 (Table 4).

191

192 The commonest finding, when comparing same-patient primary treatment-naïve and
193 mCRPC samples, was an increase in AR mutations and amplification. Other than *AR*,
194 the main differences between the two same-patient biopsies were increased *TP53*, *RBI*
195 and PI3K/AKT pathway alterations in mCRPC (Figure 2 and Appendix) suggesting that
196 these may emerge with treatment selection pressures.

197

198 In several cases, mutations in *TP53* (n=4) and *RBI* (n=4), detected in mCRPC samples,
199 were not detected in the same patient's, matched, treatment-naïve and diagnostic
200 primary tumour biopsies. Overall, there was a decrease in copy-number for both *TP53*
201 and *RBI* in mCRPC, even after adjusting for tumour purity based on low-pass WGS.
202 More deep deletions in *PTEN* were also detected in the mCRPC cohort. Mutations in
203 the WNT pathway genes *CTNNB1* and *APC*, as well as *MYC* amplification, were also
204 more common in mCRPC.

205

206 Conversely, aberrations in DNA damage repair pathway genes were relatively
207 unchanged from diagnosis to mCRPC. Eleven truncating mutations in *BRCA2*, *CDK12*,
208 *ATM*, *MSH6* and *PALB2* were identified in the mCRPC biopsies of 9/61 patients (one
209 patient had both *CDK12* and *PALB2* mutations; one patient *CDK12* and *MSH6*
210 mutations). Two patients had pathogenic germline *BRCA2* mutations; in both of these
211 cases, both the primary untreated tumour and the mCRPC biopsy presented loss of
212 heterozygosity resulting in biallelic *BRCA2* loss. The other 8 deleterious mutations (4 in
213 *CDK12*, 2 *BRCA2*, 1 *ATM*, 1 *PALB2*, 1 *MSH6*) were only detected in somatic DNA; all

214 8/8 were also detected in the patient-matched, metachronous, diagnostic, treatment-
215 naïve, biopsies. In 3 of 4 cases with CDK12 truncating mutations, there was a second
216 missense mutation in CDK12; again, these second events were also detected in both the
217 diagnostic patient-matched biopsies. However, 2 truncating mutations in ATRX and
218 FANCM were detected only in the mCRPC samples.

219

220 With regards to copy number aberrations in DNA repair genes, we identified a trend for
221 lower tumour suppressor gene copy number in mCRPC samples, only partially
222 explained by the higher tumour purity of mCRPC biopsies. No deep deletions in
223 *BRCA1/BRCA2/ATM* were identified, although changes indicating single copy loss with
224 disease evolution to mCRPC were detected.

225

226 Generally, the number of private events was small. An outlier case was P001, a patient
227 with a MMR-defective prostate cancer who had the highest mutation burden, including
228 several shared mutations between primary and mCRPC (*APC, CDK12, MSH6, ERBB4,*
229 *PTEN* and *TP53*), several private mutations only detected in mCRPC (including
230 missense, non-truncating, mutations in *APC, ATM, EZH2, JAK1*) and several private
231 mutations of the primary tumour not detected in the later mCRPC biopsy (*CTNNA1,*
232 *PRKDC, ERCC3* and *ERRC6*), suggesting the presence of different clones coming from
233 a shared origin.

234

235

236 DISCUSSION

237 Molecular stratification of prostate cancer promises to impact patient care and deliver
238 more precise treatments, but several challenges remain to be addressed including the
239 elucidation of the genomic profiles of distinct clinical states and understanding the
240 impact of drug resistance and tumour evolution (13, 14). Here, we show that the
241 primary prostatic biopsies of patients who develop metastatic prostate cancer are
242 enriched for genomic aberrations typically found in mCRPC, even prior to exposure to
243 androgen deprivation. These data may help define a subset of patients with locoregional
244 disease at diagnosis with higher risk of lethal disease; clinical trials should test if these
245 patients may benefit from more intense therapeutic approaches. Furthermore, our data
246 support the use of primary prostate biopsies to characterize metastatic hormone-naïve
247 prostate cancers, which may facilitate the implementation of genomic testing into
248 clinical practice.

249

250 Defects in some DNA damage repair genes have been identified as promising predictive
251 biomarkers for PARP inhibitors or platinum chemotherapy(15-18). The prevalence of
252 mutations and deletions in DNA repair genes in our cohorts of patients with only
253 locoregional disease detected at diagnosis or metastatic, hormone-naïve prostate cancer
254 was similar to what has been previously described for mCRPC. In a recent study,
255 Marshall et al found an increased prevalence of these mutations in higher-Gleason score
256 primary tumours, which also indirectly supports the association of these mutations with
257 more aggressive primary tumours (19). These data in a cohort of 470 primary tumours
258 suggest that lethal prostate cancer is enriched for DNA repair defects from diagnosis,
259 prior to developing castration-resistance. However, the limited number of cases with
260 DDR gene alterations in the cohort of matched primary-metastatic biopsies, including

261 only 4 cases with *BRC A2* mutations, prevents us from making broad conclusions with
262 regards to the genomic evolution of these tumours. Indeed, we and others have reported
263 sub-clonal homozygous deletions of DDR genes (20, 21). Detecting these subclonal
264 deletions is technically challenging with targeted NGS assays used for patient
265 stratification in clinical practice or in clinical trials, particularly when studying primary
266 tumour samples with low tumour content and degraded DNA.

267

268 Alterations in TP53 were common in diagnostic biopsies in this cohort. Moreover,
269 several loss-of-function alterations of *TP53*, *RB1* and *PTEN* were detected in mCRPC
270 biopsies but not in patient-matched, treatment-naïve, primary tumours. Concurrent loss
271 of RB1 and TP53 function has been postulated to drive a phenotypic change associated
272 with resistance to endocrine therapies(22, 23); additionally, TP53 mutations have been
273 associated with more aggressive disease (24-26), which may in part explain why we are
274 observing TP53 mutations more often than expected in primary prostate cancer in this
275 cohort of patients who all had lethal forms of the disease, even if many presented as
276 localized tumours.

277

278 As precision medicine strategies are developed for prostate cancer patients, our findings
279 become clinically-relevant. Firstly, our analyses indicate that *RB1* loss in the primary
280 tumour associates with poor prognosis; these data confirm recently published results
281 from two independent studies looking at genomics-clinical outcome correlations in
282 metastatic samples (27, 28). In our series, DDR defects and particularly *BRC A2*
283 mutations did not associate with shorter survival; however, most of these patients were
284 enrolled into PARPi clinical trials; data from randomized trials has confirmed the
285 improved outcome of patients with DDR defects receiving PARPi; this needs to be

286 taken into consideration when interpreting our results. Secondly, these data are critically
287 important for designing precision medicine strategies: if DNA repair defects are already
288 detectable in the primary tumour, there is a rationale for testing synthetic lethal strategies
289 with PARP inhibitors or platinum, in metastatic hormone-naïve prostate cancer, where
290 the magnitude of benefit for patients could be larger. These data also support the use of
291 diagnostic prostate cancer biopsies for the patient stratification based on DNA repair
292 gene defects in trials of men with mCRPC, as the prevalence of these alterations in
293 primary tumours from patients with lethal prostate cancer was similar to what has been
294 reported for metastatic disease, and in the small number of same-patient sample pairs
295 available, DDR mutational status was concordant (29). Conversely, trials investigating
296 novel therapeutic approaches in the TP53/RB1-deficient phenotype should take into
297 account that a proportion of genomic aberrations in *TP53* and *RB1* are not detected
298 when assessing diagnostic treatment-naïve primary tumour specimens.

299

300 The main limitation of our study comes from having only one biopsy core available per
301 time point and patient; we therefore could not assess spatial tumour heterogeneity.

302 Primary prostate cancers can be multifocal, and previous studies have reported on inter-
303 foci genomic heterogeneity(30, 31). We cannot rule out that in some cases the primary
304 tumour sample may not represent the dominant tumour clone in the primary biopsy;
305 hence, it is possible that some of the differences we observe in paired mCRPC biopsies
306 may have not resulted from treatment-selective pressure but been in other areas of these
307 primary tumours. However, genomic testing in clinical practice is largely based on the
308 analyses of single biopsy cores. With the advent of novel imaging modalities, genomic
309 stratification of prostate cancer could be improved by better identifying aggressive areas
310 of prostate cancer in clinical diagnostic pathways (32, 33). Another key limitation is the

311 inability to pursue subclonality assessments using our clinically-oriented targeted
312 sequencing assay. Hence, we cannot prove if some of the gene aberrations detected in
313 the mCRPC biopsies, but not in the treatment-naïve samples, were already present at a
314 subclonal level at the time of diagnosis. Regardless of whether these events emerge *de*
315 *novo* or as a result of expansion of a subclone, the observed enrichment for certain
316 alterations (such as *TP53* or *RBI*) in the post-treatment resistance samples supports the
317 clinical relevance of such alterations.

318

319 In conclusion, this study describes the genomic landscape of primary prostate tumours
320 that will evolve to lethal prostate cancer across a cohort of 470 cases, with this being
321 characterized by higher frequencies of *TP53* and DNA repair gene aberrations.
322 Significant differences in the detection of *AR*, *TP53*, *RBI* and *PTEN* alterations, but not
323 of DNA repair genes, was observed when comparing same patient mCRPC and
324 treatment-naïve biopsies. These data are important for the genomic stratification of
325 primary prostate cancer to identify higher risk cases, support the use of primary prostate
326 tumour biopsies for molecular stratification of metastatic hormone-naïve prostate cancer
327 and provide a rational for the study of DNA repair-targeting therapies, including PARP
328 inhibitors, in earlier stages of the disease.

329

330 **METHODS**

331 *Study design*

332 This analysis included all consecutive patients consented between March 2015 and
333 December 2017 for molecular characterization of prostate cancer biopsies at The
334 Institute of Cancer Research (London, UK). These studies involved either prostate
335 tumour samples and/or newly acquired metastatic biopsies. We report here on all
336 patients for whom a treatment-naïve primary prostate tumour sample was successfully
337 sequenced. Primary tumour samples were retrieved from referring hospitals; in most
338 cases, only one sample was made available for the study; if more than one sample from
339 the primary tumour was available, the highest Gleason lesion was used. Additionally,
340 metastatic biopsies in castrate-resistant conditions were pursued in consenting patients.

341

342 *Sample acquisition and processing*

343 All prostate cancer treatment-naïve and metastatic biopsy samples were centrally
344 reviewed by a pathologist (D.N.R). DNA was extracted from formalin-fixed and
345 paraffin embedded (FFPE) tumour blocks (average, 6 sections of 10mic each per
346 sample) using the FFPE Tissue DNA kit (Qiagen). DNA was quantified with the Quant-
347 iT high-sensitivity PicoGreen double-stranded DNA Assay Kit (Invitrogen). The
348 Illumina FFPE QC kit (WG-321-1001) was used for DNA quality control tests
349 according to the manufacturer's protocol as previously described (34). In brief,
350 quantitative polymerase chain reaction (qPCR) was performed using 4ng of sample or
351 control DNA and the average Cq (quantification cycle) was determined. The average Cq
352 value for the control DNA was subtracted from the average Cq value of the samples to
353 obtain a ΔCq . DNA samples with a $\Delta Cq < 4$ were selected for sequencing; double
354 amount of DNA was used for cases with ΔCq between 2-4.

355 *Sequencing and bioinformatic analyses*

356 Libraries for next-generation targeted sequencing were constructed using a customized
357 panel (Generead DNaseq Mix-n-Match Panel v2; Qiagen) covering 6025 amplicons
358 (398702 bp) across 113 genes used in (35) (Appendix). Libraries were run using the
359 MiSeq Sequencer (Illumina). FASTQ files were generated using the Illumina MiSeq
360 Reporter v2.5.1.3. Sequence alignment and mutation calling were performed using the
361 Qiagen GeneRead Targeted Exon Enrichment Panel Data Analysis Portal
362 (<https://ngsdataanalysis.qiagen.com>). Mutation calls were reviewed manually in IGV
363 according to the standard operating procedure for somatic variant refinement of tumour
364 sequencing data, following the principles described in (36). This manual review
365 included assessing read strand quality, base quality, read balance and sequencing
366 artefacts (high discrepancy regions, adjacent indels, multiple mismatches, start or end of
367 amplicons. Mutation annotation was based on data from publically available databases
368 (ClinVar, COSMIC, Human Genome Mutation Database, IARC TP53 Database),
369 published literature and *in silico* prediction tools, and only deleterious mutations were
370 included in the analysis.

371

372 Copy number variations (CNV) in prostatic biopsies were assessed using CNVkit
373 (v0.3.5, <https://github.com/etal/cnvkit>(37)), which we previously validated in an
374 independent cohort of prostate cancer samples(7). The read depths of tumour samples
375 were normalized and individually compared to a reference consisting of non-matched
376 male germline DNA; the circular binary segmentation (CBS) algorithm was used to
377 infer copy number segments. Quality estimation of the CNV was based on distribution
378 of bin-level copy ratios within segments. Cases were excluded from the analysis if any
379 of the following criteria were met: IQR>1, total reads<500000, <99.9% of reads on

380 target, <95% paired reads or single reads>0. Manual review of copy number calls for
381 selected oncogenes and tumour suppressors was pursued, accounting for tumour
382 content. Oncoprints and heatmaps representing mutations and copy number calls were
383 generated using R and cBioportal OncoPrinter (38-40).

384

385 Low-Pass Whole Genome Sequencing was performed on the mCRPC, and same
386 patient, treatment-naïve, diagnostic, paired samples for copy-number profiling.
387 Libraries were constructed using the NEBNext Ultra FS II DNA kit (NEB) according
388 to the manufacturer's protocol. Samples were pooled and run on the NextSeq
389 (Illumina) at 0.5X mean coverage, using the 300 cycles High Output V2.5 kit. BCL files
390 were converted to FASTQ files using BCL2FASTQ v2.17. Sequence alignments were
391 performed using Burrows-Wheeler Aligner (BWA mem v0.7.12) to the hg19 human
392 genome build. Copy number analysis was performed using IchorCNA(41). In short,
393 hg19 genomes (filtered centromeres) were divided into 500kb non-overlapping bins,
394 and the abundance of the mapped reads was counted by HMMcopy Suite in each bin
395 and predicted segments of CNAs. GC and mappability bias were corrected by loess
396 regression and based on a panel of germline DNA sequencing from healthy donors. The
397 maximum CNA detection was set to 20 copies.

398

399 Raw sequencing data has been deposited at the European Nucleotide Archive with
400 Accession number PRJEB32038. VCF files with mutation calls and CN values for the
401 targeted sequencing data are available in the appendix.

402

403

404

405 *Statistical considerations*

406 Descriptive statistics were used to summarize patient, and sample, characteristics data
407 as well as mutation frequency. The prevalence of mutations was compared between
408 cohorts using Fisher's exact test. The statistical analysis plan and the gene list to be
409 analysed was designed prior to data collection. A Bonferroni correction was applied; p-
410 values of <0.01 were considered statistically significant and all tests were two-sided
411 unless otherwise specified.

412

413 Additionally, exploratory associations between the pre-selected list of gene alterations
414 and patient outcomes were tested in a subset of the study population (n=210) with
415 available consent for clinical data collection (all at The Royal Marsden). Clinical data
416 was captured retrospectively from electronic patient records. Time to ADT progression
417 was defined from the date of starting ADT to start of first mCRPC therapy. Overall
418 survival was defined as time from the date of diagnosis, date of metastatic disease and
419 the date of CRPC to the date of death or last follow up. To account for variability
420 between patients who were diagnosed with de-novo metastatic vs localized disease,
421 survival data is presented from the first evidence of metastatic disease. Patients alive at
422 the time of last follow up were censored. Association of genomic aberrations with
423 survival are presented using Kaplan-Meier curves and log-rank test. All calculations
424 were performed using STATA v15.1(Stata Corp,TX).

425

426 *Study Approval*

427 The study included all patients with mCRPC who, between March 2015 and December
428 2017 provided written consent to participate in one of two IRB-approved molecular
429 characterization programs for prostate cancer: 1) an internal molecular characterization

430 study at The Royal Marsden Hospital (London, UK) and/or 2) a tumour next-generation
431 sequencing (NGS) pre-screening study at 17 hospitals (Appendix) for the TOPARP-B
432 study, an investigator-initiated clinical trial of the PARP inhibitor olaparib in mCPRC
433 (42) (TOPARP, CR-UK 11/029, NCT 01682772).

434 **Author contributions**

435 JM, SC, JSDB designed the study. JM, DD, NP, EH, JSDB created the study
436 methodology. JM, PR, RC, CM, SS, DB, MB, AP, ZZ, MF, RPL, NT, BF, RJ, UM, CR,
437 MV, OP, SJ, TE, SS consented patients, acquired samples and collected clinical data.
438 JM, CB, IF, SM, DNR, BG, MA, SC processed samples and generated experimental
439 data. GS, WY, SC planned and conducted bioinformatics analysis. DD, NP designed
440 and conducted the statistical analysis plan. JM, GS, WY, SC, NP, DD, JSDB analysed
441 and interpreted data. JM, GS, SC, JSDB wrote the manuscript. EH, JSDB obtained
442 funding. SC and JSDB supervised the study. All authors reviewed and approved the
443 manuscript. Order of joint first authors was determined based on their role in data
444 interpretation and manuscript preparation.

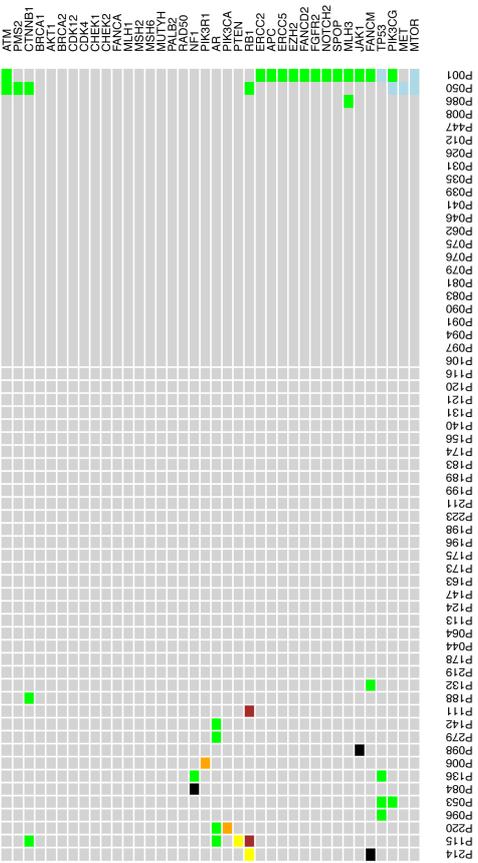
445

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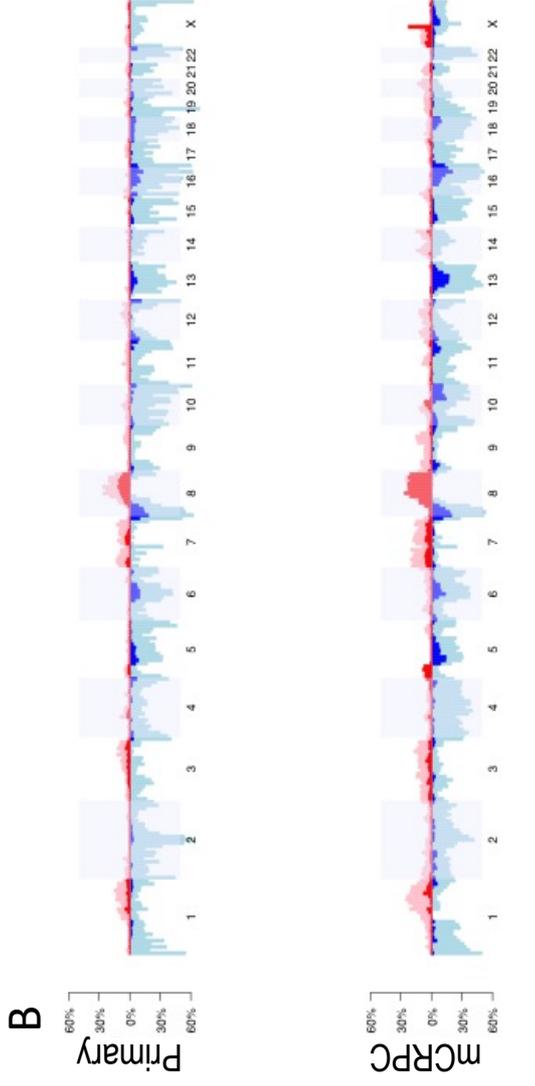
459 Clinical Trials and Statistics Unit; full list of clinical investigators involved is presented
460 in the Appendix.

465 **Figure 2.** Differences in genomic profiles between same patient, matched, primary
466 untreated and mCRPC biopsies. A) Mutation calls in genes of interest for the mCRPC
467 biopsies which were not present in the treatment-naïve primary tumour for the same
468 patient (61 pairs, full gene set in Suppl Fig 6); B) Overall copy number profiles based
469 on low-pass WGS (52 pairs); C) amplifications and deep deletions detected in the
470 mCRPC biopsies and not present in the treatment-naïve primary tumours for the same
471 patient (based on low-pass WGS, after adjusting for tumour purity and ploidy, and
472 validated by SNP data from targeted panel sequencing).



A

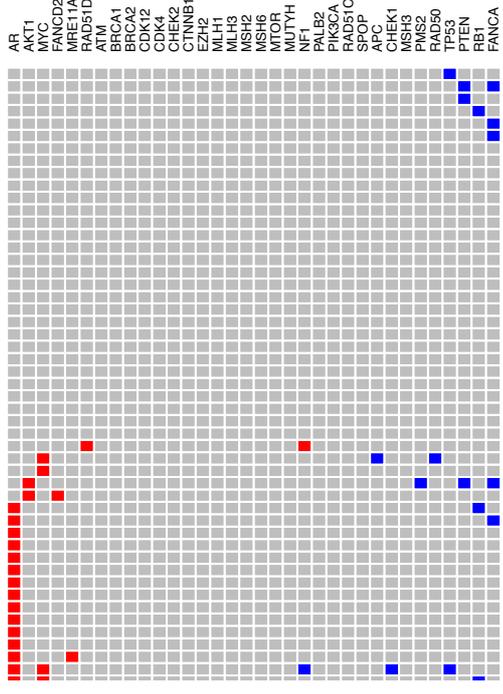
C



B

C

CNA
HomDel
Normal
Amp



474 **Table 1.** Population characteristics and sample disposition for the overall study
 475 population (n=470)

Metastatic disease at original diagnosis of prostate cancer		
No (Cohort 1)	175	37.5%
Yes (Cohort 2)	292	62.5%
Not recorded	3	
Gleason score primary tumour (overall population)		
<7	15	3.3%
7	90	19.7%
8	85	18.6%
9	245	53.5%
10	23	5.0%
Gleason not recorded	12	
Race		
Caucasian	431	96.9%
African or african-american	7	1.6%
asian	4	0.9%
Caribbean	4	0.9%
Not recorded	25	
Staging of patients in Cohort 1		
T1	6	3.7%
T2	20	12.2%
T3	131	79.9%
T4	7	4.3%
N0	114	69.5%
N1	50	30.5%
T-N not recorded	11	
Gleason score in Cohort 1		
<7	11	6.5%
7	50	29.6%
8	28	16.6%
9	76	45.0%
10	4	2.4%
Not recorded	6	

476 **Table 2.** Comparison of cohort 1 in this study (patients with primary, non-metastatic at
 477 diagnosis, prostate cancer) and the TCGA series for primary prostate cancers
 478 (distribution of genomic events per Gleason score group are available in the Appendix).

Gene	Events considered	TCGA(N=333)	Cohort 1 (N=175)	p-value (Fisher exact test)
		N (%)	N (%)	
<i>AKT1</i>	Activating mutations	3 (0.9%)	0 (0%)	0.56
<i>ATM</i>	Loss-of-function mutations and deep deletions	20 (6%)	10 (6%)	1.00
<i>BRCA1</i>	Loss-of-function mutations and deep deletions	3 (1%)	3 (2%)	0.42
<i>BRCA2</i>	Loss-of-function mutations and deep deletions	10 (3%)	14 (8%)	0.015
<i>CDK12</i>	Loss-of-function mutations and deep deletions	7 (2%)	10 (6%)	0.04
<i>CTNNB1</i>	Activating mutations	7 (2%)	3 (2%)	1.00
<i>PIK3CA</i>	Activating mutations and copy number gains	7 (2%)	7 (4%)	0.26
<i>PTEN</i>	Loss-of-function mutations and deep deletions	57 (17%)	20 (11%)	0.09
<i>RBI</i>	Loss-of-function mutations and deep deletions	3 (1%)	6 (3%)	0.07
<i>SPOP</i>	Hotspot mutations	37 (11%)	5 (3%)	0.001
<i>TP53</i>	Loss-of-function mutations and deep deletions	27 (8%)	44 (25%)	<0.001

479 **Table 3. Association of gene defects with clinical outcome. Long-rank p-values are**
 480 **presented unadjusted and adjusted for both Gleason score (≤ 7 vs ≥ 8) and**
 481 **presence/absence of metastatic disease at initial diagnosis.**

	Time to ADT progression			Overall Survival (from metastatic disease)		
	n	Median (Years)	Log-rank/Log-rank stratified p-values	n	Median (Years)	Log-rank/Log-rank stratified p-values
Overall population	202	1.17 (95%CI: 1.08-1.27)		203	4.28 (95%CI: 3.71-4.84)	
Gene alteration						
<i>TP53</i>	47	1.19 (95%CI: 1.00-1.67)	0.64/0.19	47	4.24 (95%CI: 3.06-5.00)	0.51/0.77
<i>PTEN</i>	23	1.58 (95%CI: 0.83-2.15)	0.09/0.06	22	3.78 (95%CI: 3.20-5.60)	0.38/0.48
<i>RB1</i>	13	1.17 (95%CI: 0.56-2.33)	0.89/0.79	13	2.32 (95%CI: 1.82-3.84)	0.006/0.004
<i>SPOP</i>	9	1.25 (95%CI: 0.50-2.23)	0.67/0.91	9	5.46 (95%CI: 2.07-NA)	0.63/0.47
<i>BRCA2</i>	15	0.92 (95%CI: 0.50-1.17)	0.39/0.36	15	3.84 (95%CI: 2.09-4.69)	0.25/0.13
<i>CDK12</i>	12	1.20 (95%CI: 0.58-2.82)	0.88/0.67	12	4.32 (95%CI: 2.44-NA)	0.39/0.24
<i>ATM</i>	11	1.07 (95%CI: 0.42-2.33)	0.44/0.32	10	4.73 (95%CI: 2.03-5.65)	0.98/0.77
<i>PIK3CA</i>	7	1.62 (95%CI: 0.58-2.41)	0.97/0.80	7	2.92 (95%CI: 1.02-NA)	0.14/0.24
<i>CTNNB1</i>	7	1.42 (95%CI: 0.50-2.00)	0.68/0.70	8	6.46 (95%CI: 2.53-NA)	0.22/0.27
<i>AKT1</i>	2	1.58 (95%CI: NA)	0.77/0.53	2	5.64 (95%CI: NA)	0.65/0.59
<i>BRCA1</i>	3	1.08 (95%CI: 0.42-NA)	0.66/0.62	3	2.31 (95%CI: NA)	0.07/0.17
<i>BRCA1/2 / ATM</i>	28	1.07 (95%CI: 0.83-1.21)	0.27/0.21	27	3.61 (95%CI 3.01-4.69)	0.17/0.15
<i>PIK3CA/ AKT1/PTEN</i>	32	1.59 (95%CI: 1.00-2.15)	0.11/0.05	31	4.11 (95%CI 3.20-5.60)	0.70/0.74

482 **Table 4.** Sample disposition for the patient-matched primary untreated and mCRPC
 483 biopsies (n=61 cases with paired samples). Median time between the two same-patient
 484 samples were taken was 45.2 months (range: 12 to 211 months)

		n (total 61)	%
Location Hormone-Naive Sample	Prostate	61	100
Location CRPC Sample	Bone	24	39.4%
	Lymph Node	22	36.17%
	Liver	4	6.6%
	Other	11	18.0%
Metastatic status at original diagnosis	M0	25	41.7%
	M1	35	58.3%
Treatments received between the two samples acquisition	Prostatectomy	10	16.4%
	Pelvic radiotherapy	27	44.3%
	Androgen deprivation therapy	61	100%
	First gen antiandrogen	41	67.2%
	Abiraterone acetate	34	55.7%
	Enzalutamide	33	54.1%
	Abiraterone and/or enzalutamide	55	90.2%
	Docetaxel	49	80.3%
Lines of therapy for CRPC before mCRPC biopsy	Cabazitaxel	20	32.8%
	Radium-223	4	6.5%
	Investigational agents	14	22.9%
	0	2	3.2%
	1	9	14.7%
	2	21	34.4%
	3 or more	29	47.5%

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