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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.

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

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JM has served as a consultant for AstraZeneca, Roche, Janssen, Clovis and Amgen. TE has received educational grants from Janssen. RJ reports fees/advisory role from

Astellas, AstraZeneca, BMS, Bayer, Exelixis, Janssen, Ipsen, Merck Serono, Novartis, Pfizer, Roche, Sanofi, EUSA. CR has received research grants from Oncolytics and Tusk Therapeutics, honoraria from BMS and support for travel from Roche, GSK, Viralytics, Janssen, Novartis, Pfizer, Ipsen. SJ reports fees/advisory role from Astellas, Janssen, Bayer, Boston Scientific, Almac Diagnostics. JDB has served as a consultant for Astellas, AstraZeneca, Bayer, Daiichi, Genentech, GSK, Janssen, Merck Serono, MSD, Orion, Pfizer Oncology, Sanofi-Aventis, Silicon Biosystems and Taiho. No relevant conflicts of interest were disclosed by other authors.

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Keywords: prostate cancer, genomics, targeted NGS, tumour evolution, castration-resistance.

ABSTRACT

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 non-lethal 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.

INTRODUCTION

Inter-patient genomic heterogeneity in prostate cancer is well-recognized (1) . However, molecular stratification of prostate cancer to guide treatment selection based on predictive genomic biomarkers remains an unmet clinical need. Recent genomic studies have elucidated this inter-patient heterogeneity, identifying multiple potentially actionable alterations which are now being evaluated in clinical trials. These studies have also described differences in the genomic landscape of the different clinical states of the disease (localized vs metastatic)(1, 2). Alterations in the *AR* gene (mutations, amplifications and structural variants) are increased the prevalence in mCRPC, and associated with the development of castration-resistance, as well as resistance to abiraterone acetate and enzalutamide (3, 4). Moreover, loss-of-function events in *TP53*, *RB1*, *PTEN* and DNA damage repair (DDR) genes are more common in mCRPC compared to non-metastatic, prostate cancer cohorts. It remains unclear whether these differences are the result of evolutionary processes in response to therapy exposure, or whether these reflect different disease sub-types with differing outcomes.

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

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

RESULTS

Patient and sample disposition

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

Genomic profile of lethal primary prostate tumours

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

Activating mutations in *PIK3CA* and *AKT1* were detected in 5%, with *PTEN* loss-of-function mutations or deep deletions in 12%. Deep deletions of *RB1* were uncommon in the primary tumours (5%), although shallow deletions in *RB1* were frequent. Genes in the WNT pathway (loss of *APC* or activating mutations in *CTNNB1*) were altered in 7% of cases (8, 9). SPOP mutations were identified in 7% cases(10, 11).

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

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

Clinical outcome based on primary tumour genomics.

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

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

Patients with *RB1* alterations in the primary tumour had a significantly shorter overall survival (median OS from metastatic disease 2.32 years, 95%CI 1.82-3.84; p=0.006). (Table 3 and Appendix)

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

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

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

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

The commonest finding, when comparing same-patient primary treatment-naïve and mCRPC samples, was an increase in AR mutations and amplification. Other than *AR*, the main differences between the two same-patient biopsies were increased *TP53*, *RB1* and PI3K/AKT pathway alterations in mCRPC (Figure 2 and Appendix) suggesting that these may emerge with treatment selection pressures.

In several cases, mutations in *TP53* (n=4) and *RB1* (n=4), detected in mCRPC samples, were not detected in the same patient's, matched, treatment-naïve and diagnostic primary tumour biopsies. Overall, there was a decrease in copy-number for both *TP53* and *RB1* in mCRPC, even after adjusting for tumour purity based on low-pass WGS. More deep deletions in *PTEN* were also detected in the mCRPC cohort. Mutations in the WNT pathway genes *CTNNB1* and *APC*, as well as *MYC* amplification, were also more common in mCRPC.

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

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

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

Generally, the number of private events was small. An outlier case was P001, a patient with a MMR-defective prostate cancer who had the highest mutation burden, including several shared mutations between primary and mCRPC (*APC, CDK12, MSH6, ERBB4, PTEN* and *TP53*), several private mutations only detected in mCRPC (including missense, non-truncating, mutations in *APC, ATM, EZH2, JAK1*) and several private mutations of the primary tumour not detected in the later mCRPC biopsy (*CTNNB1, PRKDC*, *ERCC3* and *ERRC6*), suggesting the presence of different clones coming from a shared origin.

DISCUSSION

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

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

261 only 4 cases with *BRCA2* 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 *BRCA2*
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

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

The main limitation of our study comes from having only one biopsy core available per time point and patient; we therefore could not assess spatial tumour heterogeneity. Primary prostate cancers can be multifocal, and previous studies have reported on inter-foci genomic heterogeneity(30, 31). We cannot rule out that in some cases the primary tumour sample may not represent the dominant tumour clone in the primary biopsy; hence, it is possible that some of the differences we observe in paired mCRPC biopsies may have not resulted from treatment-selective pressure but been in other areas of these primary tumours. However, genomic testing in clinical practice is largely based on the analyses of single biopsy cores. With the advent of novel imaging modalities, genomic stratification of prostate cancer could be improved by better identifying aggressive areas 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 *RB1*) 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*, *RB1* 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

METHODS

Study design

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

Sample acquisition and processing

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

Sequencing and bioinformatic analyses

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

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

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

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

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

Statistical considerations

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

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

Study Approval

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

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

Author contributions

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

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

Figure 1. Oncoprint of genomic aberrations (non-sense, indels, splice site mutations, relevant missense mutations and copy number changes) for 470 untreated primary prostate cancer biopsies from patients who later developed metastatic castration-resistant disease.

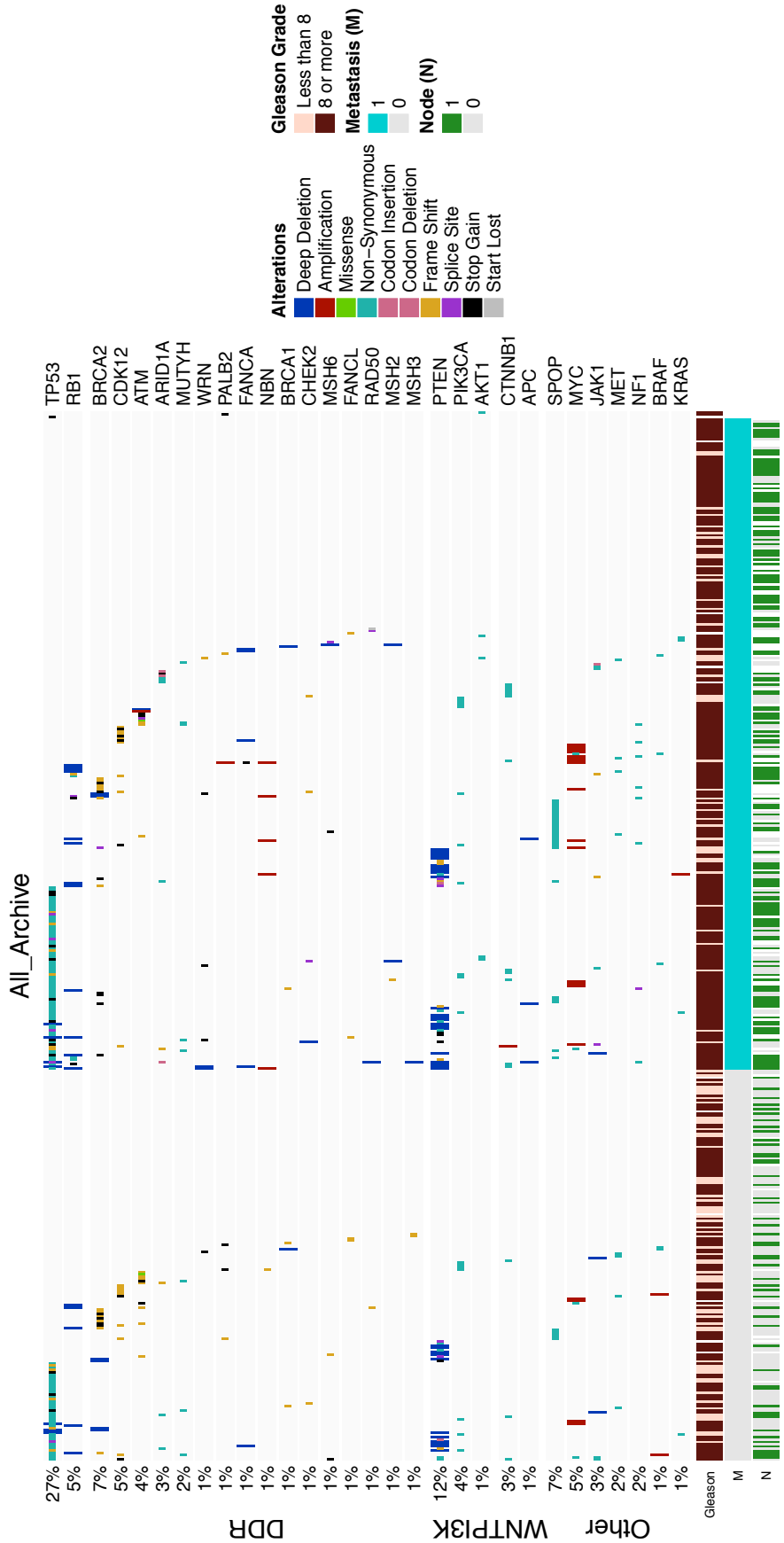
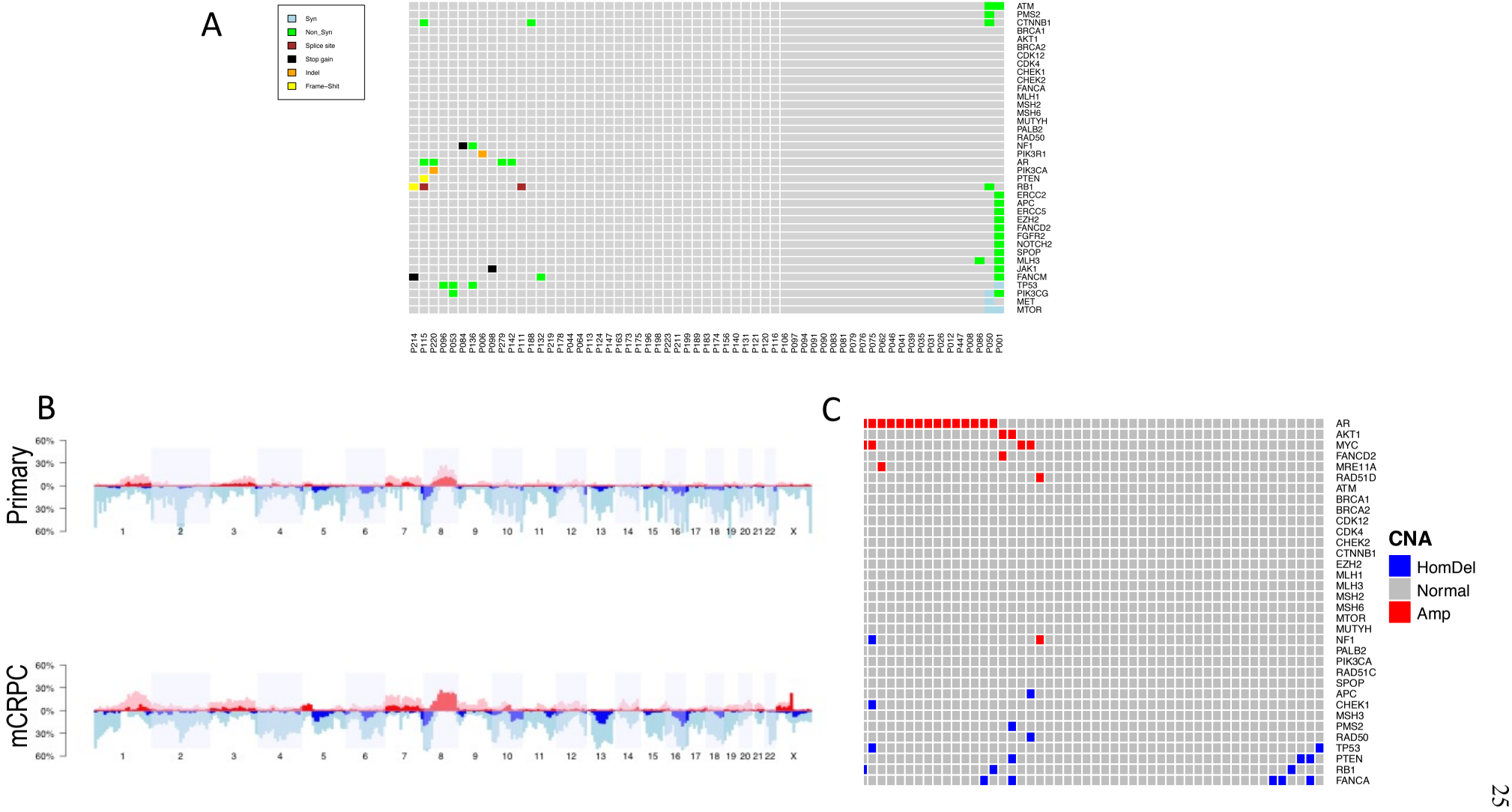


Figure 2. Differences in genomic profiles between same patient, matched, primary untreated and mCRPC biopsies. A) Mutation calls in genes of interest for the mCRPC biopsies which were not present in the treatment-naïve primary tumour for the same patient (61 pairs, full gene set in Suppl Fig 6); B) Overall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


A
Syn
Non_Syn
Splice site
Stop gain
Indel
Frame–Shit
ATM
PMS2
CTNNB1
BRCA1
AKT1
BRCA2
CDK12
CDK4
CHEK1
CHEK2
FANCA
MLH1
MSH2
MSH6
MUTYH
PALB2
RAD50
NF1
PIK3R1
AR
PIK3CA
PTEN
RB1
ERCC2
APC
ERCC5
EZH2
FANCD2
FGFR2
NOTCH2
SPOP
MLH3
JAK1
FANCM
TP53
PIK3CG
MET
MTOR
P214 P115 P220 P096 P053 P084 P136 P006 P098 P279 P142 P111 P188 P132 P219 P178 P044 P064 P113 P124 P147 P163 P173 P175 P196 P198 P220 P211 P191 P189 P183 P174 P156 P140 P131 P121 P120 P116 P106 P097 P094 P091 P090 P083 P081 P079 P076 P075 P062 P046 P041 P039 P035 P031 P026 P012 P447 P008 P086 P050 P001
B
Primary
mCRPC
60%
30%
0%
30%
60%
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 X
C
AR
AKT1
MYC
FANCD2
MRE11A
RAD51D
ATM
BRCA1
BRCA2
CDK12
CDK4
CHEK2
CTNNB1
EZH2
MLH1
MLH3
MSH2
MSH6
MTOR
MUTYH
NF1
PALB2
PIK3CA
RAD51C
SPOP
APC
CHEK1
MSH3
PMS2
RAD50
TP53
PTEN
RB1
FANCA
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 (%)	
AKT1	Activating mutations	3 (0.9%)	0 (0%)	0.56
ATM	Loss-of-function mutations and deep deletions	20 (6%)	10 (6%)	1.00
BRCA1	Loss-of-function mutations and deep deletions	3 (1%)	3 (2%)	0.42
BRCA2	Loss-of-function mutations and deep deletions	10 (3%)	14 (8%)	0.015
CDK12	Loss-of-function mutations and deep deletions	7 (2%)	10 (6%)	0.04
CTNNB1	Activating mutations	7 (2%)	3 (2%)	1.00
PIK3CA	Activating mutations and copy number gains	7 (2%)	7 (4%)	0.26
PTEN	Loss-of-function mutations and deep deletions	57 (17%)	20 (11%)	0.09
RB1	Loss-of-function mutations and deep deletions	3 (1%)	6 (3%)	0.07
SPOP	Hotspot mutations	37 (11%)	5 (3%)	0.001
TP53	Loss-of-function mutations and deep deletions	27 (8%)	44 (25%)	<0.001

Table 3. Association of gene defects with clinical outcome. Long-rank p-values are presented unadjusted and adjusted for both Gleason score (≤7 vs ≥8) and 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						
TP53	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
PTEN	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
RB1	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
SPOP	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
BRCA2	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
CDK12	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
ATM	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
PIK3CA	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
CTNNB1	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
AKT1	2	1.58 (95%CI: NA)	0.77/0.53	2	5.64 (95%CI: NA)	0.65/0.59
BRCA1	3	1.08 (95%CI: 0.42-NA)	0.66/0.62	3	2.31 (95%CI: NA)	0.07/0.17
BRCA1/2 / ATM	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
PIK3CA/ AKT1/PTEN	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

Table 4. Sample disposition for the patient-matched primary untreated and mCRPC biopsies (n=61 cases with paired samples). Median time between the two same-patient 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%
	Cabazitaxel	20	32.8%
	Radium-223	4	6.5%
	Investigational agents	14	22.9%
Lines of therapy for CRPC before mCRPC biopsy	0	2	3.2%
	1	9	14.7%
	2	21	34.4%
	3 or more	29	47.5%

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