Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Mateo J, Carreira S, Sandhu S, et al. DNA-repair defects and olaparib in metastatic prostate cancer. N Engl J Med 2015;373:1697-708. DOI: 10.1056/NEJMoa1506859

Supplementary Appendix

Supplement to:

Mateo J, Carreira S, Sandhu S, Miranda S, Mossop H, Perez-Lopez R et al. *DNA Repair Defects and PARP Inhibition In Metastatic Prostate Cancer.*

Contents:

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Eligibility criteria

Inclusion criteria

1. The subject is capable of understanding and complying with the protocol requirements and has signed the informed consent document.

2. Age ≥18 years.

3. Histologically confirmed adenocarcinoma of the prostate with archival tumour tissue available for molecular analyses. If the patient does not have a prior histological diagnosis then the planned baseline fresh biopsy may be used for both the purpose of confirming the histological diagnosis prior to trial entry and for subsequent biomarker analysis. All patients must be willing to have fresh biopsies to obtain tumour tissue for biomarker analysis

4. At least one but no more than two previous taxane-based chemotherapy regimens. If docetaxel chemotherapy is used more than once, this will be considered as one regime. Patients may have had prior exposure to cabazitaxel treatment.

5. At least 28 days since the completion of prior therapy, including major surgery, chemotherapy and other investigational agents. Additionally, clinically relevant sequelae should have resolved to grade 1 or less prior to recommencing treatment. For hormonal treatment and radiotherapy refer to the guidelines below:

5.1 At least 28 days since the completion of prior flutamide treatment. Patients whose PSA did not decline in response to antiandrogens given as a second line or later intervention will only require a 14 days washout prior to Cycle 1, Day 1.

5.2 At least 42 days since the completion of prior bicalutamide (Casodex) and nilutimide (Nilandron) treatment. Patients whose PSA did not decline for 3 or 4 months in response to antiandrogens given as second line or later intervention will require only a 14 day washout period prior to Cycle 1 Day1.

5.3 At least 14 days from any radiotherapy with the exception of a single fraction of radiotherapy for the purposes of palliation (confined to one field) is permitted.

6. Documented prostate cancer progression as assessed by the investigator with one of the following:

6.1. PSA progression defined by a minimum of three rising PSA levels with an interval of ≥1 week between each determination. The PSA values at the Screening visit should be ≥2 ug/l (2ng/ml); patients on systemic glucocorticoids for control of symptoms must have documented PSA progression by PCWG22 while on systemic glucocorticoids prior to commencing Cycle1 Day1 of treatment.

6.2. Radiographic progression of soft tissue disease by modified RECIST criteria 1.1 or of bone metastasis with two or more documented new bone lesions on a bone scan with or without PSA progression.

7. Surgically or medically castrated, with testosterone levels of < 50 ng/dL (< 2.0 nM). If the patient is being treated with LHRH agonists (patient who have not undergone orchiectomy), this therapy must have been initiated at least 4 weeks prior to Cycle 1 Day 1 and must be continued throughout the study.

8. Eastern Cooperative Oncology Group (ECOG) Performances Status of ≤2 (Karnofsky Performance Status≥50%)

9. Life expectancy > 12 weeks.

10. Patient must be able to swallow a whole tablet.

11. Patient and the patient's partner of childbearing potential, must agree to use medically accepted methods of contraception (e.g., barrier methods, including male condom, female condom, or diaphragm with spermicidal gel) during the course of the study and for 3 months after the last dose of study drug.

12. Agreeable to have all the biomarker studies including the paired fresh tumour biopsies.

13. Subject must have a CTC count of $\geq$5 cells/7.5ml blood at screening confirmed by the central laboratory.

14. Subjects must have adequate bone marrow, hepatic and renal function documented within 7 days of registration, defined as:

14.1. Hemoglobin >10g/dl independent of transfusions for 14 days if related to anemia due to advanced prostate cancer. If not, subjects should be independent of transfusions for 28 days.

14.2 White blood cells >3x 10^9/L

14.3 Absolute neutrophil count >1.5 x 10^9/L

14.4 Platelets > 100 x 10^9/L

14.5 Total bilirubin <1.5 x upper limit of normal (ULN) except for patients with Gilbert's syndrome.

14.6 Aspartate transaminase (AST) (SGOT) and alanine transaminase (ALT) (SGPT) $\leq$2.5 x ULN or $\leq$5 ULN in the presence of liver metastases.

14.7 Serum creatinine $\leq$1.5 x ULN or a calculated creatinine clearance 40mL/min for patients with creatinine levels above institutional normal. For GFR estimation, the Cockcroft and Gault equation should be used:

GFR = CrCl (ml/min) = (140 - age) × wt (kg)/(serum creatinine × 72)

14.8 Albumin >25 g/dl

Exclusion criteria

Patients who meet any of the following criteria will be excluded from the study:

1. Surgery, or local prostatic intervention (excluding a prostatic biopsy) less than 28 days of Cycle 1 Day 1.
2. Less than 28 days from any active anticancer therapy or investigational agents. For hormonal treatment and radiotherapy refer to the guidelines outlined in the inclusion criteria.
3. Prior treatment with a PARP inhibitor, platinum, cyclophosphamide or mitoxantrone chemotherapy.
4. Uncontrolled intercurrent illness including, but not limited to, active infection, symptomatic congestive heart failure (New York Heart Association Class III or IV heart disease), unstable angina pectoris, cardiac arrhythmia, uncontrolled hypertension or psychiatric illness/social situations that would limit compliance with study requirements.
5. Any acute toxicities due to prior chemotherapy and / or radiotherapy that have not resolved to a NCI-CTCAE v4.02 grade ≤1 with the exception of chemotherapy induced alopecia and grade 2 peripheral neuropathy.
6. Malignancy within the previous 2-years with a > 30% probability of recurrence within 12 months with the exception of non-melanoma skin cancer, in-situ or superficial bladder cancer.
7. Patients with myelodysplastic syndrome/acute myeloid leukaemia.

8. Patients with known symptomatic brain metastasis are not suitable for enrolment. Patients with asymptomatic, stable, treated brain metastases are eligible for study entry.

9. Patients with symptomatic or impending cord compression unless appropriately treated beforehand and clinically stable and asymptomatic.

10. Patients who have experienced a seizure or seizures within 6 months of study treatment or who are currently being treated with cytochrome P450 enzyme inducing anti-epileptic drugs for seizures (use of anti-epileptic drugs to control pain is allowed in patients not suffering from seizures unless drug is excluded due to CYP3A4 induction - phenytoin, carbamazepine, phenobarbital (see Section 12.13).

11. Patients receiving any of the following classes of inhibitors of CYP3A4

- Azole antifungals
- Macrolide antibiotics
- Protease inhibitors

12. Patients with gastrointestinal disorders likely to interfere with absorption of the study medication.

13. Initiating bisphosphonate therapy or adjusting bisphosphonate dose/regimen within 30 days prior to Cycle 1 Day 1. Patients on a stable bisphosphonate regimen are eligible and may continue.

14. Presence of a condition or situation, which, in the investigator's opinion, may put the patient at significant risk, may confound the study results, or may interfere significantly with patient's participation in the study.

15. The subject is unable or unwilling to abide by the study protocol or cooperate fully with the investigator or designee.

Criteria of progression for trial eligibility by disease manifestation according to Prostate Cancer Working Group 2.

Adapted from: Scher HI, Halabi S, Tannock I, et al. Design and end points of clinical trials for patients with progressive prostate cancer and castrate levels of testosterone: recommendations of the Prostate Cancer Clinical Trials Working Group. J Clin Oncol 2008;26(7):1148–59.

Variable	Criteria of progression for trial eligibility
PSA	• Obtain a sequence of at least 2 rising values at a minimum of 1-week intervals. • 2.0 ng/ml minimum starting value.
Target lesions	• Nodal or visceral disease progression is sufficient for trial entry independent of PSA. • Presence of measurable lesions is not required for study entry. • Use RECIST to record soft-tissue (nodal and visceral) lesions as target or non-target. • Only lymph nodes > 2cm in diameter should be used to assess for a change in size. • Record presence of nodal and/or visceral disease separately.
Prostate/prostate bed (primary site)	• Record prior treatments of the primary tumor. • Perform directed pelvic imaging to document presence or absence of disease.

Bone	• Progression is defined as appearance of 2 or more new lesions in bone scans. • Ambiguous results to be confirmed by other imaging modalities (e.g., CT or MRI).
Other sites of disease	• Patients may still be enrolled if they have epidural disease that has been treated and there is no progression in the treated area.

Safety assessments based on Common Terminology Criteria for Adverse Events (CTCAE 4.0)

Grading in CTCAE refers to the severity of the adverse event; The CTCAE displays Grades 1 to 5 with unique clinical descriptions of severity for each event, based on this general guideline:

- Grade 1: Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
- Grade 2: Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living.
- Grade 3: Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self care activities of daily living.
- Grade 4: Life-threatening consequences; urgent intervention indicated.
- Grade 5: Death related to adverse event.

The full listing of Common Terminology Criteria for Adverse Events (CTCAE 4.0) is available at: http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf.

Methods text not included in the main paper: Biomarker studies

The whole-exome sequencing was performed on the Illumina *HiSeq 2500* in paired-end mode and the primary base call files were converted into *FASTQ* sequence files using the bcl2fastq converter tool *bcl2fastq-1.8.4* in the *CASAVA 1.8* pipeline. The *FASTQ* sequence files generated were then processed through an in-house pipeline constructed for whole-exome sequence analyses of paired cancer genomes. Tumor content for each tumor exome library was estimated from the sequence data by fitting a binomial mixture model with two components to the set of most likely SNV candidates on 2-copy genomic regions. The sequencing reads were aligned to the reference genome build *hg19*, *GRCh37* using *Novoalign* Multithreaded (*Version2.08.02*) (Novocraft) and converted into BAM files using *SAMtools* (*Version 0.1.18*).48 Sorting and indexing of BAM files utilized *Novosort* threaded (*Version 1.00.01*) and duplicates reads were removed using *Picard* (*Version 1.74*). Mutation analysis was performed using *VarScan*2 algorithms (*Version2.3.2*)49 utilizing the pileup files created by *SAMtools mpileup* for tumor and matched normal samples, simultaneously performing the pairwise comparisons of base call and normalized sequence depth at each position. Copy number aberrations were quantified and reported for each gene as the segmented normalized log2-transformed exon coverage ratios between each tumor sample and matched normal sample. We used the publicly available software FastQC to assess sequencing quality. For each lane, we examine per-base quality scores across the length of the reads. Lanes were deemed passing if the per base quality score boxplot indicated that >75% of the reads had >Q20 for bases 1-80. In addition to the raw sequence quality, we also

assess alignment quality using the Picard package. This allows monitoring of duplication rates and chimeric reads that may result from ligation artifacts; crucial statistics for interpreting the results of copy number and structural variant analysis. Pathogenicity of germline variants were determined through review of the published literature, public databases including but not limited to ClinVar, Human Genome Mutation Database, and Leiden Open Variation Databases, and variant specific databases (e.g., International Agency for Research on Cancer TP53 Database, International Society for Gastrointestinal Hereditary Tumors mutation databases).

Targeted sequencing was conducted at The Institute of Cancer Research (UK) using a 113-gene panel including genes found to be mutated/deleted in the exome studies, key DNA repair genes and genes commonly aberrant in prostate cancer utilizing an orthogonal method that can serve as a high-throughput lower cost biomarker assay for prospective molecular characterization for future clinical trials.

Somatic and/or germline aberrations were identified in an unsupervised fashion. For the purposes of this study, we focused on aberrations in genes previously associated with PARP inhibition sensitivity and specifically genes involved in DNA repair. The sequencing and analyses team was blinded to the clinical outcome of the patients enrolled on this study.

Immunohistochemistry studies: PTEN immunoreactivity was assessed with a rabbit monoclonal anti-PTEN antibody 138G6 (Cell Signaling Technology Inc;

Cat no 9559) detected using the Vectastain Elite ABC kit (Vectorlabs; Cat no. PK-6101), PTEN loss was defined as an H score ≤10; ERG immunoreactivity was investigated using a rabbit monoclonal anti-ERG antibody EPR3864 (Abcam; Cat no ab92513) detected using the Dako EnVision kit (Dako; Cat no. K5007). Slides were reviewed by a trained pathologist blinded to clinical outcomes data.

Results text not included in the main paper: association between PTEN and ERG IHC and response to olaparib

Loss of PTEN was detected in 17 (34.7%) of the treated tumors; 21 (43.9%) patients had ERG positive mCRPC. There was no association between either PTEN loss, or the presence of ERG expression, and sensitivity to olaparib (Supplemental Tables 3-4); Chi-squared test; p-value 0.77 for PTEN and 0.26 for ERG).

Supplemental Figures S1-S4

Figure S1. Clinical Trial design. The number of responses in stage 1 (10/30) and at the end of the trial supported the study of molecular signatures for PARP inhibitor antitumour activity.

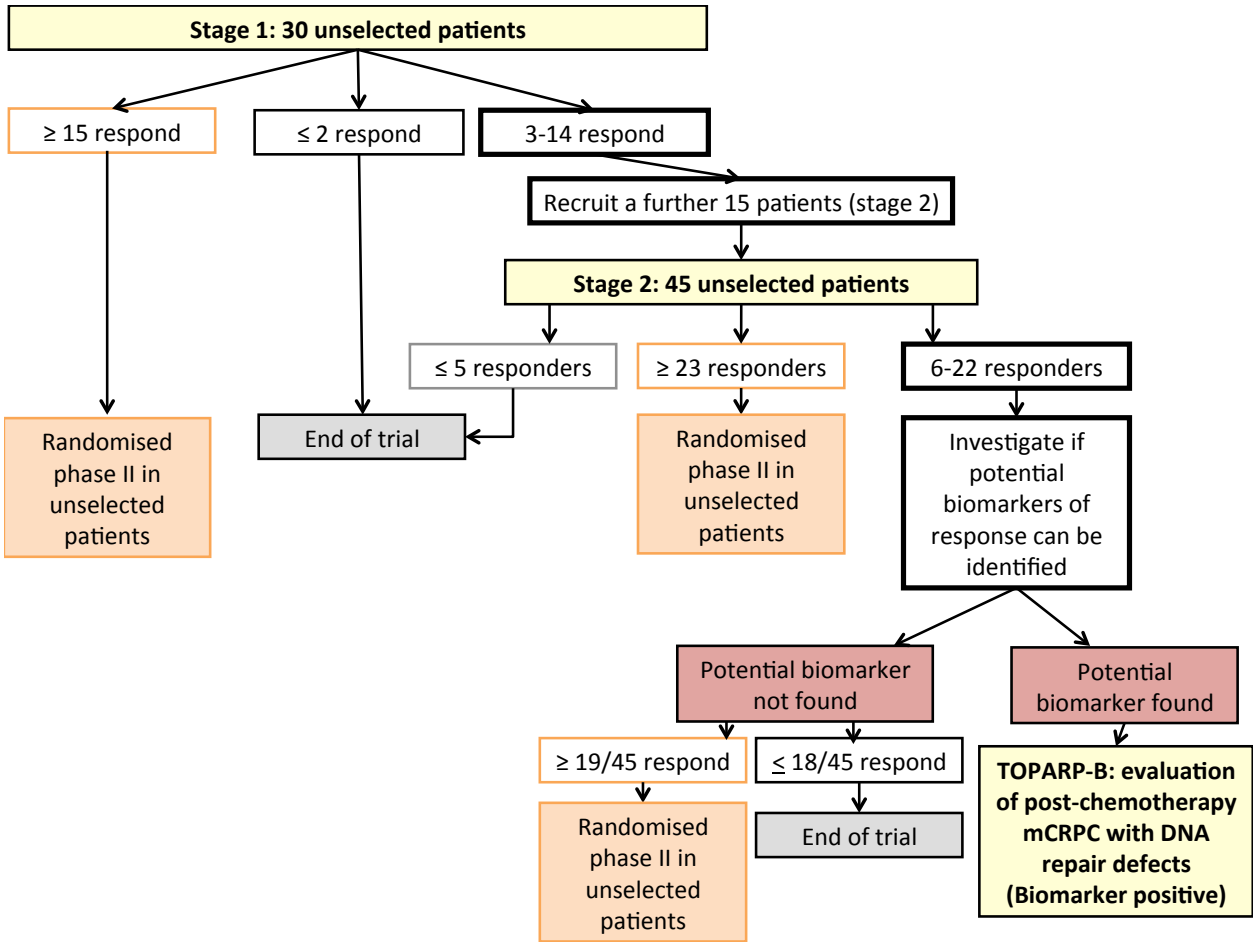


Figure S2. Consort diagram of patients participating in the study.

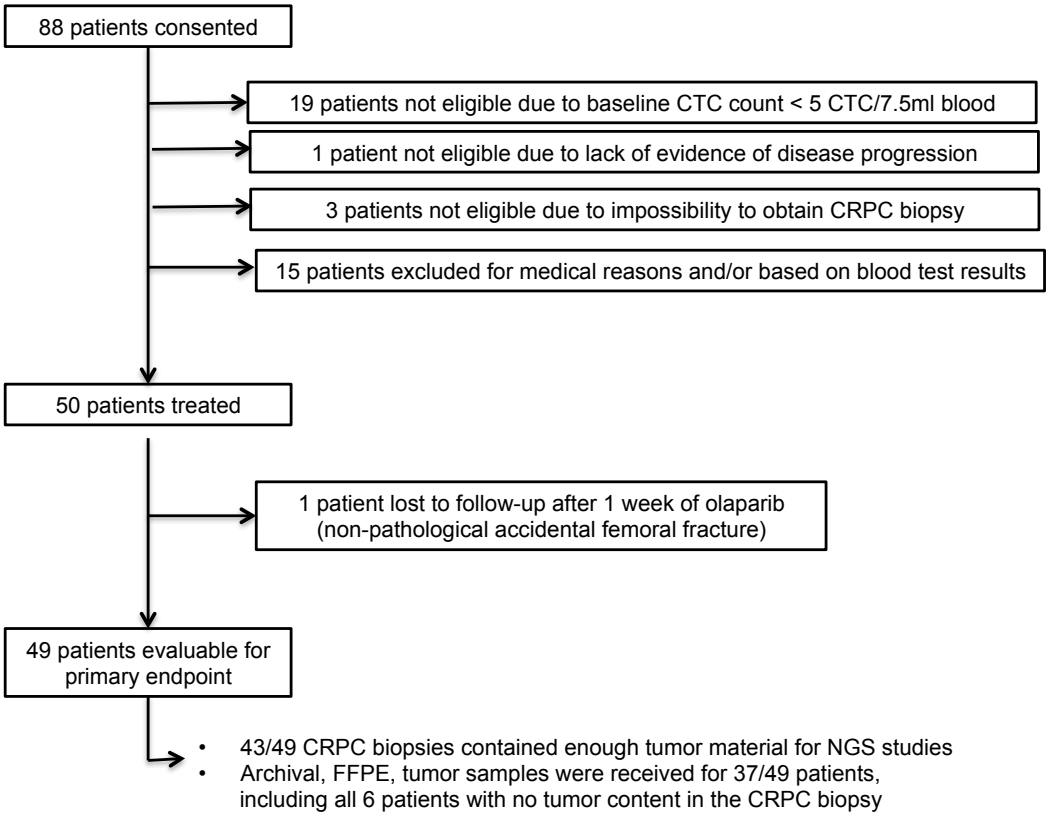
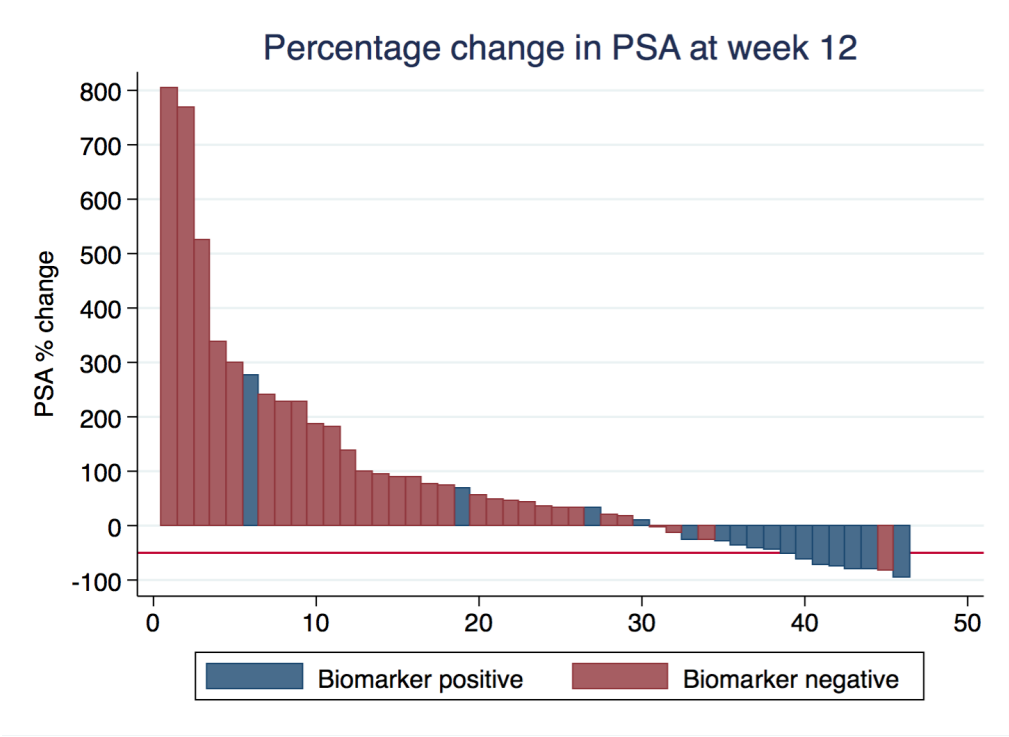


Figure S3. Waterfall plots showing changes in PSA and CTC enumeration after 12 weeks of therapy. Blue bars indicate patients with genomic defects in DNA repair genes.



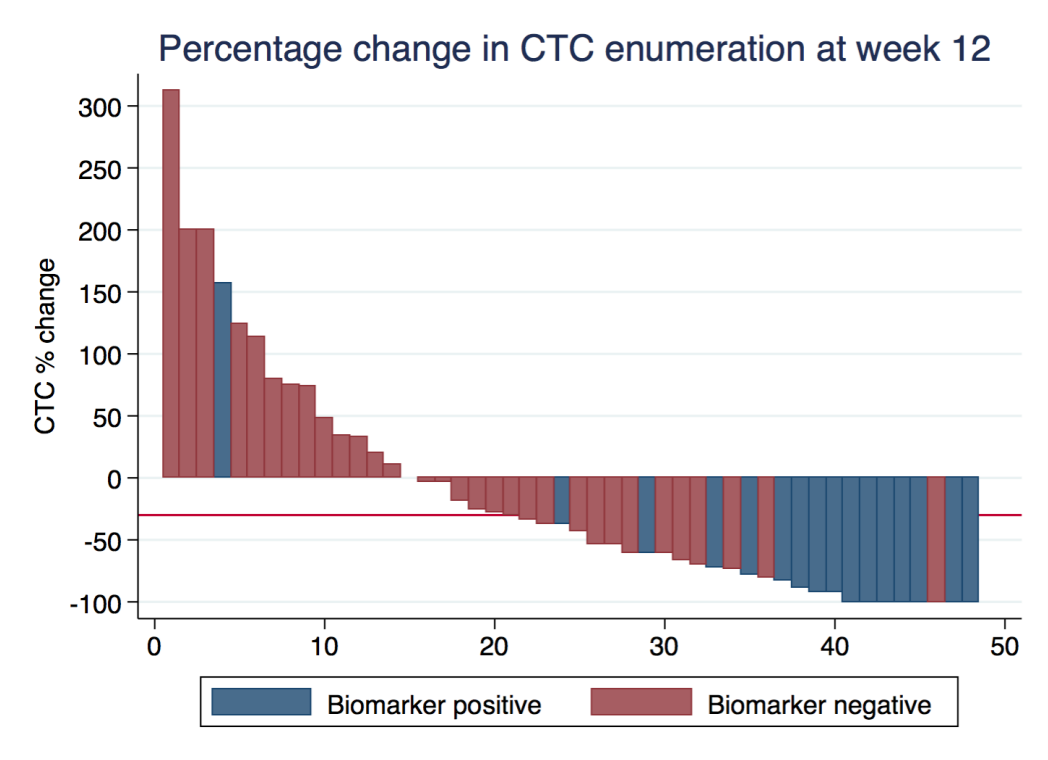
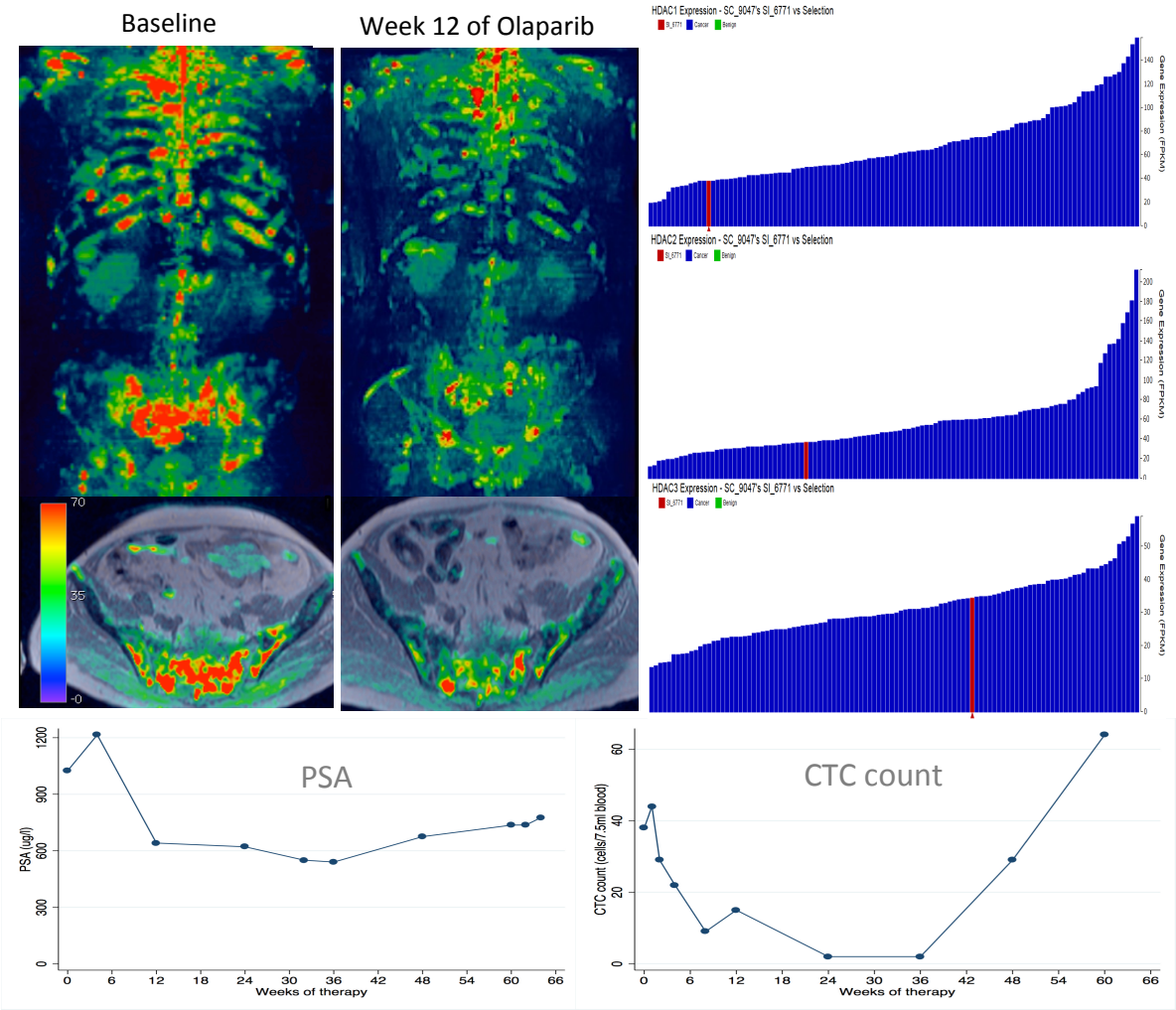


Figure S4. Coronal 3D reconstruction and selected axial images of a multiparametric whole-body magnetic resonance including diffusion-weighted imaging. The images show reduction in the water content within the skeletal metastasis, which in conjunction with other imaging parameters would be consistent with regression of metastatic disease in patient #8 after 3 months of therapy. This patient was on treatment for 60 weeks with a 47 % fall in PSA and a 95% fall in circulating tumor cells counts from a baseline CTC count of 38 CTC/7.5ml of blood. A somatic missense mutation of HDAC2 was identified, with loss of the second allele by somatic deletion; transcriptome studies showed very low expression of HDAC1 and HDAC2 mRNA which were among the lowest when compared with a cohort of 110 other mCRPC transcriptomes (right panel).



Supplemental Tables S1-S5

Table S1. Treatment disposition and median follow-up of the trial population.

Treatment disposition		
On going, n (%)	4 (8%)	*All 4 had >40 weeks of therapy at data cut-off*
Discontinued, n (%)	46 (92%)	
Reason for discontinuation	Radiological progression (RECIST or bone scan)	35 (70%)
	Clinical/PSA progression	7 (14%)
	Adverse event	3 (6%)
	Patient choice	1 (2%)
Duration of therapy (weeks), median (IQR)		12 (11-24.4)
Follow-up (months), median (range)		14.4 (1.4-21.9)

Table S2. Correlation between the presence of genomic defects in DNA repair genes (Biomarker positive/negative) and response to olaparib, including sensitivity and specificity of the biomarker suite. (OR: unadjusted odds ratio, estimated by logistic regression).

Biomarker	**Responder**		Total (n=49)
	No (n=33)	*Yes (n=16)*	
Negative	31 (93.9%)	2 (6.1%)	33
Positive	2 (12.5%)	14 (87.5%)	16
Fishers' exact p-value	p<0.001		
Sensitivity	87.5%		
Specificity	93.9%		
Accuracy	91.8%		
OR (95% CI)	108.5 (13.84-850.50)		

Table S3. Multivariable logistic regression model for association of genomic DNA repair defects and response to olaparib.

Predictive variable	Odds ratio	Multivariable analysis p-value	95% CI
DNA repair gene defect	**86.90**	**p<0.001**	**9.79-771.04**
PTEN (IHC)	1.02	p=0.989	0.10-9.92
ERG (IHC)	0.68	p=0.745	0.07-6.75
ALP	1.00	p=0.897	1.00-1.00
LDH	1.00	p=0.894	1.00-1.00
Albumin	1.18	p=0.889	0.11-12.11
CTC count	1.00	p=0.617	0.98-1.01
PSA	1.00	p=0.676	1.00-1.01

Table S4. Correlation between PTEN status by IHC (using H-value of 10 as cut-off) and response to olaparib (OR: unadjusted odds ratio, estimated by logistic regression.

PTEN status	**Responder**		Total (n=49)
	No (n=33)	*Yes (n=16)*	
Negative	11 (64.7%)	6 (35.3%)	17
Positive	22 (68.8%)	10 (31.2%)	32
Chi-squared p-value	p = 0.77		
Sensitivity	62.5%		
Specificity	33.3 %		
Accuracy	42.9%		
OR (95% CI)	0.83 (0.24-2.89)		

Table S5. Correlation between ERG status by IHC (using H-value of 0 as cut-off) and response to olaparib (OR: unadjusted odds ratio, estimated by logistic regression.

ERG status	**Responder**		Total (n=49)
	No (n=33)	*Yes (n=16)*	
Negative	17 (60.7%)	11 (39.3%)	28
Positive	16 (76.2%)	5 (23.8%)	21
Chi-squared p-value	p = 0.25		
Sensitivity	31.3%		
Specificity	51.5%		
Accuracy	44.9%		
OR (95% CI)	0.48 (0.14-1.70)		

Table S6. Baseline distribution of established prognostic factors in mCRPC among the patients positive or negative for DNA repair gene aberrations.

	Biomarker negative (n=33)		**Biomarker positive (n=16)**	
	N	%	N	%
PTEN				
Negative	11	33.3	6	37.5
Positive	22	66.7	10	62.5
Chi^2 p-value	*p=0.77*			
ERG				
Negative	17	51.5	11	68.75
Positive	16	48.5	5	31.25
Chi^2 p-value	*p=0.25*			
ALP (IU/L)				
Median (IQR)	168 (84, 363)		189.5 (99.5, 492.5)	
Mann-Whitney p-value	*p=0.52*			
LDH (IU/L)				
Median (IQR)	279 (198, 610)		247.5 (187, 500.5)	
Mann-Whitney p-value	*p=0.59*			
Albumin (g/dL)				
Median (IQR)	3.5 (3.3, 3.8)		3.3 (3.1, 3.95)	
Mann-Whitney p-value	*p=0.62*			
CTC count (cells/7.5ml blood)				
Median (IQR)	47 (15, 138)		23.5 (10.5, 93.5)	
Mann-Whitney p-value	*p=0.29*			

Table S7. Treatment-emergent adverse events reported in at least 10% of the trial population (CTCAE v4.0).

Adverse event	All grades		Grade ≥3	
	n	%	n	%
Anemia	38	76	10	20
Fatigue	29	58	6	12
Nausea	18	36	0	
Arthralgia	15	30	1	2
Anorexia	14	28	1	2
Dyspnea	14	28	1	2
Back pain	11	22	1	2
Vomiting	10	20	0	
Weight loss	9	18	0	
Diarrhea	8	16	0	
Peripheral edemas	8	16	1	2
Bone pain	8	16	1	2
Pain (extremities)	8	16	0	
Creatinine elevation	7	14	0	
Chest pain	7	14	0	
Constipation	7	14	0	
Cough	7	14	0	
Headache	6	12	0	
Hyponatremia	6	12	1	2
Leucopenia	6	12	3	6
Dizziness	5	10	0	
Neutropenia	5	10	2	4
Thrombocytopenia	5	10	2	4