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TITLE: Clinical Qualification of DNA Repair Defects as Biomarkers in Metastatic Prostate Cancer Using Integrated Genomics and Tissue-Based Functional Assays

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CONTRACTING ORGANIZATION: Vall d'Hebron Institute of Oncology, Barcelona Spain

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14. ABSTRACT

We and others previously described an enrichment for somatic and germline alterations in DNA damage repair (DDR) genes among men with metastatic prostate cancer. Several recent clinical studies have indicated many of these patients could benefit from precision medicine strategies with PARP inhibitors and DNA damaging agents. In this project, our teams would investigate genomic, transcriptomic and protein-related functional signatures for a more accurate sub-classification of prostate cancers associated to DDR defects, aiming for a more precise patient care. The project is divided in 3 main aims: 1) testing the prognostic value of somatic DDR defects in a retrospective cohort of tumor biopsies, 2) developing multi-omics signatures based on prospective analyses of metastatic biopsies and 3) clinical validation of these biomarkers in a clinical trial using carboplatin as DNA damaging chemotherapy.

15. SUBJECT TERMS-

None Listed

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1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

   We and others previously described an enrichment for somatic and germline alterations in DNA damage repair (DDR) genes among men with metastatic prostate cancer. Several recent clinical studies have indicated many of these patients could benefit from precision medicine strategies with PARP inhibitors and DNA damaging agents. In this project, our teams would investigate genomic, transcriptomic and protein-related functional signatures for a more accurate sub-classification of prostate cancers associated to DDR defects, aiming for a more precise patient care. The project is divided in 3 main aims: 1) testing the prognostic value of somatic DDR defects in a retrospective cohort of tumor biopsies, 2) developing multi-omics signatures based on prospective analyses of metastatic biopsies and 3) clinical validation of these biomarkers in a clinical trial using carboplatin as DNA damaging chemotherapy.

2. **KEYWORDS:** *Provide a brief list of keywords (limit to 20 words).*

   Genomics; Whole-exome sequencing; RNAseq; Precision Medicine; DNA repair; BRCA; PARP inhibitors; platinum chemotherapy; clinical trial.

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

   What were the major goals of the project?
   List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

   Specific Aim 1 – To correlate the presence or absence of somatic/germline alterations in DNA repair genes with overall survival from mCRPC, and specific response to taxanes, Abiraterone, Enzalutamide, and Rd223, in samples from a prospective study.

Major Task 1: Targeted NGS on all study samples	**Timeline (Months)**	**Completed (%)**
Preparation of tumor biopsies for DNA extraction	0-12	100%
Milestone 1.1 – Shipment of samples to UW Laboratory (batches)	3 to 15	100%
Library preparation for targeted NGS	3 to 20	100%
Sequencing of all samples from the PROREPAIR-B study	3 to 20	100%
Variant calling, bioinformatics analysis	3 to 20	100%

	Timeline (Months)	Completed (%)
Milestone 1.2 – Classification of each patients as "positive" or "negative" for each of the biomarkers of interest (BRCA1, BRCA2, ATM, PALB2)	3 to 20	100%
Sequencing data analysis board: identification of putative relevant calls for patient care and relatives' risk of cancer		
Statistical analysis: correlation of genomic biomarkers with previously annotated clinical outcome data	22	100%
Milestone 1.3 – Data analysis and interpretation, Manuscript Preparation	24	80%
Milestone 1. 4 - F2F meeting among participating sites to discuss progress	12	100%

Specific Aim 2 – To optimize tissue-based tests of HR functionality samples for CRPC samples, and study the correlation with genomic aberrations in HR genes.

Major Task 2: Acquisition of bone marrow metastatic biopsies	**Timeline (Months)**	**Completed (%)**
Harmonization of tissue acquisition protocol among participating sites	1 to 2	100%
Collection of 100 metastatic biopsies, samples are sent to sites 2 and 3	3 to 22	100%
Milestone 2.1 – Sample acquisition completed	23	100%
Major Task 3: Whole-exome sequencing studies		
DNA extraction from tumor and germline DNA	6 to 24	100%
Whole exome sequencing studies	12 to 26	100%
Variant calling, bioinformatics analysis	12 to 28	100%
Sequencing data analysis board: identification of putative relevant calls for patient care and relatives' risk of cancer	6 to 30	100%
Major Task 4: Expression profiling studies		
RNA extraction from frozen core of biopsies	6-24	100%

RNA-seq studies	9 to 26	100%
Bioinformatics analysis	12 to 28	100%
Major Task 5: Immunofluorescence studies		
Sample preparation	8 to 30	100%
Immunofluorescence studies	10 to 30	100%
Milestone 5.1 – Integrated analysis of sequencing and IF data	32	100%
Milestone 5.2 – Data analysis and interpretation, Manuscript Preparation	34	100%

Specific Aim 3 To clinically qualify this HR functional test in a clinical trial of carboplatin in CRPC

Major Task 6: Clinical Trial Set Up	**Timeline (Months)**	**Completed (%)**
Clinical Trial Protocol Writing and Development	1 to 5	100%
Submission of clinical trial protocol to local ethics and regulatory bodies	5	100%
Set up of clinical sites participating in the trial		100%
Milestone 6.1 – First patient enrolled in the clinical trial	12	100%
Major Task 7: Clinical Trial conduction		
Patient recruitment	12 to 30	100%
Continuous data monitoring	12-36	80%
Trial-related biopsy acquisition	12 to 30	100%
Milestone 7.1 Recruitment completed for cohort 1	26	100%
Milestone 7.2 Recruitment completed for cohort 2, stage 1	22	100%
Recruitment for cohort 2, stage 2 (depending on results from stage 1)	23-30	80%

Milestone 7.3 Recruitment completed for cohort 2, stage 2	30	50%
Major Task 7: Biomarker studies in trials samples		
Preparation of trial related biopsies for NGS studies	12 to 30	60%
Targeted sequencing in trial-related biopsies	12 to 30	30%
Variant calling, bioinformatics analysis	12 to 30	30%
Immunofluorescence studies	12 to 30	100%
Sequencing data analysis board: identification of putative relevant calls for patient care and relatives' risk of cancer	12 to 30	50%
Milestone 7.1 – Integrated analysis of clinical and biomarker data	34	50%
Milestone 7.2 – Data analysis and interpretation, Manuscript Preparation	36	50%

What was accomplished under these goals?
For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

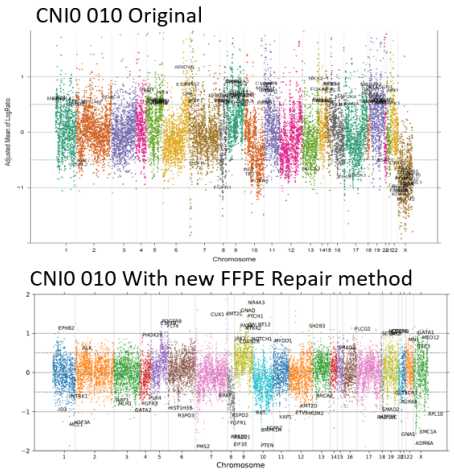
Specific Aim 1 – To correlate the presence or absence of somatic/germline alterations in DNA repair genes with overall survival from mCRPC, and specific response to taxanes, Abiraterone, Enzalutamide, and Rd223, in samples from a prospective study.

Major Task 1: Targeted NGS on all study samples

Over the course of this award, Site 1 undertook several sequencing strategies aimed at producing the highest genomics data using the clinically-validated UW-OncoPlex platform. Substantial work was done on producing quality genomics data from these patients. The optimal approach was to focus on plasma cell-free DNA, which produced high quality data. Tumor tissue and plasma cell-free DNA samples were shipped in batches from the Olmos site the Pritchard site over the course of the award, as outlined in prior reports. In total, plasma cell-free DNA or tumor tissue samples were successfully sequenced by UW-OncoPlex for 510 unique patients as part of this study, among whom 125 (25%) had DNA repair gene mutations (**Table**). Twenty-seven patient studies failed sequencing (5%) even after optimization.

Many of the tumor tissue samples sent had low amounts of residual DNA remaining (<250ng). There was availability of pre-capture libraries for most of the samples. To facilitate adequate performance on these low input samples we undertook three parallel development efforts in year 1 to modify and re-validate the UW-OncoPlex assay for clinical use with low-input samples anticipated from the PROREPAIR trial as part of this work. In year 1-3 a major focus was optimizing methods to allow clinical sequencing of very low input and low-quality DNA samples from the CNIO site. Major activities included review of an FFPE DNA repair step to improve DNA quality for sequencing (**Figure**), revision of the pooled hybridization capture protocol to include maximum input quantity in NGS, while reducing the control sample input (to avoid sinking DNA sequence), and exploration of low-input single-stranded NGS library prep protocol. In Year 3 we completed experiments using a new FFPE DNA repair protocol on low quality samples.

Figure: Effect of FFPE DNA repair on UW-OncoPlex sequencing results. Example copy number plots of CNIO sample 010 run on the UW-OncoPlex v6 panel without (top, original) or with (bottom) FFPE DNA repair. Quality metrics were improved with the FFPE DNA repair step, including copy number quality



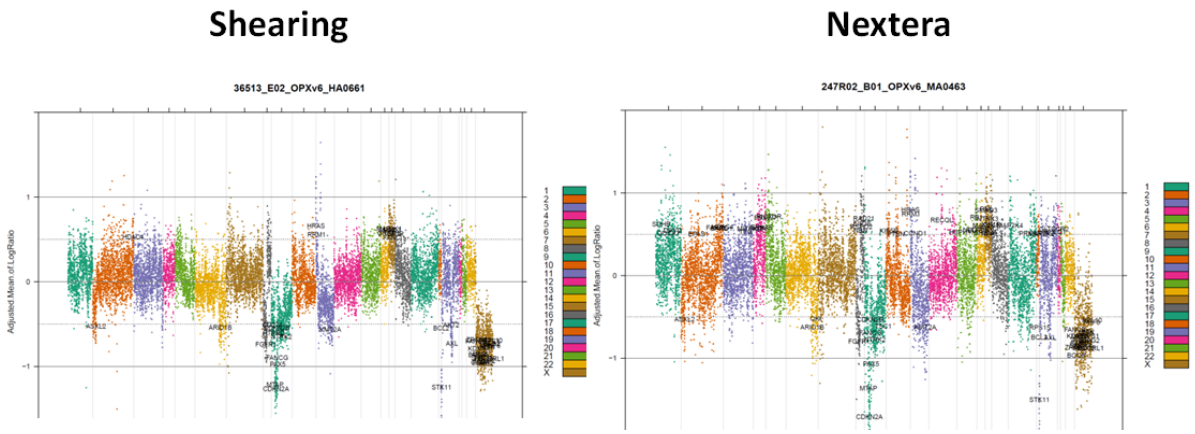


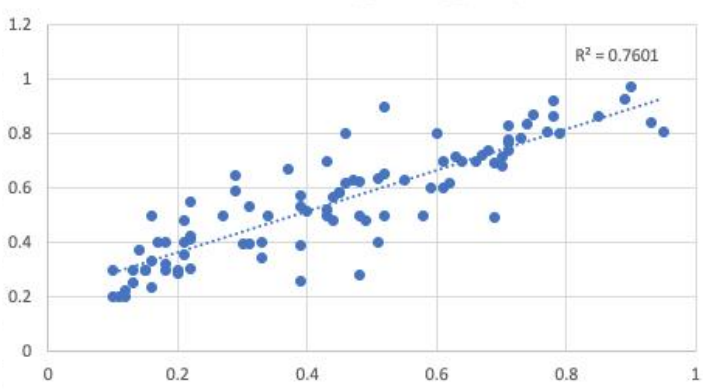
Figure: Comparison of copy number calling between the standard shearing and low input Nextera UW-OncoPlex sequencing. We observed high qualitative and quantitative concordance between the standard shearing-based library prep and Nextera low input library preparation for the UW-OncoPlex assay.

The first approach was to validate pre-capture libraries from Site 2 for use with UW-OncoPlex. To evaluate and validate pre-capture libraries as a sample type for UW-OncoPlex pilot samples were sent

to Site 1 (UW) from Site 2 (CNIO) with matched pre-cap libraries and extracted DNA. We are currently working closely with our bioinformatics team, wet-bench staff to work out the protocol to run and analyze these pre-cap library samples on our platform. Briefly, the samples are quantified on the Agilent Tape Station and pooled together for hybridization along with a HapMap control (NA12878). They are hybridized with latest UW-OncoPlex (version 6) capture, using an IDT xGen protocol. The pool is loaded on an Illumina instrument (PE101 + 8bp index read). Since the samples were previously barcoded with 6bp indexes, we added "NN" to the end of the sequences for the MiSeq sample sheet, which would allow demultiplexing and analysis of both the 6bp and 8bp indexes in the pool. Using this protocol we have successfully sequenced four pre-capture libraries, however the sequencing quality is not yet adequate using pre-capture libraries. To troubleshoot, we are attempting more pre-capture libraries with higher DNA quantity. In parallel we focused on testing samples with >250ng input DNA, prioritizing patients with radical prostatectomy first.

The second approach was to modify and re-validate the UW-OncoPlex sequencing assay for use with Nextera NextFlex enzymatic tagmentation-based sequencing library preparation rather than using DNA shearing with the Covaris. This NextFlex method allows the assay to take as little as 10ng DNA input rather than the 250ng input desired with Covaris shearing method. Also, less DNA is lost in wash steps using the NextFlex method. Briefly, to validate this method at Site 1, we selected a total of 57 tumor DNA samples that had been previously characterized by UW-OncoPlex and re-ran these using the Nextera low input protocol. All reportable mutations, copy number variants, and structural variants were identified using the Nextera protocol. Between run and within reproducibility was assessed for 3 tumor samples and for the NA12878 HapMap control with perfect concordance. MSI status was also 100% concordant. An example of the qualitive concordance of copy number calling is given in the Figure below.

Finally, as a third approach, we will explore testing plasma cell-free DNA for patients <250ng input DNA remaining. The CNIO group at Site 2 has frozen plasma available from most of these patients and is currently exploring whether it may be feasible to use these samples. The UW-OncoPlex assay has recently been extensively clinically-validated for use with plasma cell-free DNA in patients with metastatic prostate cancer (Schweizer et al. 2019 PMID:30865311, DOD support acknowledged). In parallel, and for those PROREAPIR-B cases without cell-free DNA samples and poor quantity/quality DNA yields, Site 2 (CNIO) will explore to complement the results with shallow whole genome sequencing (WGS) which may yield results satisfactory enough to detect chromosomal deletions which cause loss of function in the genes of interest, in some genes as *BRCA2* this large deletion are the commonest somatic change. At the present a small cross validation of both UW-OncoPlex sequencing at site 1 and shallow WGS has been completed as part of an initial PROREPAIR report in ASCO and ESMO annual meetings (Lozano et al. 2021, DOD support acknowledged) and which a manuscript has been submitted. During year 3, We implemented a technical improvement to the UW-OncoPlex panel that included the additional of a validated Homologous Recombination DNA repair (HRD) signature analysis using global burden of LOH, in the UW-OncoPlex version 7 panel update that went live at the end of Year 3 Q3 (**Figure**).



Major activities in Year 4-5: we decided to start the alternative plan to sequence the plasma circulating DNA from those patients which lack adequate tumor tissue, or in which extracted DNA did not yield the minimum quantity or quality for NGS analysis or quality. At site 1 we have started identifying those cases in which we have available plasma samples for processing and DNA extraction. This samples will be then sent to UW (Site 1). A total of 11 batches of samples were sent from CNIO to the UW site and UW-OncoPlex sequencing and 510 had successful sequencing using our more optimized low input protocols as outlined in the Table. Among these 62 had *BRCA2* mutations, 4 had *BRCA1* mutations, 18 had *ATM* mutations, 1 had an *NBN* mutation, X had *CHEK2* mutations, 14 had *MUTYH* mutations, 3 had a *FANCA* mutation, 6 had *CDK12* mutation, 3 had *BRIP1* mutation, 2 had *MLH1* mutation (MSI-high), 7 had evidence of MMR deficiency (3 *MSH2*, 2 *MLH1* and 2 without without underlying MMR gene mutation detected), 4 had *MRE11A* mutations, 5 had *FANCD2* mutation, and 1 had a *FANCC* mutation, 1 had *FANCM* mutation, 1 had *RAD51B* mutation, and 1 had *RAD51D* mutation (**Table**).

Table: Prostate Cancer Samples with DNA Repair Gene Mutations Detected by UW-OncoPlex

CNIO_OLM_ID	UW Dataset_ID	DRD Mutation?	Interpretation
OLM_03.035	198R16_H02_OPXv5_NB0187	ATM	1. POSITIVE for a pathogenic ATM
CNIOUW 012	272R10_B02_OPXv6_NA0414	ATM	1. POSITIVE for a pathogenic ATM
CNIOUW 028	276R04_D01_OPXv6_NB0352	ATM	1. POSITIVE for two pathogenic AT
OLM,03.012	281R08_H01_OPXv6_NB0365	ATM	1. POSITIVE for ATM exon 25-63 de
PROS05002	396R14_F02_OPXv7_ND0563	ATM	1. POSITIVE for a likely pathogenic
OLM,13-BC-01	435R02_B01_OPXv7_NA0688	ATM	1. POSITIVE for pathogenic ATM mu
OLM,13-BC-04	435R06_F01_OPXv7_NA0688	ATM	1. POSITIVE for bi-allelic ATM muta
OLM,07-3-04	435R42_B06_OPXv7_ND0632	ATM	POSITIVE for ATM mutation with LC
OLM,11-2-08	435R60_D08_OPXv7_ND0635	ATM	POSITIVE for ATM mutation with LC
OLM,20-1-06	448R26_B04_OPXv7_ND0650	ATM	1. POSITIVE for likely pathogenic A
OLM,13-1-09	448R29_E04_OPXv7_ND0650	ATM	1. POSITIVE for a pathogenic muta
OLM,13-1-20	481R29_E04_OPXv7_NB0109	ATM	ATM, BRAF K601E
OLM,41-3-14	482R05_E01_OPXv7_ND0741	ATM	ATM with LOH, AR amplification
OLM,41-3-17	482R08_H01_OPXv7_ND0741	ATM	ATM, AR amplification, TP53, low tu
OLM,20-1-10	486R09_A02_OPXv7_NA0814	ATM	ATM mutation
PROS05003	396R05_E01_OPXv7_ND0563	ATM (favor CHIP)	POSITIVE for AR amplification, SPO
PROS02067	396R24_H03_OPXv7_ND0563	ATM (VUS)	1. POSITIVE for ATM p.I2401T VUS
OLM,05-BC-07	435R22_F03_OPXv7_NA0688	ATM, FANCA	POSITIVE for a pathogenic ATM mu
OLM_03.047	198R17_A03_OPXv5_NB0187	ATM (VUS)	1. POSITIVE for a pathogenic TP53
OLM,30-BC-03	435R18_B03_OPXv7_NA0688	BRCA1	1. POSITIVE for AR amplification, TI
OLM,47-3-03	448R15_G02_OPXv7_ND0649	BRCA1	POSITIVE for pathogenic BRCA1 mu
OLM,01-1-19	483R35_C05_OPXv7_NB0113	BRCA1	BRCA1 and PTEN copy loss, TMPRS
OLM_01.039	198R07_G01_OPXv5_NB0187	BRCA2	1. POSITIVE for a pathogenic muta
CNIOUW 005	272R03_C01_OPXv6_NA0414	BRCA2	1. POSITIVE for BRCA2 focal deletio
CNIOUW 018	275R06_F01_OPXv6_NB0350	BRCA2	1. POSITIVE for BRCA2 exon 1-24 de
CNIOUW 032	276R08_H01_OPXv6_NB0352	BRCA2	1. POSITIVE for a pathogenic BRCA2
OLM,FIVO.012	286R25_A04_OPXv6_NB0365	BRCA2	1. POSITIVE for a pathogenic BRCA
PROS02043	396R01_A01_OPXv7_ND0563	BRCA2	POSITIVE for bi-allelic BRCA2 mutat
PROS02053	396R20_D03_OPXv7_ND0563	BRCA2	1. POSITIVE for a pathogenic somat
Duplicated PROS02	396R21_E03_OPXv7_ND0563	BRCA2	1. POSITIVE for a pathogenic somat
PROS03036	396R22_F03_OPXv7_ND0563	BRCA2	1. POSITIVE for a pathogenic BRCA2
PROS02031	396R27_C04_OPXv7_NA0630	BRCA2	1. POSITIVE for a pathogenic BRCA2
PROS02055	396R34_B05_OPXv7_NA0630	BRCA2	1. POSITIVE BRCA2 and RB1 copy lo
OLM,13-BC-03	435R04_D01_OPXv7_NA0688	BRCA2	1. POSITIVE for a pathogenic BRCA
OLM,20-BC-03	435R13_E02_OPXv7_NA0688	BRCA2	POSITIVE for BRCA2 exon 15 deletio
OLM,61-BC-02	435R29_E04_OPXv7_ND0632	BRCA2	POSITIVE for BRCA2 rearrangement
OLM,07-BC-03	435R36_D05_OPXv7_ND0632	BRCA2	POSITIVE for BRCA2 complex rearra
OLM,07-BC-04	435R38_F05_OPXv7_ND0632	BRCA2	POSITIVE for pathogenic BRCA2 mu
OLM,10-1-07	435R52_D07_OPXv7_ND0635	BRCA2	POSITIVE for pathogenic BRCA2 mu
OLM,04-1-04	435R53_E07_OPXv7_ND0635	BRCA2	POSITIVE for a BRCA2 rearrangeme
OLM,13-1-06	435R59_C08_OPXv7_ND0635	BRCA2	POSITIVE for BRCA2 exon 1-24 dele
OLM,13-3-12	481R47_G06_OPXv7_NB0109	BRCA2	BRCA2 wtih LOH (bi-allelic), MYC ar
OLM,52-5-01	483R04_D01_OPXv7_NA0807	BRCA2	POSITIVE for CTNNB1, PIK3CA/PIK3
OLM,07-1-02	483R20_D03_OPXv7_NA0807	BRCA2	AR amplification, possible BRCA2 co
OLM,10-1-02	483R21_E03_OPXv7_NA0807	BRCA2	AR and MYC amplification, possible
OLM,13-1-03	483R23_G03_OPXv7_NA0807	BRCA2	Pathogenic BRCA2 mutation
OLM,11-1-13	483R26_B04_OPXv7_NB0113	BRCA2	BRCA2 copy loss, HRAS
OLM,13-1-05	483R29_E04_OPXv7_NB0113	BRCA2	BRCA2 and PTEN copy loss, TMPRS
OLM,28-2-04	483R32_H04_OPXv7_NB0113	BRCA2	BRCA2/RB1 co-deletion, PTEN copy
OLM,28-2-06	483R33_A05_OPXv7_NB0113	BRCA2	BRCA2/RB1 co-deletion, PTEN copy
OLM,04-2-01	483R36_D05_OPXv7_NB0113	BRCA2	AR amplification, focal bi-allelic TP5
OLM,01-3-15	484R04_D01_OPXv7_NA0809	BRCA2	AR amplification, BRCA2 copy loss a
OLM,01-3-18	484R05_E01_OPXv7_NA0809	BRCA2	BRCA2/RB1 co-deletion, PTEN and
OLM,12-3-02	484R27_C04_OPXv7_NB0115	BRCA2	AR amplification, BRCA2/RB1 co-de
OLM,13-3-35	485R09_A02_OPXv7_NA0811	BRCA2	AR amplification, BRCA2 and PTEN
OLM,05-4-04	485R18_B03_OPXv7_NA0811	BRCA2	AR amplification, BRCA2/RB1 co-de
OLM,28-4-08	485R31_G04_OPXv7_NB0118	BRCA2	AR amplification, BRAC2 copy loss a
OLM,07-5-06	486R03_C01_OPXv7_NA0814	BRCA2	AR amplification, BRCA2/RB1 co-de
OLM,01-5-07	486R06_F01_OPXv7_NA0814	BRCA2	AR amplification, BRCA2 and PTEN
OLM,48-1-12	486R21_E03_OPXv7_NB0122	BRCA2	AR amplification, BRCA2 copy loss,
OLM,58-1-01	486R23_G03_OPXv7_NB0122	BRCA2	MYC amplification, BRCA2/RB1 co-
OLM,27-1-01	487R06_F01_OPXv7_ND0748	BRCA2	Possible BRCA2/RB1 co-deletion an
OLM,11-1-37	487R14_F02_OPXv7_ND0748	BRCA2	Possible BRCA2/RB1 co-deletion an
OLM,40-1-09	487R26_B04_OPXv7_NA0818	BRCA2	AR amplification, bi-allelic BRCA2 c
OLM,07-1-01	490R12_D02_OPXv7_NA0822	BRCA2	BRCA2 copy loss and PIK3CA mutat
OLM,48-1-01	490R19_C03_OPXv7_NA0822	BRCA2	CHD8 amplification, BRCA2/RB1 co
OLM,13-1-28	490R39_G05_OPXv7_NB0132	BRCA2	AR amplification, BRCA2/RB1 co-de
OLM,48-5-05	483R03_C01_OPXv7_NA0807	BRCA2	POSITIVE for pathogenic BRCA2 mu

OLM,11-2-18	481R17_A03_OPXv7_NA0804	**BRCA2 possible**	1) Low tumor content limits the study
OLM,07-2-06	483R15_G02_OPXv7_NA0807	**BRCA2 Possible**	Likely pathogenic TP53, possible BRCA2
OLM,47-2-01	483R16_H02_OPXv7_NA0807	**BRCA2 Possible**	AR, MYC amplification, possible BRCA2
OLM,20-4-04	482R17_A03_OPXv7_ND0741	**BRCA2, CDK12**	BRCA2 (bi-allelic), CDK12 (bi-allelic), A
OLM,16-2-01	483R24_H03_OPXv7_NA0807	**BRCA2, CHEK2, ATM**	BRCA2/RB1 co-deletion, CHEK2 exon 3
OLM,45-1-08	487R30_F04_OPXv7_NA0818	**BRCA2, FANCD2**	AR and FANCD2 amplification, BRCA2/
OLM,11-2-06	483R27_C04_OPXv7_NB0113	**BRCA2, MLH1**	BRCA2 copy loss, TP53 and MLH1 mut
OLM,13-4-22	485R23_G03_OPXv7_NA0811	**BRCA2, MSH2**	BRCA2/RB1 possible copy loss, pathog
PROS02025	396R02_B01_OPXv7_ND0563	**BRCA2, PALB2 (sucblon**	POSITIVE for bi-allelic BRCA2 mutation
OLM_01.006	198R01_A01_OPXv5_NB0187	**BRCA2**	1. Very low quality limits the study. 2
OLM,02.007	281R11_C02_OPXv6_NB0365	**BRCA2**	1. POSITIVE for TP53 mutation, BRCA2
OLM,41-BC-01	435R07_G01_OPXv7_NA0688	**BRCA2**	1. POSITIVE for AR amplification, BRCA
OLM,01-4-12	435R62_F08_OPXv7_ND0635	**BRCA2**	POSITIVE for ERBB2 (HER2) amplificati
OLM,12-1-08	490R29_E04_OPXv7_NB0132	**BRCA2**	AR amplification, MAP2K4 bi-allelic co
OLM,41-3-11	484R02_B01_OPXv7_NA0809	**BRIP1**	AR and BRIP1 amplification and TP53 m
OLM,01-1-25	490R16_H02_OPXv7_NA0822	**BRIP1**	BRCA2 copy loss and BRIP1 mutation
PROS03058	396R16_H02_OPXv7_ND0563	**BRIP1 (subclonal)**	1. POSITIVE for MED12 hotspot mutat
OLM,03.065	286R23_G03_OPXv6_NB0365	**CDK12**	1. POSITIVE for CDK12 bi-allelic patho
PROS02051	396R17_A03_OPXv7_ND0563	**CDK12**	1. POSITIVE for CDK12 mutation (favor
OLM,61-BC-05	435R33_A05_OPXv7_ND0632	**CDK12**	POSITIVE for CDK12 mutation (bi-allelic
OLM,41-2-01	481R09_A02_OPXv7_NA0804	**CDK12**	POSITIVE for CDK12 exon 1-5 del + pos
OLM,51-1-03	487R37_E05_OPXv7_NA0818	**CDK12**	CDK12 mutation (bi-allelic) BRCA2/RB1
OLM,02.009	281R01_A01_OPXv6_NB0365	**CHEK2**	1. Low sample quality limits the study,
OLM,01-2-05	435R63_G08_OPXv7_ND0635	**CHEK2**	1. POSITIVE for a pathogenic CHEK2 m
OLM,13-2-34	481R15_G02_OPXv7_NA0804	**CHEK2**	POSITIVE for CHEK2 p.F169Lfs*2 with L
OLM,48-4-01	485R32_H04_OPXv7_NB0118	**CHEK2**	CHEK2 mutation
CNIOUW 034	277R02_B01_OPXv6_NB0354	**CHEK2**	1. POSITIVE for SPOP p.F102C mutation
OLM,FIVO.228	286R37_E05_OPXv6_NB0365	**FANCA**	1. POSITIVE for FANCA pathogenic mut
PROS03086	396R12_D02_OPXv7_ND0563	**FANCA**	1. POSITIVE for AKT1 p.E17K, FANCA m
CNIOUW 039	277R07_G01_OPXv6_NB0354	**FANCC**	1. Low sample quality limits the study,
OLM,05-BC-12	435R25_A04_OPXv7_ND0632	**FANCD2**	1. POSITIVE for FANCD2 mutation (cou
OLM,01-1-20	448R06_F01_OPXv7_ND0649	**FANCD2**	POSITIVE for FANCD2 p.R794* (could b
OLM,14-2-06	481R18_B03_OPXv7_NA0804	**FANCD2**	1) POSITIVE for FANCD2 mutation, CT
OLM,01-3-17	481R30_F04_OPXv7_NB0109	**FANCD2**	FANCD2, MYC amplification, PTEN, CTN
OLM,40-4-06	482R20_D03_OPXv7_ND0741	**FANCD2**	FANCD2, BRCA2 loss (uncertain if bi-all
OLM,13-1-19	481R24_H03_OPXv7_NA0804	**FANCM**	POSITIVE for FANCM mutation and gen
PROS02083	396R36_D05_OPXv7_NA0630	**MRE11A**	1. POSITIVE for MRE11A p.R605* (path
OLM,13-1-02	435R45_E06_OPXv7_ND0632	**MRE11A**	POSITIVE for likely pathogenic MRE11A
OLM,33-1-02	486R27_C04_OPXv7_NB0122	**MRE11A**	AR amplification, CHD1 copy loss, APC,
OLM,12-1-09	487R32_H04_OPXv7_NA0818	**MRE11A**	MRE11A mutation (favor germline)
OLM_02.033	198R12_D02_OPXv5_NB0187	**MLH1, MSI-high**	1. Low quality limits the study. 2. Favo
CNIOUW 010	272R08_H01_OPXv6_NA0414	**MSI/MMRd**	1. MSI-high likely (limited analysis due
PROS02033	396R26_B04_OPXv7_NA0630	**MSI-high**	1. POSITIVE for MSI-high and HIGH tum
OLM,14-1-06	490R40_H05_OPXv7_NB0132	**MSH2**	MSH2 mutation
OLM,55-1-02	487R02_B01_OPXv7_ND0748	**MSH2, FANCA**	NOTCH3 amplification, TP53 copy loss
OLM,54-3-02	485R04_D01_OPXv7_NA0811	**MSH2, MSI-high**	POSITIVE for MSH2 gene rearrangemer
OLM,11-1-34	487R25_A04_OPXv7_NA0818	**MSH2, MSI-high**	POSITIVE for MSH2 mutation (c.942+3A
OLM,20-1-07	490R23_G03_OPXv7_NA0822	**MSH2, MSI-high**	POSITIVE for MSH2 mutation with asso
CNIOUW 033	277R01_A01_OPXv6_NB0354	**MUTYH**	1. Very low sample quality limits the st
OLM,20-BC-01	435R12_D02_OPXv7_NA0688	**MUTYH**	1. POSITIVE for pathogenic MUTYH mu
OLM,01-5-15	435R43_C06_OPXv7_ND0632	**MUTYH**	1. POSITIVE for pathogenic germline M
OLM,09-2-03	481R22_F03_OPXv7_NA0804	**MUTYH**	POSITIVE for AR amplification, PTEN lo
OLM,14-4-05	482R19_C03_OPXv7_ND0741	**MUTYH**	MYC amplification, MUTYH G396D gen
OLM,29-3-02	483R47_G06_OPXv7_NB0113	**MUTYH**	Pathogenic MUTYH mutation
OLM,34-5-02	486R04_D01_OPXv7_NA0814	**MUTYH**	MUTYH mutation
OLM,2-1-01	490R03_C01_OPXv7_NA0822	**MUTYH**	AR amplification, RB1 copy loss and AP
CNIOUW 009	272R07_G01_OPXv6_NA0414	**MUTYH (carrier)**	1. Very low sample quality limits the st
OLM,FIVO.009	286R32_H04_OPXv6_NB0365	**MUTYH (carrier), BRCA**	1. POSITIVE for germline heterozygous
OLM,36-1-03	490R15_G02_OPXv7_NA0822	**MUTYH, BRCA2**	BRCA2 copy loss, AR resistance, MUTYH
OLM,55-1-01	490R24_H03_OPXv7_NA0822	**MUTYH, BRCA2**	MUTYH amplification, possible BRCA2/
CNIOUW 008	272R06_F01_OPXv6_NA0414	**NBN**	1. Very low sample quality limits the st
OLM,24-3-02	482R03_C01_OPXv7_ND0741	**NTHL1**	NTHL1 germline carrier, low tumor con
OLM,05-2-04	481R16_H02_OPXv7_NA0804	**RAD51B**	POSITIVE for RAD51B exon 10-11 delet
OLM,13-BC-02	435R03_C01_OPXv7_NA0688	**RAD51D**	1. POSITIVE for pathogenic RAD51D mu

Table: Prostate Cancer Samples with DNA Repair Gene Mutations Detected by UW-OncoPlex (Continued)

In this reporting period we applied the validated HRD assay to 49 prostate cancer paired samples, which included both tumor tissue and cell-free DNA (**Figure**). LOH scores were highest overall in tumor with bi-allelic mutations in *CHEK2, BRCA1*, and *BRCA2*.

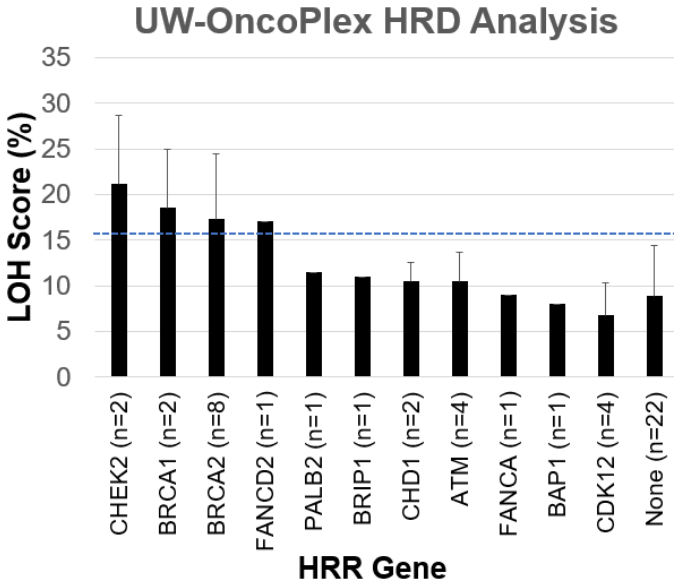


Figure: UW-OncoPlex HRD Analysis in Prostate Cancer by Gene. Mean LOH scores are depicted by gene, error bars are standard deviation. The dashed line indicates the threshold for calling a sample HRD mutation signature positive by this approach.

In addition, a *JAMA Oncology* study led by the Pritchard Group with regard to cell-free DNA sequencing in prostate cancer acknowledged support from this award was featured in many news media outlets.

HRPO approvals: The research for Aim 1 at Site 1 (UW) was determined to be not human subjects by the UW IRB, with HRPO concurrence on 10/17/2018. This facilitated use of de-identified samples from Site 2 in year 1 and year 2 for optimization of the UW-OncoPlex sequencing assay in the context of limited sample quantity. HRPO approval was obtained at Site 2 (CNIO) on 9/30/19 for research on aims 1 and 2. In years 1 and 2 Site 1 (UW) received representative de-identified extracted DNA specimens from the Site 2 (CNIO) for UW-OncoPlex sequencing in batches to optimize sequencing protocols.

Summary of progress on milestones related to Aim 1 in Year 4

Milestone 1.1 Shipment of samples From CNIO laboratory to UW laboratory (batches) (Month 3-15): In summary, four batches of samples were shipped from CNIO to the UW Laboratory, focusing on samples with the highest quantity of residual DNA. Batches of de-identified samples were initially shipped for the purpose of assay and protocol optimization from Site 2 to Site 1 in year 1 (not human subjects research) while HRPO approval at site 2 was pending. The PROREPAIR-B trial in which aim 1 was based, was an already approved and completed protocol in Spain. There were some unanticipated delays in obtaining HRPO approvals at Site 2 (CNIO) due in part to requirements of independent evaluation of this work by our reference IRB, and review of several iterations of verified English translations from original study documents produced in Spanish between January and July 2019. After submission of the final required documents in July 2018, HRPO approval at Site 2 (CNIO) was granted on September 30th, 2019.

Since receiving HRPO approval at Site 2 (CNIO), 217 samples were reviewed by a trained GU pathologist, macro-dissected from tumor sections and processed for DNA extraction at the CNIO Lab. These were archived biopsies from multiple participating sites (38) which were obtained primary for pathology diagnosis a median of 2 years (range 4-21 years) before developing mCRPC and entering the study the tumor tissue availability was scarce in many previously devastated tumour blocks, or the DNA quantity and quality yield by these samples was low in most cases. After discussion with the Site 1 UW laboratory, and following progress in improving the UW-OncoPlex assay to work with samples with lower DNA quantity/quality as expected from PROREPAIR-B FFPE sample collection. Shipments were organised according to quality/quantity starting with best samples from initial 120 extracted samples.

Milestone 1.2 – Classification of each patients as "positive" or "negative" for each of the biomarkers of interest (BRCA1, BRCA2, ATM, PALB2): To date, we have identified 23 patients as "positive for the biomarker" of interest, using sequencing done at site 1 (see Table 1), 12 additional patients with alterations limited to *BRCA2* (*gene* deletions) has been identified at site 2 using alternative approaches as described above.

Milestone 1.3 – Data analysis and interpretation, Manuscript Preparation
(24 months; Site 1, 2 and 3):
Two initial communications related to aim 1 have been presented at international meetings in which the DOD funding has been acknowledged:

1. Meeting: 27th Prostate Cancer Foundation Scientific Retreat, October 20-23, 2020
- Title: *Association between BRCA2 alterations and intraductal and cribriform histologies in prostate cancer*
- Authors: E. Castro, D.C. Salles; R. Lozano, H. Thorne, F. López-Campos, J. Rubio-Briones, Ana M. Gutierrez-Pecharroman, M.I. Pacheco, T. Garcés, N. Romero-Laorden, F. Zambrana1, P.P. López-Campos, S. Sandhu, **J. Mateo, C. Pritchard**, E. Antonarakis, **D. Olmos**, T. Lotan.
- Reference: https://www.morressier.com/article/association-brca2-alterations-intraductal-cribriform-histologies-prostate-cancer/5f69edb69b74b699bf38c600?

2. Meeting: European Society of Medical Oncology annual meeting 2020, September 19-21, 2020 (also presented at the American Society of Clinical Oncology annual meeting, May 27-Jun 1, 2020)
- Title: *Clinical impact of somatic alterations in prostate cancer patients with and without previously known germline BRCA1/2 mutations: Results from PROREPAIR study*
- Authors: R. Lozano Mejorada, E. Castro Marcos, I.M. Aragon, H. Thorne, F. Lopez Campos, A. Sanz, C. Alonso, U. Anido, M.J. Juan Fita, A.M. Gutierrez Pecharromán, M. Ramirez-Backhaus, J. Balmana, I. Chirivella Gonzalez, G. Llort, N. Romero Laorden, S. Arevalo Lobera, J. Rubio Briones, **J. Mateo, C.C. Pritchard,** S. Sandhu, **D. Olmos Hidalgo**
- Reference: https://doi.org/10.1016/j.annonc.2020.08.872

A manuscript based on the PROREPAIR samples and the results in abstract 1 was accepted for publication in the European Journal of Cancer in 2021:

- Title: Association between BRCA2 alterations and intraductal and cribriform histologies in prostate cancer
- Authors: R. Lozano, D.C. Salles, S. Sandhu, I.M. Aragón, H. Thorne, F. López-Campos, J. Rubio-Briones, A.M. Gutierrez-Pecharroman, L. Maldonado, T. di Domenico, A. Sanz, J.D. Prieto, I. García, M.I. Pacheco, T. Garcés, C. Llacer, N. Romero-Laorden, F. Zambrana, P.P. López-Casas, D. Lorente, J. Mateo, C.C. Pritchard, E.S. Antonarakis, D. Olmos, T.L. Lotan, E. Castro
DOI: https://doi.org/10.1016/j.ejca.2021.01.027

DOD funding was acknowledged as part of the submitted manuscript.

A manuscript related to Abstract 2 has been submitted with DoD funding acknowledged.

Milestone 1. 4 - F2F meeting among participating sites to discuss progress (12 months; Site 1, 2 and 3): A project Kick-Off meeting with three PIs (Pritchard, Olmos, and Mateo) and with some co-investigators (Cheng and Castro) was held in San Diego, CA in Oct 2018. An end-of-year 1 meeting to discuss progress was held Oct 25th 2019 in San Diego, California, that included the three PIs, according to the planned timelines. A grant review meeting that included the three partnering PIs and key team members was held September 21, 2020. The PIs met in person annually at the PCF Scientific Retreat.

During Year 1-5 we had several virtual meetings, 1 regular bi-monthly meeting between site 2 and site 3 to improve coordination for aims 2 and 3, and 2 meetings with teams from site 1, site 2 and site 3 in June and September 2021.

Specific Aim 2 – To optimize tissue-based tests of HR functionality samples for CRPC samples, and study the correlation with genomic aberrations in HR genes.

Major Task 2: Acquisition of metastatic biopsies

For Site 2 (CNIO): IRB approval for the participation of site 2 at this major task (2.2) was received on November 26th, 2018 with the approval to proceed with aim 1. As outlined in the section above HRPO approval for aim 1 and 2 was received September 30th, 2019. No samples from Site 2 were included in this Aim due to early discontinuation of the project at Site 2.

For Site 3 (VHIO), the research protocol for acquisition and analysis of patient biopsies was approved by the local ethics board. As of Dec 2023, 276 patients have been consented for consideration of biopsies. After discussion of suitability with interventional radiology, 118 patients have successfully undergone a metastatic biopsy procedure, collecting at least 1 fresh frozen core and 1 FFPE core for the study. Additionally, archival prostate primary tumor biopsy material has been retrieved from the diagnostic hospital for 155 cases. Saliva samples for correlative germline analyses were collected for all patients at the time of consent.

The study included 219 samples from 187 advanced prostate cancer patients. At diagnosis, most patients presented tumors with high Gleason grade (group 4-5 tumors, n=133, 71.1%) and de novo metastatic disease (n=117, 62.6%) (Table below).

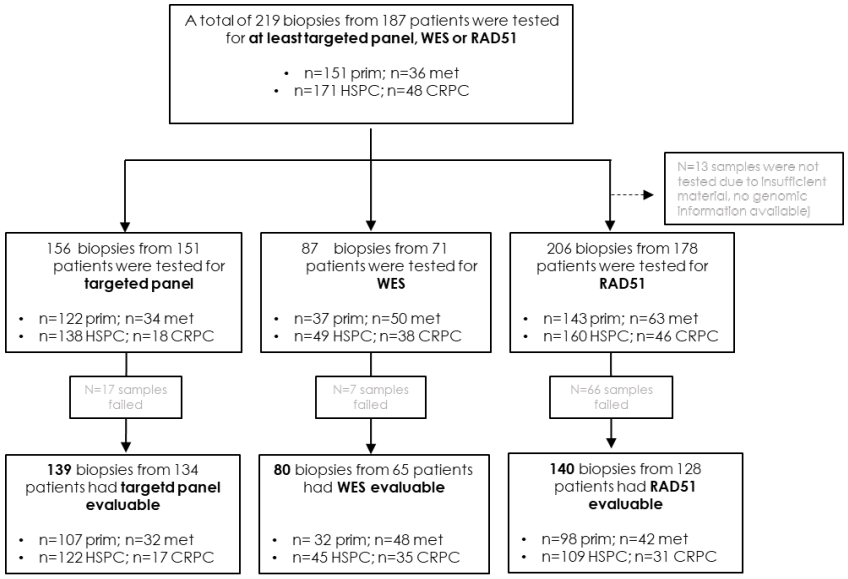
The samples were acquired from primary (n=151, 68.9%) and metastatic tumors (n=68, 31.1%). Out of the 219 samples, 67.6% (n=148) were prostate primary tumor biopsies (n=148, 67.6%), 15.1% (n=33) were bone metastases, and 10.5% lymph node biopsies (n=23). In 169 cases (77.2%), the

Table 1. Disease characteristics at the time of diagnosis

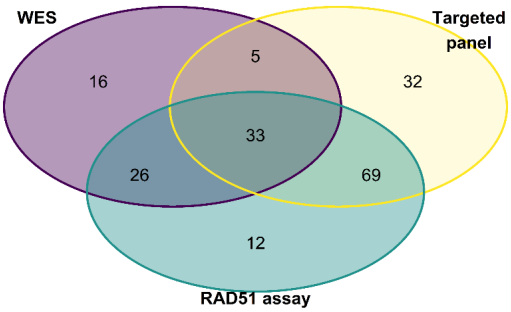
Characteristic	Value
n	187
Histology, n (%)	
Adenocarcinoma	135 (72.2)
Adenocarcinoma acinar	45 (24.1)
Other	7 (3.7)
Histologic Grade Group, n (%)	
1-3	37 (19.7)
4	56 (29.9)
5	77 (41.2)
Unknown	17 (9.1)
PSA (ng/ml), median [IQR]	38.89 [12.28, 264.75]
Tumour category, n (%)	
T1	5 (2.7)
T2	18 (9.7)
T3	75 (40.5)
T4	41 (22.2)
Tx	46 (24.9)
Nodal stage, n (%)	
N0	51 (27.4)
N1	94 (50.5)
Nx	41 (22.0)
Metastases, n (%)	
M0	68 (36.4)
M1	117 (62.6)
Mx	2 (1.1)

REFERENCES. PSA: Prostate-specific antigen, IQR: interquartile range.

A



B



biopsy had been collected prior to hormonal therapy (hormone-sensitive, HSPC), whereas in 50 cases (22,8%), the biopsy was acquired upon castration-resistant (CRPC) .

Out of 219 samples, RAD51 immunofluorescence was pursued on 206 biopsies from 178 patients, as for the remaining 13 samples there was insufficient material for IF after NGS. The success rate was 68% (140/206). RAD51 IF results were obtained for 98 primary tumors and 42 metastatic biopsies. Reasons for non-evaluability included low levels of γH2AX positive cells, bad quality/conservation of the sample, low proliferative tumors (insufficient geminin-positive cells), or insufficient tumoral cells.

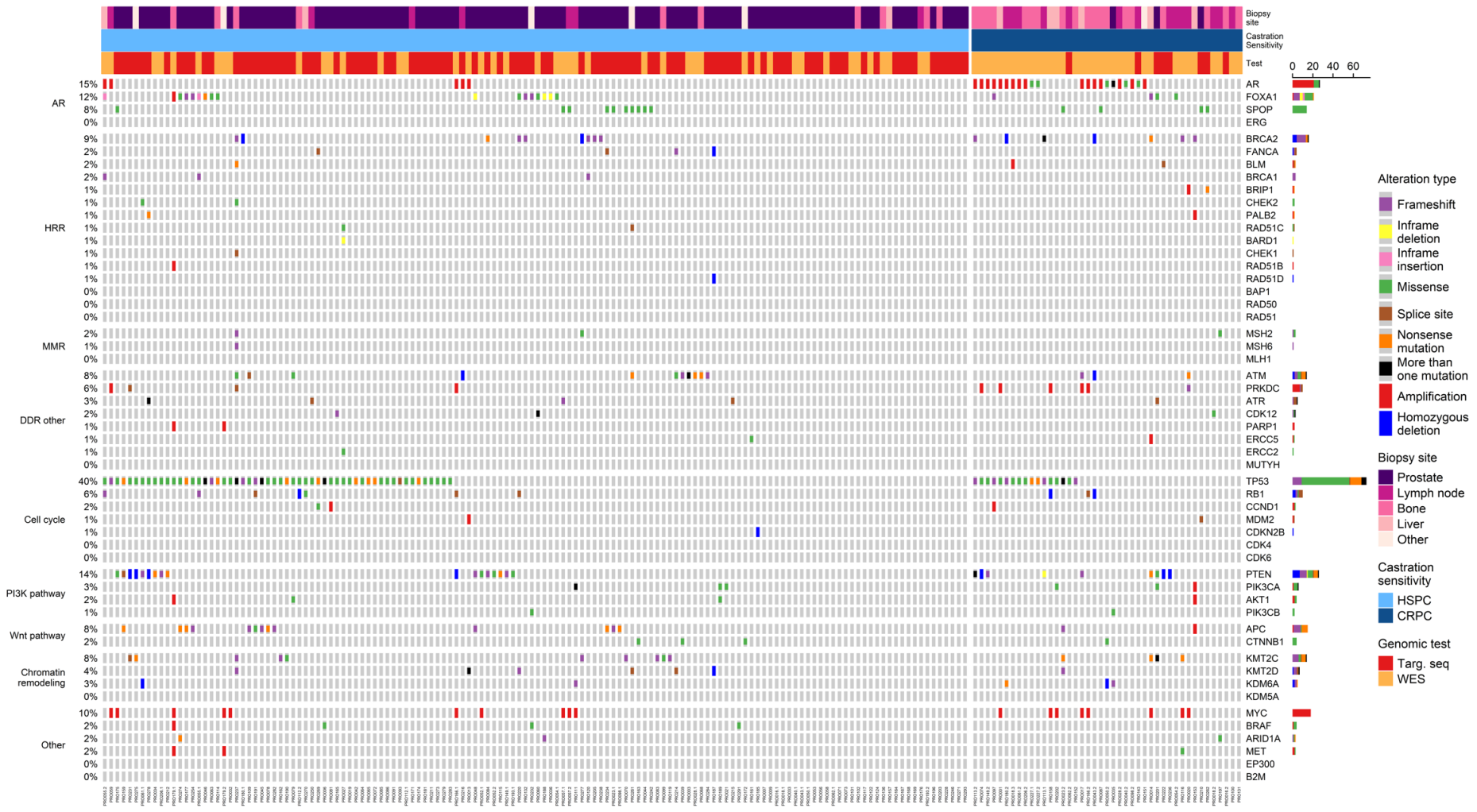
Major Task 3: Whole-exome sequencing studies

All samples (n=219) underwent NGS (targeted sequencing was pursued in 156 biopsies from 151 patients and WES in 87 biopsies from 71 patients; 38 biopsies underwent both targeted and whole-exome sequencing) (Fig.1A). Genomics data was obtained for 181 samples (157 patients): 139 samples (134 patients) had targeted sequencing results, whereas WES data was successfully obtained for 80 biopsies (65 patients).

Libraries were generated using the KAPA HyperPrep kit (Roche) following manufacturer's instructions and captured with KAPA HyperExome following manufacturer's instructions (KAPA HyperCap workflow v3, Roche). Sequencing of paired tumor and nomal libraries was performed on Illumina HiSeqX or NovaSeq6000 (Illumina) with 150 bp paired-end reads. Reads were mapped to the human reference genome (GRCh38) using the BWA-MEM algorithm (v0.7.15) (Li & Durbin, 2009). BAM files were generated, all duplicate reads were marked, and the quality scores were recalibrated by using Picard (v2.26.2, https://broadinstitute.github.io/picard) and Genome Analysis Toolkit's Table Recalibration tool (v4.2.5.0) (McKenna et al., 2010), respectively. Somatic mutations were called (tumor versus matched normal) using Mutect2 (Genome Analysis Toolkit (GATK) v4.2.5.0) (McKenna et al., 2010), Strelka2 (v2.9.2) (Kim et al., 2018), and Varscan2 (v2.4.3) (Koboldt et al., 2012) and left-aligned and normalized using bcftools (v1.17) (Danecek et al., 2021a). Mutations detected by at least two algorithms were retained. Annotation of variants was performed using Cancer Genome Interpreter (CGI, https://www.cancergenomeinterpreter.org/analysis) followed by manual curation. Germline mutations were called with Varscan (v2.4.3) and annotated with Annovar (v2.27.1) (Wang et al., 2010). Allele-specific copy-number profiles were estimated from WES data by using ASCAT (v3.1.2) (Van Loo et al., 2010). Low-Pass WGS (LP-WGS, 0.5x) was used for validation of copy-number alterations. Tumor-only capture-based targeted sequencing was performed using the ISO-accredited VHIO-300 targeted panel (Saura et al., 2023). In brief, libraries were prepared using SureSelect XT Human (Agilent) and captured using a costumized panel covering exonic regions of 435 genes.

Loss of heterozygosity (HRD-LOH), large-scale state transitions (LST), number of telomeric allelic imbalances (NtAI) and the unweighted numeric sum of LOH, tAI, and LST, called HRD-sum, were determined from WES using the scarHRD R package (Sztupinszki et al., 2018). Additionally, these three genomic scars were also determined from the capture-based NGS panel sequencing data applying an algorithm previously described by Marquard et al (Marquard et al., 2015) (test adapted from https://rdcu.be/c2727) using the B-allele fraction calculation determined with the 105 SNPs distributed throughout the genome from the backbone of the VHIO-CARD-300 panel.

We investigated the presence of pathogenic mutations and copy number changes on relevant genes involved in AR pathway, DDR (including HRR, MMR, and others), cell cycle regulation, PI3K and Wnt pathways, among others (Figure below). The most frequently altered genes in included TP53 (40%), PTEN (14%), AR (15%), FOXA1 (12%), MYC (12%) and BRCA2 (9%). As expected, AR amplifications were observed primarily in samples collected at the castration-resistant setting (n=16, 37%) compared to HSPC (n=5, 4%). Regarding alterations in HRR-related genes, the most frequently altered was BRCA2 (9%, including frameshift mutations and homozygous deletions), followed by BRCA1 and FANCA (2% each). Among other DDR genes, ATM was the most commonly altered (8%). The prevalence of DDR mutations was similar among HSPC and CRPC samples.


Biopsy site
Castration Sensitivity
Test
0 20 40 60
AR
FOXA1
SPOP
ERG
BRCA2
FANCA
BLM
BRCA1
BRIP1
CHEK2
PALB2
RAD51C
BARD1
CHEK1
RAD51B
RAD51D
BAP1
RAD50
RAD51
MSH2
MSH6
MLH1
ATM
PRKDC
ATR
CDK12
PARP1
ERCC5
ERCC2
MUTYH
TP53
RB1
CCND1
MDM2
CDKN2B
CDK4
CDK6
PTEN
PIK3CA
AKT1
PIK3CB
APC
CTNNB1
KMT2C
KMT2D
KDM6A
KDM5A
MYC
BRAF
ARID1A
MET
EP300
B2M
HRR
MMR
DDR other
Cell cycle
PI3K pathway
Wnt pathway
Chromatin remodeling
Other
Alteration type
Frameshift
Inframe deletion
Inframe insertion
Missense
Splice site
Nonsense mutation
More than one mutation
Amplification
Homozygous deletion
Biopsy site
Prostate
Lymph node
Bone
Liver
Other
Castration sensitivity
HSPC
CRPC
Genomic test
Targ. seq
WES

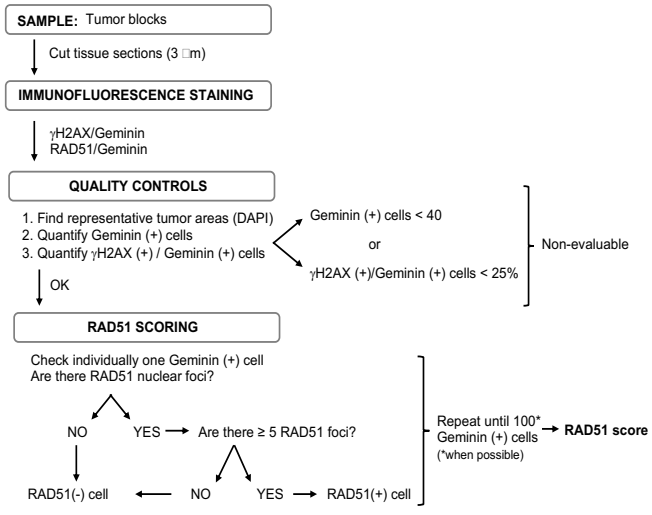
Major Task 4: Expression profiling studies

RNA extraction from the frozen blocks of the metastatic biopsies was started in Sept 2020. Unfortunately, this represented a significant delayed from the original planned calendar, resulting from the complete shutdown of our lab at site 3 (VHIO) for over 3 months and later partial re-opening, due to the COVID19 pandemic-related restrictions, that made us prioritize other projects with prospective sample collection. Similarly, work for this task at site 2 (CNIO) was severely disrupted due to the Covid-19 pandemic in Spain, the lab was closed from March 7th, 2020 until July 1st, 2020 under the government regulations. During the rest of year 2 and the most of year 3 the work site 2 for this task was delayed to due to staffing (see section 5 Changes and problems during the project at site 2). Finally, RNAseq from 80 biopsies including primary and metastatic samples was pursued at Site 3. Unfortunately, no samples from Site 2 were made available for this work due to early termination of the project at CNIO. The 80 samples analyzed belong to the cohort of NGS in Major Task 3.

Gene set enrichment analysis were performed for samples with or without loss of RAD51 foci formation (Major Task 5) but no signatures associated to RAD51 loss were found. Due to the need to proceed with only samples from one site, we had to include both FFPE and frozen biopsies in the cohort; that resulted in significant differences in RNAseq profiles inherent to the technical challenges to deliver RNAseq from FFPE blocks. Subgroup analysis for FFPE and FF samples separately were conducted but due to small numbers in each cohort, no significant associations with BRCA alterations or RAD51 loss were found.

Major Task 5: Immunofluorescence studies

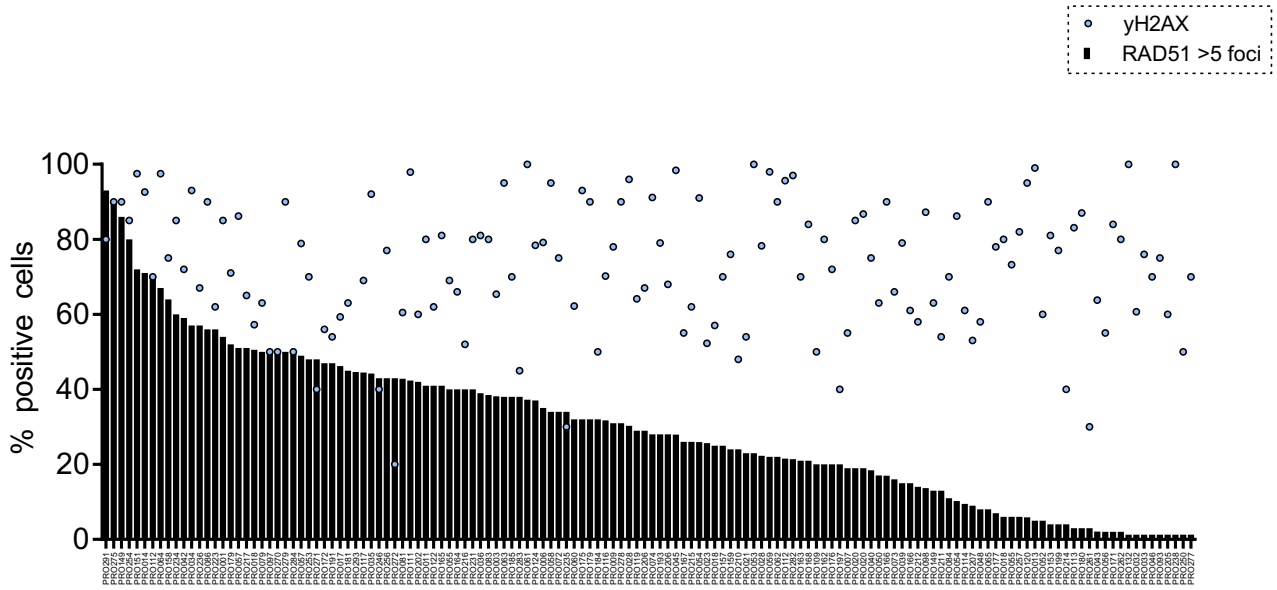
As planned, we have re-optimized now an IF-based test initially developed in breast cancer patient-derived xenoimplant models and then validated in breast cancer biopsies (Cruz et al, Ann Onc 2018; Castroviejo-Bermejo et al, EMBO Med 2019). We are using FFPE slides from prostate cancer primary and metastatic biopsies. An overview of the assay procedure and interpretation workflow is presented below:



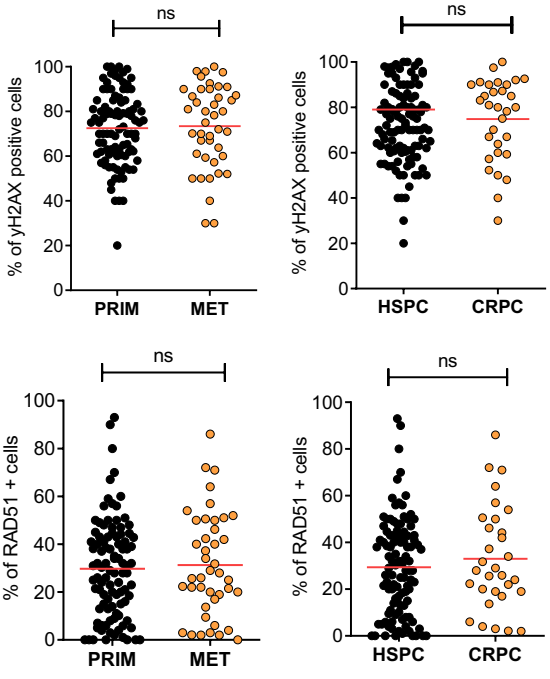
We evaluated baseline HRR function based on detection of RAD51 and yH2AX foci in geminin-positive tumor cells by immunofluorescence (IF). All samples were scored by two trained readers

blinded to genomic and clinical data. Samples were considered HRR deficient (HRD) when RAD51 scores were low, pre-defined as <10% tumor cells presenting ≥5 RAD51 foci/cell.

Out of 219 samples, RAD51 immunofluorescence was pursued on 206 biopsies from 178 patients, as for the remaining 13 samples there was insufficient material for IF after NGS. The success rate was 68% (140/206). RAD51 IF results were obtained for 98 primary tumors and 42 metastatic biopsies. Reasons for non-evaluability included low levels of γH2AX positive cells, bad quality/conservation of the sample, low proliferative tumors (insufficient geminin-positive cells), or insufficient tumoral cells. The prevalence of tumor cells with RAD51 foci nuclear formation was quantified in 140 biopsies from 128 patients. Median RAD51 IF score was 28.5 (IQR 13.9 - 43.3). No differences were



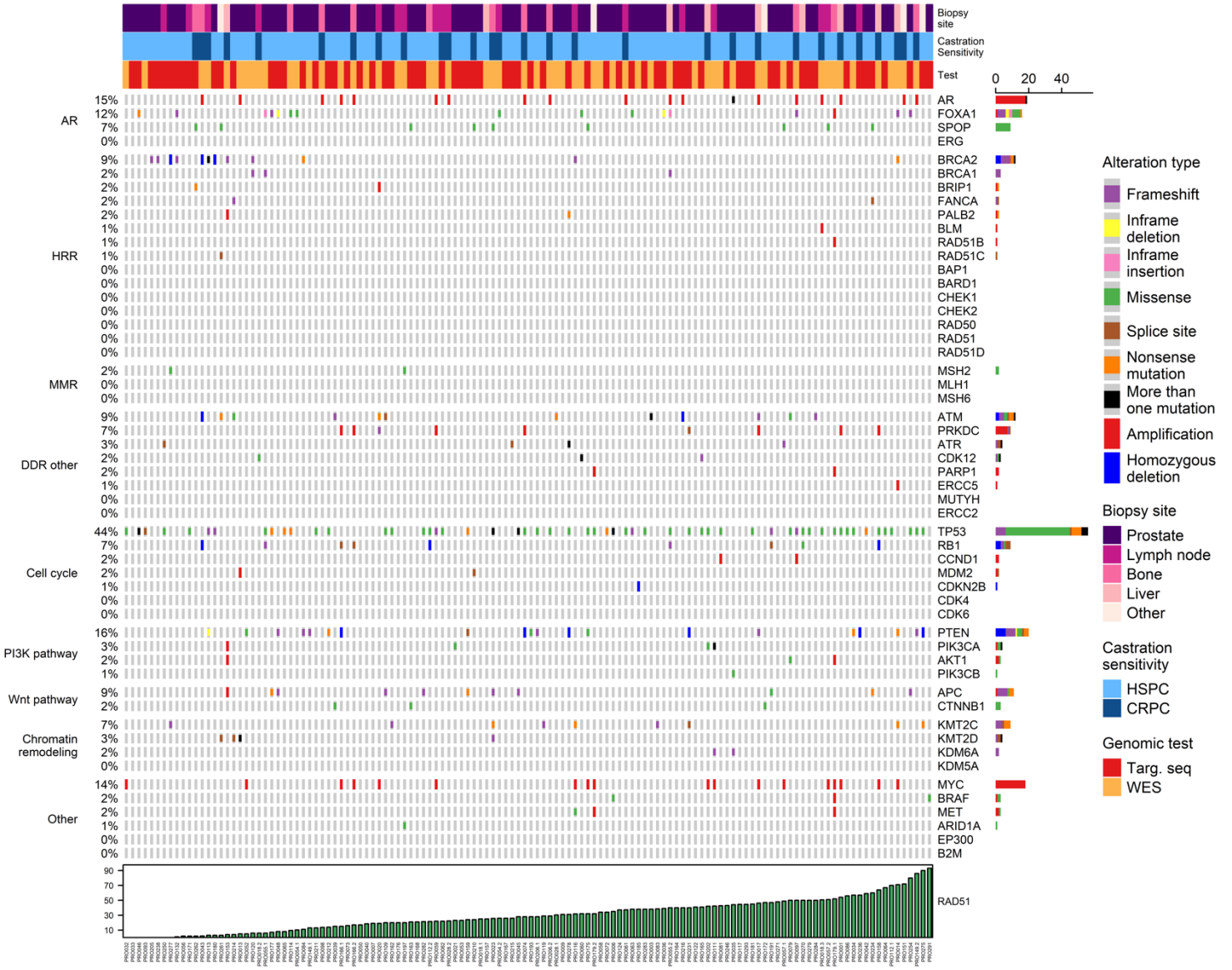
observed when comparing primary (n=98) versus metastatic (n=42) tumors (median 29.6 and 27.0 respectively; p-value=0.704), nor hormone-sensitive (n=109) versus castration-resistant (n=31) (29.0 and 28.0 respectively; p-value=0.49) biopsies. Applying a previously defined threshold of ≤10% RAD51-positive cells to be considered HRR deficient, 21% of the samples (30/140) were classified as HRD.



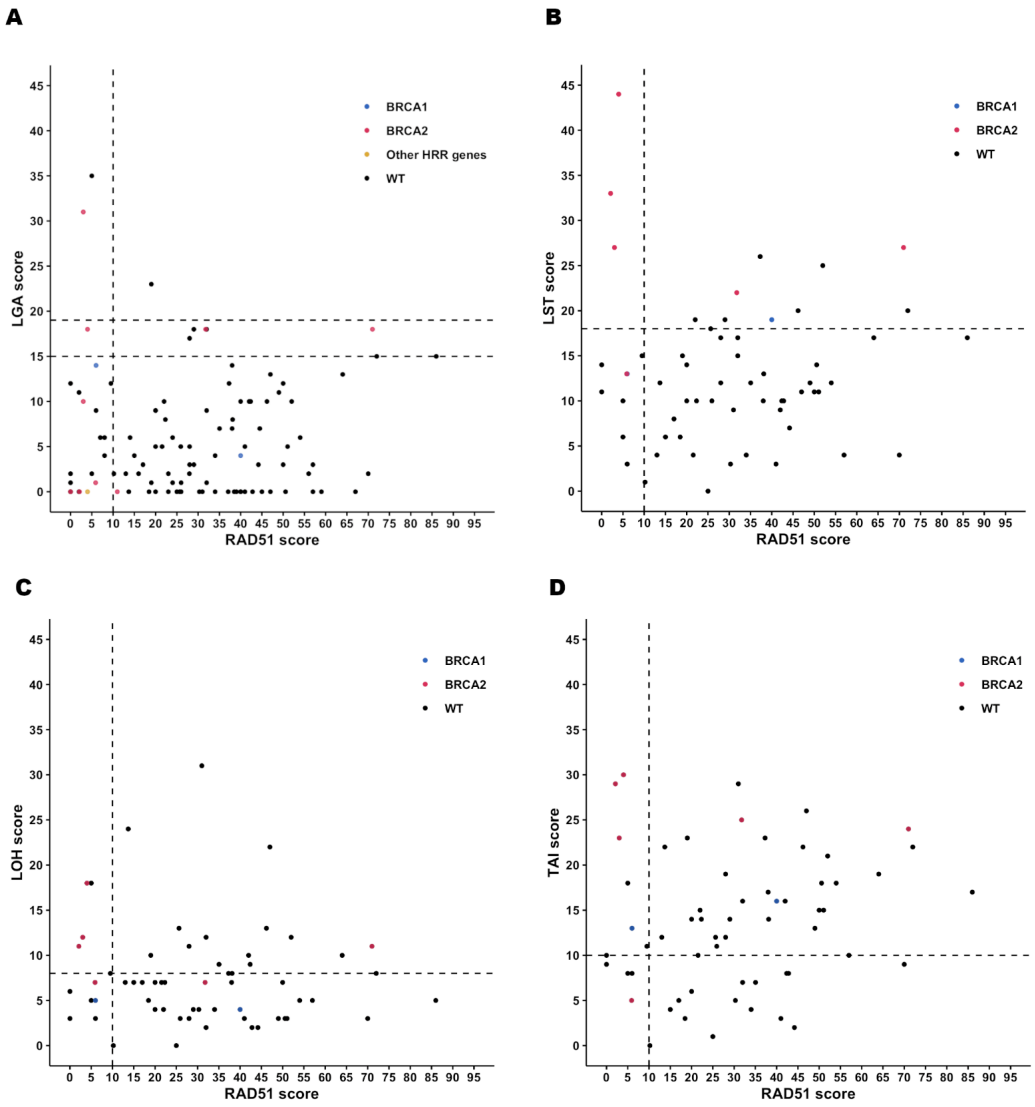
The oncoprint below shows the genomic landscape among the 128 samples with evaluable RAD51 IF, sorted by RAD51 IF score. TP53 was altered in 44% of the cases followed by AR (15%), PTEN (16%), FOXA1 (12%), BRCA2 (9%), and ATM (9%), as the more common alterations.

Cases with pathogenic BRCA1/2 alterations associated with lower RAD51 IF scores, with a

median score of 3.5 (IQR 9.8 – 8.5) for BRCA1/2 altered (n=14) and 29.7 (IQR 19.0 - 44.5) for BRCA1/2-WT (n=114). Considering the threshold of 10% RAD51 positive cells, the sensitivity and specificity of the RAD51 IF assay in identifying BRCA1/2 altered cases was 0.71 and 0.85 respectively. When including other HRR genes (BRIP1, FANCA, PALB2, BLM, CHEK2, RAD50), the sensitivity and specificity were 0.68 and 0.87, respectively. Delving into the alteration type of these BRCA1/2 cases, all samples with BRCA2 deep deletions were identified by the RAD51 low. Four cases with BRCA1/2 pathogenic mutations had RAD51 scores of 11, 31, 40 and 71 respectively, although only in 2/4 we could confirm loss of the second allele by LOH.

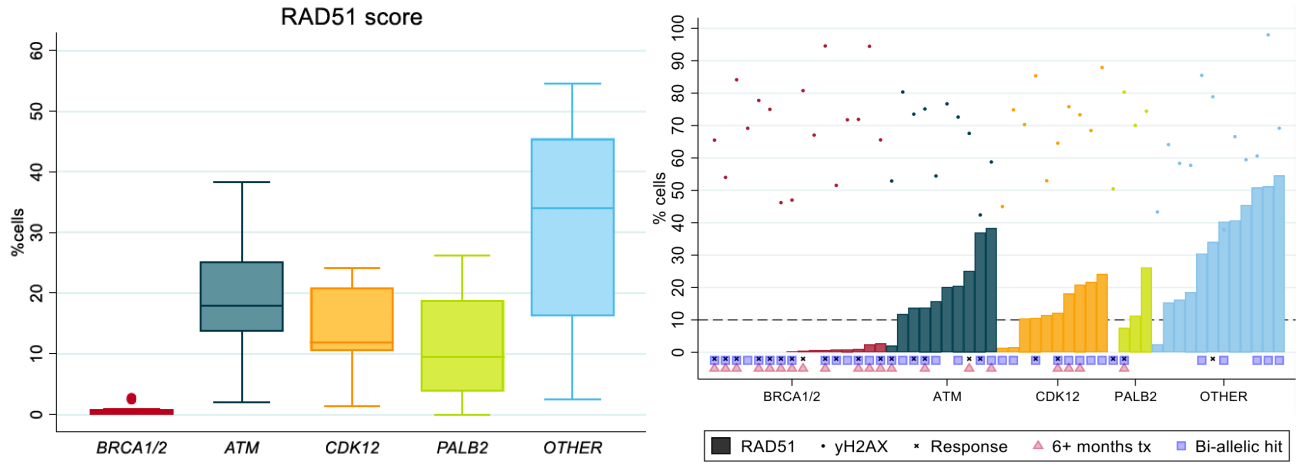


We studied the distribution of genomics scars derived from WES (LOH, LST, NtAI, and HRD-sum; n=80), LP-WGS (LGA; n=133), and targeted panel (HRD-sum; n=134) and their correlation with RAD51 IF scores. HRD-sum derived from targeted panel was found to significantly associate with RAD51 IF status (n=99; categorical classification as low vs high: OR 1.03, 95% CI 1.005 - 1.062; p-value=0.021). When looking at HRD-sum based on WES (n=59), there was a non-significant trend for association (OR 10.2, 95% CI 0.988 – 1.061, p-value=0.2) that became significant when adjusting for hormone-sensitive vs castration-resistant status (p-value=0.035), in line with the described impact of castration-sensitivity status in HRR scars in prostate cancer.



Overall, this study represents the first implementation of a RAD51-based functional assay in a molecularly-unselected cohort of advanced prostate cancer, demonstrating the feasibility in clinical routine samples and potential to complement genomic testing for patient stratification in metastatic prostate cancer.

Also during the time of this award, we managed to test the assay in a further cohort of samples from metastatic prostate cancer patients enriched for DNA repair gene mutations. In particular, primary or metastatic biopsies from 52 men with metastatic prostate cancer who participated in the phase II TOPARP clinical trial of olaparib (results published in Mateo et al, Lancet Onc 2020) were made available to us. The methodology for the gHA2X and RAD51 evaluation is the same as in the previous cohort. All tumors showed abundant DNA damage (yH2AX scores >40%). The intra-class correlation score (ICC) between the two blinded readers was 0.88. Overall, 22 of 52 (42%) cases were considered as HRD based on low RAD51 scores. Response rate (based on the composite RECIST/PSA/CTC trial criteria) was 15/22 (68%) vs 7/30 (23%) for patients with low vs high RAD51 scores (p=0.001). Patients with low RAD51 scores also had longer rPFS (median 9.3 vs 2.9 months p=0.002) and overall survival (median 17.4 vs 9.5 months, p=0.05) from initiation of olaparib. All 16/16 cases with BRCA1/2 alterations were identified as RAD51 low (Figure below). For patients with PALB2 mutations, 2/2 patients with biallelic loss showed RAD51 low scores and responded to olaparib, whereas 2/2 patients with monoallelic PALB2 mutations showed RAD51 high scores and did not respond to olaparib. Mutations in ATM and CDK12 did not associate with low RAD51. Indeed, 10/11 ATM-mutated and 8/10 CDK12-mutated tumors presented high RAD51 scores; RECIST/PSA responses were observed in two patients with ATM mutations and high RAD51 scores. The results on RAD51 IF in the TOPARP-B cohort were published in Carreira et al, Cancer Discovery 2021.



Specific Aim 3 – To clinically qualify this HR functional test in a clinical trial of carboplatin in CRPC

Tasks for Aim 3 were pursued by Site 2 (CNIO) and joint PI Dr David Olmos who did not obtain the Year 5 extension period of this award. Hence, no updates in these two tasks apply since the last annual report by Site 2.

Major Task 6: Clinical Trial Set Up

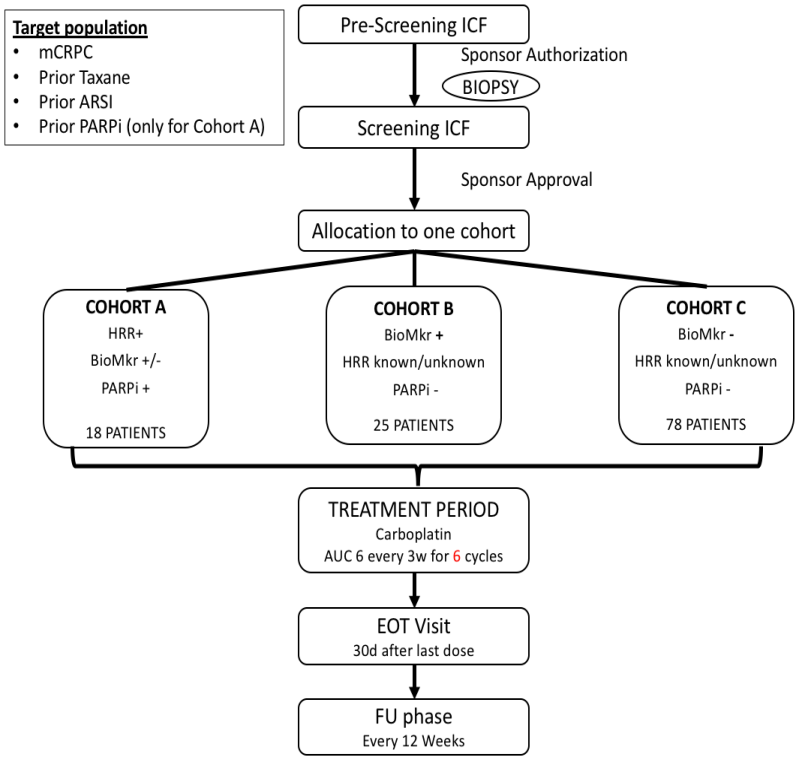
In year 1, we completed the trial protocol which was initially submitted to site 2 reference IRB (CEI Provincial de Malaga) and the AEMPS (Spanish regulatory agency) and initial review and proposed amendments were received by October 2020. The protocol was submitted to the HRPO before regulatory submission, but final feedback from HRPO was received in January 2020. These feedbacks were implemented together with the initial feedback from reference IRB and AEMPS and resubmitted for evaluation to both. Final IRB and AEMPS approvals of amended clinical trial documents were granted on March 27th and April 20th, 2020, respectively. The original documents and their verified translations of these documents were submitted to HRPO.

Following these approvals contract negotiations with participating sites were initiated by the CNIO team from July 1st, 2020, as the trial office was also in shutdown until July due to the government restrictions related to the COVID pandemic and the effects in the Spanish National Health System.

The first patient on trial started screening in March and was enrolled in study. However, initiation of some sites was delayed due to an unexpected sick leave of the study trial manager. By September 2021 all sites except 1 (H.U. La Princesa) were initiated (see site status list below in the next major task)

Major Task 7: Clinical Trial conduction

By end of October 2021, 28 patients have entered pre-screening, 24 patients have been screened and 20 patients have been successfully enrolled and received at least 1 dose of Carboplatin in the study. Two these 20 patients were enrolled in Cohort A (post-PARPi), 4 in Cohort B and 14 in Cohort C (see trial design below).



The summary of the clinical trial status is as follows:

1. 10 sites have consented and/or enrolled at least 1 patient

Trial site / Hospital	**Consented**	**Screening Failure**	**Enrolled**
Hospital Universitario 12 de Octubre, Madrid	20	6	14
Hospital Universitario Virgen de la Victoria de Málaga	9	2	7
Hospital Universitario Vall D'Hebron, Barcelona	6	1	5
Instituto Valenciano de Oncología, Valencia	6	1	5
Hospital Provincial de Castellón, Castellón	7	2	5
Instituto Catalan de Oncología, L'Hospitalet	6	3	3
Hospital Clínico San Carlos, Madrid	5	0	5
Centro Oncológico de Galicia, La Coruña	3	1	2
Hospital del Mar, Barcelona	2	0	2
Instituto Oncológico de Donostia – Onkologicoa, San Sebastian	3	1	2
Instituto Catalan de Oncología, L'Hospitalet	1	0	1

2. 2 sites have not consented any patient by end of Year 4
    - Hospital Universitario de Santiago, Santiago de Compostela
    - Hospital Universitario Puerta del Hierro, Madrid

By end of Year 4 we achieved 80% enrolment. As described in prior reports, we were not able to fulfil the anticipated plans described in the quarterly reports during year 3 and Y4Q1. CNIO as institutional recipient rejected to transfer sponsorship to another sponsor and rejected to support anew NCE request to complete the trial recruitment. In this context and despite improving trial recruitment, the trial management and monitoring were behind schedule. Site 2 PI, David Olmos, anticipated in prior reports the need to hire an external CRO in order to complete it adequately. After several meetings between CNIO managing director and Site 2, David Olmos (site 2 PI) and Imas12 (David Olmos new institution), an agreement was reached to complete the grant work:

a) David Olmos as PI accepted the following demands from CNIO

1- The trial should finalize enrolment by the end of 2022 and David Olmos as PI undertake the necessary protocol changes to complete such deadline
2- There will not be additional sites and sites that have not recruited patients will be closed
3- No additional NCE of the grant will be supported institutionally for site 2 (CNIO).

b) CNIO agreed to support David Olmos to execute the remaining work in his grant, especially supporting subcontracting of external services where CNIO cannot offer support at the present:

1- allow the hiring of an external CRO to support trial monitoring and to execute trial activities fees invoicing and payments from sites to CNBIO and *vice versa*

2- allow the hiring of support (external services) for managing the trial human samples collection, processing and biobanking
3- Support all changes and expenses related to trial insurance, trial electronic clinical data forms, document translations and fees in order to comply with regulatory demands in Spain and EU as well as with HRPO

Following this agreement, a public tender process was initiated by CNIO, in which EXPERIOR/ClinScience (www.experior.es) a Spanish CRO which previously supported the pharmacovigilance of Biochip trial) was selected to perform this task. In addition, IBIMA research Institute (www.ibima.eu) was contracted to support trial samples management (collection, shipments, processing and biobanking). An intensive monitoring plan and sample management plan was designed, and was initiated by the end of Year 4 Q3 in order to complete the pending trial work by the end of Q4.

To fulfil the requirement of CNIO to complete the trial recruitment by the end of September 2022, David Olmos organised a "Biochip Trial" virtual meeting with all trial sites PI on May 13th, 2022. Three conclusions were obtained from this meeting:

- First, it was not feasible to reach the initially required sample size in less than a year, since the % of patients entering cohort C (Biomarker negative: HRR deficient) was between 15-20% of all recruitment, and we initially estimated 25-30%.
- Second, a feasible sample size would be below 80 patients, up to 25-26 additional patients between May 15th and September 30th.
- Third, sites needed more support from the sponsor or if that would not be possible, then from a CRO.

Next we decided to amend the study design from 3 different cohorts, each with an independent sample size calculation based on probability of success for each biomarker status/cohort, to a single-stage Phase II design in which the biomarkers of response would be analysed as secondary endpoints, leaving the possibility in the future to add an adaptative design to validate the "winner" biomarker but not as part of this grant. On this basis, we submitted for initial review to the Spanish regulatory agency and the reference ethics committee the following sample size amendment:

"*Up to 90 patients will need to be screened to enroll up to 70 patients with a minimum of 64 eligible for the efficacy analyses. As carboplatin is commonly used off-label palliative treatment for mCRPC who fail other standard treatment options and the overall response-rate and symptomatic benefit has been established we elected a one-stage phase 2 design to test our hypothesis. A multi-stage designs, rather than the proposed single-stage designs, would only be preferable in a situation in which early termination is desirable if a new proposed treatment or combination is ineffective 51; but not in a setting like this trial where we wish to identify populations in which a known treatment option may be more effective using biomarkers.*

To minimize the sample size for this study allowing a minimum number of patients enough to explore potential biomarkers of carboplatin sensitivity based on HRR deficiency proposed to use the A'Hern Tables for single- stage phase 2 trials 51 which is based on the binomial exact distribution and therefore are preferable to those models based on the normal distribution, such as Flemming and others, which could rise more anomalous results.

To calculate the sample size of the study we fixed a significant-level (α or error type I) of 0.05 and a power (1-β or error type II) of 0.90. In addition, we considered a threshold 15% for the null hypothesis (p0), whilst the probability of success or alternative hypothesis (p1) was adjusted to 30%.

With this, we will require to see 15 responses out of 64 patients enrolled. In addition, we have estimated an 8% over-recruitment (up to 70 patients) to allow the final efficacy analysis population is similar to the a priori calculated sample size"

To preserve the initial scientific aims, the original primary trial endpoint:
"*To estimate the efficacy of single agent carboplatin, as measured by response rate, in three different cohorts of patients with progressive metastatic CRPC*" namely: biomarker negative, biomarker positive/Unknown and post-PARPi

We proposed the following aims:

- Primary aim: "*To estimate the efficacy of single agent carboplatin, as measured by response rate, in patients with progressive metastatic CRPC independently of DNA repair gene status*"
- New secondary aims: "*To estimate the efficacy of single agent carboplatin, as measured by response rate in CRPC patients accordingly to its DNA repair status defined by the γ-histone-2AX–RAD51 immunofluorescent assay or the prior use of PARPi*"

Finally, we are subcontracting the performance of RNAseq to derive signatures associated to response in the clinical trial with GENYO (www.genyo.es). Initially in the scientific plan we proposed to derive transcriptomic signatures from biopsies using RNAseq or arrays.

Major Task 8: Biomarker studies in trials samples

Prospective allocation of patients to the different study cohorts is based on the RAD51-IF assay performed at Site 3 (VHIO).

In total, Site 3 received 91 tumor samples from 72 individual patients who consented for trial participation as part of their prescreening or screening procedures. For some patients, more than one sample was tested due to 1) the trial biopsy block received was deemed not evaluable and a second block was sent; or 2) some trial sites sent to the central lab the archival biopsy and the fresh biopsy in parallel. For those cases, the result on the fresh sample was prioritized for trial enrolment.

Of 91 samples, 57 were prostate biopsies, 13 were bone metastasis biopsies, 11 were lymph node biopsies and 5 were liver biopsies. The remaining 5 samples were labelled as "other". 10 samples were returned after pathology review, so the RAD51 test was performed in 81 samples. The breakdown of results were as follows:

- 28 samples not evaluable (34%)
- 12/52 evaluable samples were HR Deficient as per RAD51 test criteria (23%)
- 41/52 evaluable samples HR proficient as per RAD51 test criteria (77%)

Additionally samples from biopsies and plasm has been gathered to preform NGS analysis with the UW-OncoPlex assay, they will be submitted during Y4Q1 to site 12 to complete analyses.

Finally, exploratory RNAseq analysis is undergoing in a small subset of tumor samples from the first 10 clinical trial patients enrolled. These analyses are performed in collaboration with GENYO-GFranada University (Spain), as a potential future new partner/subawardee. As explained during aim 2 achievements narrative, Site 2 was hampered to continue this work onsite by reasons largely explain in section 5.

- ***Summary of progress on milestones related to Aim 3 in Year 4***

Milestone 6.1 – First patient enrolled in the clinical trial (12 m): completed
Milestone 7.1 Recruitment completed for cohort A (n=18) – (48): not completed
Milestone 7.2 Recruitment completed for cohort B (n=25) – (46): not completed
Milestone 7.3 Recruitment completed for cohort C (n=78) – (48): not completed
Milestone 8.1 – Integrated analysis of clinical and biomarker data (48): not completed
Milestone 8.2 – Data analysis and interpretation, Manuscript Preparation (48+6): planned for additional year

What opportunities for training and professional development has the project provided?

If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

- **Site 1 (UW):** Gavin Ha, PhD recent junior faculty member recruit at the Fred Hutchinson Cancer Center who had collaborated with the Pritchard site on the UW-OncoPlex assay was awarded a 2019 Prostate Cancer Foundation Young Investigator Award. Jonathan Reichel, PhD, a postdoctoral fellow in the Pritchard group has received mentorship in bioinformatics for UW-OncoPlex. In June 2020, Heather Cheng, MD, PhD, co-investigator received a special NCI career development award for Cancer Clinical Investigator Team Leadership to make complex cancer research information more approachable. A Laboratory Medicine Masters Student, Mohammad Adil has continued training to learn how to analyze UW-OncoPlex data. A molecular genetic pathology fellow, Regina Kwon MD has been trained on UW-OncoPlex prostate cancer variant interpretation and leading the molecular tumor board. Colin Pritchard, site 1 PI was awarded the C2 Catalyst for Precision Medicine Award from Scientific American.

- **Site 2 (CNIO):** Elena Castro, MD, PhD, investigator at site 2 was awarded a Juan Rodés Clinician Scientist fellowship from ISCIII (Spanish NIH) to continue working in the area of this project and DNA repair in Prostate Cancer during Year 1. At year 3, her fellowship was

evaluated and renewed for an additional year. During the 4th quarter of Year 3 Q3, Daniel Alameda joined site 2 team with an EU funded post-doctoral researcher fellowship.

- **Site 3 (VHIO):** Sara Arce-Gallego has completed his PhD in part baased on the work described in Major Task 5. In 2020, she was awarded a PhD fellowship from the Spanish Ministry of Health to complete this work. She successfully defended her thesis on 19 Jan 2024. . She also presented part of this work at the 2021 AACR Annual Meeting. Pablo Cresta, MD, is a clinicall fellow at Site 3 who joined the team to pursue some of the tasks related to HRR scars analysis; he has submitted an abstract for the 2024 ASCO Annual Meeting based on this work. They are both co-lead authors of a manuscript reporting the main results from this grant, currently under review.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

- **Site 1 (UW):** Nothing to report.

- **Site 2 (CNIO):** This project has been discussed with other projects at a virtual Patient Engagement Event hold in Málaga in September 2020 co-organized by the CNIO team and the CRIS foundation, a cancer research charity. The attendance to this virtual meeting was estimated in 115.

- **Site 3 (VHIO):** J Mateo has participated in virtual Dissemination Events organized by the FERO Foundation directed at employees of Mango and CocaCola Europe, talking about prostate cancer in general and this project in particular. Also, Sara Arce, PhD student in this proposal, participated in an event annually organized by VHIO for primary and secondary schools in Barcelona, where she presented her group to undergraduate students.

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Nothing to report – this is the final technical report.

4. **IMPACT:** D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

There has been significant interest in our results demonstrating 1) the clinical utility of functional RAD51 foci assays to predict homologous recombination DNA repair deficiency, 2) our work on the PROREPAIR study as it relates to novel insights into the predictive value of *BRCA2 a*nd other homologous recombination DNA repair genes in prostate cancer, and 3) our work with UW-OncoPlex assay has garnered attention through highlighting the issue of false positives among HRD genes in cell-free plasma DNA testing in prostate cancer due to clonal hematopoiesis interference.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Site 1 PI Dr. Colin Pritchard received a prestigious award from Scientific American, the 'C2 Catalyst for Precision Medicine Award', in recognition for his leadership in molecular diagnostics – particularly in the area of DNA repair gene assays and dissemination into the community.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:
- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to report

What was the impact on society beyond science and technology?

If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:
- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to report

5. **CHANGES/PROBLEMS:** *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

The COVID-19 pandemic impacted the progress of this project at different levels: firstly, as our laboratories were working at reduced capacity, or even under strict lockdown for some time, some of the analysis run behind schedule. Secondly, the capacity to pursue research biopsies from patients at Site 2 and 3 were severely reduced during 2020 due to the restrictions in our hospitals and the need for reducing the non-COVID related clinical activities and concerns about patient safety. Last, the lockdown also has reduced the activates of our trials offices, delaying the setup of the clinical trial in Aim 3. The granted additional no-cost extensions were used to complete the proposed work.

In order to minimize the impact of these restrictions in our progress, we have implemented diverse measures such as: 1) pursuing the validation of RAD51 IF assay in a separate cohort of metastatic biopsies with targeted genomics data available at Site 3; 2) prioritize exploiting publicly available transcriptomics databases, so the analysis can be conducted faster once we acquire the necessary biopsies.

Thanks to the additional no-cost extensions granted by the research office, were used to complete the proposed work.

For Aim 3, the clinical trial design was amended due to the low recruitment rate in the initial years. Details on the revised study designed and the impact in the statistical plan was describe above in the section dedicated to Aim 3 results.

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

None

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Not applicable

Significant changes in use or care of vertebrate animals

Not applicable

Significant changes in use of biohazards and/or select agents

Not applicable

6. PRODUCTS: *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

Examples of products include:

- *publications, conference papers, and presentations;*
- *website(s) or other Internet site(s);*
- *technologies or techniques;*
- *inventions, patent applications, and/or licenses; and*
- *other products, such as data or databases, biospecimen collections, germplasm, audio or video products, software, models, educational aids or curricula, instruments or equipment, data and research material, clinical or educational interventions, or new business creation.*

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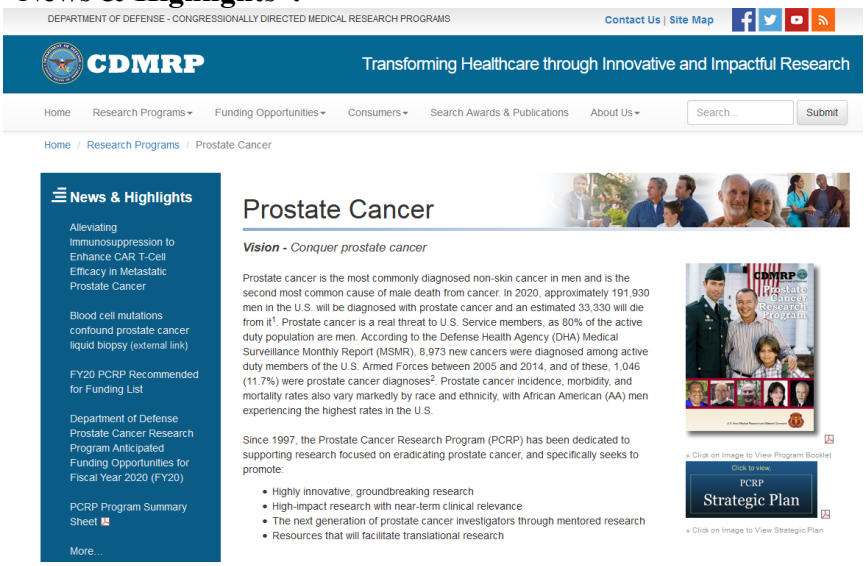

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Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

BOOK CHAPTER (in press): Germline and Somatic Defects in DNA Repair Pathways in Prostate Cancer. Book Title: Prostate Cancer - Cellular and Genetic Mechanisms of Disease Development and Progression. Authors: Sara Arce, Alejandro Athie, **Colin C. Pritchard, Joaquin Mateo**

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

- Sara Arce-Gallego, Alba Llop-Guevara, Suzanne Carreira, Nuria Porta, Roberta Fasani, Diletta Bianchini, George Seed, Pasquale Rescigno, Alec Paschalis, Claudia Bertan, Chloe Baker, Jane Goodall, Susana Miranda, Ruth Riisnaes, Ines Figueiredo, Ana Ferreira, Rita Pereira, Bora Gurel, Daniel Nava Rodrigues, Wei Yuan, Jan Rekowski, Emma Hall, Violeta Serra, Johann S. de Bono, Joaquin Mateo. A homologous recombination repair (HRR) functional assay to stratify patients with metastatic prostate cancer for PARP inhibitor treatment in the TOPARP-B clinical trial [abstract]. In: Proceedings of the American Association for Cancer Research Annual Meeting 2021; 2021 Apr 10-15 and May 17-21. Philadelphia (PA): AACR; Cancer Res 2021;81(13_Suppl):Abstract nr CT161.

- **Website(s) or other Internet site(s)**

List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to report

- **Technologies or techniques**

Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

Nothing to report

- **Inventions, patent applications, and/or licenses**

Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to report

- **Other Products**

Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

- *data or databases;*
- *physical collections;*
- *audio or video products;*
- *software;*
- *models;*
- *educational aids or curricula;*
- *instruments or equipment;*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other.*

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Example:

Name: *Mary Smith*
Project Role: *Graduate Student*
Researcher Identifier (e.g. ORCID ID): 1234567
Nearest person month worked: *5*

Contribution to Project: *Ms. Smith has performed work in the area of combined error-control and constrained coding.*
Funding Support: *The Ford Foundation (Complete only if the funding support is provided from other than this award.)*

SITE: VHIO

Name: Joaquin Mateo
Project Role: Principal Investigator
Nearest person month worked: 12
Contribution to Project: Dr. Mateo is the PI of this award. Work in Aim 2 Patient Recruitment and Sample Acquisition.
FUNDING SUPPORT: Prostate Cancer Foundation, European Commission H2020 Programm, CRIS Cancer Foundation, FERO Foundation and this award.

Name: Daniel Aguilar
Project Role: Postdoctoral Researcher
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 6
Contribution to Project: Optimization of NGS protocols and bionformatic analysis for Aim 2.
Funding support: this award and an award from Spanish Cancer Research Society (AECC)

Name: Sara Arce
Project Role: Laboratory Technician
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 2
Contribution to Project: Task 5
Funding support: Spanish Ministry of Science

Name: Sarai Cordoba (left the group in April 2023)
Project Role: Laboratory Technician
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 3,4

Contribution to Project: Task 5
FUNDING SUPPORT: this award

Name: Luisa Delgado
Project Role: Bionformatician
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 1
Contribution to Project: Tasks 3-4-5
Funding support: this award and CRIS CANCER FOUNDATION

Name: Pablo Cresta
Project Role: Clinical Fellow
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 1
Contribution to Project: Task 5
FUNDING SUPPORT: ESMO Translational Research Fellowship

Name: Violeta Serra
Project Role: Collaborator
Nearest person month worked: 2
Contribution to Project: Dr Serra collaborates with Dr Mateo in development of Task 5.
FUNDING SUPPORT: Spanish Ministry of Science, Asociacion Española contra el Cancer

Name: Raquel Perez-Lopez
Project Role: Collaborator
Nearest person month worked: 3
Contribution to Project: Dr Perez-Lopez oversees patient evaluation for pursuing biopsies and has participated in set up of Aim 3.
FUNDING SUPPORT: Prostate Cancer Foundation, CRIS Foundation, FERO Foundation

Name: Sara Simonetti
Project Role: Collaborator
Nearest person month worked: 3
Contribution to Project: Dr Simonetti is a pathologist who provides advise with sample processing and is responsible for final assessment of immunofluorescence assays and approving reports for the biomarker test in the clinical trial in Aim 3.
FUNDING SUPPORT: institutional core funding

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

If there is nothing significant to report during this reporting period, state "Nothing to Report."
If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending

changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

OTHER SUPPORT
JOAQUIN MATEO

PREVIOUS SUPPORT (5 YEARS)

a) Title of the project; "Defining the landscape and clinical relevance of ATM defects in lethal prostate cancer"
b) Project ID and Funding agency; 2016YI1172 Prostate Cancer Foundation
c) Goals of the project; To study the landscape of ATM aberrations in CPRC, assess the functional impact of these, and ultimately to better understand the role of ATM defects as a predictive biomarker of response to PARPi and platinum chemotherapy**.**
d) Specific aims/tasks;
A) To pursue DNA and protein expression studies by IHC of CRPC biopsies to describe the landscape of ATM aberrations. B) To study the functional impact and clinical relevance of ATM aberrations in DNA repair function and sensitivity/resistance to PARPi, both in in-vitro experiments as through prospective clinical trials. C) To assess the clinical implications of these ATM aberrations in clinical trials of PARPi for patients suffering from CRPC and analysing the family risk of cancer in ATM germline mutation carriers. D) To identify mechanisms of secondary resistance to PARPi in ATM deficient cancers, using plasma and tumor samples from patients receiving these therapies.
e) Est. start and end date (month/ year - month/ year); September 2016-September 2019
f) Level (%) of effort in the project; now completed; previously 25%
g) Point of contact at the funding agency Audrey Gardner (agardner@pcf.org)
Overlap: None

a) Title of the project; CARACTERIZACION DEL IMPACTO FUNCIONAL DE MUTACIONES EN GENES DE LA REPARACION DEL DNA EN PACIENTES CON CANCER DE PROSTATA.
b) Project ID and Funding agency; FSEOM2017 Spanish Society of Medical Oncology (SEOM)
c) Goals of the project; To study the impact in transcriptional profiles of DNA repair defects in preclinical models of prostate cancer
d) Specific aims/tasks;
1- to study transcriptional changes induced by ATM KO and pharmacological inhibition in prostate cancer cell lines models.
2- to assess drug sensitivity in cell line models with and without DNA repair gene loss.
e) Est. start and end date (month/day/year - month/year); January 2018-January2019
f) Level (%) of effort in the project; now completed, previously 15%
g) Point of contact at the funding agency Marina Casanueva marinacasanueva@seom.org
Overlap: None

a) Title of the project; Impact of gene mutations in DNA damage repair and metastatic prostate cancer

b) Project ID and Funding agency MSCA-IF-EF-ST-837900 European Research Council – MSCA Program
c) Goals of the project; To study the impact of different gene alterations in the DNA repair capacities of prostate cancer preclinical models
d) Specific aims/tasks;
•Obj1: To assess the impact of ATM aberrations in DDR function, transcriptional regulation and sensitivity to PARPi, DNA-PKi, ATRi, using prostate cancer models, in AR active/blocked conditions and TP53 WT/loss conditions. (WP1)
•Obj2: To explore potential tumour vulnerabilities based on the identification of overlapping transcriptional regulatory functions for AR and PARP1. (WP1)
•Obj3: To study the impact of AR blockade in DDR function, applying transcriptome ad tissue-based functional assays to biopsies from CRPC patients receiving AR-targeting drugs, in parallel to clinical data emerging for AR blockade-PARPi combinations. (WP2)
e) Est. start and end date (month/year - month/year); April 2018-March 2020
f) Level (%) of effort in the project; now completed, previously 40%
Overlap: None

g) Point of contact at the funding agency: Sandrine Jacobs <Sandrine.JACOBS@ec.europa.eu>

a) Title of the project; Molecular signatures associated to genomic DNA repair defects in prostate cancer
b) Project ID and Funding agency PI18/01384 Spanish Ministry of Science – Instituto de Salud Carlos III
c) Goals of the project; To implement a DNA sequencing program in our institution for prostate cancer patient stratification for clinical trials.
d) Specific aims/tasks;

- Assesment of quality parameters of primary prostate tumor biopsies for NGS
- Mutation calling and copy number assessment in a cohort of primary prostate tumors from patients who later develop CRPC

e) Est. start and end date (month/year - month/year); January 2019 to December 2021
f) Level (%) of effort in the project; now completed, previously 20%
g) Point of contact at the funding agency: Carmen Aranda <caranda@isciii.es>
Overlap: None

CURRENT SUPPORT

a) Title of the project; "Genomic evolution of advanced prostate cancer under selective pressure from novel therapeutic strategies
b) Project ID and Funding agency PI21/00430 - Spanish Ministry of Science – Instituto de Salud Carlos III
c) Goals of the project; To study genomic evolution of prostate cancers upon progression to castration-resistant through longitudinal liquid biopsy NGS.
d) Specific aims/tasks

1.- To optimize a targeted NGS panel assay for liquid biopsies for metastatic prostate cancer patients that can be implemented for clinical practice
2.- To compare the genomic profile at castration-resistance of those patients who previously received ADT vs ADT+docetaxel vs ADT+ARSI in the hormone-naive setting. This objective will be analyzed first in a cohort of patients at Vall d'Hebron using a liquid biopsy assay, and then in a large cohort of tumor biopsies collected at the time of CRPC.
3.- To study the impact of genomic alterations (focusing in TP53, RB1, AR alterations) at the time of castration-resistance (CRPC) in the response/resistance to subsequent therapy lines and overall survival from the time of castration-resistance.
e)Overlap: NO
e) Est. start and end date (month/ year - month/ year); January 2022-December 2024
f) Level (%) of effort in the project: 5%
g) Point of contact at the funding agency: Carmen Aranda <caranda@isciii.es>
Overlap: None

a) Title of the project; "Leveraging the AR-DDR interaction in de-novo metastatic prostate cancer towards precision combination therapies with PARP inhibitors"
b) Project ID and Funding agency LABAE20019MATE - AECC Foundation
c) Goals of the project; To study the modulation of DDR gene transcription upon AR inhibition in clinical samples.
d) Specific aims/tasks;
Aim 1. To study how androgen-deprivation therapy modulates DDR gene transcription in hormone-naïve
prostate cancer using longitudinal biopsies from a presurgical clinical trial.
Aim 2. To investigate the impact of intra-patient subclonality of mHNPC in response/resistance to AR inhibition
with and without PARP inhibitors, evaluating solid and liquid biopsies.
e)Overlap: There is no direct overall; however, some of the samples in this project were analyzed through bulk RNAseq and now in this award will be analyzed using spatial transcriptomics (the samples are the same, but the assay and analysis is a new one, so new data will be generated)
e) Est. start and end date (month/ year - month/ year); October 2020-October 2023
f) Level (%) of effort in the project: 5%
g) Point of contact at the funding agency: Patricia Nieto Cantero patricia.nieto@contraelcancer.es
Overlap: None

a) Title of the project; "Prostate cancer genomic evolution and signatures of dna damage repair deficiencies"
b) Project ID and Funding agency; PR_TCL_2020-10 - CRIS Cancer Foundation
c) Goals of the project To study enrichments for genomic signatures associated to DDR defects along prostate cancer progression to endocrine therapies**.**
d) Specific aims/tasks;
- To study the association between genomic signatures in mPC and different DDR gene mutations.
- To develop NGS signatures and assays that can be implemented in clinical practice.
- To study the impact of ATM-loss in genomic evolution of mPC models and treatment resistance.
e)Overlap: NO
e) Est. start and end date (month/ year - month/ year); July 2021-June 2025

f) Level (%) of effort in the project: 10%
g) Point of contact at the funding agency: Tamara Mondejar tmondejar@criscancer.org
Overlap: None

a) Title of the project; "A Multicenter, Randomized, Open-Label, Two-Arm, Phase II Clinical Trial of enZalutamide and talaZoparib for the Treatment of metastatic hormonena.ve PCa– The ZZ-First Study"
b) Project ID and Funding agency MEDOPP234 **-** Pfizer Oncology
c) Goals of the project; To conduct an investigator-initiated phase II clinical trial of enzalutamide and talazoparib combined in mHNPC.
d) Specific aims/tasks;
A) To study the antitumor activity of enzalutamide combined with talazoparib in mHNPC.
e)Overlap: No direct overall, but some of the samples acquired in this clinical trial will be included in this new project but the analysis here proposed are new and not part of this other grant.
e) Est. start and end date (month/ year - month/ year); October 2019-December 2022 (recruitment finished, we are discussing a No Cost Extension for longer patient follow-up)
f) Level (%) of effort in the project: 5%
g) Point of contact at the funding agency: Belen Sanz: Belen.SANZCastillo@pfizer.com
Overlap: None

a) Title of the project; "Developing synergistic combinations of olaparib with other DNA repair targeting agents and BET inhibition in prostate cancer"
b) Project ID and Funding agency; POC5270 AstraZeneca
c) Goals of the project; To test combinations of novel DNA repair targeting drugs in preclinical models of metastatic prostate cancer
d) Specific aims/tasks;
- Study antitumor activito of AZD5153 alone and in combination with other DDR-targeting agents such as AZD1390, AZD1390, AZD6738 and AZD7648. These studies will be pursued in in in-vitro and in-vivo models of prostate cancer, pre and post-castration resistance. Our models will recapitulate the diverse molecular subgroups of the disease (focusing in ERG-rearranged, AR-amplified/SV+, SPOP-mutant/CHD1 loss, ATMloss, BRCAloss)
- To study the impact of AZD5153 in AR signaling, and HR repair (RNAseq, RAD51foci immunofluorescence, DR-GFP reporter assays), aiming to establish transcriptomic and functional signatures that predict response to later interrogate these signatures in patients WES/RNAseq data.
Overlap: None

- To study epigenetic changes derived from exposure to AZD5153 in prostate cancer models, particularly looking for epigenetic silencing of HR genes and other genes of potential interest (integrating H3K27Ac and BRD4 ChIP data with RNAseq data)
- To study if AZD5153 as single agent or in combination DDR targeting inhibitors can rescue sensitivity of BRCA2-loss models to olaparib after development of acquired resistances.
e)Overlap: NO
e) Est. start and end date (month/ year - month/ year); November 2020-October 2023
f) Level (%) of effort in the project: 5%
g) Point of contact at the funding agency: Patricia Casbas, patricia.casbas-hernandez@astrazeneca.com

Overlap: None

a) Title of the project; "RAD51 immunofluorescence to identify homologous recombination deficient (HRD) metastatic prostate cancers in a real-world population"
b) Project ID and Funding agency; ESCR21-21360, AstraZeneca
c) Goals of the project; To study the sensitivity and specificity of the RAD51PREDICT immunofluorescence assay to identify metastatic prostate cancers with pathogenic BRCA1/2 alterations
d) Specific aims/tasks;
•To determine and cross-compare the failure rates (non-evaluable cases) for the RAD51PREDICT test and the customized NGS assay in FFPE blocks from 1) archival primary prostate tumor biopsies and 2) metastatic biopsies including bone biopsies.
•To explore the intra-patient (spatial and temporal) heterogeneity in the RAD51PREDICT test in a subset of patients with longitudinal biopsies.
•To explore the prognostic value of the RAD51PREDICT test in a real-world metastatic prostate cancer population, exploring the association with patient outcome to standard of care treatments.
e)Overlap: NO
e) Est. start and end date (month/ year - month/ year); November 2021-October 2024
f) Level (%) of effort in the project: 5%
g) Point of contact at the funding agency Patricia Casbas, patricia.casbas-hernandez@astrazeneca.com
Overlap: None

a) Title of the project; "Multimodal imaging biomarkers for precision management of prostate cancer"
b) Project ID and Funding agency CPP2021-009002, Spanish Ministry of Economy (MINECO)
c) Goals of the project; To study the landscape of ATM aberrations in CPRC, assess the functional impact of these, and ultimately to better understand the role of ATM defects as a predictive biomarker of response to PARPi and platinum chemotherapy**.**
d) Specific aims/tasks;
Aim 1. To evaluate PSMA expression changes upon treatment with AR signaling inhibitors using advanced functional imaging with ^{18}F -PSMA-PET.
Aim 2. To develop a non-invasive functional assay using whole-body MRI and ^{18}F -PSMA-PET to monitor metastatic prostate cancer upon systemic therapy.
Aim 3. To study the correlation of functional imaging and novel liquid-biopsy based endpoints of tumor evolution and response to therapy.
Aim 4. To study tumor heterogeneity and its clinical relevance through an integrative analysis of functional imaging, genomics and transcriptomics of tumor and liquid biopsies.
e)Overlap: NO
e) Est. start and end date (month/ year - month/ year); Jan 2023-Dec 2025
f) Level (%) of effort in the project: 5%
g) Point of contact at the funding agency: justieco@aei.gob.es

a) Title of the project; " *Studying intra-tumor heterogeneity in the evolution of metastatic hormone-naïve prostate cancer to castration-resistance after intensified hormonal therapy"*

b) Project ID and Funding agency Department of Defense CDMRP PCRP, **PC220307**
c) Goals of the project; To study heterogeneity in prostate cancer response to AR inhibition though spatial transcriptomics analysis of preclinical models and clinical samples.
e)Overlap: Some of the samples collected in this current award for which there is remanent tissue after WES/RNAseq will be used for spatial transcriptomics analysis in Aim 2 of this new award.
e) Est. start and end date (month/ year - month/ year); May 2023-Apr 2026
f) Level (%) of effort in the project: 50%
g) Point of contact at the funding agency: Jason Wong, jason.wong5.civ@health.mil

What other organizations were involved as partners?

If there is nothing significant to report during this reporting period, state "Nothing to Report."
Two other organizations are involved in this Impact Award:
Organization Name: University of Washington
Award # W81XWH-18-1-0756
PC170510
PI: Colin Pritchard
Location of Organization: Seattle, WA

Organization Name: Centro Nacional Investigaciones Oncologicas (CNIO)
Award # W81XWH-18-1-0770
PC170510P2
PI: David Olmos
Location of Organization: Madrid, Spain

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to* ***https://ers.amedd.army.mil*** *for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.*

See attached

9. APPENDICES:

Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.

None