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14. ABSTRACT

Metastatic castration-resistant prostate cancer (mCRPC) is an incurable disease despite several agents being approved over the last decade. Understanding the inter-patient genomic heterogeneity in this disease is critical to advance to personalized cancer care based on predictive biomarkers. We and others have identified enrichment of homologous recombination (HR) mediated DNA repair defects in mCRPC, accounting for 20-25% cases, with inheritable defects in almost half of these cases. Ongoing clinical trials are studying the role of PARP inhibitors in this subpopulation. Particularly, BRCA2 mutations are known to be an independent poor prognostic factor for relapse in localized disease. Here, we propose to elucidate the prognostic and predictive impact of these mutations with regards to outcome from standard-of-care treatments for mCRPC, and to develop and clinically qualify functional tests to stratify mCRPC patients based on DNA repair damage proficiency, to improve the care of men with advanced prostate cancer.

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

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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	70%
Library preparation for targeted NGS	3 to 20	70%
Sequencing of all samples from the PROREPAIR-B study	3 to 20	70%
Variant calling, bioinformatics analysis	3 to 20	70%

	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)		
Sequencing data analysis board: identification of putative relevant calls for patient care and relatives' risk of cancer	3 to 20	70%
Statistical analysis: correlation of genomic biomarkers with previously annotated clinical outcome data	22	20%
Milestone 1.3 – Data analysis and interpretation, Manuscript Preparation	24	20%
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	80%
Milestone 2.1 – Sample acquisition completed	23	75%
Major Task 3: Whole-exome sequencing studies		
DNA extraction from tumor and germline DNA	6 to 24	80%
Whole exome sequencing studies	12 to 26	60%
Variant calling, bioinformatics analysis	12 to 28	40%
Sequencing data analysis board: identification of putative relevant calls for patient care and relatives' risk of cancer	6 to 30	10%
Major Task 4: Expression profiling studies		
RNA extraction from frozen core of biopsies	6-24	60%

RNA-seq studies	9 to 26	30%
Bioinformatics analysis	12 to 28	10%
Major Task 5: Immunofluorescence studies		
Sample preparation	8 to 30	90%
Immunofluorescence studies	10 to 30	75%
Milestone 5.1 – Integrated analysis of sequencing and IF data	32	50%
Milestone 5.2 – Data analysis and interpretation, Manuscript Preparation	34	0%

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	0%
Major Task 7: Clinical Trial conduction		
Patient recruitment	12 to 30	17%
Continuous data monitoring	12-36	4%
Trial-related biopsy acquisition	12 to 30	17%
Milestone 7.1 Recruitment completed for cohort 1	26	0%
Milestone 7.2 Recruitment completed for cohort 2, stage 1	22	0%
Recruitment for cohort 2, stage 2 (depending on results from stage 1)	23-30	0%

Milestone 7.3 Recruitment completed for cohort 2, stage 2	30	18%
Major Task 7: Biomarker studies in trials samples		
Preparation of trial related biopsies for NGS studies	12 to 30	3%
Targeted sequencing in trial-related biopsies	12 to 30	3%
Variant calling, bioinformatics analysis	12 to 30	0%
Immunofluorescence studies	12 to 30	15%
Sequencing data analysis board: identification of putative relevant calls for patient care and relatives' risk of cancer	12 to 30	0%
Milestone 7.1 – Integrated analysis of clinical and biomarker data	34	0%
Milestone 7.2 – Data analysis and interpretation, Manuscript Preparation	36	25%

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

In year 3, a major focus was to continue to optimize 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. Following completion of these additional optimization experiments Site 1 will plan to complete sequencing of samples from CNIO.

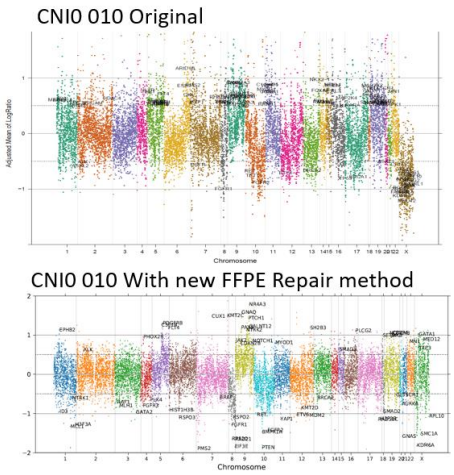


Figure: Effect of FFPE DNA repair on UW-OncoPlex sequencing results. Example copy number plots of CNIO sample 010 run on the UW-OncoPlex v6 panel without (top, original) or with (bottom) FFPE DNA repair. Quality metrics were improved with the FFPE DNA repair step, including copy number quality

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.

Four batches of samples have been sent from CNIO to the UW site and UW-OncoPlex sequencing and 97 have had sequencing using our more optimized low input protocols as outlined below. Of these, 70 had adequate studies to call mutations despite very low input quantities and low DNA quality. Among these 7 had *BRCA2* mutations, 5 had *ATM* mutations, 1 had an *NBN* mutation, 2 had *CHEK2* mutations, 2 had *MUTYH* mutations, 1 had a FANCA mutation, and 1 had a FANCC mutation (**Table**).

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

CNIO_OLM_ID	UW Dataset_ID	DNA Repair Gene Mutation	Interpretation
OLM_03.035	198R16_H02_OPXv5_NB0187	**ATM**	POSITIVE for a pathogenic ATM mutation with associated LOH (p.R521*), CHD1 focal homozygous copy loss, possible MYC amplification
CNIOUW 012	272R10_B02_OPXv6_NA0414	**ATM**	POSITIVE for a pathogenic ATM mutation (p.R531*) with LOH (bi-allelic), CHD1 homozygous copy loss (bi-allelic), possible MYC amplification
CNIOUW 028	276R04_D01_OPXv6_NB0352	**ATM**	POSITIVE for two pathogenic ATM mutations (bi-allelic).
OLM,03.012	281R08_H01_OPXv6_NB0365	**ATM**	POSITIVE for ATM exon 25-63 del mutation with LOH (bi-allelic), CHD1 homozygous copy loss, SPOP p.F102I mutation
OLM_03.047	198R17_A03_OPXv5_NB0187	**ATM VUS**	POSITIVE for a pathogenic TP53 mutation, ATM VUS in the FAT domain (p.I2401T), and PTEN copy loss
OLM_01.006	198R01_A01_OPXv5_NB0187	**BRCA2**	POSITIVE for BRCA2 copy loss, cannot determine if 1 or 2 copies. Possible MYC amplification.
OLM_01.039	198R07_G01_OPXv5_NB0187	**BRCA2**	POSITIVE for a pathogenic mutation in BRCA2 (c.6650_6654del); cannot tell if germline or somatic
CNIOUW 005	272R03_C01_OPXv6_NA0414	**BRCA2**	POSITIVE for BRCA2 focal deletion (favor bi-allelic) and FOXA1 mutation
CNIOUW 018	275R06_F01_OPXv6_NB0350	**BRCA2**	POSITIVE for BRCA2 exon 1-24 deletion + LOH (bi-allelic) and possible MYC amplification.
CNIOUW 032	276R08_H01_OPXv6_NB0352	**BRCA2**	POSITIVE for a pathogenic BRCA2 mutation (c.3264dup), with possible BRCA2 copy loss.
OLM,FIVO.012	286R25_A04_OPXv6_NB0365	**BRCA2**	POSITIVE for a pathogenic BRCA2 mutation (cannot determine if mono-allelic or bi-allelic)
OLM,02.007	281R11_C02_OPXv6_NB0365	**BRCA2?**	POSITIVE for TP53 mutation, BRCA2 single copy loss, possible MYC amplification, and additional alterations
OLM,03.065	286R23_G03_OPXv6_NB0365	**CDK12**	POSITIVE for CDK12 bi-allelic pathogenic mutation with associated tandem duplication signature, MYC amplification, FOXA1 mutation
OLM,02.009	281R01_A01_OPXv6_NB0365	**CHEK2**	POSITIVE for a pathogenic mutation in CHEK2 (exon 11-12 deletion), cannot determine if mono- or bi-allelic
CNIOUW 034	277R02_B01_OPXv6_NB0354	**CHEK2 VUS**	POSITIVE for SPOP p.F102C mutation, KDM6A mutation, MYC amplification, and CHEK2 VUS.
OLM,FIVO.228	286R37_E05_OPXv6_NB0365	**FANCA**	POSITIVE for FANCA pathogenic mutation (cannot tell if mono-allelic or bi-allelic), TP53 mutation, and additional alterations
CNIOUW 039	277R07_G01_OPXv6_NB0354	**FANCC**	POSITIVE for a pathogenic mutation in FANCC (c.455dup, carrier only)
OLM_02.033	198R12_D02_OPXv5_NB0187	**MLH1, MSI-high**	MSI-high due to MLH1 loss, high total mutation burden.
CNIOUW 010	272R08_H01_OPXv6_NA0414	**MSI/MMRd**	MSI-high likely (limited analysis due to low sample quality)
CNIOUW 033	277R01_A01_OPXv6_NB0354	**MUTYH (carrier)**	POSITIVE for a pathogenic mutation in MUTYH (p.G396D, carrier only)
CNIOUW 009	272R07_G01_OPXv6_NA0414	**MUTYH (carrier)**	POSITIVE for a pathogenic mutation in MUTYH (p.G396D, carrier only)
OLM,FIVO.009	286R32_H04_OPXv6_NB0365	**MUTYH (carrier), I**	POSITIVE for germline heterozygous MUTYH mutation (p.Y179C carrier), TP53 mutation, BRCA2 single copy loss, and additional alterations
CNIOUW 008	272R06_F01_OPXv6_NA0414	**NBN**	POSITIVE for a pathogenic mutation in NBN (p.R43*)

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.

Many of the samples had low amounts of residual DNA remaining (<250ng). There is 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.

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.

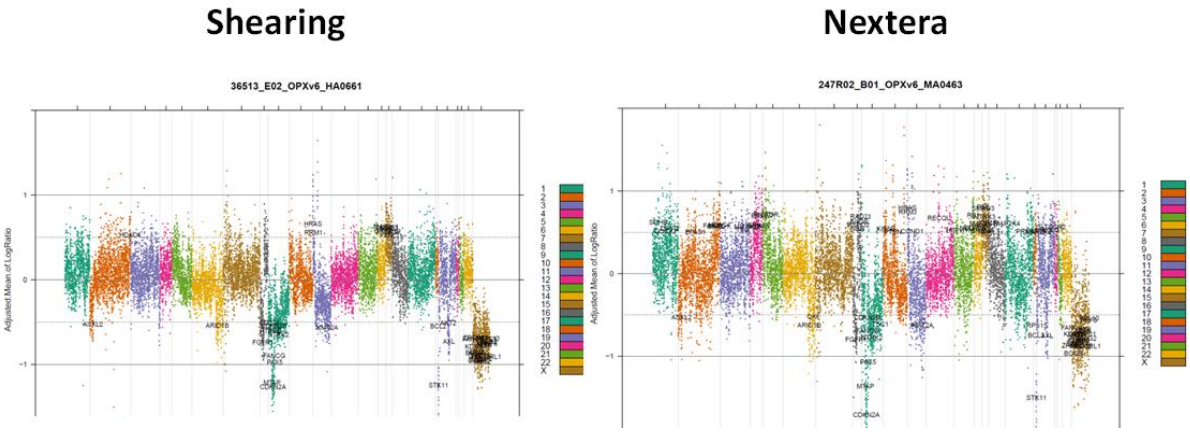


Figure: Comparison of copy number calling between the standard shearing and low input Nextera UW-OncoPlex sequencing. We observed high qualitative and quantitative concordance between the standard shearing-based library prep and Nextera low input library preparation for the UW-OncoPlex assay.

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.

For the no cost extension period 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)

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**).

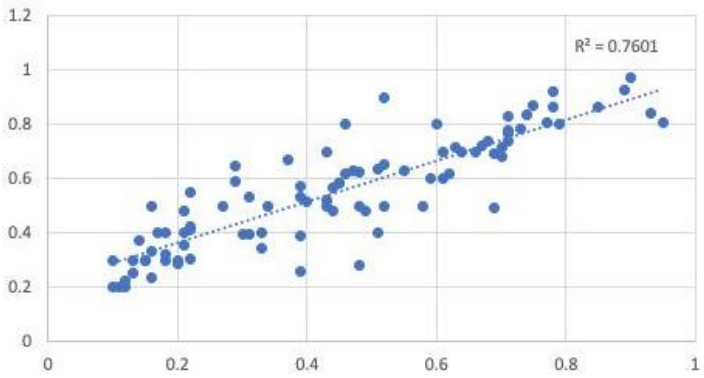


Figure: Comparison of HRD analysis by UW-OncoPlex (X-axis) compared to a commercial lab approach (Y-axis)

In summary, major activities in year 3 included review of an FFPE DNA repair step to improve DNA quality for sequencing, 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), exploration of low-input single-stranded NGS library prep protocol, preparation for cell-free plasma DNA testing, and improvement of the UW-OncoPlex panel by the addition of HRD mutation signature analysis.

Summary of progress on milestones related to Aim 1 in Year 3

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.

During Year 3 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 bone marrow 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. Biopsies from twenty-three cases with metastatic disease that underwent biopsy of their metastatic disease has been identified at site 2,

patient has been consented to use remnant tissue under the DoD protocol and samples will be shipped to Site 3 during the first semester of Y4.
For Site 3 (VHIO), the research protocol for acquisition and analysis of patient biopsies was approved by the local ethics board. As of 20th Jan 2022, 196 patients have been consented for consideration of biopsies. After discussion of suitability with interventional radiology, 68 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 120/196 cases. Saliva samples for correlative germline analyses were collected for all patients at the time of consent

Major Task 3: Whole-exome sequencing studies

DNA has been extracted from both tumor and saliva samples for all acquired biopsies, and low-pass whole-genome sequencing has been performed in all of them. Samples with a tumor content over 20%, estimated by low-pass WGS bioinformatics analysis have been selected for WES. As of Feb 2022, we have completed and analyzed whole-exome sequencing for 43 fresh biopsies included in this study.

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).

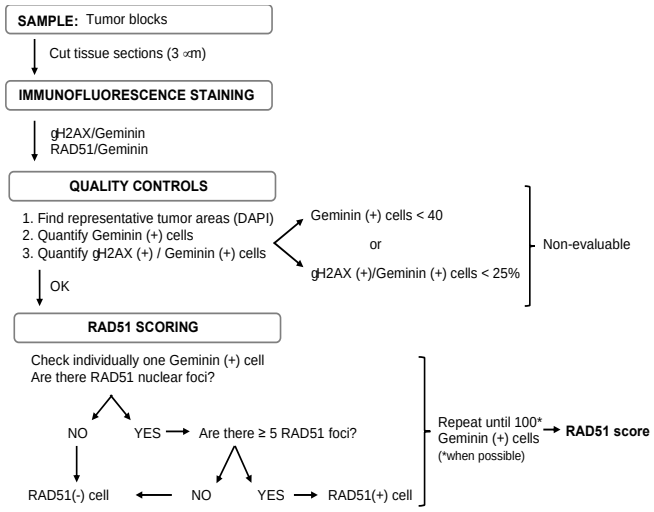
At site 3, during year 3 we have extracted RNA and prepared RNA libraries for all acquired biopsies in the study. As of Feb 2022, we have successfully completed RNAseq on 51 fresh biopsies. of June 2021.

To maximize the number of evaluable samples in this study, during Year 3 we worked in optimizing protocols for RNAseq from RNA extracted from FFPE material. In our study, both FFPE and fresh-frozen blocks are acquired for each biopsy; however, the tumor content and RNA yield may vary from one core to another in the same biopsy procedure. Hence, implementing FFPE-RNAseq may increase the number of evaluable samples.

During no cost extension year (year 4) we will pursue the correlative analysis of the WES and RNAseq studies as detailed in the research plan. We will try to increment the cohort size by adding biopsies collected in Q1-2022 that have not been yet processed and with samples that may be provided by Site 2, as stated in the research plan.

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:

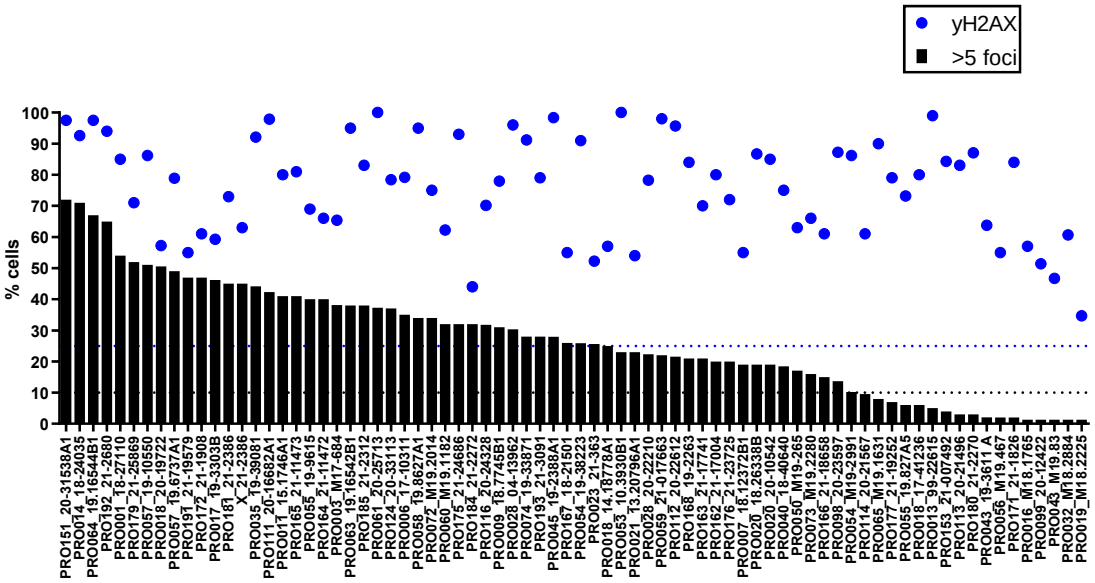


We evaluated baseline HRR function based on detection of RAD51 and yH2AX foci in geminin-positive tumor cells by immunofluorescence (IF). All samples were scored by two trained readers blinded to genomic and clinical data. Samples were considered HRR deficient (HRD) when RAD51 scores were low, pre-defined as <10% tumor cells presenting ≥5 RAD51 foci/cell.

We have now completed the RAD51 IF studies in 137 samples corresponding to 114 individual patientss from this study. RAD51 IF has been completed for all patients for whome WES/RNAseq analysis were pursued, from the same biopsy block when feasible. In cases where the biopsy was insufficient, had low tumor content, or also for those patients who consented but did not have a biopsy done, we pursued RAD51 IF in the archival material available (in those cases, we are pursuing targeted NGS to complement the genomics-IF correlation).

Of 137 samples tested, 29 samples (21%) were deemed not evaluable due to low tumor content or insufficient number of germline-positive cells for RAD51 evaluation, as per the QC criteria described in the figure above. Additionally, there are 32 samples for which the staining has been done but the final report with results has not been yet issued at the time of writing this report. Hence, this report includes results for 76 samples from 68 individual patients.

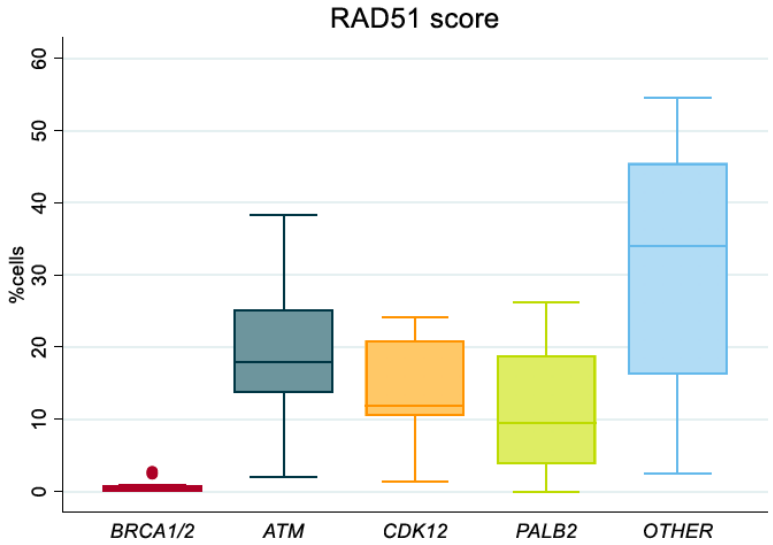
As observed in the graph below, 22% of the tested samples had a RAD51 score lower than 10%, being considered then HR-deficient for the criteria of this study. The high levels of gHA2X, a marker of DNA damage, across the cohort (represented with blue dots in the plot below) are reassuring that the cases with RAD51 negativity represent probably true HR-deficient cases and are not an artifact due to low baseline damage.



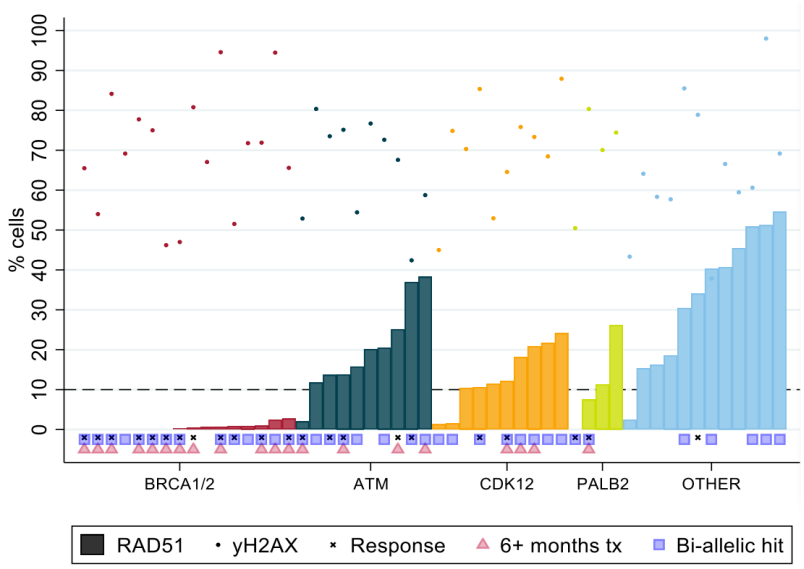
In parallel, we managed to test the assay in a further cohort of samples from metastatic prostate cancer patients enriched for DNA repair gene mutations. In particular, primary or metastatic biopsies from 52 men with metastatic prostate cancer who participated in the phase II TOPARP clinical trial of 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. The association of the RAD51 score, response to olaparib and survival (radiographic progression-free survival, rPFS, and overall survival, OS) was analyzed by Chi-Square and log-rank tests.

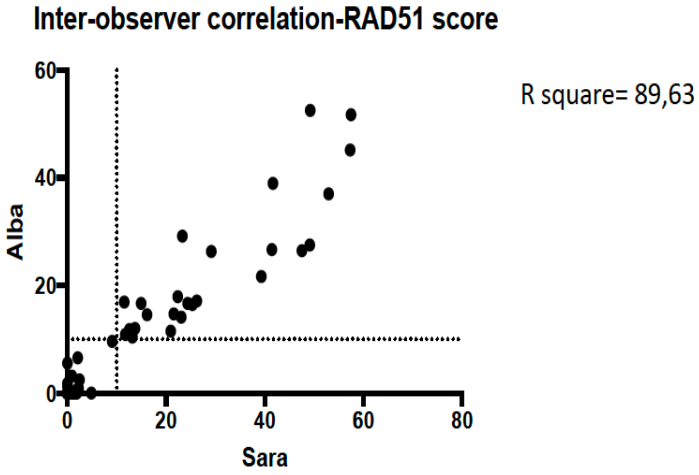
Results: RAD51 and yH2AX were successfully scored in 52 cases, in the same biopsies previously used for NGS in the clinical trial. All tumors showed abundant DNA damage (yH2AX scores >40%). The intra-class correlation score (ICC) between the two blinded readers was 0.88. Overall, 22 of 52 (42%) cases 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.



Additionally, we have analyzed the inter-reader reproducibility of the assay, finding a 100% concordance using a dycotomic positive/negative calling, and high correlation (R=0.896) using a continuous variable calling between two blinded operators. At present, we are working in automatizing the reporting of results, in collaboration with our Pathology core services at VHIO; in order to expedite the development of the assay, we have now employed a pathologist (Dr Maria



Urbanowicz) who work part-time in this project, but costs of this additional personnel will be covered by other sources, and not from this award.

These results were presented at the 2021 AACR Meeting, and a manuscript has been published in Cancer Discovery:

- Carreira S, Porta N, Arce-Gallego S et al. Biomarkers Associating with PARP Inhibitor Benefit in Prostate Cancer in the TOPARP-B Trial. 2021. Cancer Discovery. doi: 10.1158/2159-8290.CD-21-0007

Summary of progress on milestones related to Aim 2 in Year 2

Milestone 2.1 – Sample acquisition completed (month 23): Not completed, currently at 80%
Milestone 5.1 – Integrated analysis of sequencing and IF data (*month 32*): 25%
Milestone 5.2 – Data analysis and interpretation, Manuscript (month 34): To be pursued in Year 4.

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

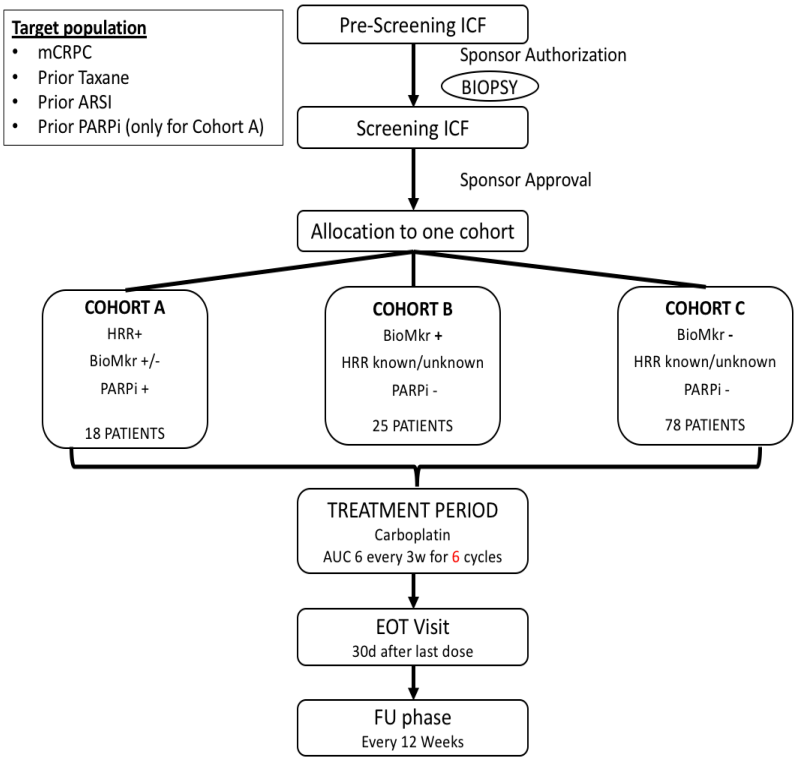
In year 1, we completed the trial protocol which was initially submitted to site 2 reference IRB (CEI Provincial de Malaga) and the AEMPS (Spanish regulatory agency) and initial review and proposed amendments were received by October 2020. The protocol was submitted to the HRPO before regulatory submission, 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. <u>10 sites have consented and/or enrolled at least 1 patient</u>

- Hospital Universitario Virgen de la Victoria de Málaga: 6 patients consented, 1 screening failure (SF), 4 enrolled
- Hospital Universitario Vall D'Hebron, Barcelona (site 3): 2 patients consented and 1 enrolled
- Hospital Clínico San Carlos, Madrid, 1 patient consented and 1 enrolled
- Hospital Provincial de Castellon, 1 patient consented and 1 enrolled
- Instituto Catalan de Oncología, L'Hospitalet, 4 patients consented, 1 SF and 3 enrolled
- Centro Oncológico de Galicia, La Coruña; 2 patients consented and 2 enrolled
- Hospital del Mar, Barcelona, 2 patients consented, 1 enrolled
- Instituto Oncológico de Donostia, San Sebastian, 2 patients consented and 2 enrolled
- Instituto Valenciano de Oncología, Valencia, 2 patient consented and 2 enrolled
- Hospital Universitario 12 de Octubre, Madrid, 5 consented, 1 SF and 3 enrolled

2. <u>3 sites have not consented any patient by end of Year 3</u>
    - Instituto Catalan de Oncología, Badalona
    - Hospital Universitario de Santiago, Santiago de Compostela
    - Hospital Universitario Puerta del Hierro, Madrid

By end of Year 3 we have achieved at least 17% enrolment, although we cannot yet anticipate the potential impact in the recruitment of the 5th and futures COVID-19 waves in Spain, as restriction policies have been relaxed since June 2021 and especially after the end of the summer the recruitment in the trial is taking speed.

Trial conduction has not been initiated pending on completing contracts signatures with the participating sites. On other hand pre-initiation on site or virtual visits to train and evaluate the research team at each trail site has been completed.

Following the anticipated plans described in the quarterly reports during year 3, we have the support of CRIS Cancer Foundation and site 2 institution to initiate the sponsor transfer as soon as we gain approval from HRPO/CDMRP. This sponsor change will not involve additional cost over the budget. We have also contacted and confirmed 6 new additional sites in Spain: Hospital Universitario de Salamanca, Hospital Universitario Marques de Valdecilla-Santander, Hospital Costa del Sol – Marbella, Hospital Universitario de Valme – Sevilla, Hospital San Pedro de Alcántara - Cáceres , and Complejo Hospitalario de Navarra. In addition, we have contacted 4 potential sites in Italy: Azienda Ospedaliero Universitaria Maggiore della Carità, Novara; Istituto Oncologico Romagnolo, Meldola; Istituto Nazionale di tumori, Milano; and Candiolo Cancer Institute, Turin

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).

As of November 2021, Site 3 has received 36 tumor samples from 24 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 36 samples, 22 were prostate biopsies, 8 were bone metastasis biopsies, 4 were lymph node biopsies and 2 were liver biopsies.

For the 24 patients enrolled, RAD51-IF negativity (suggestive of HR defects) was detected in 3 patients (5 samples from 3 patients), and RAD51-IF positivity (suggestive of no HR defects) was observed in 15 cases (19 samples from 15 cases). For the remaining 6 patients, results were deemed as inconclusive or samples were considered not evaluable for technical reasons.

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 3***

Milestone 6.1 – First patient enrolled in the clinical trial (12 m): achieved at month 30
Milestone 7.1 Recruitment completed for cohort A (n=18) – (48): 11%
Milestone 7.2 Recruitment completed for cohort B (n=25) – (46): 17%
Milestone 7.3 Recruitment completed for cohort C (n=78) – (48): 18%
Milestone 8.1 – Integrated analysis of clinical and biomarker data (48): Not/A
Milestone 8.2 – Data analysis and interpretation, Manuscript Preparation (48+6): N/A

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. He will support them with bioinformatics and genomics work in aims 1 and aims 2 from Hospital 12 de Octubre, the new site that Dr. Olmos will be joining as PI by the end of 2022

- **Site 3 (VHIO):** Sara Arce, laboratory technician at Site 3 participating in this project, has been awarded a PhD fellowship to conduct her PhD in part related to this project under the mentorship of PI J. Mateo, and her role as technician in this project was taken over by Sarai Cordoba, PhD. Sara Arce remains involved in the project by acting as 2nd reader for all the RAD51 IF assays. Dr Daniel Aguilar has joined the team as bionformatician dedicated to this project, starting May 2021. Dr Sara Simonetti, MD PhD, Pathologist, has joined the project as senior researcher (part-time dedicated to this project), to provide support in evaluating challenging cases from the pathology-immunofluorescence perspective.

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."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

Site 1 (UW): For Aim 1, we anticipate completing UW-OncoPlex testing for PROREPAIR-B samples with the available and adequate DNA is available. For Aim 3, we anticipate beginning to receive samples for targeted sequencing from Site 3 during Y4 (no cost extension year). As we progress toward the characterization of 100% of cases in aim 1 and aim 3, we will shift our focus to data analysis and manuscript preparation.

Site 2 (CNIO): The team at site 2 will be moving during Y4Q1 from CNIO to a new institution, and once this is complete we probably would need to ask to transfer the grant to the new institution. From our new institution (Hospital 12 de Octubre), we plan to send to site 1 the samples required to

complete the analysis in aim 1 (between Q1 and Q3). At our new institution, we anticipate access to extra resources (including pathology and technicians' hours) to accelerate sample review and sample shipment. We will also finish the collection of additional samples for aim 2 to support the work led in aim 2 at site 3, and finally we will focus our greatest effort in advancing the clinical trial in aim 3 by transferring the sponsorship to CRIS foundation, enrolling new sites and contacting a professional CRO to overcome potential limitations at CNIO.

Site 3 (VHIO): During this last year of award, we will prioritize the combined analysis of WES+RNAseq data for the samples already analyzed. In order to expand the RAD51-IF tested cohort, we are pursuing targeted sequencing for those sample where the archival material did not allow for WES. That way, we anticipate being able to present integrative genomics-IF report for a larger cohort that initially planned.

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 current COVID-19 pandemic we are suffering worldwide has impacted the progress of this project at different levels: firstly, as our laboratories have been working at reduced capacity, or even under strict lockdown for some time, some of the analysis have been delayed. Secondly, the capacity to pursue research biopsies from patients at Site 2 and 3 were severely reduced during the period March-July due to the restrictions in our hospitals and the need for reducing the non-COVID related clinical activities and concerns about patient safety. At present, our sites still suffer from some limitations with regards to pursuing research biopsies, albeit not as strict as during Q2-Q3 2020. However, it is envisioned the second wave of COVID cases, currently affecting Europe severely may result again in more strict restrictions to the acquisition of research biopsies in the next few months. Last, the lockdown also has reduced the activates of our trials offices, delaying the setup of the clinical trial in Aim 3.

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.

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

As outlined above, we developed additional protocols and alternative strategies for use with low input DNA quantity as many of the PROREPAIR-B samples have limited DNA. In parallel, we prioritized sequencing of samples from patients with high input DNA. As third option, in the no cost extension year we will now prioritize ctDNA from plasma samples when available.

Due to the COVID pandemic, the recruitment of patients and biopsy acquisition was severely restricted during 2020. After having been awarded a no-cost extension, recruitment was accelerated again in Q3-Q4 2021.

An additional problem came to the number of samples were the fresh-frozen core of the biopsy had insufficient tumor content for RNAseq analysis. At site 3, we have been working to optimize RNAseq from FFPE material testing different reagents kits and optimizing the bioinformatics pipeline, and are now capable to include both FFPE or fresh-frozen blocks in this study, which will allow us to increase the number of evaluable samples.

In addition, and as described above and in prior reports the activation of the Clinical Trial embedded in Aim 3 has delayed due to the Covid-19 pandemic in addition to recent changes in the legal frame for conducting clinical trials which difficult role as sponsor of CNIO.

Finally, in year 3 the institution of site 2 (CNIO) decided to deprioritize in investment in prostate cancer research, and therefore site 2 PI (David Olmos) and his team has been invited to move to a new institution by September 2021. Site 2 (CNIO) scientific management also experienced significant challenges in supporting the tasks in this grant in year 3, especially the clinical trial embedded in aim 3, which was exacerbated by the crisis provoked by COVID-19 pandemic in Spain. Due to the increasing difficulties to continue the grant of CNIO (site 2), the Site 2 PI and the prostate research team have proposed to change the site 2 sponsor to a third party "CRIS Cancer Research Foundation" a non-for-profit cancer research-oriented NGO. As part of the planned amendment to complete the sponsor transfer, we will add new trial sites in order to achieve the study enrollment trial in a shorter timeframe.

With regard to Dr. David Olmos as PI for site 2 and his team moving institutions, negotiations with the new institution "Research Institute Hospital 12 de Octubre (i+12)" in Madrid has advanced positively and the team is aiming to move to this new institution by December 2021. The CDMRP grant officer has been informed and the possibility to transfer the grant to this new institution has been proposed.

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:

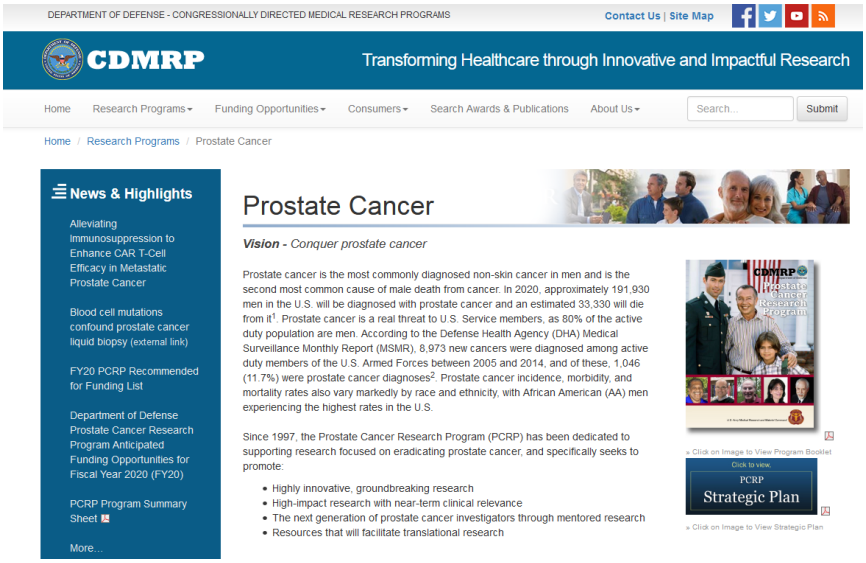
- *publications, conference papers, and presentations;*
- *website(s) or other Internet site(s);*
- *technologies or techniques;*
- *inventions, patent applications, and/or licenses; and*
- *other products, such as data or databases, biospecimen collections, germplasm, audio or video products, software,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

Year 3:

Schweizer MT, Sivakumar S, Tukachinsky H, Coleman I, De Sarkar N, Yu EY, Konnick EQ, Nelson PS, **Pritchard CC**, Montgomery B. Concordance of DNA Repair Gene Mutations in Paired Primary Prostate Cancer Samples and Metastatic Tissue or Cell-Free DNA; JAMA Oncology; 7: 2021; 1-5; acknowledgement of federal support (yes).

Rebeca Lozano, Daniela C. Salles, Shahneen Sandhu, Isabel M. Aragón, Heather Thorne, Fernando López-Campos, José Rubio-Briones, Ana M. Gutierrez-Pecharroman, Tomas di Domenico, Alejandro Sanz1, Juan Daniel Prieto, Isabel García, María I. Pacheco, Teresa Garcés, Casilda Llacer, Nuria Romero-Laorden, Francisco Zambrana, Pedro P. López-Casas, David Lorente, **Joaquin Mateo, Colin C. Pritchard**, Emmanuel S. Antonarakis, **David Olmos**, Tamara L. Lotan, **Elena Castro**. Association between BRCA2 alterations and intraductal and cribriform histologies in prostate cancer; European Journal of Cancer; 147: 2021; 74-83; acknowledgement of federal support (yes).

Jensen K, Konnick EQ, Schweizer MT, Sokolova AO, Grivas P, Cheng HH, Klemfuss NM, Beightol M, Yu EY, Nelson PS, Montgomery B, **and Pritchard CC**. Clonal Hematopoiesis in DNA Repair Genes Substantially Interferes with Prostate Cancer Plasma Cell-free DNA Testing. JAMA Oncology; 7:2021:107-110; published; acknowledgement of federal support (yes). **This study is currently featured on the CDMRP PCPR website homepage under "News & Highlights".**



Michael T. Schweizer, Smruthy Sivakumar, Hanna Tukachinsky, Ilsa Coleman, Navonil De Sarkar, Eric Q. Konnick, Peter S. Nelson, **Colin C. Pritchard**, R. Bruce Montgomery. Concordance of DNA Damage Repair (DDR) Gene Mutations in Paired Primary and Metastatic Prostate Cancer Samples. (2021). American Society for Clinical Oncology (ASCO) annual meeting.

<u>Year 1 and 2:</u>

Dines JN, Shirts BH, Slavin TP, Walsh T, King MC, Fowler DM, and Pritchard CC. Systematic misclassification of missense variants in BRCA1 and BRCA2 "coldspots". Genet Med; 22: 2020; 825-830; published; acknowledgement of federal support (yes).

Nyquist MD, Corella A, Coleman I, De Sarkar N, Kaipainen A, Ha G, Gulati R, Ang L, Chatterjee P, Lucas J, Pritchard C, Risbridger G, Isaacs J, Montgomery B, Morrissey C, Corey E, Nelson PS. Combined TP53 and RB1 Loss Promotes Prostate Cancer Resistance to a Spectrum of Therapeutics and Confers Vulnerability to Replication Stress. Cell Rep; 31; 107669; published; acknowledgement of federal support (yes).

Graham LS, Montgomery B, Cheng HH, Yu EY, Nelson PS, Pritchard C, Erickson S, Alva A, Schweizer MT. Mismatch repair deficiency in metastatic prostate cancer: Response to PD-1 blockade and standard therapies. PLoS One; 15; 2020 5:e0233260; published; acknowledgement of federal support (yes).

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Schweizer MT, Gulati R, Beightol M, Konnick EQ, Cheng HH, Klemfuss N, DeSarkar N, Yu EY, Montgomery RB, Nelson PS, and Pritchard CC. Clinical determinants for successful circulating tumor DNA analysis in prostate cancer. Prostate; 79: 2019; 701-708; published; acknowledgement of federal support (yes).

Abida W, Cyrta J, Heller G, Prandi D, Armenia J, Coleman I, Cieslik M, Benelli M, Robinson D, Van Allen EM, Sboner A, Fedrizzi T, Mosquera JM, Robinson BD, DeSarkar N, Kunju LP, Tomlins S, Wu YM, Nava Rodrigues D, Loda M, Gopalan A, Reuter VE, Pritchard CC, Mateo J, Bianchini D, Miranda S, Carreira S, Rescigno P, Filipenko J, Vinson J, Montgomery RB, Beltran H, Heath EI, Scher HI, Kantoff PW, Taplin ME, Schultz N, deBono JS, Demichelis F, Nelson PS, Rubin MA, Chinnaiyan AM, Sawyers CL. Genomic correlates of clinical outcome in advanced prostate cancer. Proc Natl Acad Sci; 116: 2019; 11428-11436; published; acknowledgement of federal support (yes).

Schweizer MT, Antonarakis ES, Bismar TA, Guedes LB, Cheng HH, Tretiakova MS, Vakar-Lopez F, Klemfuss N, Konnick EQ, Mostaghel EA, Hsieh AC, Nelson PS, Yu EY, Montgomery RB, True LD, Epstein JI, Lotan TL, and Pritchard CC. Genomic Characterization of Prostatic Ductal Adenocarcinoma Identifies a High Prevalence of DNA Repair Gene Mutations. JCO Precis Oncol. 2019; PMID: 31123724; published; acknowledgement of federal support (yes).

Khani F, Wobker SE, Hicks JL, Robinson BD, Barbieri CE, De Marzo AM, Epstein JI, Pritchard CC, Lotan TL. Intraductal carcinoma of the prostate in the absence of high-grade invasive carcinoma represents a molecularly distinct type of in situ carcinoma enriched with oncogenic driver mutations. J Pathol; 2019; PMID:30993692; published; acknowledgement of federal support (yes).

Abida W, Cyrta J, Heller G, Prandi D, Armenia J, Coleman I, Cieslik M, Benelli M, Robinson D, Van Allen EM, Sboner A, Fedrizzi T, Mosquera JM, Robinson BD, DeSarkar N, Kunju LP, Tomlins S, Wu YM, Nava Rodrigues D, Loda M, Gopalan A, Reuter VE, Pritchard CC, Mateo J, Bianchini D, Miranda S, Carreira S, Rescigno P, Filipenko J, Vinson J, Montgomery RB, Beltran H, Heath EI, Scher HI, Kantoff PW, Taplin ME, Schultz N, deBono JS, Demichelis F, Nelson PS, Rubin MA, Chinnaiyan AM, Sawyers CL. Genomic correlates of clinical outcome in advanced prostate cancer. Proc Natl Acad Sci; 116: 2019; 11428-11436; published; acknowledgement of federal support (yes).

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.*

Nothing to report

- **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 1 (UW)

Name:	Pritchard, Colin
Project Role:	Initiating PI
Research Identifier:	cpritch (eRA Commons)
Nearest person month worked:	1.28 calendar months
Contribution to Project:	Colin Pritchard coordinates UW-OncoPlex sequencing and focuses on interpreting the sequencing data for this project. He is guiding experiments and participating in manuscript preparation and review.

Name:	Cheng, Heather
Project Role:	Co-Investigator
Research Identifier:	hhcheng (eRA Commons)
Nearest person month worked:	0.40 calendar months

Contribution to Project:	Heather Cheng reviews sequencing data at molecular tumor boards to identify relevant findings for patient care and relatives' risk of cancer.

Name:	Salipante, Stephen
Project Role:	Co-Investigator
Research Identifier:	stevesal (eRA Commons)
Nearest person month worked:	0.60 Calendar Months
Contribution to Project:	Stephen Salipante directed the development and implementation of the data analysis pipeline, assists with UW-OncoPlex data interpretation, and in guiding and preparation of manuscripts.

Name:	Beightol, Mallory
Project Role:	Research Tech
Research Identifier:	N/A
Nearest person month worked:	2.00 Calendar Months
Contribution to Project:	Mallory Beightol is responsible for preparing genomic libraries and UW-OncoPlex sequencing for this project.

Name:	Reichel, Jonathan
Project Role:	Bioinformaticist
Research Identifier:	N/A
Nearest person month worked:	0.60 Calendar Months
Contribution to Project:	Jonathan Reichel is responsible for UW-OncoPlex bioinformatics pipeline development and data analysis.

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

PRITCHARD, COLIN – SITE: UNIVERSITY OF WASHINGTON

Active

Title: Pacific NW Prostate Cancer SPORE (68-6188)
Time Commitments: 0.36 Calendar Months
Supporting Agency: Fred Hutch
Address: 1100 Fairview Ave N, Seattle WA 98109
Contracting/Grants Officer: Mackenzie Krouse
Performance Period: 9/1/2020 – 8/31/2023
Level of funding: Total Award
Project Goals: The University of Washington (PI: Dr. R. Bruce Montgomery, MD; Co-Investigator: Colin C. Pritchard, MD, PhD) will design and conduct the clinical trials described in this proposal that include platinum-based chemotherapy and the maintenance therapy with PARP inhibitors. Drs. Montgomery and Pritchard will work closely with Dr. Nelson to facilitate the molecular assays from biospecimens acquired on the clinical studies and will work closely with Biospecimen Core B for sample collection and Clinical Core D for trial management.
Specific Aims:
1) Conduct Phase 2 clinical trials of FDA-approved genotoxic therapeutics and PARPi in patients with mCRPC to determine response rates, identify resistance mechanisms, and establish associations between those specific genomic defects predicted to result in HRD and the depth and duration of clinical responses.
2) Systematically assess tumor responses to rational combinations of genetic and pharmacological targeting DNA repair pathways using Patient Derived Xenograft (PDX) models with inherent or engineered HRD aberrations.
3) Develop minimally-invasive biomarkers involving the capture and analysis of circulating tumor DNA capable of distinguishing patients for therapeutics targeting DNA repair pathways.
Overlap: None

Title: Bringing OncoPlex Tumor Genomic Data to the BBI Community (68-3861)
Time Commitments: 0.12 Calendar Months
Supporting Agency: Brotman Baty Institute
Address: University District Magnuson Health Sciences Building H-564, 1959 NE Pacific St, Seattle, WA 98195
Contracting/Grants Officer: Nola Klemfuss
Performance Period: 02/01/2021 – 01/31/2023
Level of funding: Total Award
Project Goals: The proposed plan the University of Washington Medical Center (UWMC) in partnership with the Seattle Cancer Care Alliance (SCCA) routinely performs a clinical next-generation sequencing assay called UW-OncoPlex for the molecular profiling of tumors from SCCA patients, with over 10,000 patient samples tested since 2011. This in-system tumor genomic data is very valuable for translational and clinical research, but there are currently not effective methods for sharing it broadly with Brotman-Baty Institute members and the larger community. Improved access

to OncoPlex data for BBI members will advance precision medicine by accelerating research on tumor genome alterations and their role in cancer risk and response to therapy.
Overlap: None

Title: Northwest Genomics Center for All of Us (61-8385)
Time Commitments: 0.60 calendar
Supporting Agency: National Institutes of Health; 1 OT2 OD 002748-01
Address: 9000 Rockville Pike, Bethesda, Maryland 20892
Contracting/Grants Officer: Irene Haas (grissomi@mail.nih.gov)
Performance period: 9/25/18 – 8/31/2023
Level of funding: Total Award
Project Goals: The goal of the proposal is to establish a Genome Center for the All of Us Research Program. The NWGC for All of Us will provide whole genome sequencing, genotyping and clinical validation of variants in the ACMG 59 genes.
Specific Aims: To advance the goals and objectives of the All of Us Research Program we will produce and interpret variants from genotyping arrays for up to 100,000 samples in year 1 and up to 200,000 samples in years 2 - 5. We will also produce and interpret variants on more than 10,000 samples by WGS in year 1; up to 100,000 samples in year 2; and up to 200,000 samples in years 3-5 using the Illumina NovaSeq platform. To accomplish this, we will:
1- Work with the All of Us program, the DRC, the Biobank, and other groups to deliver an efficient and effective process for evaluating and completing high-throughput genotyping and WGS, call variants, and interpret the impact of variants in the ACMG 59 genes and other genes as indicated by the program in a CLIA-certified environment.
2- Interact directly with the Biobank to carefully develop the logistics and methods for preparing and receiving samples.
3- Track all samples and data transfers for all samples at every stage of the process (from project initiation to data delivery using our secure, completely interactive, and integrated laboratory information management system (LIMS)) and provide reports to the program, the DRC, and other groups as required.
4- Provide genotype and WGS data of the highest quality, in formats required by the program such as IDAT files for genotyping and CRAMs and VCFs for WGS.
5- Provide a team of specialized personnel and staff versed in the workflow of a well-established high throughput CLIA-certified genome center. These include individuals specifically trained in DNA sample receipt, quality control, and large-scale bioinformatics analysis and variant interpretation.
6- Assist as needed with additional data interpretation (beyond the ACMG genes), with publications (i.e., materials and methods), and other activities as required for the program.
7- Provide secure backup of raw sequence data from the samples and all metadata associated with the project (i.e., sample tracking, storage, and QC information).
Overlap: None

Title: Project 1: Molecular Predictors of Prostate Cancer Progression and Mortality (63-0945)
Time Commitments: 1.32 calendar
Supporting Agency: Fred Hutchinson Cancer Center through NIH
Address: 1100 Fairview Ave N, Seattle, WA 98109
Contracting/Grants Officer: Mackenzie Krouse
Performance period: 09/18/2018 – 08/31/2023

Level of funding: Total Award
Project Goals: Prostate cancer (PCa) is the most common solid tumor in men and is a major cause of cancer-related morbidity and mortality. Prostate-specific antigen (PSA) testing is controversial, and current consideration of high risk men is inadequate. Also, clinicopathological criteria are insufficient to differentiate indolent vs aggressive disease. The recent discovery of high prevalence of high to moderate penetrance germline cancer risk mutations in metastatic PCa will lead to increased testing and cascade testing of unaffected male relatives, thus identifying men at high risk for developing aggressive PCa. Preliminary evidence suggests the need for refined cancer screening in this high risk group. The overall intent of this population sciences research is to find men at high genetic risk for aggressive prostate cancer and to conduct an early Pca detection study and incorporate novel PCa biomarkers.
The proposed plan builds on our prior SPORE work, taking advantage of our experience to prospectively recruit a population-based PCa cohort with germline mutations (index cases) and their male first degree relatives (high risk cohort) with the goal of conducting a PCa early detection study that will incorporate germline DNA sequencing to characterize risk, novel PCa biomarkers, clinical and PCa-specific outcomes data. Univariate, stratified, and multivariate analyses will be completed to evaluate sensitivity and specificity of new biomarkers. The Cox proportional hazards model will be used to calculate hazard ratios, 95% CIs, and p-values to examine the association of individual and combinations of germline genetic biomarkers and with PCa outcomes. The overall goal is to identify and validate prognostic genetic-epigenetic biomarkers and begin to translate these findings into better patient management by investigating novel screening and detection approaches for men at high risk for aggressive PCa.
Specific Aims:
1) To ascertain and recruit men at high genetic risk for developing aggressive prostate cancer.
2) To test new approaches to early detection of prostate cancer in men with high genetic risk for aggressive prostate cancer.
3) To identify and evaluate new prostate cancer biomarkers in men with high genetic risk for aggressive prostate cancer.
Overlap: None

Title: Clinical qualification of DNA repair defects as biomarkers in metastatic prostate cancer using integrated genomics and tissue-based functional assays (61-7639)
Time Commitments: 3.6 calendar
Supporting Agency: Department of Defense US Army; W81XWH-18-1-0756
Address: 820 Chandler ST, Fort Detrick, MD 21702-5000
Contracting/Grants Officer: Elena Howell (elena.g.howell.civ@mail.mil)
Performance period: 9/30/2018 – 9/29/2022
Level of funding: Total Award
Project Goals: The major goals we propose will provide physicians tools to develop more effective treatment strategies for men with mCRPC, by assessing DNA repair defects as predictive biomarkers of patient outcome to standard therapies. In the near term, developing and validating functional biomarkers of HR functionality would facilitate implementation of personalized treatment-decisions in mCRPC into clinical practice in the community and also provide valuable information to address mechanisms of drug resistances to PARP inhibitors and DNA damaging chemotherapy in this subclass of the disease. Eventually these data could be relevant for men with localized disease too, and help personalizing treatment to prevent progression to lethal disease.

Specific Aims: 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 Ra-223, in samples from a prospective study.
Aim 2: To optimize tissue-based tests of HR functionality samples for CRPC samples, and study the correlation with genomic aberrations in HR genes.
Aim 3: To clinically qualify this HR functional test in a clinical trial of carboplatin in CRPC.
Overlap: None

Changes/Ended

Title: Advanced development and validation of genome-scale molecular diagnostics for microsatellite instability using targeted molecular counting methods (61-3459)
Time Commitments: 0.60 calendar
Supporting Agency: National Institute of Health; 5R33CA222344-03
Address: 9000 Rockville Pike, Bethesda, Maryland 20892
Contracting/Grants Officer: Rao L Divi (divir@mail.nih.gov)
Performance period: 2/8/2018 – 1/31/2022
Level of funding: Total Award
Project Goals: New forms of cancer treatment are extremely effective against tumors affected by genomic instability, but our ability to practicably identify and classify tumors with this feature in clinical practice is limiting. This proposal outlines the advanced development and validation of ultrasensitive, cost effective, and highly accurate methods for detecting, classifying, and analyzing genomic instability in tumor specimens. This technology will be suitable for use as a clinical diagnostic, and will provide enhanced capabilities including improved prognostic approximation and generality across tumor types, while offering improved performance characteristics over existing standards of care.
Specific Aims: An ideal diagnostic for MSI would have the following properties that are absent from existing paradigms: 1) scalability – thousands of microsatellites could be examined, far in excess of the five loci queried by current multiplexed PCR approaches; 2) generality – the assay would work for all cancer types, whereas current methods are optimized only for colorectal cancers; 3) quantitative precision – a quantitative measure of MSI would be provided, rather than simple positive or negative classification; 4) sensitivity and specificity – the correct diagnosis would be reliably and accurately achieved, even in heterogeneous specimens.
Overlap: None

Title: Accelerating the development and validation of liquid biopsy assays (66-0743; previously 63-6770)
Time Commitments: 0.48 calendar
Supporting Agency: Fred Hutchinson Cancer Research Center
Address: 1100 Fairview Ave N, Seattle WA 98109
Contracting/Grants Officer: Pamela Allen (pgallen@fredhutch.org)
Performance Period: 11/1/2019 – 12/03/2020
Level of funding: DC
Project Goals: Dr. Colin Pritchard with the University of Washington Medical Center (UWMC) Clinical Diagnostic Platform will facilitate rapid translation of both analytical innovations and biomarker findings into the clinic and to advance clinical research of cfDNA for precision medicine.

He will interface with the Clinical Diagnostics Platform Laboratory at the UW Medical Center, which he directs, to develop new cfDNA assays and informatics approaches to improve the detection of tumor-specific aberrations in patient cfDNA, and to identify potential biomarkers for inclusion in their clinical sequencing platforms.
Specific Aims: Aim 1(a) Complete the computational design and development of methodologies for analyzing linked-read tumor genome sequencing. Aim 1(b) Perform an initial genomic analysis of 53 tumor samples sequenced using 10X Genomics, including profiling structural variation, copy number alteration, and somatic point mutations. Aim 2(a) Complete the design, development, and benchmarking of the methodology for multi-sample analysis of low-pass sequencing of cfDNA. Aim 2(b) On-going low-pass WGS sequencing of new mCRPC cfDNA samples. Aim 2(c) On-going exploration and design of algorithm for tissue-of-origin of origin analysis using nucleosome profiling to enhance detection of ctDNA.
Overlap: N/A

Title: Targeting the Subtype of Metastatic Prostate Cancer Deficient in DNA Repair Capacity (61-6227)
Time Commitments: 2.52 calendar
Supporting Agency: US Army Department of Defense
Performance period: 8/15/2018 – 8/14/2021
Level of funding: Direct Costs
Project Goals: The objectives are supported by compelling data derived from the PCF/SU2C Precision Medicine project, other sequencing efforts that assessed the molecular landscape of mCRPC, and striking clinical observations. We will aggressively target the subtype of DNA Repair Deficient (HRD) mCRPC to test the hypothesis that aberrations in key genes that repair DNA strand breaks by homologous recombination (HR) are predictive of meaningful clinical responses to FDA-approved genotoxic therapeutics (e.g carboplatin) and to emerging therapeutics (PARP and WEE1 inhibitors). We will also test the hypothesis that men with mPC represent a population highly enriched for germ-line aberrations in DNA repair genes irrespective of racial background. The proposal will also develop strategies to enhance initial responses and assess mechanisms of resistance to genotoxic agents.
Overlap: None

Title: Returning clinically actionable results to MVP participants with metastatic prostate cancer: a pilot study (62-0740)
Time Commitments: 0.60 calendar
Supporting Agency: VA Puget Sound Health Care System
Address: 1660 S Columbian Way, Seattle, WA 98108
Contracting/Grants Officer: Bruce Montgomery
Performance period: 01/01/2020 – 12/31/2021
Level of funding: Direct Costs
Project Goals: The Pritchard Lab will perform the clinical retest for all participants in the study. He will interpret the results of the retest as to whether the results indicate the presence or absence of a pathogenic germline BRCA1, BRCA2 or PALB2 allele. He will assist in the interpretation of the final data addressing the objectives of the study
Specific Aims: Analyzing the results of the retest as to whether the results indicate the presence or absence of pathogenic germline BRCA1, BRCA2 or PALB2 allele. He will assist in the interpretation

of the final data addressing the objectives of the study.
Overlap: None

CHENG, HEATHER – SITE: UNIVERSITY OF WASHINGTON

CURRENT

Title: A Phase 1, open label study evaluating the safety, pharmacokinetics and clinical effects of intravenously administered PT-112 injecting in patients with advanced solid tumors and subsequent expansion cohorts
Effort: 5%
Supporting Agency: Phosplatin Therapeutics LLC
Contracting/Grants Officer:
Performance Period: 09/28/2021 – 07/31/2026
Level of Funding:
Project Goals: The major goal of this study is to define the recommended dose level for PT-112, administered on Days 1 and 15 of each 28-day cycle, for pivotal studies based on the risk/benefit ratio of 360 mg/m2 (Arm 1) and 250 mg/m2 (Arm 2) dose levels.
Overlap: None

Title: 67652000PCR3002
Effort: 5%
Supporting Agency: Janssen Research & Development, LLC
Contracting/Grants Officer: Sean Murphy: smurph41@its.jnj.com
Performance Period: 8/16/21 – 1/31/2026
Level of Funding:
Project Goals: The major goal of this study is to assess the primary endpoint, rPFS, and defined as the time from the date of the randomization to the date of radiographic progression, or death.
Overlap: None

Title: A ph I/II trial of concurrent chemohormonal therapy using enzalutamide (MDV-3100) and cabazitaxel in patients with metastatic castration resistant prostate cancer
Effort: 0.60 calendar
Supporting Agency: PCCTC, LLC (Medivation and Sanofi)
Contracting/Grants Officer: Casey Sisco, siscoc@mskcc.org, (646) 888-0404
Performance Period: 07/14/16 to 11/30/22
Level of Funding:
Project Goals: The major goal of this project is to test the safety and efficacy of combination treatment with enzalutamide (MDV3100) and cabazitaxel chemotherapy of prostate cancer.
Specific Aims: To determine safe dosing level. To collect correlative biospecimens to understand the biological effects of the treatment and to evaluate for potential prognostic biomarkers.
Overlap: None

Title: Prostate Cancer Outcomes: An International Registry to Improve Outcomes in Men with Advanced Prostate Cancer (IRONMAN)
Effort: 0.30 calendar

Supporting Agency: Movember (via PCCTC, LLC)
Contracting/Grants Officer: Casey Sisco, siscoc@mskcc.org, (646) 888-0412
Performance Period: 08/18/17 – 07/31/22
Level of Funding:
Project Goals: The major goal of this study is to create an international, population-based, prospective registry of at least 5,000 men with advanced prostate cancer.
Overlap: None

Title: PROSTATE CANCER CLINICAL TRIALS CONSORTIUM, W81XWH-17-2-0043 (Cheng)
Effort: 1.80 calendar
Supporting Agency: DOD
Contracting/Grants Officer:
Performance Period: 09/30/2017 to 09/29/2022
Level of Funding:
Project Goals: The Department of Defense provides funding for infrastructure to support participation as a clinical site in the Prostate Cancer Clinical Trials Consortium
Overlap: None

Title: A phase 1b study of enzalutamide plus CC-115 in men with castration-resistant prostate cancer
Effort: 0.60 calendar
Supporting Agency: PCCTC, LLC (Celgene)
Contracting/Grants Officer: Casey Sisco, siscoc@mskcc.org, (646) 888-0404
Performance Period: 10/01/17 to 12/31/22
Level of Funding:
Project Goals: The major goal of this study is to determine the safety, pharmacokinetics, and the Maximum Tolerated Dose and/or Recommended Phase 2 Dose of the combination of CC-115 plus enzalutamide.
Overlap: Dr. Cheng will be the site PI for this study, which will provide the biospecimens used in Aim 4 of the Movember-PCF proposal to develop a predictive biomarker. Dr. Cheng's effort on the CC-115 trial will be paid for by the Movember-PCF grant, and remaining costs for running the CC-115 trial will be paid on the PCCTC budget.

Title: PLATI-PARP: A phase 2 study of induction docetaxel and carboplatin followed by maintenance rucaparib in treatment of patients with metastatic castration resistant prostate cancer with homologous recombination DNA repair deficiency
Effort: 0.60 calendar
Supporting Agency: Clovis Oncology, Inc
Contracting/Grants Officer: Vivian Chen, vchen@clovisoncology.com, (310) 803-0334
Performance Period: 07/26/2018 to 08/30/2022
Level of Funding:
Project Goals: The major goal of this trial is to determine radiographic progression free survival with 4 cycles of docetaxel with carboplatin followed by maintenance rucaparib in the treatment of patients with metastatic castration resistant prostate cancer with homologous recombination DNA repair deficiency.
Overlap: None

Title: Clinical qualification of DNA repair defects as biomarkers in metastatic prostate cancer using integrated genomics and tissue-based functional assays
Effort: 0.24 calendar
Supporting Agency: US Department of Defense (DOD)
Contracting/Grants Officer: Janet P. Kuhns
Performance Period: 09/30/18 to 09/29/22
Level of Funding:
Project Goals: We aim to evaluate tissue-based tests of HR proficiency to stratify patients to receive DNA repair targeting agents. In a two-step approach, we will optimize the test and study the correlation with genomic data in a cohort of mCRPC biopsies, to then implement the assay into a clinical trial to stratify patients for receiving treatment with carboplatin, a DNA damaging chemotherapy.
Specific Aims: 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 Ra-223, in samples from a prospective study. 2) To optimize tissue-based tests of HR functionality samples for CRPC samples, and study the correlation with genomic aberrations in HR genes. 3) To clinically qualify this HR functional test in a clinical trial of carboplatin in CRPC.
Overlap: None

Title: Pacific Northwest (PNW) Prostate Cancer Sponsored Program of Research Excellence (SPORE) Project 1: Molecular Predictors of Prostate Cancer Progression and Mortality
Effort: 1.20 calendar
Supporting Agency: NIH/NCI
Contracting/Grants Officer: Samantha Farrell, farrellsa@mail.nih.gov
Performance Period: 09/01/18 to 08/31/23
Level of Funding: (subaward Y2)
Specific Aims: The proposed study will ascertain and recruit germline cancer risk mutation carriers from: 1) population- and clinic-based incident cases of metastatic PC to find index cases with germline cancer risk mutations; 2) to conduct a PC early detection study incorporating novel biomarkers for unaffected, male germline mutation carriers (including first degree relatives of those with metastatic PC who are mutation carriers); and 3) to understand the cascade genetic testing process what will facilitate an innovative recruitment strategy for recruiting men at highest genetic risk of aggressive prostate cancer.
Overlap: None

Title: ACT PROMISE
Effort: 1.51 calendar
Supporting Agency: DOD PROSTATE CANCER CLINICAL TRIALS CONSORTIUM
Contracting/Grants Officer: ADVANCING CANCER TREATMENT
Performance Period: 06/19/2020 to 06/30/2022
Level of Funding:
Project Goals: The major goal of this study, in collaboration with Dr. Channing Paller, is to design, implement, recruit patients and identify prostate cancer patients who carry germline pathogenic variants, assessing frequency, family history, outcomes, longitudinal treatment response, treatment sequences and therapy combinations.

Specific Aims: 1) Identify and recruit subjects to a prospective registry of men with localized, biochemically recurrent, and metastatic prostate cancer with a germline pathogenic or likely pathogenic variant in one of the following cancer risk genes of interest — *ATM, BRCA1, BRCA2, BRIP1, CHEK2, MLH1, MSH2, MSH6, NBN, PALB2, PMS2, PTEN, RAD51C, RAD51D, and TP53* — using public education programs, outreach, and no-cost germline cancer risk testing. 2) Identify and recruit subjects with a variant of uncertain significance (VUS) in one of the cancer risk genes of interest. 3) Capture family history to assist with the interpretation of germline genetic testing results

Title: Technology-Enhanced Acceleration of Germline Evaluation for Therapy - The TARGET Study
Effort: 0.6 calendar
Supporting Agency: Prostate Cancer Foundation
Contracting/Grants Officer: Brigid Czyszczon, Brigid.Czyszczon@jefferson.edu
Performance Period: 08/06/20 – 12/31/22
Level of Funding:
Specific Aims: The proposed study will 1) evaluate understanding of providers around genetic testing in prostate cancer patients and uncover barriers to identifying patients who meet the NCCN guidelines for genetic testing. 2) develop a mobile app to assist providers in educating patients and identifying candidates for genetic testing. 3) devise a randomized clinical trial comparing mobile-assisted app to traditional, in-person genetic counseling for men with metastatic prostate cancer in different practice settings.
Overlap: None

Title: Enhanced Genetic Awareness and Genetic Evaluation for Men Through Technology - The ENGAGEMENT Study
Effort: 0.60 calendar
Supporting Agency: DOD W81XWH2010310
Contracting/Grants Officer: Jennifer Shankle, jennifer.e.shankle.civ@mail.mil
Performance Period: 09/30/2020-09/29/2023
Level of Funding: (subaward)
Specific Aims: The project will 1) Develop and implement a web-based virtual PCA genetics board across academic, community, and VA settings. Perceived usefulness, acceptability, self-efficacy for genetically-based recommendations, and genetics knowledge from dynamic case-based learning will be assessed. 2) Establish a web-based, national, patient-driven registry for any male who has undergone PCA genetic testing to assess men's experience with the genetic evaluation process and inform patient centered genetics practice and resource development. 3) Utilize digital media to share updated information on genetic testing and precision management of PCA through a public-facing podcast series.
Overlap: None

Title: c16_174 PCCTC MSK DORA
Effort: 0.0 calendar
Supporting Agency: Bayer Healthcare Pharmaceuticals, Inc.
Contracting/Grants Officer: Drew Davis: davisa1@mskcc.org
Performance Period: 10/1/18 – 9/30/22
Level of Funding:

Project Goals: The major goal of this study is to compare overall survival for subjects treated with docetaxel versus subjects treated with docetaxel plus radium-223.
Overlap: None

Title: CO-338-063
Effort: 0.00 calendar
Supporting Agency: Clovis Oncology
Contracting/Grants Officer: Ben Shoemaker; bshoemaker@clovisoncology.com
Performance Period: 8/21/2018 – 4/30/2023
Level of Funding:
Project Goals: The major goal of this study is to assess the efficacy of rucaparib versus physician's choice of treatment based on radiographic progression free survival (rPFS) in mCRPC patients with HRD who progressed on prior AR-directed therapy and have not yet received chemotherapy in the castration-resistant setting.
Overlap: None

PREVIOUS

Title: CCITLA: Cancer Clinical Investigator Team Leadership Award
Effort: 1.51 calendar
Supporting Agency: NIH/NCI
Contracting/Grants Officer: Fred Hutchinson Cancer Research Center (FHCRC)
Performance Period: 03/04/2020 to 02/28/2022
Level of Funding:
Project Goals: The major goal of this award is to develop access to clinical trials for patients using media and expand genetic care using new methods of delivery such as telehealth and molecular tumor boards, disseminating research opportunities to cancer center catchment and region.
Specific Aims: 1) to consider the genitourinary cancer clinical trial portfolio and translate individual clinical trials into different media-friendly format. 2) to expand cancer genetics care delivery to newer formats: telehealth and molecular tumor boards to expand delivery of care and disseminate research opportunities to our cancer center catchment and region
Overlap: None

Title: The Galahad Study: A phase 2 efficacy and safety study of niraparib in men with metastatic castration-resistant prostate cancer and DNA-repair anomalies
Effort: 0.60 calendar
Supporting Agency: Janssen Research & Development, LLC
Contracting/Grants Officer: Danielle Green, JD; dgreen32@its.jnj.com, (919) 803-6375
Performance Period: 12/28/16 to 12/31/21
Level of Funding:
Project Goals: The major goal of this project is to assess the efficacy of niraparib in men with mCRPC and DNA-repair anomalies who have measurable disease by looking at the objective response rate.
Specific Aims: To assess the efficacy of niraparib in subjects with mCRPC and DNA-repair anomalies.
Overlap: None

Title: Targeting the Subtype of Metastatic Prostate Cancer Deficient in DNA Repair Capacity Instability Using Targeted Molecular Counting Methods
Effort: 0.60 calendar
Supporting Agency: US Department of Defense (DOD)
Contracting/Grants Officer: Janet P Kuhns, Nrusingha C. Mishra (nrusingha.mishra.civ@mail.mil)
Performance Period: 08/01/2018 to 11/30/2021
Level of Funding:
Specific Aims: 1) Determine if germ-line and somatic aberrations in homologous recombination DNA re-pair pathways associate with responses to FDA-approved therapeutics in men with mCRPC. 2) Develop minimally-invasive biomarkers capable of distinguishing patients for therapeutics targeting homologous recombination DNA repair pathways and ascertaining resistance mechanisms. 3) Identify rational drug combinations that exploit DNA repair vulnerabilities to eradicate prostate cancers with homologous recombination repair deficiency.
Overlap: None

Title: CRISPR-excision and long-read sequencing of BRCA1, BRCA2, PALB2 and ATM to identify previously undetectable classes of mutations in families severely affected with advanced prostate cancer (PIs Tom Walsh, Heather Cheng)
Level (%) of effort: 1.20 calendar
Funding Agency: Brotman Baty Institute
Point of contact at the funding agency: Nola Klemfuss

Performance period: 02/01/2020 to 01/31/2021

Total level of Funding:
Goals of the project: The goal is to identify complex structural mutations in advanced prostate cancer families that have been missed by current sequencing approaches.
Specific Aims. 1. Using CATCH (Cas9-Assisted Targeting of Chromosomal segments), excise complete genomic regions (~200kb) of BRCA1, BRCA2, PALB2, and ATM from lymphoblast DNA of 80 prostate cancer patients from 40 families. 2. CATCH libraries and SMRT sequencing. Construct low-input barcoded libraries, then sequence to high depth of coverage, with reads up to 40kb in length, on a Sequel II instrument at the UW PacBio core. 3. Structural mutations and their consequences. Identify all genomic rearrangements >50bp in the four gene regions. For rare or private rearrangements, resolve genomic breakpoints and evaluate effects on gene expression and splicing using patients' lymphoblast RNA. 4. Clinical follow-up. Inform patients with positive test results and integrate new genetic information into their care following NCCN guidelines for mutation carriers.

Title: Genitourinary Cancer Clinical Research Database Support
Effort: 0.60 calendar
Supporting Agency: Institute for Prostate Cancer Research (IPCR)
Contracting/Grants Officer: Nola Klemfuss, klemfuss@uw.edu, (206) 667-3042
Performance Period: 09/01/14 – 12/31/19
Level of Funding:
Project Goals: The GU Cancer Clinical Research Database (GUCCRD) is designed to inventory, display, aggregate, and integrate comprehensive clinical information derived from patients with prostate cancer (and potentially other GU malignancies).

Specific Aims: Modify the existing database to integrate templated clinic notes from electronic medical record systems, develop/implement a system for long-term follow-up of patient outcomes, develop a system for typical database queries for research feasibility assessments, ensure all GU patient consent and data are entered, and numerous other enhancements.
Overlap: none

Title: Cancer Center Support Grant: New Investigator Award
Effort: 0.60 calendar
Supporting Agency: NIH/NCI (P30 CA015704)
Contracting/Grants Officer: cancerconsortium@fredhutch.org
Performance Period: 12/15/14 – 12/31/19
Level of Funding:
Project Goals: The major goal of this project is to recruit new investigators who will further the strategic objectives of the University of Washington/Fred Hutchinson Cancer Consortium. Specifically, this project will develop infrastructure to study the underlying genetic causes of early onset prostate cancer and familial prostate cancer.
Specific Aims: To be used at the PI's discretion to fund or supplement research projects as needed.
Overlap: none

Title: A precision clinical trial targeting DNA repair defects
Effort: 0.60 calendar
Supporting Agency: Institute for Prostate Cancer Research (IPCR)
Contracting/Grants Officer: Nola Klemfuss, klemfuss@uw.edu, (206) 667-3042
Performance Period: 07/01/15 – 12/31/19
Level of Funding:
Project Goals: The major goal of this project is to determine if men with tumors that harbor DNA repair defects will exhibit the hypothesized enhanced sensitivity to platinum-based chemotherapy.
Specific Aims: Correlative blood samples are collected prior to and during therapy (docetaxel and carboplatin), and at time of progression for analysis.
Overlap: None

Title: 2015 PCF Young Investigator Award: Identifying high-penetrance prostate cancer risk genes: leveraging families for next generation discovery and prevention
Effort: 2.40 calendar
Supporting Agency: Prostate Cancer Foundation
Contracting/Grants Officer: Andrea Miyahira, PhD; amiyahira@pcf.org, (310) 570-4705
Performance Period: 10/01/15 – 09/30/18
Level of Funding:
Project Goals: The major goals of this project are to collect families affected by prostate cancer and discover new prostate cancer risk genes, to collect men with prostate cancer who are found via tumor testing to carry high-penetrance germline cancer risk mutations, and to provide both groups of men and their family members with access to better educational materials, genetic counseling resources, and research opportunities.
Specific Aims: Recruit families with 2 or more relatives with prostate cancer, at least one with early-onset and/or aggressive prostate cancer; use next generation sequencing approaches to discover new high-penetrance prostate cancer risk genes in families; recruit men (and their families) with prostate

cancer who are known to carry mutations; develop and disseminate educational materials, genetic counseling guidelines, screening strategies/guidance and updated resources and research opportunities for men and their families who may have a high-penetrance prostate cancer risk gene.
Overlap: none

Title: PCa-001: Phase I, open-label trial to evaluate the safety and immugenicity of INO-5150 alone or in combination with INO-9012 in men with biochemically relapsed (PSA) prostate cancer
Effort: 0.60 calendar
Supporting Agency: Inovio Pharmaceuticals
Contracting/Grants Officer: Zane Yang, MD; zyang@inovio.com, (267) 440-4248
Performance Period: 11/12/15 – 11/11/18
Level of Funding:
Project Goals: The major goal of this study is to test the study drug INO-5150 (plasmid DNA vaccine) for prostate specific proteins alone or in combination with INO-9012 (plasmid DNA vaccine for human interleukin 12) to see how safe they are and if they cause any side effects or generate an immune response against prostate cancer cells when given by intramuscular injection followed by electroporation.
Specific Aims: Evaluate safety and tolerability, and cellular and hormonal immune responses to the treatment.
Overlap: none

Title: Pharmacogenetic dissection of protein synthesis control across the spectrum of PI3K pathway mutations in prostate cancer
Effort: 0.60 calendar
Supporting Agency: Movember Foundation & Prostate Cancer Foundation (2016CHAL1523)
Contracting/Grants Officer: Audrey Gardner, agardner@pcf.org, (310) 570-4792
Performance Period: 10/01/16 – 09/30/18
Level of Funding:
Project Goals: The major goal of this project is to delineate the biology of various PI3K pathway mutations that occur in CRPC and develop strategies to effectively target tumors harboring these mutations.
Specific Aims: 1) Determine the pervasiveness and necessity of aberrant protein synthesis across the spectrum of PI3K-AKT-mTOR pathway mutations in prostate cancer. 2) Determine the effects of highly specific PI3K pathway inhibitors on feedback and downstream translation activation in the context of distinct mutations. 3) Determine the therapeutic efficacy of targeting both the PI3K signaling pathway and oncogenic mRNA translation. 4) Define new biomarkers of specific PI3K pathway mutant CRPC and predictors of drug sensitivity.
Overlap: Dr. Cheng will be the site PI for the CC-115 study, which will provide the biospecimens used in Aim 4 of this proposal to develop a predictive biomarker. Dr. Cheng's effort on the CC-115 trial will be paid for by the Movember-PCF grant, and remaining costs for running the CC-115 trial will be paid on the PCCTC budget.

SALIPANTE, STEPHEN – SITE: UNIVERSITY OF WASHINGTON

Active

Title: PROMISE-OB-18 (68-4440)
Effort: 0.36 calendar months
Supporting Agency: Cystic Fibrosis Foundation
Performance Period: 06/01/2021 – 05/31/2022
Level of Funding: Total Award
Project Goals: This is a prospective, multi-center observational study. The study is designed to measure the clinical effectiveness of triple combination modulator therapy (TCT) in people with one or more copies of the F508del mutation, study the effects of TCT across a number of CF disease manifestations, and collect specimens for future research. Subjects in the study will have one "before TCT" visit within 30 days before initiation of the therapy and five "after TCT" visits over a 24-month follow-up period. Most participating sites will be divided into sub-study groups; each sub-study group will have specific non-optional procedures conducted in addition to the "Core" procedures. Finally, there are four optional procedures (pH pill, transient elastography, chest CT, and nasal cell procurement) that will be offered to subjects at certain sites. The duration of participation for each subject is 25 months.
Overlap: None
Role: Co-Investigator

Title: Defining the intrinsic cystic fibrosis respiratory phageome (63-1099)
Effort: 0.12 calendar months
Supporting Agency: Cystic Fibrosis Foundation
Performance Period: 11/01/2021 – 10/31/2022
Level of Funding: Total Award
Project Goals: We propose to define both the phageomes and microbiota in longitudinal sputum samples from people with CF during periods of stability, exacerbations, and antibiotic treatment. We hypothesize that changes in CF sputum bacterial microbiota during clinical change or treatment will be accompanied by corresponding changes in sputum phageomes within subjects. The results of this study will establish reliable methods for sampling and defining the CF sputum phageome and will have important implications for the predicted durability and efficacy of phage therapy for CF infections.
Overlap: None
Role: Co-Investigator

Title: Development and Implementation of a Tumor Type-Specific LOH Assay for the Clinical Determination of Homology Directed Repair Deficiency (68-3860)
Effort: 0.60 Calendar Months
Supporting Agency: Brotman Baty Institute
Contracting/Grants Officer: Nola Klemfuss
Performance Period: 2/1/2021 – 1/31/2023
Level of Funding: Total Award
Project Goals: In this proposal we will develop approaches for quantitating LOH using NGS assays, determine sensitive and specific LOH scores for the tumor type-specific HRD classification, and

integrate this assay into the UW-OncoPlex pipeline for the routine clinical assessment of HRD from tumor biopsies.
Overlap: None
Role: Co-Investigator

Title: Cystic fibrosis microbiological outcomes advancement core (63-5154)
Effort: 0.12 calendar months
Supporting Agency: Cystic Fibrosis Foundation
Performance Period: 4/1/2021 – 3/31/2022
Level of Funding: Total Award
Project Goals: This application is for support for a new research core specifically dedicated to non-standard microbiological tests for infections due to the disease cystic fibrosis. Specifically, this core will provide clinical isolates of bacteria to interested researchers, as well as performing tests for microbiological outcomes of novel CF therapies that are not performed by standard CF clinical laboratories.

Overlap: None

Role: Co-Investigator

Title: Combined Methylation and Mutation to Predict Response to PARP Inhibitors (62-1023)
Effort: 0.396 Calendar Months
Supporting Agency: National Institutes of Health
Performance Period: 5/1/2020 – 3/31/2025
Level of Funding: Total Award
Project Goals: The overall goal of the current proposal is to develop and validate a combined mutation and methylation assay as a clinical predictor of PARP inhibitor response. We will develop and refine a quantitative methylation assay, then test whether combining methylation and mutation analyses can predict PARP inhibitor sensitivity in patients with BRCA wildtype cancers using clinical samples from 4 large randomized controlled trials in ovarian and breast cancer.

Overlap: None
Role: Co-Investigator

Title: Understanding Staphylococcus aureus host-bacterium interactions that drive chronic infection in CF patients (66-2477)
Effort: 0.12 calendar months
Supporting Agency: Vertex Pharmaceuticals Inc
Contracting/Grants Officer: Emily Matusiak
Performance Period: 2/5/2020 – 2/4/2023
Level of Funding: Total Award
Project Goals: The major goals we propose are we hypothesize that polygenic mutations arising in S. aureus during CF infections can increase bacterial tropism for host airway cells and produce phenotypes that promote persistent infection. We will test this hypothesis and identify genes involved using a novel, cross disciplinary approach combining methods from evolutionary biology, population genetics, genomic sequencing, and genome editing.
Specific Aims:
Aim 1: Identify spontaneous mutations in S. aureus that promote increased persistence phenotypes in

CF. (Years 1-2)
Aim 2: Define variants associated with persistence phenotypes in S. aureus isolates from chronic CF infection. (Years 1-2)
Aim 3: Determine the function of mutations associated with persistence phenotypes in S. aureus using high throughput genome editing techniques. (Years 2-3)
Overlap: None
Role: PI

Title: Contribution of altered lipid metabolism to resistance to cell envelope-targeting antimicrobials in MRSA (61-6832)
Effort: 0.60 Calendar Months
Supporting Agency: National Institute of Health
Contracting/Grants Officer:
Performance Period: 8/15/2018 – 7/31/2022
Level of Funding: Total Award
Project Goals: The major goals we propose to comprehensively interrogate the mechanisms of resistance and cross-resistance to GP, LP, and LGP antimicrobials in MRSA by integrated lipidomics, genomics, and transcriptomics.
Overlap: None
Role: Co-Investigator

Title: Microbial Cell-Free DNA sequencing to Diagnose Respiratory Infection (63-0222)
Effort: 0.60 Calendar Months
Supporting Agency: Cystic Fibrosis Foundation
Performance Period: 08/01/2021 – 07/31/2022
Level of Funding: Total Award
Project Goals: Diagnosis of respiratory infection in people with cystic fibrosis (CF) has remained a persistent challenge by conventional techniques using in vitro microbiological culture of patient sputum, which is variably sensitive, specific, and informative. Moreover, with the rise of highly effective CFTR modulator therapies, CF patients are increasingly unable to generate sputum for diagnostic purposes. There is consequently an increasing need for alternative methods to diagnose respiratory infection in the CF patient population. This proposal will define robust methodology for identifying cfDNA from the circulation of CF patients, and will provide foundational data for establishing microbial cfDNA as a biomarker of infection and/or exacerbation in CF patients. Collectively, these efforts have great potential to enhance CF patient care through improved diagnosis of respiratory pathogens, enabling improved therapeutic interventions and patient outcomes.
Overlap: None
Role: PI

Title: Clinical qualification of DNA repair defects as biomarkers in metastatic prostate cancer using integrated genomics and tissue-based functional assays (61-7639)
Effort: 0.60 Calendar Months
Supporting Agency: National Institute of Health
Performance Period: 9/30/2018 – 9/29/2022
Level of Funding: Total Award
Project Goals: The major goals we propose will provide physicians tools to develop more effective

treatment strategies for men with mCRPC, by assessing DNA repair defects as predictive biomarkers of patient outcome to standard therapies. In the near term, developing and validating functional biomarkers of HR functionality would facilitate implementation of personalized treatment-decisions in mCRPC into clinical practice in the community and also provide valuable information to address mechanisms of drug resistances to PARP inhibitors and DNA damaging chemotherapy in this subclass of the disease. Eventually these data could be relevant for men with localized disease too, and help personalizing treatment to prevent progression to lethal disease.
Overlap: None
Role: PI

Title: A Prospective Study to Evaluate Effects of Corrected CFTR Function BEGIN (63-4915)
Effort: 0.48 Calendar Months
Supporting Agency: Cystic Fibrosis Foundation
Performance Period: 01/01/2020 – 12/31/2026
Level of Funding: Total Award
Project Goals: Cystic fibrosis (CF) is a result of mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) protein. CFTR dysfunction leads to disease in multiple organ systems, including the lungs, pancreas, liver, intestines, and blood. A triple-combination therapy for restoring CFTR function was approved in the US (elexacaftor/tezacaftor/ivacaftor [ETI]) for people with CF and one copy of the F508del mutation 12 years of age and older and is soon expected to be available to younger people with CF.
A Prospective Study to Evaluate Effects of Corrected CFTR Function (BEGIN) is designed to measure the direct and indirect effects of ETI by collecting and analyzing clinical research outcomes and biomarkers on infants and toddlers with CF both before and after they begin this treatment, focusing on the earliest stages of disease. The primary objective of Part A is to describe and define the natural history of growth, gastrointestinal health, and pulmonary function in infants and young children with CF without CFTR modulators, while Part B will describe changes in growth, gastrointestinal health, and pulmonary function in this age group following initiation of CFTR modulators.
Measures selected for analysis in this study include those for endocrine function, bone and body composition, gastrointestinal and respiratory symptoms, microbiology and inflammation, liver and pancreatic function, and sweat chloride. Blood, urine and stool will be collected to enable future research in this modulator-naive and exposed population. BEGIN will also provide a platform for a detailed imaging ancillary study.
Overlap: None

Title: Field Study to Understand Progression of Chronic Airway Infection (62-0010)
Effort: 0.60 Calendar Months
Supporting Agency: National Institute of Health
Performance Period: 8/1/2019 – 7/31/2023
Level of Funding: Total Award
Project Goals: This proposal investigates the contribution of genetic variation that evolves in Pseudomonas aeruginosa strains that infect cystic fibrosis (CF) patients to lung function decline in CF. This work will provide proof of principle for a new idea to explain disease variability that could have implications for many chronic infections.
Overlap: None

Title: UW RDP - Center for Basic and Translational Research in Cystic Fibrosis Respiratory Disease (63-1421)
Effort: 0.48 Calendar Months
Supporting Agency: Cystic Fibrosis Foundation
Performance Period: 10/1/2020 – 09/30/2022
Level of Funding: Total Award
Project Goals: This proposal will focus on providing instrumentation, computational infrastructure, technical and analytic expertise, and guidance to broadly enable and enhance the use of genomic analysis for research of bacteria important in CF airway infections. The Aims of the Core are to: 1) Provide sequencing and computational resources that advance research on important CF pathogens, with a focus on organisms under intense study at our center, 2) Provide sequencing and computational resources to facilitate understanding of airway microbial communities, and 3) Develop novel technologies for the genome-scale analysis of CF infections.
Overlap: None

Title: P30 Cystic Fibrosis Research Translation Center (68-4390)
Effort: 2.40 Calendar Months
Supporting Agency: Seattle Children's Hospital through NIH
Contracting/Grants Officer: Donna Crist
Performance Period: 6/1/2021 – 5/31/2022
Level of Funding: Total Award
Project Goals: The goals of the Genomics Core are to provide instrumentation, computational infrastructure, technical and analytic expertise, and guidance in order to broadly enable and to enhance the use of genomic analysis in Cystic Fibrosis (CF) research. Among other areas of research focus, the Genomics Core will support studies of microbial communities in the CF gut, will advance research on pathogens associated with CF disease states, and will develop novel technologies for the genome-scale analysis of CF microbiology.
Overlap: None

Changes/Ended

Title: Tuberculosis urine cell-free DNA: mapping biomarker fragments for development of point of care TB tests (68-2798)
Effort: 0.60 Calendar Months
Supporting Agency: Brotman Baty Institute
Contracting/Grants Officer: Nola Klemfuss
Performance Period: 2/1/2020 – 7/31/2021
Level of Funding: (Direct Costs)
Project Goals: Tuberculosis (TB) kills more people globally than any other infectious disease, and it is estimated that over ¼ of the global population is infected. Diagnosis using standard techniques requires a difficult process of expectorating mucus from deep in the lungs, and the resulting sputum (thick mucus) sample requires special processing that can be done only by trained professionals (at their own risk) or expensive instruments, both of which are lacking in low-income regions where TB is most prevalent.
Specific Aims:

Aim 1: Map TB cfDNA fragments from patient urine (and plasma) by next-generation sequencing (**NGS**) using extraction, library preparation, and target enrichment techniques designed specifically for short DNA.
Aim 2: Confirm NGS fragment characterization and develop assays for selected TB targets.
Overlap: None
Role: PI

Title: Cystic Fibrosis Microbiological Outcomes Advancement Core (66-3690)
Effort: 0.12 calendar
Supporting Agency: Cystic Fibrosis Foundation
Performance Period: 4/20/2020 – 3/31/2021
Level of Funding: (Direct Costs)
Project Goals: We propose to create a Core that will develop and provide special and clinically-relevant microbiological tests and resources for CF research and therapy development, including clinical specimens and microbial isolates, uncommon culture media and conditions, and molecular (DNA-based) microbiological identification and quantitation. This Core, the CF Microbiological Outcomes Advancement Core (CF-MOAC), will serve as a repository of the specimens and isolates collected by CF studies, as a facility that can perform existing, nonstandard tests (such as sputum binding and in vitro susceptibity testing for new candidate treatments), and as a laboratory that develops new approaches and resources and performs new tests, such as DNA-based analyses. These services will be provided to researchers in academia and industry developing new therapies and studying the microbial determinants of disease and response to treatment, complementing the standard services currently provided by CF clinical laboratories.
Overlap: None
Role: Co-Investigator

Title: Advanced development and validation of genome-scale molecular diagnostics for microsatellite instability using targeted molecular counting methods (61-3459)
Effort: 0.48 calendar
Supporting Agency: National Institute of Health
Contracting/Grants Officer: Rao Divi
Address of Funding Agency: 6705 Rockledge Drive, Bethesda, MD 20892-7986
Performance Period: 2/8/2018 – 1/31/2021
Level of Funding: (Direct Costs)
Project Goals: New forms of cancer treatment are extremely effective against tumors affected by genomic instability, but our ability to practicably identify and classify tumors with this feature in clinical practice is limiting. This proposal outlines the advanced development and validation of ultrasensitive, cost effective, and highly accurate methods for detecting, classifying, and analyzing genomic instability in tumor specimens. This technology will be suitable for use as a clinical diagnostic, and will provide enhanced capabilities including improved prognostic approximation and generality across tumor types, while offering improved performance characteristics over existing standards of care.
Specific Aims:
1) scalability – thousands of microsatellites could be examined, far in excess of the five loci queried by current multiplexed PCR approaches
2) generality – the assay would work for all cancer types, whereas current methods are optimized only

for colorectal cancers
3) quantitative precision – a quantitative measure of MSI would be provided, rather than simple positive or negative classification
4) sensitivity and specificity – the correct diagnosis would be reliably and accurately achieved, even in heterogeneous specimens.
Overlap: None
Role: PI

Title: Cross-validation of University of Washington OncoPlex NGS panel tumor mutation burden analysis against Foundation One CDx (63-5907)
Effort: 0.12 calendar
Supporting Agency: Bristol-Myers Squibb Company
Contracting/Grants Officer: Kimberly Gray
Address of Funding Agency: Route 206 and Province Line Road, Princeton, New Jersey 08543
Performance Period: 10/31/2018 – 7/29/2021
Level of Funding: (Direct Costs)
Project Goals: TMB is a predictive biomarker of response to immune checkpoint blockade therapies. We have developed a 261 gene next-generation sequencing (NGS) panel capable of determining TMB and microsatellite instability (MSI) status, as well as somatic mutations in cancer-related genes (e.g. targetable oncogene driver mutations). Our TMB determination has been validated via strong correlation with mutations in hypermutation genes, microsatellite instability burden, and mutations in DNA repair genes and POLE.
Overlap: None
Role: PI

Title: Development and clinical validation of quantitative and ultrasensitive chimerism detection by single-molecule molecular inversion probe capture of copy number deletion polymorphisms (68-2352)
Effort: 0.12 calendar
Supporting Agency: Brotman Baty Institute
Contracting/Grants Officer: Nola Klemfuss
Performance Period: 1/1/2019 – 12/31/2020
Level of Funding: (Direct Costs)
Project Goals: Here, we propose a set of methodological and computational improvements to smMIP-based chimerism detection which will dramatically improve its ability to accurately quantitate minor cell populations. In brief, we will incorporate smMIP probes targeting number constant control loci, and will subsequently utilize compositional data analysis and computational modeling to predict the abundance of chimeric cells in an experimental population. Following these enhancements, we will perform clinical grade validation of the assay to enable its diagnostic use for patient care. smMIPs are inexpensive and scalable to high sensitivity and large numbers of informative markers, and will enable ultrasensitive and quantitative chimerism detection for many clinical applications.
Specific Aims:
Aim 1: Improve chimerism quantitation by smMIP
Aim 2: Clinical validation using patient specimens
Overlap: None
Role: PI

Title: Resistance Selection Potential of the Long Acting Lipoglycopeptides Dalbavancin and Oritavancin (61-3910)
Effort: 0.60 calendar
Supporting Agency: National Institute of Health
Address of Funding Agency: 6705 Rockledge Drive, Bethesda, MD 20892-7986
Performance Period: 5/1/2019 – 4/30/2020
Level of Funding: (Direct Costs)
Project Goals: This proposal will focus on the recently approved long-acting lipoglycopeptides, dalbavancin and oritavancin, which have the longest half-lives of any available antibacterial (8-16 days). This slow clearance means that dalbavancin and oritavancin persist at low levels in the body for months after a single dose. These prolonged low-level exposures carry a high theoretical risk to select for resistance to the lipoglycopeptides but also cross-resistance to related drugs, including vancomycin, the standard of care for treating the "superbug" methicillin-resistant Staphylococcus aureus (MRSA). We have demonstrated that in vitro dalbavancin exposure can select for resistance to vancomycin and daptomycin, and observed this cross-resistance emerge from a patient who received dalbavancin for an MRSA infection. Using a novel and comprehensive lipidomic approach, we observed for the first time that dalbavancin resistance was associated with a loss of phosphatidylglycerol and digalactosyldiacylglycerol species from the cell membrane. Using whole genome sequencing, we identified mutations in six genes, two of which strongly linked to the emergence of vancomycin and daptomycin-nonsusceptible MRSA.
Overlap: None
Role: Co-Investigator

Title: A precision medicine approach to understand antibiotic action (68-2352)
Effort: 0.36 calendar
Supporting Agency: Brotman Baty Institute
Contracting/Grants Officer: Nola Klemfuss
Performance Period: 1/1/2019 – 12/31/2020
Level of Funding: (Direct Costs)
Project Goals: This study uses sputum samples and data from an ongoing NIH-funded clinical trial to identify mechanisms that may decrease antibiotic effectiveness when drugs are co-administered.
Specific Aim:
Aim 1: Improve chimerism quantitation by smMIP
Aim 2: Clinical validation using patient specimens
Overlap: None
Role: Co-Investigator

Title: UW RDP - Center for Basic and Translational Research in Cystic Fibrosis Respiratory Disease (66-6597; 63-5597)
Effort: 0.60 calendar
Supporting Agency: Cystic Fibrosis Foundation
Contracting/Grants Officer: Donna Crist
Address of Funding Agency: 2001 Eighth Ave, Suite 500; Seattle, WA 98121
Performance Period: 10/1/2019 – 09/30/2020
Level of Funding: (Total and Direct Costs)

Project Goals: This proposal will focus on providing instrumentation, computational infrastructure, technical and analytic expertise, and guidance to broadly enable and enhance the use of genomic analysis for research of bacteria important in CF airway infections.
Specific Aims:
The Aims of the Core are to: 1) Provide sequencing and computational resources that advance research on important CF pathogens, with a focus on organisms under intense study at our center, 2) Provide sequencing and computational resources to facilitate understanding of airway microbial communities, and 3) Develop novel technologies for the genome-scale analysis of CF infections.
Overlap: None
Role: PI

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: Vall D'Hebron Institute of Oncology (VHIO)
Award # W81XWH-18-1-0758
PC170510P1
PI: Joaquin Mateo
Location of Organization: Barcelona, Spain

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