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Pritchard, Colin

E-Mail: cpritch@uw.edu

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University of Washington
4333 Brooklyn Ave NE
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14. ABSTRACT

This proposal will address the challenge of effectively treating mPC by exploiting specific tumor vulnerabilities conferred by defects in HR DNA repair. 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.

15. SUBJECT TERMS
Prostate cancer, CRPC, DNA repair, cell-free DNA, ctDNA, germline mutation, VUS, HDR-D, BRCA1, BRCA2, Lynch

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

   This proposal is designed to address the challenge of effectively treating men with metastatic castration resistant prostate cancer (mCRPC) by exploiting specific tumor vulnerabilities conferred by defects in a specific type of DNA repair: homology-directed repair (HDR). 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 homology-directed repair deficient (HDR-D) mCRPC to test the hypothesis that aberrations in key genes that repair DNA strand breaks by homology-directed repair (HDR) are predictive of meaningful clinical responses to FDA-approved genotoxic therapeutics (e.g carboplatin) and to emerging therapeutics (PARP inhibitors, WEE1 inhibitors and other drugs). The proposal will also develop approaches to identify men with tumors exhibiting HDR-D, and strategies to enhance initial responses and assess mechanisms of resistance to genotoxic agents.

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

   Prostate cancer, CRPC, DNA repair, cell-free DNA, ctDNA, germline mutation, VUS, HDR-D, *BRCA1, BRCA2*, Lynch

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?
   This is a partnering PI award with research sites at SIBCR/VA Puget Sound, Fred Hutchinson Cancer Research Center, and the University of Washington. The aims and applicable sub-tasks:

   AIM 1: Determine if germ-line and somatic aberrations in homologous recombination DNA repair pathways associate with responses to FDA-approved therapeutics in men with mCRPC.

   Subtask 1: IACUC, ACURO, IRB and HRPO approval (1-4)
   ***100% complete*. Nelson, Pritchard and Montgomery teams/sites have approvals for the proposed work. Note that IACUC and IRB (and ACURO/HRPO) modifications will likely be made/requested to exploit research findings in accordance with the SOW.**

   Subtask 2: Initiate study start-up procedures (staff and physician training). (1-2)
   ***100% complete*. Montgomery Team.**

   Subtask 3: Identify patients with mCRPC and germ-line and/or somatic defects in HDR. (2-24)
   ***50% complete.* Montgomery and Pritchard Teams have identified patients with mCRPC and have identified a subset with germline and/or somatic HDR-D (see Accomplishments).**

   Subtask 4: Extract DNA, complete genomic sequencing, and identify variants conferring HDR-D and variants with uncertain significance. (3-24 months)
   ***10% complete.* Montgomery and Pritchard Teams have extracted DNA and completed genomic sequencing on mCRPC patients during routine clinical care and identified variants deemed to confer HDR-D and others with uncertain significance (see Accomplishments).**

Subtask 5: Obtain tumor biopsies prior to initiating treatment and at the time of treatment resistance. (2-34 months)

5% complete. **Montgomery Team has organized and trained clinical staff to collect tumor biopsies in a manner that preserves nucleic acids/analytes for molecular analyses. IRB approvals were obtained.**

Subtask 6: Sequence tumor DNA from study patients and identify aberrations in DNA repair and possible resistance alterations. (3-34 months)

10% complete. **All Teams. During monthly Team meetings and a sequencing tumor board, we have identified aberrations in DNA repair genes from the Oncoplex clinical assay (see Accomplishments)**

Subtask 7: Integrate VUS data with databases from breast cancer, ovarian cancer, and other malignancies associated with hereditary DNA repair aberrations. (3-34).

30% completed. **Pritchard Team-see Accomplishments.**

Subtask 8: Conduct cause-effect studies of HDR-D and VUS using preclinical models to ascertain pathogenicity. (3-34)

20% completed. **The Nelson Team has completed DNA sequencing on 30 preclinical models and identified a subset with VUS and HDR-D using a novel assay for HDR-D termed iHRD (see Accomplishments).**

Milestone #1: Prepare and submit manuscript detailing clinical response rates (depth and duration) to agents targeting HDR-D in men with documented aberrations in HDR (germ-line and somatic). (24-30)

10% completed. **All Teams. Data (HDR gene mutations) that will be used in the manuscript are being collected by all teams.**

Milestone #2: Present data and prepare and submit manuscript detailing the resistance mechanisms to Platinum in men with documented aberrations in HDR (germ-line and somatic). (33-35)

10% completed. **All Teams. Data (HDR gene mutations) that will be used in the manuscript are being collected by all teams.**

AIM 2: Develop minimally-invasive biomarkers capable of distinguishing patients for therapeutics targeting homologous recombination DNA repair pathways and ascertaining resistance mechanisms.

Subtask 1: Assemble a panel of genes involved in homology directed DNA repair comprising current data from SU2C, UWMC TAN, clinical sequencing and studies of breast and ovarian cancer.

100% completed. **Nelson Team and Pritchard Team. The gene panel is now included in the next iteration of the Oncoplex clinical assay (see Accomplishments).**

Subtask 2: Develop a NextGen sequencing strategy comprehensively targeting relevant DNA repair genes and suitable for minimally-invasive assessments (germ-line and tumor). (2-3)

100% completed. **Nelson Team and Pritchard Team. The gene panel is now included in the current iteration of the Oncoplex clinical assay (see Accomplishments).**

Subtask 3: Confirm metrics of sensitivity and specificity using blood, saliva, tissue for germ-line assessments. (4-12)

100% completed. **Pritchard Team-see Accomplishments. The Pritchard team will continue to refine the assay to increase efficiency and throughput and reduce cost.**

Subtask 4: Determine concordance and discordance of assay performance comparing minimally-invasive assessments with tumor assessments for clinical trial participants. (12-34)

100% completed. **Nelson Team and Pritchard Team. See Accomplishments.**

Subtask 5: Determine assay performance in longitudinal assessments of tumor responses and assessing resistance mechanisms. (6-34)

10% Completed. **Nelson Team and Pritchard Team. Samples (biospecimens) from existing University of Washington tissue banking protocols are banked for the planned retrospective studies designed to assess longitudinal alterations.**

Subtask 6: Submit data for CLIA/CAP approval of assays. (18)

100% Completed. **Pritchard Team-see Accomplishments. The Oncoplex tissue and plasma assays are CLIA/CAP approved.**

Milestone #3: Prepare and submit manuscript detailing the performance characteristics of minimally- invasive assays for DNA repair aberrations. (16-20)

80% Completed. **All Teams. We assembled and published a manuscript detailing the performance characteristics of ctDNA Oncoplex (led by Dr. Pritchard). See Accomplishments and Products. Additional manuscripts may be forthcoming as assays are modified.**

Milestone #4: Prepare and submit manuscript detailing the utility of minimally-invasive assessments of DNA repair aberrations to impact patient care: identification of appropriate patients for treatment and monitoring responses. (30-34)

10% Completed. **All Teams. The first generation assay is now available and the preliminary results indicate that it will be useful for identifying a subset of patients with DNA repair aberrations. See Accomplishments.**

AIM 3: Identify rational drug combinations that exploit DNA repair vulnerabilities to eradicate prostate cancers with homologous recombination repair deficiency.

Subtask 1: Develop and characterize preclinical models and systems that recapitulate mechanisms of HDR-D identified in human CRPC. (1-24)

100% Complete. **Nelson Team – 18 preclinical models established and characterized –**

Subtask 2: Conduct pre-clinical studies comparing concurrent vs sequential chemotherapy plus PARPi treatment strategies. (1-12)

30% Complete. **Nelson Team – PDX lines propagated and 4 therapies initiated. Analysis of 2 therapies were completed – pharmacological testosterone and inhibition of DNAP-Kcs. (see Accomplishments).**

Subtask 3: Conduct pre-clinical studies evaluating rational combinations of drugs that co-target HDR-D tumor vulnerabilities. (8-30)

PDX lines propagated and 4 therapies initiated. Analysis of 2 therapies were completed – pharmacological testosterone and inhibition of PARP. A manuscript detailing these results was published (see Accomplishments and Products).

Subtask 4: Develop informatics-based signature of HR DNA repair defect to benchmark with known DRG mutations and with clinical responses. (1-6)

100% Complete. **Nelson and Pritchard Teams. An integrated 'signature' of HR deficiency was constructed termed iHRD (see Accomplishments).**

Subtask 5: Conduct yearly discussions with the PCCTC investigators regarding opportunities for trials designs in HDR-D PC. (12-36)

20% Complete. **Nelson and Montgomery Teams-interacted with clinical colleagues/collaborators regarding trial designs/opportunities.**

Milestone #5: Prepare and submit manuscripts detailing the development and characterization of new preclinical models for sharing with the scientific community. (12-24)

10% Complete. **All teams. Data from model systems developed and manuscript writing initiated.**

Milestone #6: Prepare and submit manuscript detailing the effects of drug combinations targeting HDR-D PC. (18-34 months)

10% completed**. All Teams. Data detailing the first drug combinations of supraphysiological testosterone and the PARPi olaparib was published (see Accomplishments and Products).**

Milestone #7: Prepare and submit final report. (month 36)

All Teams – 0% completed.

What was accomplished under these goals?

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1. MAJOR ACTIVITIES. The major activities this first annual reporting period followed the approved statement of work. In summary, the activities to date have been largely focused on: Obtaining required approvals for the clinical and preclinical studies (Aim 1), training team personnel on clinical protocols and biospecimen acquisition/handling (Aim1), identifying sequence alterations associating with HDR-D (Aim 1), the development and validation activities for minimally-invasive assays to detect HDR-D (Aim 2), developing model system for testing therapeutics targeting HDR-D (Aim 3), conducting experiments to target HDR-D cancers (Aim 3).

2. SPECIFIC OBJECTIVES. The specific objectives for Y1 of the project are in alignment with addressing the three specific aims:

Aim 1. Determine if germ-line and somatic aberrations in homology-directed DNA repair pathways associate with responses to FDA-approved therapeutics in men with mCRPC.

Aim 2. Develop minimally-invasive biomarkers capable of distinguishing patients for therapeutics targeting defective homology-directed repair pathways and ascertaining resistance mechanisms.

Aim 3. Identify rational drug combinations that exploit tumor homology-directed DNA repair deficiency to eradicate prostate cancers with these defects.

3. SIGNIFICANT RESULTS AND KEY OUTCOMES.

AIM 1: Determine if germ-line and somatic aberrations in homologous recombination DNA repair pathways associate with responses to FDA-approved therapeutics in mCRPC.

Dr. Pritchard has a study currently IRB approved and open locally that is accruing men with metastatic prostate cancer and is enriched for men who have HDR in their tumors (UW-OncoPlex Prostate Cancer Precision Medicine Program, **Figure 1**). Patients participating in this study provide consent for future research including the work involved in the present proposal.

This study was open prior to receiving the award and will be appropriate for the endpoints laid out in the project as stated once the relevant modifications are approved. To date, tumor, germline, and cell-free DNA samples have been collected from a total of 210 men with metastatic prostate cancer. Germline sequencing through BROCA and tumor sequencing using UW-OncoPlex has identified 45 men with pathogenic or likely pathogenic HDR-D variants and 15 men with VUS HDR-D variants (germline or somatic) in this cohort to date. BROCA is a 60-gene panel focused on detecting germline HDR-D and UW-OncoPlex (version 6) is a >350 gene comprehensive tumor sequencing panel that includes HDR-D. Both panels are performed in Dr. Pritchard's CLIA-certified lab and have been validated for use in patient care decision making.

In year 1 the UW-OncoPlex targeted gene capture panel was validated for use as a minimally invasive assay in men with metastatic prostate cancer in the CLIA-lab setting and is now available for clinical use. This validation involved paired tumor, cell-free plasma DNA and whole blood (germline) testing in 93 prostate cancer patients. In parallel, a new targeted ~200kb "mini-panel" that includes full exon sequencing of 23 key HDR genes as well as *AR*, and key oncogenic driver coverage has been developed and has been analytically validated in the CLIA laboratory setting using anonymized samples with known mutations in HDR. This DNA probe capture-based panel has been used to successfully blindly detect circulating tumor DNA alterations in CAP cell-free DNA proficiency testing surveys to date.

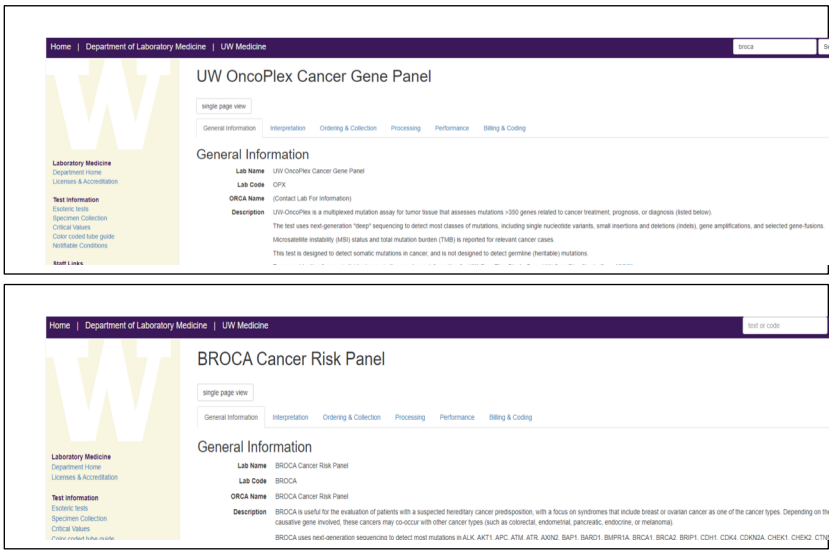


Figure 1. *Portals for the UW OncoPlex and BROCA cancer panels.*

Pritchard, Nelson and Montgomery participate together in a monthly sequencing tumor board where clinical assays are used to identify DNA repair gene (and other actionable genomic events) in tumor/germline of men with advanced prostate cancer (**Figure 2**). A subset of men are eligible for the studies comprising the present proposal – e.g. the intervention trials that comprise Aim 1. Further, variants of uncertain significance identified through these studies are compiled into the variant database.

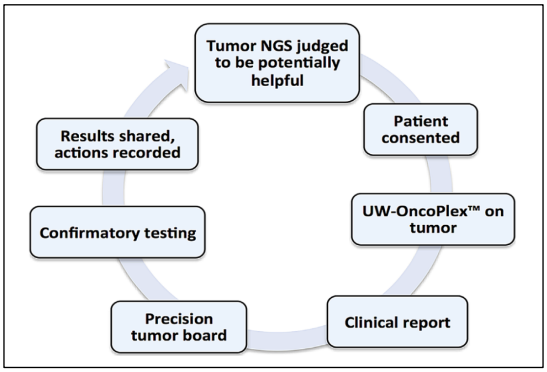


Figure 2. *Process for molecular assessments of mCRPC for sequencing, precision tumor board, and interventional trials.*

The Pritchard group has created a searchable genetic variant database **(Figure 3**) that includes all genetic variants detected in prostate cancer patients tested. This database is used to interface with ClinVar and will interface with other disease-specific database of genetic variants

The Pritchard group has been working on new and innovative approaches to classifying VUS in HDR-D genes, with emphasis on VUS in *BRCA1* and *BRCA2*. This work focuses on two areas: 1) Incorporation of high-throughput functional assays and 2) Defining new criteria for variant classification. Recently the Shendure and Starita groups at the University of Washington published a comprehensive functional analysis of all genetic variants in key regions of *BRCA1* they term "saturation genome editing" (Findlay et al. *Nature* PMID:30209399). Using the Pritchard lab genetic variant database, and with direct help from first author of the study Greg Findlay during an extended training rotation in the Pritchard lab, we annotated all prostate cancer variants in *BRCA1* according to the functional assay scores (**Figure 4**).

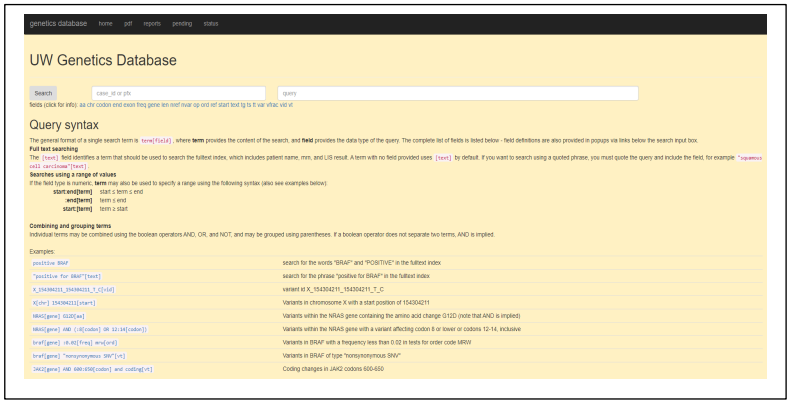


Figure 3. *Germline variant database (Pritchard Lab).*

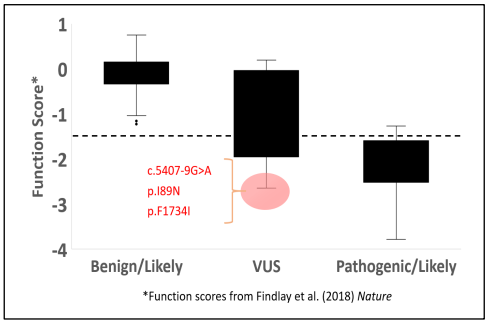


Figure 4: *BRCA1 function Scores from Findlay et al. 2018 associated with clinical variant interpretations in the Pritchard Lab. Highlighted are variants classified as VUS, but are likely pathogenic based on a low functional score.*

Updating variant classification guidelines: To assist in defining new and improved ways to perform genetic variant classification of HDR-D genes we employed Baysian approaches to identify "coldspots" in *BRCA1* and *BRCA2* genes where pathogenic missense variants (MVs) are extremely infrequent. We used this reasoning to re-classify 1,627 missense VUS in *BRCA1* and 3,150 missense VUS in *BRCA2* as likely benign (**Figure 5**, manuscript under review, with acknowledgement of federal funding from this award). We anticipate that coldspots will become a part of formal variant classification guidelines to improve variant interpretation.

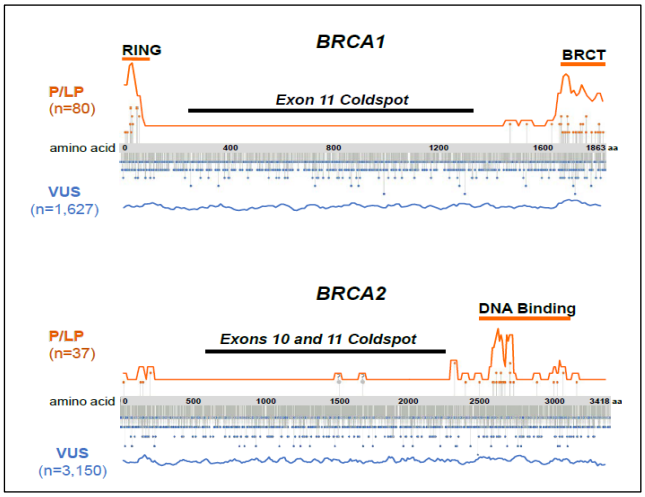


Figure 5: Distributions of missense variants in *BRCA1* and *BRCA2*. Missense variants (MVs) are indicated by lollipops. For each gene, distributions of pathogenic and likely pathogenic (P/LP) MVs are shown in orange above the gene and of VUS in blue below the gene. *BRCA1* exon 11 and *BRCA2* exons 10 and 11 harbor virtually no P or LP MVs and are defined as coldspots. We suggest reclassifying the 2500 VUS in these coldspots as likely benign. Two MVs in the *BRCA2* coldspot classified as P in ClinVar are indicated with "?" and may be misclassified.

AIM 2. Develop minimally-invasive biomarkers capable of distinguishing patients for therapeutics targeting homologous recombination DNA repair pathways and ascertaining resistance mechanisms.

We (Pritchard, Nelson, Montgomery) assembled a set of a set of 23 key DNA repair genes for complete coding sequencing coverage with targeted regions in an additional 38 genes relevant to prostate cancer treatment decision making (**Figure 6**). This panel uses IDT DNA-based probes and hybrid-capture next-generation sequencing methods on an Illumina instrument that are well-established in our CLIA-certified laboratory. The total capture size of this mini-panel is ~200kb.

DNA Repair*		Additional Genes		
ATM	*MSH6*	*AKT1*	*H3F3A*	*NRAS*
ATR	*NBN*	*ALK*	*HIST1H3B*	*NTRK1*
BARD1	*PALB2*	*AR*	*HIST1H3C*	*PDGFRA*
BLM	*PARP1*	*BRAF*	*HRAS*	*PIK3CA*
BRCA1	*PMS2*	*CDKN2A*	*IDH1*	*PTCH1*
BRCA2	*POLE*	*CTNNB1*	*IDH2*	*PTEN*
BRIP1	*POLQ*	*EGFR*	*JAK2*	*RB1*
CDK12	*RAD51C*	*ERBB2*	*KIT*	*RET*
CHEK2	*RAD51D*	*ESR1*	*KRAS*	*ROS1*
MLH1	*TP53BP1*	*FGFR1*	*MAP2K1*	*SETBP1*
MRE11A	*XRCC2*	*FGFR2*	*MET*	*SPOP*
MSH2		*FGFR3*	*MYC*	*TERT*
			MYCN	*TP53*

*All coding regions sequenced

Figure 6. *Genes Included in the Minimally-Invasive Mini-Oncology Panel ("MONC")*

We have taken three parallel approaches to developing NGS strategies for minimally invasive assessment of DNA repair genes in advanced prostate cancer: First, Validate our large comprehensive UW-OncoPlex NGS panel for use with cell-free DNA (~2Mb panel); Second, Develop and validate and DNA repair-focused "Mini-panel" as described above (200kb panel); Third, improve the accuracy of HDR-D by incorporating other parameters indicative of HDR-D such as copy number alterations and mutation signatures. We term this iHRD for 'integrated HRD.

Each approach has strengths and limitations and is appropriate for different prostate cancer clinical scenarios. The Mini-panel is more cost-effective and is sequenced to very high depth (we are targeting ~2,000x coverage) due to its relatively smaller size. The UW-OncoPlex panel, by contrast, has the advantage of being far more comprehensive, being already well-established as a clinical assay (it has been used by our lab in tumor tissue testing in the CLIA setting for over 8 years), and having more accurate determination of total mutation burden and microsatellite instability due to larger capture size. The disadvantage of UW-OncoPlex is that it is not cost-effective to sequence to high depth of coverage, although we still target ~800x coverage. Therefore the limit of detection is not as good at the mini-panel. The iHRD approach has not been validated in a clinical setting

For each approach we are pursuing paired cell-free DNA/whole blood sequencing that enables germline and somatic mutation calling, while filtering out clonal hematopoiesis of indeterminate potential mutations (CHIP) that are very common in older individuals and often interfere with commercial assays.

Validation of UW-OncoPlex CT*:* UW-OncoPlex has been previously extensive validated for use in whole blood, saliva, fresh tissue, and formalin-fixed tissue (see Pritchard et al. Journal of Molecular Diagnostics 2014 PMID:24189654), including sensitivity, specificity, within- and between run reproducibility, and limit of detection.

For validation of UW-OncoPlex for use in plasma cell-free DNA ("UW-OncoPlex CT test") in men

with metastatic prostate cancer blood plasma, tumor tissue, and paired whole blood (germline) samples were obtained from patients with advanced prostate cancer. UW-OncoPlex was optimized for detection of cfDNA mutations using low-input samples by modification in the library prep that forgo the shearing step and included the KAPA hyperprep method to allow low input quantities down to 5ng. Multivariate logistic regression was performed to determine the clinical characteristic that associated with successful detection of somatic cfDNA alterations (defined as detecting at least one clearly somatic PC mutation).

Under a previously IRB approved protocol the Pritchard group obtained plasma for cfDNA sequencing from 93 prostate cancer patients with tumor tissue (N=67) and germline (N=93) controls and included data from 76 patients (72 prostate adenocarcinoma; 4 variant histology) in the analysis. Thirty-four cfDNA samples were successfully sequenced from patients with prostate adenocarcinoma, with detection of somatic DNA aberrations, including copy number changes. High PSA level, high tumor volume, and castration-resistance were significantly associated with successful detection of somatic cfDNA alterations. Among samples with somatic mutations detected, the cfDNA assay detected 93/102 (91%) somatic alterations detected in tumor tissue and yielded a clustering-corrected sensitivity of 92% (95% confidence interval 88%–97%). All germline pathogenic variants found in lymphocyte DNA were detected in cfDNA (N=12). Overall, somatic mutations from cfDNA were detected in 30/33 (93%) instances when PSA was >10ng/ml.

Disease burden, including a PSA >10ng/ml, is strongly associated with detecting somatic mutations from cfDNA specimens (**Figure 7**). We have validated UW-OncoPlex for cfDNA analysis in the CLIA/CAP lab environment in advanced prostate cancer patients with high burden disease.

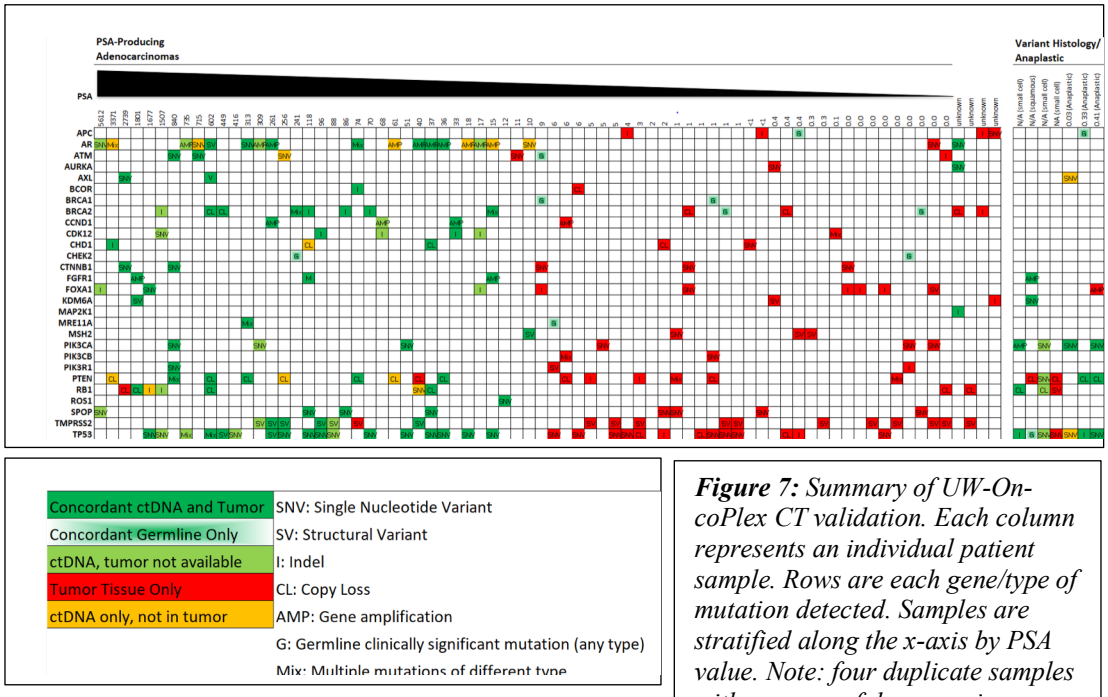


Figure 7: *Summary of UW-OncoPlex CT validation. Each column represents an individual patient sample. Rows are each gene/type of mutation detected. Samples are stratified along the x-axis by PSA value. Note: four duplicate samples with unsuccessful sequencing are not presented in this figure. Aggressive-variant (AV) prostate cancers were defined based on previously described clinical features (e.g. predominately visceral metastases, low PSA level compared to disease burden, short interval to developing CRPC.*

Validation of the "MONC" mini-panel: For validation of the DRD oriented mini-panel we first focused on germline blood and saliva samples from patients with known DNA repair mutations based on clinical testing by BROCA or other clinically-validated assays. We identified 55 patient samples with known pathogenic or likely pathogenic germline mutations in HDR-D

genes including *BRCA1, BRCA2, MLH1, MSH2, MSH6, PALB2,* and *PMS2*. We focused on cases with analytically challenging-to-detect mutations such as copy number variants and indel mutations. We paid particular attention the distal exons of PMS2 (exon 13-15) which have a highly homologous pseudogene that makes this region especially challenging for short-read NGS methods. Overall 54/55 cases the germline mutations were accurately picked up by the mini-panel (98.1% sensitivity). (**Figure 8**). The one case that was not called correctly had a deletion of exons 12-15 of *PMS2* in the pseudogene region. For this case, there was a call of pathogenic *PMS2* mutation, but only exon 12 was called as deleted due to the pseudogene interference. In practice, our protocol is to validate all copy number variants in *PMS2* by orthogonal methods, so this case would also have been correctly identified following orthogonal confirmation.

Table: Validation of Germline Pathogenic DNA Repair Variants by the "MONC" mini-panel

Gene	p.	c.	VAF	VAF from BROCA	Mutation Validated?
BRCA1	p.E23Vfs*17	NM_007294.3:c.68_69del	0.48	0.4	yes
BRCA1	p.K381Efs*3	NM_007294.3:c.1140dup	0.45	0.48	yes
BRCA1	p.Q1756Pfs*74	NM_007294.3:c.5266dup	0.45	0.5	yes
BRCA1	p.V233Nfs*4	NM_007294.3:c.697_698del	0.47	0.48	yes
BRCA1	exon 17 deletion	copy number variant	NA	NA	yes
BRCA1	exon 13 duplication	copy number variant	NA	NA	yes
BRCA1	exons 21-24 deletion	copy number variant	NA	NA	yes
BRCA1	p.Q563*	NM_007294.3:c.1687C>T	0.47	0.5	yes
BRCA1	p.T276Afs*14	NM_007294.3:c.815_824dup	0.19	0.33	yes
BRCA2	p.S1230Yfs*2	NM_000059.3:c.3689_3690del	0.45	0.47	yes
BRCA2	p.Y1655*	NM_000059.3:c.4965C>G	0.47	0.44	yes
BRCA2	p.S1882*	NM_000059.3:c.5645C>A	0.46	0.44	yes
MLH1	p.L73P	NM_000249.3:c.218T>C	0.47	0.48	yes
MLH1	p.L559R	NM_000249.3:c.1676T>G	0.49	0.45	yes
MLH1	p.I276Nfs*31	NM_000249.3:c.826dup	0.47	0.48	yes
MLH1	p.D12Efs*4	NM_000249.3:c.36_39del	0.46	0.47	yes
MLH1	exon 10 deletion	copy number variant	NA	NA	yes
MLH1	exon 16-19 deletion	copy number variant	NA	NA	yes
MLH1	p.G67R	NM_000249.3:c.199G>A	0.51	0.46	yes
MSH2	splicing	NM_000251.2:c.942+3A>T	0.12	0.42?	yes
MSH2	p.H428Tfs*15	NM_000251.2:c.1281dup	0.47	0.49	yes
MSH2	exon 7 duplication	copy number variant	NA	NA	yes
MSH2	exons 3-16 deletion	copy number variant	NA	NA	yes
MSH2	exon 13-14 deletion	copy number variant	NA	NA	yes
MSH2	exon 1-6 deletion	copy number variant	NA	NA	yes
MSH2	exon 8 deletion	copy number variant	NA	NA	yes
MSH2	exon 1-2 deletion	copy number variant	NA	NA	yes
MSH2	p.A636P	NM_000251.2:c.1906G>C	0.49	0.53	yes
MSH2	p.I356Mfs*5	NM_000251.2:c.1068del	0.49	0.56	yes
MSH2	p.Q885*	NM_000251.2:c.2653C>T	0.47	0.5	yes
MSH2	deletion of exons 5-7	copy number variant	NA	NA	yes
MSH6	p.V907Rfs*10	NM_000179.2:c.2719_2720del	0.46	0.49	yes
MSH6	p.A1320Sfs*5	NM_000179.2:c.3939_3957dup	0.06	0.2	yes
MSH6	p.R298*	NM_000179.2:c.892C>T	0.49	0.48	yes
MSH6	p.S256*	NM_000179.2:c.766_767del	0.47	0.45	yes
MSH6	p.E1023*	NM_000179.2:c.3067G>T	0.47	0.49	yes
MSH6	p.F1088Lfs*5	NM_000179.2:c.3261dup	0.32	0.43	yes
MSH6	exon 2-6 deletion	copy number variant	NA	NA	yes
MSH6	p.Y1256*	NM_000179.2:c.3768T>G	0.49	0.5	yes

*Each row represents a unique patient sample tested. VAF= variant allele fraction; BROCA= clinically-validated HDR-D germline assay

Figure 8. Validation of Germline Pathogenic DNA Repair Variants by the "MONC" mini-panel. Note, additional variants not shown.
*Each row represents a unique patient sample tested. VAF= variant allele fraction; BROCA= clinically-validated HDR-D germline assay

We next turned out attention to validation of the "MONC" mini-panel in formalin-fixed paraffin embedded (FFPE) tissues. We assessed sensitivity by evaluating a total of 90 FFPE patients samples from tumors that had previously been clinically testing using UW-OncoPlex (as gold standard). Of the genes overlapping the MONC minipanel and UW-OncoPlex, there were a total of 138 expected single nucleotide variant (SNV) mutations, 33 expected small insertion/deletion variants (indel) and 32

Table: Sensitivity of the "MONC" minipanel in FFPE tissues

	SNV		Indel		CNV/SV	
	Expected	Observed	Expected	Observed	Expected	Observed
	138	138	33	31	32	31
Sensitivity	100.0%		93.9%		96.9%	

Figure 9. Sensitivity of the "MONC" minipanel in FFPE tissues.

expected copy number or structural variants (CNV/SV). The MONC panel accurately detected all 138 expected SNV variants (100% sensitivity), 31/33 indel variants (94% sensitivity) and 31/32 CNV/SV variants (97% sensitivity) (**Figure 9**). The two indel variants not detected by MONC were low VAF large indels in exon 19, and 20 of *EGFR*. The one missed CNV/SV variant was a non-canonical *ALK* fusion with breakpoints in a region not targeted by the MONC capture design.

We next assessed specificity by looking at genotyped "clinically flagged sites in each of the 55 patient samples used for germline validation as well as the 90 patient samples used for FFPE tissue validation of the assay. Among 9,570 genotyped calls, there were 2 low level false positives by MONC that passed quality filters, for a specificity of 99.9%.

We next assessed between-run reproducibility in 6 FFPE samples harboring a total of 22 known mutations and rare variants (**Figure 10**). All mutations were correctly called in both runs, and variant allele fractions (VAF) varied <5% for each sample and were qualitatively concordant with the gold standard UW-OncoPlex values (**Figure 10**).

To validate the MONC mini-panel assay for using in plasma cell-free DNA as a minimally invasive assay we started by running blinded cell-free DNA College of American Pathologists (CAP) proficiency testing samples from a formal survey in which our lab was recognized as a leader in this field and granted early access. Among these 6 CAP samples with low variant allele-fraction mutations 5 were accurately detected by

Between-run reproducibility of mini-oncology panel "MONC" in FFPE tissue

Gene	p.	c.	VAF From Run 1	VAF From Run 2	VAF from OPXv5	Mutation Validated?
ATM	p.E2096G	NM_000051.3:c.6287A>G	0.76	0.78	0.79	Yes
BRCA2	p.K3326X	NM_000059.3:c.9976A>T	0.46	0.47	0.4	Yes
CHEK2	p.S140I	NM_007194.3:c.419G>T	0.38	0.39	0.42	Yes
CHEK2	p.D203*	NM_007194.3:c.606dup	0.18	0.19	0.18	Yes
H3F3A	p.K28M	NM_002107.4:c.83A>T	0.38	0.38	0.41	Yes
H3F3A	p.K28M	NM_002107.4:c.83A>T	0.5	0.51	0.49	Yes
H3F3A	p.G35R	NM_002107.4:c.103G>A	0.45	0.4	0.48	Yes
KRAS	p.G13C	NM_004985.3:c.37G>T	0.33	0.35	0.31	Yes
PIK3CA	p.E545K	NM_006218.2:c.1633G>A	0.08	0.08	0.07	Yes
PMS2	p.P794S	NM_000535.5:c.2380C>T	0.08	0.08	0.04	Yes
PTEN	p.R159S	NM_000314.4:c.477G>T	0.14	0.14	0.14	Yes
PTEN	p.G36V	NM_000314.4:c.107G>T	0.11	0.11	0.11	Yes
PTEN	p.G44D	NM_000314.4:c.131G>A	0.2	0.19	0.19	Yes
PTEN	p.R130Qfs*4	NM_000314.4:c.389del	0.06	0.06	0.08	Yes
PTEN	p.R335X	NM_000314.4:c.1003C>T	0.15	0.15	0.17	Yes
PTEN	p.W274X	NM_000314.4:c.821G>A	0.8	0.78	0.61	Yes
RAD51C	p.E218Vfs*33	NM_058216.1:c.653_654del	0.44	0.45	0.38	Yes
TP53	p.R282W	NM_000546.5:c.844C>T	0.7	0.67	0.68	Yes
TP53	p.K132Q	NM_000546.5:c.394A>C	0.82	0.82	0.85	Yes
TP53	p.R273H	NM_000546.5:c.818G>A	0.09	0.1	0.07	Yes
TP53	p.R273C	NM_000546.5:c.817C>T	0.03	0.04	0.04	Yes
TP53	p.R248W	NM_000546.5:c.742C>T	0.89	0.91	0.87	Yes

Figure 10. Reproducibility of the "MONC" panel in FFPE tissues.

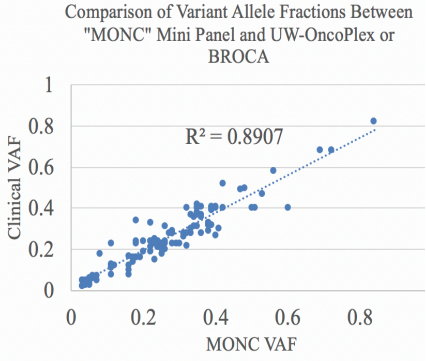
Results of blinded CAP proficiency testing* on cell-free DNA with low variant allele-fraction somatic mutations using the "MONC" Mini-Panel

CFDNA-01					CFDNA-02				
BRAF V600E 0.5%					*EGFR* T790M 0.5%				
BRAF V600E	*EGFR* T790M	*IDH1* R132C	*KRAS* G12D	*NRAS* Q61R	*BRAF* V600E	*EGFR* T790M	*IDH1* R132C	*KRAS* G12D	*NRAS* Q61R
0.4%	ND	ND	ND	ND	ND	0.4%	ND	ND	ND
CFDNA-03					**CFDNA-04**				
IDH1 R132C 1%					*EGFR* T790M 0.1%				
BRAF V600E	*EGFR* T790M	*IDH1* R132C	*KRAS* G12D	*NRAS* Q61R	*BRAF* V600E	*EGFR* T790M	*IDH1* R132C	*KRAS* G12D	*NRAS* Q61R
ND	ND	1.3%	ND	ND	ND	Below assay Limit of Detection	ND	ND	ND
CFDNA-05					**CFDNA-06**				
KRAS G12D 0.5%					*NRAS* Q61R 1%				
BRAF V600E	*EGFR* T790M	*IDH1* R132C	*KRAS* G12D	*NRAS* Q61R	*BRAF* V600E	*EGFR* T790M	*IDH1* R132C	*KRAS* G12D	*NRAS* Q61R
ND	ND	ND	0.4%	ND	ND	ND	ND	ND	1.1%

*In Green are the known mutations and VAF after un-blinding. In Pink is the mutation detected by MONC, with the corresponding VAF.

Figure 11. Reproducibility of the "MONC" panel in FFPE tissues.

Figure 12: To evaluate concordance of variant allele fraction (VAF) between the MONC minipanel the clinically-validated UW-OncoPlex and BROCA panels, we compared VAFs among all overlapping samples. The concordance was high, with an R^2 value of 0.89 and no substantive outliers



the MONC minipanel at VAF consistent with the expected value (**Figures 11,12**). The one mutation that was missed was below the anticipated limit of detection of the assay at 0.1% VAF.

Aim 3. Identify rational drug combinations that exploit tumor homology-directed DNA repair deficiency to eradicate prostate cancers with these defects.

To initiate the studies designed to identify rational drug combinations that exploit HR DNA repair deficiency we characterized a panel of patient derived xenografts, the LuCaP series, for mutations and signatures of HR gene loss (Subtask 1). These xenografts, along with cell lines, will serve as the substrate for drug testing (positive and negative for HRD). The PDX lines represent a spectrum of tumors with intact and mutated HR genes, and also reflect a spectrum of functional HR deficiency determined by mutation signatures (iHRD) (Subtask 4) (**Figure 13**).

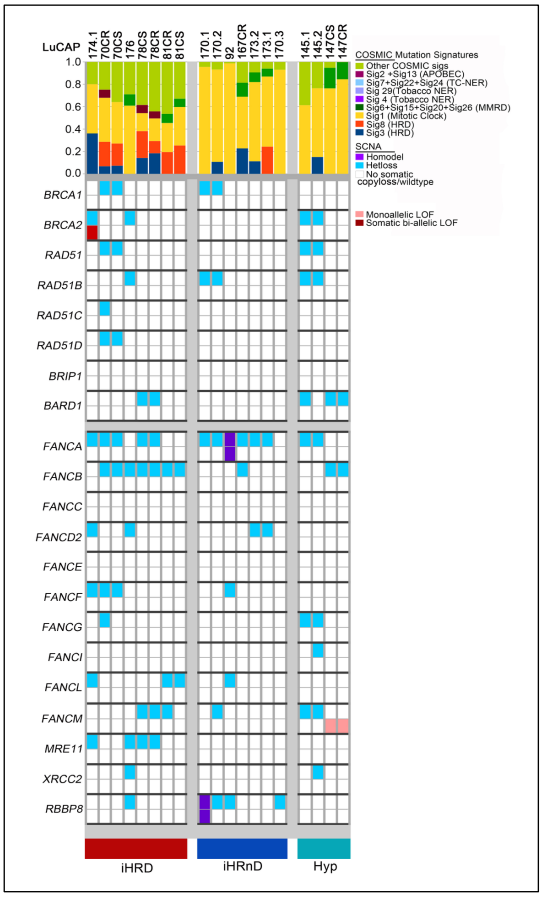


Figure 13. HRD status determination in LuCaP PDX series.

To test and confirm the functional consequences of HR deficiency determination, we exposed tumor cells to ionizing radiation and measured DNA damage by H2AX foci. Notably, iHRD status associated with delayed repair of DNA damage (**Figure 14**).

We next initiated pre-clinical studies comparing concurrent vs sequential DNA damaging therapy plus PARPi treatment strategies. We initially evaluated the use of supraphysiological androgens (SPA) which we and others have shown can induce DNA damage via enhanced androgen receptor (AR) activity. We confirmed previous findings demonstrating that SPA induces DNA double strand breaks (DSBs) in the setting of AR expression, and found that elevated AR levels, as observed in a subset of men treated with ADT and progressing to CRPC, potentiate SPA-induced DSBs and rates of apoptosis. Further, PC cell lines or PDXs deficient in *BRCA2* exhibit elevated DNA damage and cell death when exposed to SPA. The pharmacological inhibition of PARP1 or DNA-PKcs augmented these SPA effects (Subtask 3) (**Figure 15**). In support of these observations, metastatic CRPC patients with germline or somatic mutations in genes mediating homology-directed DNA repair were more likely to exhibit clinical responses to SPA administered in the form of monthly testosterone injections. These results support specific clinical strategies designed to optimize the use of SPA for the treatment of men with CRPC. These results were published (see Chatterjee *et al*) and set the stage for additional combination therapeutics.

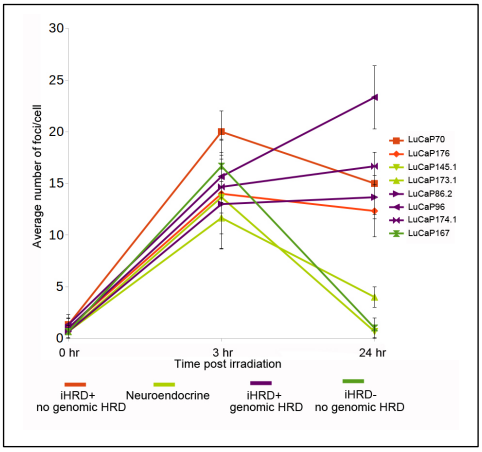


Figure 14. DNA damage and repair in PDX lines by HRD status.

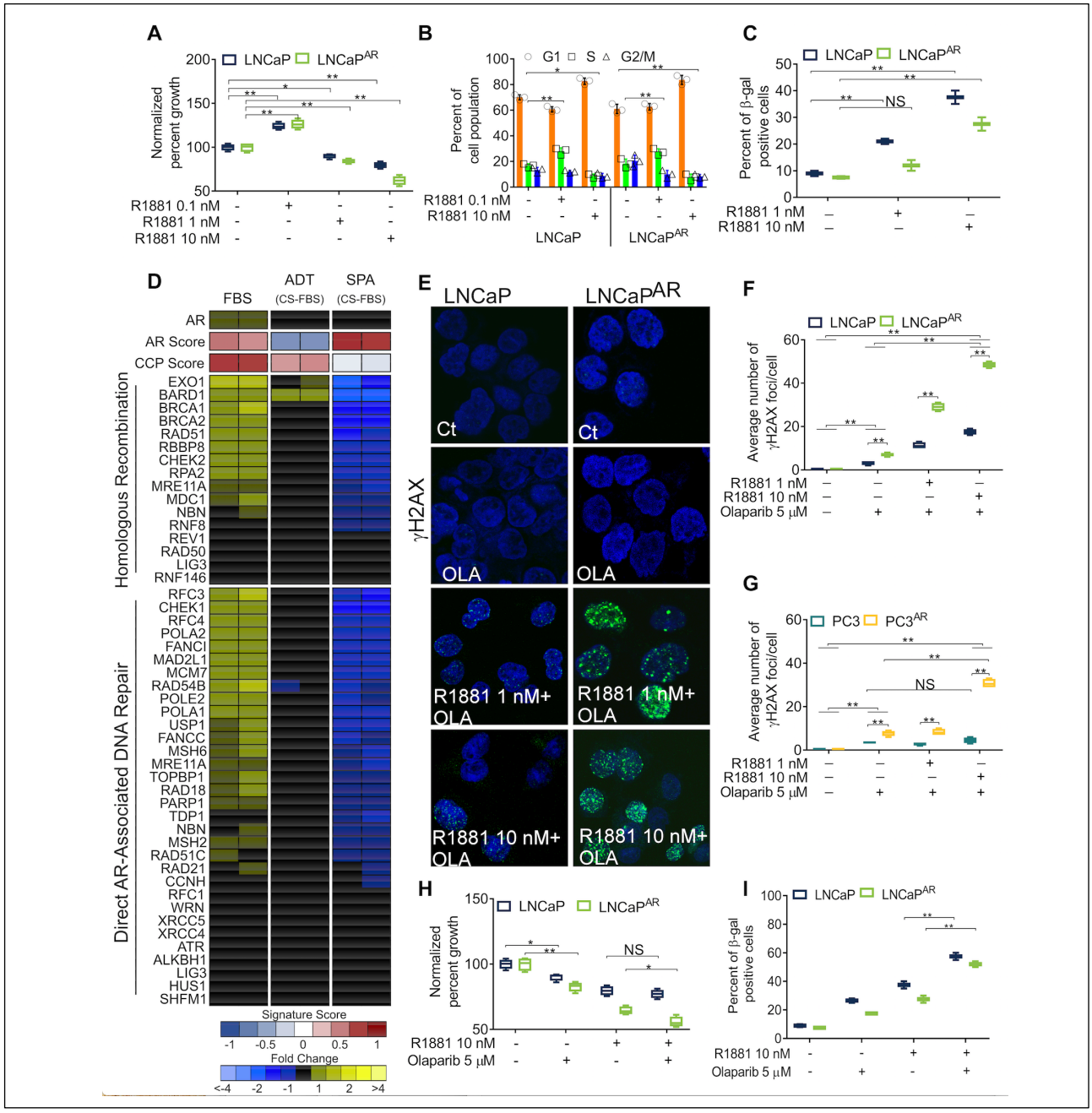


Figure 15. Supraphysiological androgen concentrations influence the growth of prostate cancer cells, alter the expression of DNA repair genes, and augment the effects of PARP inhibition. **A**. Quantitation of prostate cancer cell growth 72 hours after treatment with concentration ranges of R1881. **B**. Assessment of cell cycle phase by flow cytometry 72 hours following treatment with R1881. **C**. Assessment of cellular senescence by quantitation of B-galactosidase staining 72 hours after androgen treatment. **D**. Transcript levels determined by RNAseq analysis in LNCaP cells in standard growth medium (FBS) or in androgen depleted medium, ADT (CS-FBS), or androgen depleted medium supplemented with 10 nM R1881, SPA. **E**. Confocal immunostaining assay for □H2AX foci in prostate cancer cells in control medium alone (Ct) or supplemented with Olaparib (Ola) alone or with R1881. **F**. Quantitation of □H2AX foci per LNCaP cell. **G**. Quantitation of □H2AX foci per PC3 cell. **H**. Quantitation of prostate cancer cell growth after treatment with olaparib and/or R1881. **I**. Assessment of cellular senescence by quantitation of β-galactosidase staining. In A, C, and F-I, data represent the mean ± SD; n=4 replicates per experiment; p≤0.05 (*), p<0.01 (**) by ANOVA.

The preclinical results detailed above have prompted discussions by Montgomery and Nelson with clinical colleagues to begin exploiting these findings in clinical studies. A clinical trial of SPT with PARPi has been launched by our clinical colleague Dr. Michael Schweizer. Discussions are also underway to develop clinical studies combining ATR inhibitors and PARP inhibitors.

What opportunities for training and professional development has the project provided?
Nothing to report.

How were the results disseminated to communities of interest?
We have disseminated the results of the findings to date through publications in peer-reviewed journals and in presentations to the research community (see the 'Products' section for manuscript citations and a listing of research conferences where we have presented research findings).

What do you plan to do during the next reporting period to accomplish the goals?

We plan to follow the Statement of Work (SOW) that details the specific objectives and milestones to achieve the original specific aims.

In the next period of support we are focusing on validating the MONC mini-panel for use with cell-free DNA in prostate cancer patients and will establish sensitivity, specificity, and limit of detection using existing cell-free DNA samples as well as prospectively collected samples from the trials anticipated to open in the next year as part of this award.

We plan to have HRPO approval within the next 4 months. We will continue to work on the minimally invasive biomarker assay, next working on methods to sensitively detect HR signatures. Following trial opening, we will begin prospective tumor sequencing patients to identify HRD, in our CLIA laboratory.

We plan to open and accrue patients to the prospective trials of therapeutics that are hypothesized to be particularly effective in tumors with HR repair deficiency and confirm that specific genomic aberrations will serve as accurate biomarkers of response.

We plan to continue studies of drug combinations that exploit specific tumor vulnerabilities conferred by HR repair deficiency. A specific focus is to understand the mechanism(s) of resistance and consider approaches that address such resistance pathways and programs.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?
In year 1 we published our validation study of the UW-OncoPlex assay for use in metastatic prostate cancer patients and included this assay on our CAP activity menu for clinical use through the Genetics and Solid Tumors Laboratory at the University of Washington. This assay is currently available only for men with metastatic prostate cancer and PSA>10, or with variant histology such as small cell (**Figure 16**).

Our work to date has led to two important findings for the field of minimally-invasive biomarkers in prostate cancer patients:

1) Disease burden at the time of plasma cell-free DNA measurement is especially important to ensure there is adequate tumor content and that mutations assessed in blood plasma accurately reflect prostate cancer biology
2) Paired whole blood germline control testing is important in prostate cell-free DNA testing for DNA repair defects to exclude interfering mutations from unrelated processes such as age-related clonal hematopoiesis.

Our work to date has led to the important finding that supraphysiological androgen induces DNA damage and is particularly effective in tumors with HR repair deficiency (e.g. mutated BRCA2). Our findings also determined that SPA is augmented by PARP inhibitors and inhibitors of DNA-PKcs. These studies have led to the development of a clinical trial testing the combination of SPA and PARPi (olaparib).

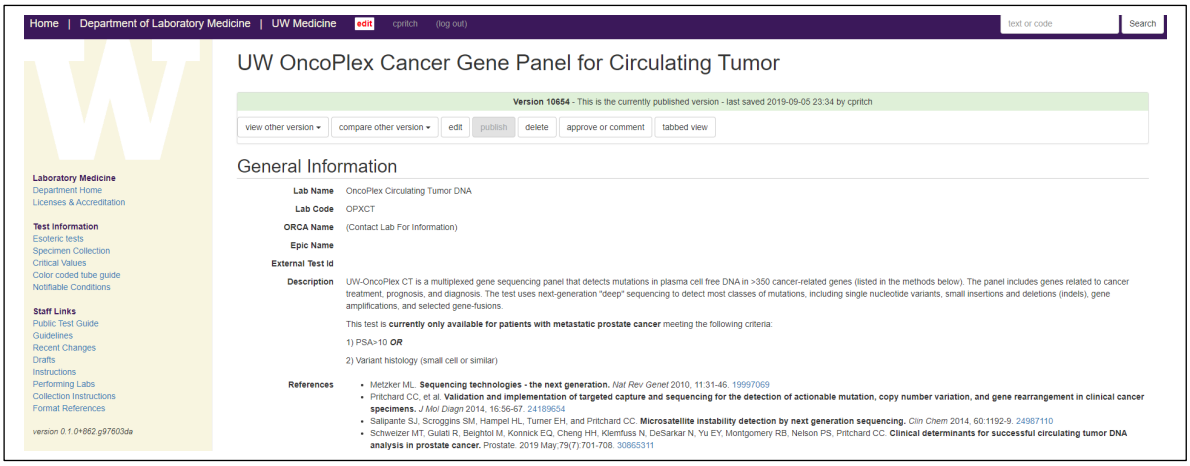


Figure 16: UW-OncoPlex CT Assay is Available for Men with Metastatic Prostate Cancer and High Burden Disease.

What was the impact on other disciplines?
The findings from our UW-OncoPlex CT validation study that tumor burden is a key determinant of a successful plasma cell-free DNA sequencing are broadly applicable across cancer types, and may help the emerging field of minimally invasive plasma cell-free DNA diagnostics ("liquid biopsy") in multiple cancer types.

What was the impact on technology transfer?
Nothing to report.

What was the impact on society beyond science and technology?
Nothing to report.

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change
No changes.

Actual or anticipated problems or delays and actions or plans to resolve them
HRPO approval of the protocol has taken longer than anticipated. We anticipate approval shortly and will begin the prospective clinical components of the project.

Changes that had a significant impact on expenditures

Services direct costs budget currently remains unexpended. Supplies expenditures exceeded anticipated budgeting costs. Supplies as purchased: UW-OncoPlex capture design, Illumina sequencing reagents.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Nothing to report.

Significant changes in use or care of human subjects
Nothing to report.

Significant changes in use or care of vertebrate animals
Nothing to report.

Significant changes in use of biohazards and/or select agents
Nothing to report.

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."*

Publications, conference papers, and presentations
Report only the major publication(s) resulting from the work under this award.

Journal publications.

- 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).

- Chatterjee P, Schweizer MT, Lucas JM, Coleman I, Nyquist MD, Frank SB, Tharakan R, Mostaghel E, Luo J, **Pritchard CC**, Lam HM, Corey E, Antonarakis ES, Denmeade SR, **Nelson PS**. Supraphysiological androgens suppress prostate cancer growth through androgen receptor-mediated DNA damage. J Clin Invest. 2019 Jul 16;130. pii: 127613.PMID: 31310591 (Acknowledgement of Federal Support-Yes).

Books or other non-periodical, one-time publications.
Nothing to report.

Other publications, conference papers and presentations.

- *Lotan T and **Pritchard CC**. Predictive biomarkers: DNA damage repair. International Society of Urologic Pathologists Annual Meeting, 2019.

- *Dines JD, Shirts BH, Walsh T, King M-C, Fowler DM, and **Pritchard CC**. Systematic Misclassification of Missense Variants in *BRCA1* and *BRCA2* Coldspots. American Society of Medical Genetics Annual Meeting, 2019.

- Schweizer MT, Gulati R, Cheng HH, de Sarkar N, Yu EY, Montgomery RB, Nelson PS, **Pritchard CC.** Cell-free Circulating Tumor DNA is a Reliable Specimen for Mutation Profiling in Prostate Cancer Patients with High-Burden Disease. (2018) STTR Scientific Retreat.

- **Pritchard CC**. UW-OncoPlexCT. Brotman Baty Institute cell-free DNA Symposium 2019.

- ***Pritchard CC**. State of the art on molecular characterization in advanced prostate cancer (APC). Advanced Prostate Cancer Consensus Conference (APCCC) 2019 meeting.

- **Nelson PS**. The Evolution of Metastatic Prostate Cancer Under Treatment Pressure: Anticipating and Exploiting Pathways of Resistance. UCSF Prostate Cancer Program Retreat. San Francisco, CA 11/2018

- **Nelson PS**. The Emergence of New Species: Targeting the Molecular Diversity and Evolution of Advanced Prostate Cancer. Institute for Oncology Research. Bellinzona, Switzerland 2/2019

- **Nelson PS**. Targeting the Molecular Diversity and Evolution of Advanced Prostate Cancer. Frontiers in Oncology Lecture. University of Maryland, Baltimore, MD 2/2019

- **Nelson PS**. Identifying and Preventing Progression to Lethal Disease. CISNET Annual

Meeting. Fred Hutchinson Cancer Research Center. Seattle, WA 4/2019.

Website(s) or other Internet site(s)
Nothing to report.

Technologies or techniques
Nothing to report.

Inventions, patent applications, and/or licenses
Nothing to report.

Other Products
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".

Name:	Pritchard, Colin
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	cpritch (eRA Commons)
Nearest person month worked:	1.20 calendar months
Contribution to Project:	Colin Pritchard interpreted the sequencing data for this project and assisted in developing non-invasive molecular assays for somatic and germline alterations involving DNA repair genes.
Funding Support:	N/A

Name:	Cheng, Heather
Project Role:	Co-Investigator
Researcher Identifier (e.g. ORCID ID):	hhcheng (eRA Commons)
Nearest person month worked:	0.80 calendar months
Contribution to Project:	Heather Cheng developed and led the Phase 2 Study of Induction Docetaxel and Carboplatin followed by Switch-Maintenance Rucaparib, also for men with metastatic castration resistant prostate cancer whose tumors harbor DNA repair defects. She will work with Drs. Nelson and Pritchard to identify patients for the clinical

	study, coordinate recruitment and treatment, adverse events, analyze data and prepare reports and presentations.
Funding Support:	
Name:	True, Lawrence
Project Role:	Co-Investigator
Researcher Identifier (e.g. ORCID ID):	lawrencetrue (eRA Commons)
Nearest person month worked:	0.69 calendar months
Contribution to Project:	Lawrence True has assisted in the acquisition and assessment of tumors from men with localized and advanced prostate cancer.
Funding Support:	N/A
Name:	Beightol, Mallory
Project Role:	Research Tech
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	4.98 calendar months
Contribution to Project:	Mallory Beightol is responsible for preparing genomic libraries and BROCA, UW-OncoPlex, and mini-panel sequencing for this project.
Funding Support:	N/A
Name:	Jacobson, Angela
Project Role:	Genetic Counselor
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	0.60 calendar months
Contribution to Project:	Angela Jacobson has assisted with consults on test report interpretation and with genetic counseling of patients. She will facilitate follow up with family members, where appropriate, for additional studies.
Funding Support:	N/A

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.

SUPPORT
PRITCHARD, COLIN

Changes: New Funding Added Since Submission
Title: Accelerating the development and validation of liquid biopsy assays (63-6770)
Time Commitments: 1.02 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: 11/1/2018 – 10/31/2019
Level of funding: $11,545 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: Developing an analytical framework for clinical genome sequencing of cell-free DNA in metastatic prostate cancer (68-2345)
Time Commitments: 0.72 calendar
Supporting Agency: Brotman Baty Institute
Address: UWMC, Health Sciences Building H-564, Seattle WA 98195-8047
Contracting/Grants Officer: Nola Klemfuss (nklemfus@seattlecca.org), Hart Edmonson (hart@brotmanbaty.org)
Performance period: 1/1/2019 – 12/31/2019
Level of funding: $50,116 DC
Project Goals: The major goals we propose building the necessary analytical framework to characterize the whole genomes of mCRPC from patient cfDNA. We will develop novel computational approaches and pipelines for high-sensitivity copy number and tissue-of-origin analysis from low-

pass genome sequencing of cfDNA. Then, we will analyze both tumor and cfDNA to identify recurrent non-coding genomic alterations affecting regulatory elements in mCRPC patients. Finally, we will perform longitudinal analysis from serial blood samples collected during therapy to identify alterations that may be implicated in treatment resistance.
Specific Aims: Aim 1. To analyze the landscape of genomic alterations in mCRPC from WGS of tumors. 1a. Develop and establish copy number and structural rearrangements analysis for tumor WGS data. 1b. Analyze the landscape of genomic alterations in mCRPC patient tumor samples. Aim 2. To develop a sensitive approach for detecting tumor-specific genomic alterations in cfDNA. 2a. Develop an approach for modeling serial cfDNA samples to improve sensitivity for predicting genomic alterations from low-pass WGS. 2b. Develop an approach to integrate the tissue-of-origin analysis of cfDNA to improve tumor DNA detection. 2c. Validate and benchmark the algorithm in serial cfDNA and tissue samples from prostate cancers. Aim 3. To characterize non-coding genomic alterations in mCRPC from cfDNA. 3a. Characterize the genome alteration signatures associated with impaired DNA damage response. 3b. Identify the recurrent alterations in non-coding regions containing gene enhancers and germline risk loci. 3c. Perform longitudinal analysis using serial cfDNA samples to monitor AR enhancer duplication, and other predicted alterations, to investigate the resistance to second-generation androgen blockade.
Overlap: N/A

Title: Northwest Genomics Center for All of Us (61-8385)
Time Commitments: 0.72 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: 9/25/18-8/31/23
Level of funding: $5,553,067 DC
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: N/A

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: 1.2 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 – 9/29/2021
Level of funding: $256,264 DC
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: N/A

Title: Project 1: Molecular Predictors of Prostate Cancer Progression and Mortality (63-9456)
Time Commitments: 1.14 calendar
Supporting Agency: Fred Hutch Cancer Research Center through NIH
Address: 1100 Fairview Ave N, Seattle WA 98109
Contracting/Grants Officer: Lillian Furlong (lfurlong@fredhutch.org)
Performance period: 9/18/2018 – 8/31/2019
Level of funding: $182,307 DC
Project Goals: 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: AIM 1: Recruit men with mPC and gDRG (index cases) to determine factors associated with uptake of ge-netic testing and to identify clinical, pathologic and molecular predictors of gDRG status. (Corollary: Men with mPC and gDRG will be offered targeted clinical trials proposed in SPORE Project 4.) AIM 2: Evaluate stepwise process involved in cascade genetic testing from index cases to their at-risk first-degree relatives (FDRs). AIM 3: Conduct a high-risk prostate cancer early detection study incorporating imaging, novel biomarkers and statistical modeling in at-risk male gDRG carriers.
Overlap: N/A

SUPPORT
CHENG, HEATHER

Changes: New Funding Added Since Submission
Title: Project 1: Molecular Predictors of Prostate Cancer Progression and Mortality (63-9456)
Time Commitments: 1.20 calendar
Supporting Agency: Fred Hutch Cancer Research Center through NIH
Address: 1100 Fairview Ave N, Seattle WA 98109
Contracting/Grants Officer: Lillian Furlong (lfurlong@fredhutch.org)
Performance period: 9/18/2018 – 8/31/2020
Level of funding: $182,307 DC
Project Goals: 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: AIM 1: Recruit men with mPC and gDRG (index cases) to determine factors associated with uptake of ge-netic testing and to identify clinical, pathologic and molecular predictors of gDRG status. (Corollary: Men with mPC and gDRG will be offered targeted clinical trials proposed in SPORE Project 4.) AIM 2: Evaluate stepwise process involved in cascade genetic testing from index cases to their at-risk first-degree relatives (FDRs). AIM 3: Conduct a high-risk prostate cancer early detection study incorporating imaging, novel biomarkers and statistical modeling in at-risk male gDRG carriers.
Overlap: N/A

SUPPORT
TRUE, LAWRENCE

Changes: New Funding Added Since Submission
Title: Nondestructive volumetric pathology of prostate biopsies for accurate prognostication and treatment stratification
Time Commitments: 1.2 calendar
Supporting Agency: Department of Defense US Army; W81XWH-18-1-0359
Address: 820 Chandler ST, Fort Detrick, MD 21702-5000
Contracting/Grants Officer:
Performance period: 08/15/18 – 08/14/21
Level of funding: $68,775 DC
Project Goals: This project aims to improve the consistency and accuracy of pathological analyses of prostate biopsy specimens in order to optimize patient treatments and outcomes. Dr. True will provide clinical oversight, education, and guidance through the project, and will be responsible for ensuring quality of data.

Title: Pacific Northwest Cancer SPORE
Time Commitments: 0.96 calendar
Supporting Agency: National Institutes of Health
Address: 9000 Rockville Pike, Bethesda, Maryland 20892
Performance period: 09/01/18 – 08/31/23
Level of funding: $186,641 DC
Project Goals: The Specimen Core provides part of the infrastructure support for Projects 1-4, as well as future pilot and developmental projects. It has been designed to meet the needs of these projects, plus serve as a stand-alone system of specimen collection, storage, distribution and related clinical/research information dissemination that is based on over two decades of experience. Dr. True will serve as the Pathologist and Co-Director of Core B.

Title: Androgen Receptor Action In Castration Resistant Prostate Cancer
Time Commitments: 0.36 calendar
Supporting Agency: National Institutes of Health
Address: 9000 Rockville Pike, Bethesda, Maryland 20892
Performance period: 02/12/19 – 01/31/24
Level of funding: $81,133
Project Goals: The major goal of the Biospecimen Core is to provide a well-organized and standardized system of specimen collection, storage, distribution and related clinical/research information dissemination that is based on over two decades of experience. The Core will ensure consistency and quality assurance in the pathological analysis of tissue specimens. It will maintain a large series of prostate cancer xenograft lines developed by Core investigators, which will be used for proposed studies by the P01 investigators. Dr. True is Co-Director of the PDX/Biospecimen Core and will participate in tissue acquisition, rapid autopsies, and characterization of PDXs studies.

Title: Noninvasive histotripsy ablation of fibrotic tissue in benign prostatic hyperplasia
Time Commitments: 0.50 calendar
Supporting Agency: National Institutes of Health

Address: 9000 Rockville Pike, Bethesda, Maryland 20892
Performance period: 07/01/19 - 06/30/24
Level of funding: $728,044
Project Goals: This project proposes to develop physical and numerical models for bubble-fiber interaction and characterize the physical effects of histotripsy exposures on fibrous tissue structures using imaging and histological methods.

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Nothing to Report

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.*

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.*

Targeting the Subtype of Metastatic Prostate Cancer Deficient in DNA Repair Capacity

Log Number PC170503P2 Award Number W81XWH-18-1-0356

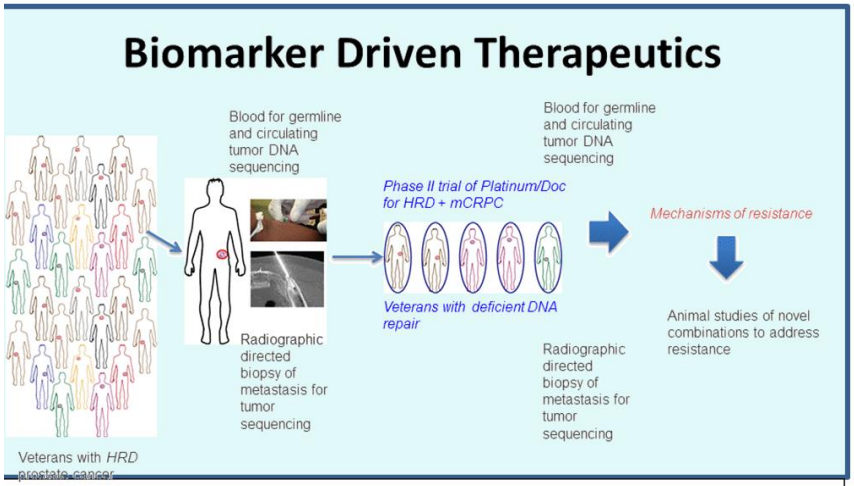
PI: Pritchard, Colin C. MD, PhD **Org:** University of Washington **Award Amount:** $1,012,019

Study/Product Aim(s)

- Aim 1: Determine if germ-line and somatic aberrations in homologous recombination DNA repair pathways associate with responses to FDA-approved therapeutics in men with mCRPC.
- Aim 2: Develop minimally-invasive biomarkers capable of distinguishing patients for therapeutics targeting homologous recombination DNA repair pathways and ascertaining resistance mechanisms.
- Aim 3: Identify rational drug combinations that exploit DNA repair vulnerabilities to eradicate prostate cancers

Approach

Identify men with tumors exhibiting HDR-D and develop strategies to enhance initial responses and assess mechanism of resistance to genotoxic agents.



Accomplishment: A DNA-repair-focused panel has been designed and undergone initial analytical validation for minimally invasive detection of HR DNA repair defects. Model systems developed and initial drug combinations analyzed.

Timeline and Cost

Activities CY	18	19	20	21
Assay Development/Validation				
DNA sequencing on subjects				
Biomarker Development				
Identify rational drug combinations				
Estimated Budget ($K)	**$000**	**$126K**	**$132K**	**$244K**

Updated: 29 October 2019

Goals/Milestones

CY18 Goals – Assay Development/Validation

☑ cfDNA panel design ☑ cfDNA panel validation

CY19 Goals – Sample acquisition

□ Identify patients with HRD defects
□ Sequence tissue
□ Biomarker study
□ Develop models
□ Test drug combinations

CY20 Goals

□ Identify patients with HDR defects
□ Sequencing tissue
□ Biomarker assay performance analysis, Manuscript preparation
□ Test drug combinations

CY21 Goals

□ Final data analysis and manuscript preparation

Comments/Challenges/Issues/Concerns

Budget Expenditure to Date

YR19 Projected Remaining Expenditure: $127K

W81XWH1810356: Targeting the Subtype of Metastatic Prostate Cancer Deficient in DNA Repair Capacity

PI: Pritchard, Colin, University of Washington, WA **Budget:** $1,012,019

Topic Area: TBD **Mechanism:** FY17 Prostate Cancer Research Program – Impact Award

Research Area(s):
DNA Repair Deficiency in Prostate Cancer
Cancer Therapeutics

Award Status: 15 August 2018 – 18 October 2019

Study Goals:

This proposal will address the challenge of effectively treating mCRPC by exploiting specific tumor vulnerabilities conferred by defects in HDR. 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 homology-directed repair deficient (HDR-D) mCRPC to test the hypothesis that *aberrations in key genes that repair DNA strand breaks by homology-directed repair (HDR) are predictive of meaningful clinical responses to FDA-approved genotoxic therapeutics (e.g carboplatin) and to emerging therapeutics (PARP inhibitors, WEE1 inhibitors and other drugs)*. The proposal will also develop approaches to identify men with tumors exhibiting HDR-D, and strategies to enhance initial responses and assess mechanisms of resistance to genotoxic agents.

Specific Aims:

Aim 1. Determine if germ-line and somatic aberrations in homology-directed DNA repair pathways associate with responses to FDA-approved therapeutics in men with mCRPC.
Aim 2. Develop minimally-invasive biomarkers capable of distinguishing patients for therapeutics targeting homology-directed DNA repair pathways and ascertaining resistance mechanisms.
Aim 3. Identify rational drug combinations that exploit DNA repair vulnerabilities to eradicate prostate cancers with homology-directed DNA repair deficiency

Key Accomplishments and Outcomes:

Publications: Please see Annual Report

Patents: Please see Annual Report

Funding Obtained: Please see Annual Report