AWARD NUMBER: W81XWH-21-1-0264

TITLE: Defining and Modulating BRCAness to Improve the Precision of Prostate Cancer Therapy

PRINCIPAL INVESTIGATOR: Dr. Peter Nelson, MD

CONTRACTING ORGANIZATION: Fred Hutchinson Cancer Center

REPORT DATE: August 2023

TYPE OF REPORT: Annual Technical Report

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	**2. REPORT TYPE**	**3. DATES COVERED**
AUGUST 2023	Annual Technical Report	1AUG2022 - 31JUL2023

4. TITLE AND SUBTITLE

Defining and Modulating BRCAness to Improve the Precision of Prostate Cancer Therapy

5a. CONTRACT NUMBER
W81XWH-21-1-0264

5b. GRANT NUMBER
PC200262

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Fred Hutchinson Cancer Center
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Seattle, WA 98109-1024

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Purpose: This proposal is focused on exploiting a specific subtype of metastatic CRPC, termed *Homology Directed DNA Repair Deficient* (HDR-D) prostate cancer (PC) to enhance treatment outcomes, reduce morbidity and improve survival. HDR-D represents at least 20% of metastatic PC and is most commonly identified through the genomic analysis of biopsies from metastatic tumors and identifying mutations in *BRCA1*, *BRCA2* and related genes.

Scope: This proposal is designed to address two challenges: First, to improve the accuracy of detecting PCs with functional HDR-D for appropriate treatment allocation. It is clear from prospective studies that simply evaluating the mutation status of HDR-associated genes lacks precision for predicting treatment responses: a high percentage (>50%) of biomarker 'positive' patients fail to respond. Second, to increase the number of men with PC that could benefit from therapeutics targeting HDR-D by promoting 'conditional haploinsufficiency' converting HDR-competent tumors to a 'BRCAness' phenotype.

Major Findings: (1) We have developed and refined a new tissue and blood based assay, termed OncoplexHRD, that represents a read out of functional HDR-D; (2) We determined that alterations in the transcriptional kinase CDK12 – previously associated with HDR-D – confers HDR-D when lost acutely, but does not confer HDR-D in cells adapted to survive with CDK12 loss. This finding has important implications for patient selection and treatment with PARP inhibitors and DNA damaging agents; (3) We determined that tumors with CDK12 loss were differentially sensitive to CDK13 loss and drugs that interfere with DNA synthesis.

15. SUBJECT TERMS
Prostate cancer, metastasis, castrate-resistant prostate cancer, DNA repair, homology-directed DNA repair deficiency, PARP inhibitor, chemotherapy, biomarker

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRDC
U	U	U	UU	34	**19b. TELEPHONE NUMBER** *(include area code)*

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1. INTRODUCTION

This proposal is focused on exploiting a specific subtype of metastatic CRPC, termed *Homology Directed DNA Repair Deficient* (HDR-D) prostate cancer (PC) to enhance treatment outcomes, reduce morbidity and improve survival. HDR-D represents at least 20% of metastatic PC and is most commonly identified through the genomic analysis of biopsies from metastatic tumors and identifying mutations in *BRCA1*, *BRCA2* and related genes. Recent clinical studies have determined that mCRPCs with these mutations are responsive to two types of therapy, PARP inhibitors and platinum-based chemotherapy. However, the current biomarkers, based on identifying gene mutations, are imprecise: many men determined to be biomarker positive do not respond, and the gene mutation-based biomarkers fail to identify other patients that will respond. Further, only about 20% of men with CRPC have a tumor with functional HDR-deficiency, consequently many men will not benefit from the 'synthetic lethality' treatment approaches that leverage this important tumor vulnerability.

This proposal is designed to address two challenges: First, to improve the accuracy of detecting PCs with functional HDR-D for appropriate treatment allocation. It is clear from prospective studies that simply evaluating the mutation status of HDR-associated genes lacks precision for predicting treatment responses: a high percentage (>50%) of biomarker 'positive' patients fail to respond. Second, to increase the number of men with PC that could benefit from therapeutics targeting HDR-D by promoting 'conditional haploinsufficiency' converting HDR-competent tumors to a 'BRCAness' phenotype.

2. KEYWORDS.

Prostate cancer, metastasis, castrate-resistant prostate cancer, DNA repair, homology-directed DNA repair deficiency, PARP inhibitor, chemotherapy, biomarker

3. ACCOMPLISHMENTS.

Project Goals:

This is a partnering PI award with research sites at Fred Hutchinson Cancer Center and the University of Washington (FH). The Aims, applicable sub-tasks, and work accomplished to date for each site are listed below:

TITLE: DEFINING AND MODULATING BRCAness TO IMPROVE THE PRECISION OF PROSTATE CANCER THERAPY	**Timeline (Months) 1-36**	**FHCRC** (Nelson)	**UW Lab. Medicine** (Pritchard)	**Completed (%) Year 1 Annual Report**
AIM 1. Develop and test clinical grade assays that define prostate cancers with functional homology directed DNA repair deficiency to improve sensitivity and specificity relative to HR gene mutations.				
Subtask 1: Obtain institutional (Fred Hutch / UW) IACUC and IRB review and approvals.	1	Nelson Team	Pritchard Team	100%
Subtask 2: Submit compliance documents from institutional offices to DOD ACURO/HRPO for review, and obtain approvals.	2	Nelson Team	Pritchard Team	100%
Subtask 3: Initiate study start-up procedures (staff training).	1-2	Nelson Team	Pritchard Team	100%
Subtask 4: Identify biospecimens from mCRPC models with germ-line and/or somatic defects in HRR.	2-12	Nelson Team		100%
Subtask 5: Extract DNA, complete genomic sequencing, and identify variants conferring HRR-D and variants with uncertain significance.	3-24		Pritchard Team	90%

Subtask 6: Identify and obtain tumor biospecimens and ctDNA samples from patients with mCRPC with and without HRR-D	2-34	Nelson Team	Pritchard Team	90%
Subtask 7: Develop a NextGen assay targeting relevant DNA repair genes and genomic parameters of HRR-D (tumor and ctDNA)	2-6		Pritchard Team	100%
Subtask 8: Sequence tumor and ctDNA from patient biospecimens and identify aberrations in DNA repair: genes and genomic scars.	3-34		Pritchard Team	80%
Subtask 9: Identify and obtain biospecimens collected from patients on clinical trials of therapies exploiting HRR-D	3-34	Nelson Team	Pritchard Team	80%
Subtask 10: Sequence tumor and ctDNA from patient biospecimens from HRR-D directed therapy and identify aberrations in DNA repair: genes and genomic scars.	3-34		Pritchard Team	80%
Subtask 11: Confirm metrics of sensitivity and specificity using tumor tissue and ctDNA.	4-12		Pritchard Team	100%
Subtask 12: Determine concordance and discordance of assay performance comparing minimally-invasive assessments with tumor assessments for clinical trial participants.	12-34		Pritchard Team	100%
Subtask 13: Determine assay performance in longitudinal assessments of tumor responses and assessing resistance mechanisms.	24-35		Pritchard Team	50%
Subtask 14: Submit data for CLIA/CAP approval of assays.	18-24		Pritchard Team	80%
Milestone #1: Prepare and submit manuscript detailing the performance characteristics of assays for accurate determination of HRR-D.	24	Nelson Team	Pritchard Team	80%
Milestone #2: Prepare and submit manuscript detailing the utility of orthogonal assays of HRR-D to impact patient care: identification of appropriate patients for treatment and monitoring responses.	30-34	Nelson Team	Pritchard Team	80%
AIM 2. Identify specific combinations of DNA repair gene and metabolic parameters that confer functional homology directed DNA repair deficiency.				
Subtask 1: Identify combinations of HR related genes with single copy loss and concurrent parameters indicating HRR-D – PDX lines.	1-6	Nelson Team		100%
Subtask 2: Identify combinations of HR related genes with single copy loss and concurrent parameters indicating HRR-D – tumor biospecimens.	1-12	Nelson Team		100%
Subtask 3: Identify variations in metabolic gene expression and activity.	3-14	Nelson Team		60%
Subtask 4: Develop models with combinations of single copy loss genes and metabolic alterations.	4-18	Nelson Team		70%
Subtask 5: Evaluate effects of metabolites and metabolic parameters on HRR-D and treatment responses.	6-24	Nelson Team	Pritchard Team	70%
Subtask 6: Conduct preclinical cell line and PDX studies evaluating gene combinations and agents	6-30	Nelson Team		70%

altering metabolic parameters: 6 drug/treatment studies – 4 lines x 3 arms x 8 mice/arm = 576 mice.				
Milestone #3: Prepare and submit manuscripts detailing the effects of haploinsufficiency and metabolic features inducing conditional HRR-D.	12-24	Nelson Team	Pritchard Team	70%
AIM 3. Identify pharmacological agents that promote HRR-D and that enhance the effects of genotoxic drugs and PARPi.				
Subtask 1: Test 3 PARPi for effects against tumors with HRR-D due to biallelic HRG loss and against tumors with multiple-monoallelic loss.	6-18	Nelson Team		70%
Subtask 2: Test platinum therapy for effects against tumors with HRR-D due to biallelic HRG loss and against tumors with multiple-monoallelic loss.	8-20	Nelson Team		70%
Subtask 3: Test drug combinations that a) induce HRR-D and b) target HRR-D for effects against tumors with HRR-D due to biallelic HRG loss and against tumors with multiple-monoallelic loss.	8-34	Nelson Team		60%
Subtask 4: Evaluate tumors resisting therapy for mechanisms of treatment resistance.	12-35	Nelson Team		80%
Milestone #4: Prepare and submit manuscripts detailing the effects of inducing HRR-D with targeting HRR-D.	32-26	Nelson Team	Pritchard Team	30%
Milestone #5: Prepare and submit final report.	36	Nelson Team	Pritchard Team	0%

Accomplishments Toward Goals:
To accomplish the Specific Aims, we developed a bi-institutional collaboration between Dr Peter Nelson (PI; Fred Hutchinson Cancer Center, Seattle, Washington USA) and Dr Colin Pritchard (PI; University of Washington, Seattle, Washington USA).

1) Major Activities:
The major activities conducted during Year 2 of this project are outlined above in the SOW according to each Specific Aim and Subtask partitioned by partnering site. The activities centered on accomplishing these aims/objectives. The results of these activities are detailed below.

2) Specific Objectives:
The specific objectives followed the Specific Aims: AIM 1. Develop and test clinical grade assays that define prostate cancers with functional homology directed DNA repair deficiency to improve sensitivity and specificity relative to HR gene mutations; AIM 2. Identify specific combinations of DNA repair gene and metabolic parameters that confer functional homology directed DNA repair deficiency; AIM 3. Identify pharmacological agents that promote HDR deficiency and that enhance the effects of genotoxic drugs and PARPi.

3) Significant Results or Key Outcomes, Including Major Findings, Developments, and Conclusions:

AIM 1. Develop and test clinical grade assays that define prostate cancers with functional homology directed DNA repair deficiency to improve sensitivity and specificity relative to HR gene mutations.

Progress to Date: The major activities for this aim continued work to develop a clinical-grade assay – CLIA/CAP approved - for ascertaining HRD status in prostate cancer. In Project Year 1 (PY1), HRPO and internal IRB protocol approvals were obtained, a research coordinator hired, and validation of the assay commenced with both prospective and retrospective molecularly-characterized prostate cancer biospecimens. In Project Year 2 (PY2), over 120 prostate cancer pairs were sequenced by the paired OncoPlex*HRD* assay for prospective validation of the approach and a threshold for LOH score established.

Protocol Approvals and Study Start Up: During the first year we obtained both HRPO and internal IRB approvals for the work. HRPO approval numbers E02119.1a and E02120.1a, approved 2/14/2022. Internal approval Fred Hutch IRB, IR# 8130, RG5118000, UW Study: DEFINING AND MODULATING BRCAness TO IMPROVE THE PRECISION OF PROSTATE CANCER THERAPY: UW IRB STUDY00014494. During PY2, a second research coordinator was hired and study training performed.

OncoPlex Assay Background: In brief (described in PY1), UW-OncoPlex is a ~3Mb, 362-gene comprehensive cancer sequencing panel developed by the Pritchard group which has been in continuous clinical use in the CLIA-laboratory setting for prostate cancer patients since 2011, with over 15,000 total patients tested to date. In collaboration with Dr. Nelson, the OncoPlex assay has been validated for prostate cancer use in *both* tumor tissue and for circulating cell-free DNA (ctDNA) (PMID:30865311, PMID:27324988, PMID:24189654). OncoPlex currently detects single nucleotide variants, all sizes of indels, copy number variants, structural rearrangements, total mutation burden (TMB), and microsatellite instability (MSI) (PMID:24987110). OncoPlex has unique features designed to accurately detected DNA repair gene mutations, including the capture of *BRCA1/2* coding and intronic and structural alterations in MMR genes leading to their inactivation.

OncoPlexHRD Development: Cancers with HRD accumulate large deletion and duplication events that lead to genomic LOH which can serve as a biomarker for detection of functional HRD. During PY1, we modified OncoPlex to measure LOH mutational signatures for HRD through analysis of paired tumor and normal samples (OncoPlex v7) by adding 3,076 single 120bp IDT lockdown capture probes at sites of carefully selected common SNPs designed to tile evenly across the genome to serve as a backbone for genomic LOH profiling. LOH is detected using the R package Sequenza, which performs probabilistic analysis of sample pairs through estimation of tumor cellularity and ploidy to calculate copy number variation and variant allele frequency in the paired tumor sample to determine overall genomic % LOH (PMID:25319062).

OncoPlexHRD Prostate Cancer Validation: Collectively across PY1 and PY2, we have performed validation analyses for OncoPlex*HRD* on a collection of 254 prostate cancer tumor-normal tissue sample pairs according to the following categories: 80 primary prostate cancer samples, 64 metastatic site tumor tissue samples, 59 ctDNA samples from men with mCRPC, and 51 LuCaP PDX prostate cancer samples. Samples with insufficient quantity or tumor cellularity below validated cutoffs were dropped from analysis (n=75). The breakdown of samples according to HR gene mutation is given in the updated **Table 1 and 2** and **Figure 1** below. Although not strictly defined at HR genes, we including *CDK12* and *CHD1* in these analyses as they have been shown to interact in important ways with HR genes. CDK12 is included in the FDA approval for the PARP inhibitor olaparib. Overall, samples tested with identified mutations in HR genes had significantly higher % LOH (AVG: 13%, SD: 7%, t-test two-tailed p=0.0004) than samples lacking HR mutations (AVG: 8%, SD: 6%) (**Table 2**).

HRR Gene	Ave. LOH Score	SD LOH Score
ATM (n=14)	0.10	0.05
BAP1 (n=1)	0.08	NA
BRCA1 (n=4)	0.15	0.06
BRCA2 (n=28)	0.13	0.06
BRIP1 (n=1)	0.11	NA
CDK12 (n=8)	0.06	0.03
CHD1 (n=18)	0.10	0.05
CHEK2 (n=4)	0.10	0.05
FANCA (n=7)	0.09	0.04
FANCD2 (n=2)	0.15	0.03
PALB2 (n=4)	0.10	0.03
RAD51B (n=1)	0.25	NA
RAD51D (n=1)	0.08	NA
None (n=79)	0.09	0.06
QC failure (n=75)	NA	NA

Table 1. Average LOH score by OncoPlex*HRD* in prostate cancer cases according to HR gene mutation

HRR Gene Mutation	**Average LOH score**	**SD LOH Score**
Present	0.12	0.07
Absent	0.08	0.06
t-test, two tailed	p= 0.004	

Table 2. Overall Average LOH score by OncoPlex HRD in prostate cancer cases with or without HR gene mutations.

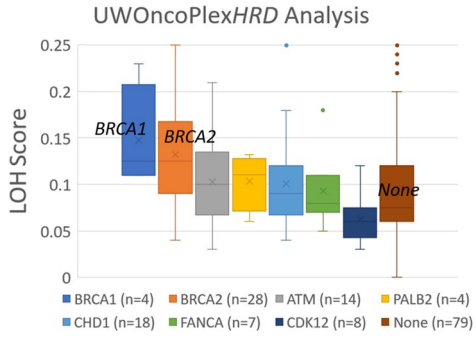

FIG 1. OncoPlexHRD results according to underling HRR gene mutation. Excluded from this box boxplot graph are cases with fewer than 4 samples (CHEK2 n=4, BRIP1 n=1, BAP1 n=1, RAD51B n=1, RAD51D n=1, FANCD2 n=2).

***TP53* Mutation**	**Average LOH score**	**SD LOH Score**
Absent	0.10	0.06
Present	0.12	0.08
t-test, two tailed	p= 0.24	

Table 3. LOH Score according *TP53* mutation status

TP53 mutation is associated with increased chromosome instability and has been suggested to raise overall LOH scores independent of HRD status in other cancer types (e.g. serous ovarian cancer), we analyzed the LOH score according to TP53 mutation status (**Table 3**). We observed a slightly higher overall average LOH score (11%) in *TP53* mutant cancer compared to TP53 wild type (9%), which was statistically significant (t-test, two tailed, p=0.009).

Assessments of multi-modal HRD detection strategies for OncoPlexHRD: HRD genomic hallmarks include increased genomic LOH, as well as SNV substitution signatures (COSMIC signature 3), indels at regions of microhomology, and characteristic rearrangement signatures (PMID:28288110). Due to limitations of a targeted panel approach, we focused on quantitative LOH as the primary measure of HRD for the clinical assay, but we will also explore incorporating COSMIC signature 3, and indel signatures. Our paired normal approach results in "clean" calls for both SNVs and indels, facilitating incorporation of this data even though it will be sparse in comparison to a whole exome or genome approach.

Our results to date identified an optimal prostate cancer-specific LOH% threshold for positivity. Following the data-lock we will establish this threshold empirically based on Receiver Operator Curve (ROC) analysis and define an indeterminate LOH% range. Within- and between run reproducibility will be performed across at least 3 runs for 20 sample pairs. Lower limit of detection will be established by mixing studies using a low-positive and high-positive HRD tumor. Validation of this cut-point is planned to occur in PY3.

AIM 2. Identify specific combinations of DNA repair gene and metabolic parameters that confer functional homology directed DNA repair deficiency.

Progress to Date: During PY2, we focused on developing a deeper understanding of the role of the transcriptional kinase CDK12 in mediating HR deficiency. As noted previously, CDK12 loss has been associated with HRD through dependencies identified in screens for PARPi susceptibility. CDK12 mutations are currently criteria for treatment with the PARPi olaparib for metastatic prostate cancer. The major clinical question is whether CDK12 does confer functional HRD and whether tumors with CDK12 alterations exhibit enhanced responses to agents that promote DNA damate (e.g. xrt, platinum chemotherapy) or that repress repair (e.g. PARPi).

Prostate cancers with CDK12 inactivation do not exhibit genomic mutation signatures associated with HRd or reduced expression of genes involved in DNA repair. To ascertain genomic alterations that associate with CDK12 loss in PC, we analyzed several large datasets where deep molecular assessments of tumors included metrics of both gene expression by RNAseq and genomic alterations by whole exome sequencing (WES) or whole genome sequencing (WGS). Five datasets were evaluated: the TCGA PRAD study of localized PC comprising 330 tumors (PRAD), the SU2C/PCF International study of mCRPC comprising 360 tumors (SU2C-I), the SU2C/PCF West Coast study of mCRPC comprising 110 tumors (SU2C-WC), 200 tumors from the Hartwig Foundation molecular data from PC (HARTWIG-PC) and the University of Washington Autopsy study of mCRPC comprising 160 tumors from 80 patients (UW-A). Collectively, 8% of patients were identified to have biallelic *CDK12* inactivation by biallelic copy loss, single copy loss with a pathogenic second allele mutation, or biallelic pathogenic mutation. CDK12 inactivation is well documented to be associated with a tandem duplicator phenotype (TDP) reflected by numerous copy gains of duplications averaging 1.3-2.5 Mb across the genome. Of the tumors with biallelic CDK12 loss by genomic assessments, 90% had genomic alterations consistent with a TDP. Four tumors were identified with a TDP that did not have CDK12 alterations.

Having identified cohorts of PCs with and without *CDK12* biallelic loss, we next sought to determine if *CDK12* loss tumors exhibited evidence of compromised HR. One method for determining HRd is by analyzing tumor genomes for mutations and/or structural alterations that result ineffective HR. CDK12mut mCRPC cases from the Stand Up 2 Cancer data set were analyzed, revealing that only 20.8% (5 of 24) bi-allelic CDK12 mutant cases were positive for the iHRD signature, a marker of functional HRD based on genomic mutation scar patterns. For comparison, 27.5% of all tumors and 78.0% of bi-allelic HR mutated mCRPC cases in the SU2C set are positive for the iHRD signature.

We also sought to look specifically for long gene downregulation and alternate polyadenylation in CDK12mut cases. Previous reports showed that CDK12 loss leads to selective 3' transcript loss in long genes, so we analyzed differentially expressed mRNAs between CDK12mut and control (non-CDK12mut, non-HRD) mCRPC samples.

The distribution of downregulated genes by size (gene length) in the CDK12mut tumors was not significantly different from the controls. To specifically measure alternate polyadenylation (APA) usage, the APAlyzer package was used to analyze various datasets. Analysis of previously published data confirmed the reported increase in APA usage upon acute Cdk12 loss in mouse embryonic stem cells. However, no such dramatic preferential upregulation of APA sites was seen in CDK12mut prostate samples from the TCGA and University of Washington Rapid Autopsy datasets, though there was a slight enrichment of APA usage in the SU2C samples. Analysis of ovarian cancer datasets from the TCGA appeared to show a moderate skew towards upregulated APA usage. Taken together, these results show that CDK12mut patients show limited APA upregulation, which does not lead to dramatic downregulation of HR genes or sensitivity to PARPi.

HR gene downregulation occurs with acute CDK12 loss. We next sought an explanation for the discrepancy between the reported mechanisms of CDK12-loss leading to HRD and the observations in patient data. First, we chose to replicate acute loss conditions, which have used in previous studies. LNCaP and 22Rv1 cells were treated for 6, 24 or 48h with a CDK12/13 inhibitor (SR4835) and protein expression was analyzed by western blot. In agreement with previous studies, BRCA2, ATM, and ATR decreased at 24h and 48h post treatment with 200nM SR4835. DNA damage (γH2A.X) increased by 48h (LNCaP) and 24h (22Rv1) but was largely ablated with the addition of Z-VAD, a pan-caspase inhibitor. Similar results were observed with an ovarian cancer line, Skov3. Thus, while SR4835 does cause slight decreases in DNA repair gene protein expression, most of the corresponding γH2A.X is likely due to apoptosis and not impaired DNA repair directly.

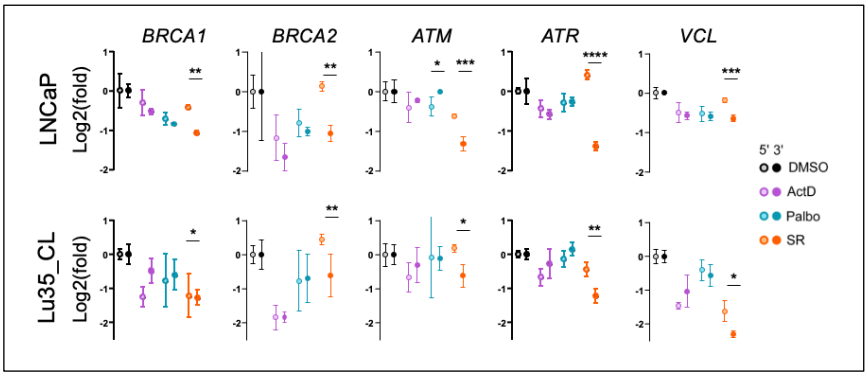

FIG 2. Acute CDK12 loss causes DNA repair gene downregulation. qPCR analysis with RNA samples from above using two sets of primers for each target to show selective loss of 3' transcripts. Vinculin (*VCL*) is a long gene not involved in DNA repair. Plots show mean Log2(fold) vs vehicle -/+stdev. Data were standardized to centered average of four housekeeping genes (18S, RPL19, ACTB, GAPDH) and significance between 5'/3' primer sets was determined by two-way ANOVA.

To measure the consequences of CDK12 loss on DNA repair gene mRNA expression, we ran a short 6h treatment with SR4835, palbociclib (CDK4/6i), or actinomycin D (ActD) on LNCaP and LuCaP35 cells and performed RNAseq (FIG 2). Some DNA repair genes, including *BRCA1* and *BRCA2*, show cell cycle linked expression so palbociclib served as a control for DNA repair gene decreases due solely to G1 arrest. ActD served as a control for non-specific RNA-Pol II inhibition. SR4835 caused a small decrease in the HR pathway, though not as dramatic as palbociclib. Dozens of pathways showed enrichment differences with both palbociclib and SR4835, leading to some difficulty in untangling which effects are due more broadly to cell arrest vs CDK12/13i-specific effects. As expected, SR4835 did lead to dramatic decreases in some key DNA repair genes (ATM and BRCA1 in LNCaP, BRCA1, BRCA2, and ATR in LuCaP 35).

We next wanted to specifically inspect whether the CDK12/13 inhibition caused long gene downregulation via transcript shortening, as would be expected from the APA phenotype. Genes downregulated by SR4835 skewed longer than those downregulated by palbociclib or ActD. When analyzed based on transcript length, the effect is not as apparent. SR4835 also caused a clear shift towards upregulation of APA site usage, which was not seen with ActD or palbociclib. qPCR was performed with specific primers for 5' and 3' regions to further validate the preferential loss of 3' transcripts of long genes, including BRCA1 and BRCA2, upon SR4835 treatment. Together, these results confirm the mRNA shortening and APA activation phenotype in two prostate lines under acute CDK12 loss conditions.

Cells adapted to CDK12 loss do not show dramatic HR gene downregulation. CDK12 is classified as a 'common essential' gene (https://depmap.org/portal/) and CDK12/13 inhibitors cause apoptosis after 24-48h. Despite the essentiality of this gene, some tumors tolerate and adapt to the loss of CDK12. We next investigated the possibility that cells adapted to CDK12 loss might not show the same phenotype as cells undergoing acute CDK12 depletion. LuCaP 189.4 is a *de novo* CDK12 mutant PDX line that we established as a cell line (LuCaP 189.4_CL). LuCaP 189.4_CL does not express CDK12 protein and exhibits a classic tandem duplicator phenotype (TDP) that is a

hallmark of CDK12mut tumors. qPCR was used to measure 5' vs 3' transcript levels and found that 189.4_CL displays some 3' decrease in *ATM* and *ATR*, but not *BRCA1* or *BRCA2*. qPCR comparison of LuCaP 35 and 189.4 PDX tumor samples showed no statistical decreases in 5' vs 3' levels of *BRCA1*, *BRCA2*, *ATM*, or *ATR*.

To further study the effect of cells adapted to CDK12 loss, CRISPR-mediated KO clones were generated in 22Rv1 (two clones: KO2 and KO5) and Skov3 cells (one clone: KO1) and 189.4_CL were engineered to re-express CDK12. Very few cells tolerated CDK12 KO, and these three rare clones all grow slower than the parental lines. At the protein level, CDK12-KO clones showed slight increases in CDK13 and decreases in CyclinK but no obvious decrease in p-Ser2 RNA Polymerase II levels. 22Rv1 and Skov3 CDK12-KO clones did not show decreases in ATR, BRCA1, or BRCA2 protein but did show decreased ATM. Interestingly, LuCaP 189.4_CL shows comparable levels of these DNA repair genes, and although the re-expression of CDK12 was not especially high, it did appear to increase ATM protein slightly. As with the 189.4_CL qPCR results, the 22Rv1 CDK12-KO clones also showed persistent 2-3 fold 3' vs 5' transcript decreases in *ATM* and *ATR*, but minimal 5'/3' difference in *BRCA1* or *BRCA2*. The 22Rv1 CDK12-KO clones did show lower overall *BRCA1* and *BRCA2* mRNA levels, but this is likely due to cell-cycle linked expression and the slower growth of these clones.

RNA-seq was performed on the CDK12 isogenic models. Analysis of downregulated genes showed no significant enrichment of longer genes in the 22Rv1 clones, but there was enrichment in the Skov3 clone. LuCaP 189.4_CL with CDK12 re-expressed only had 58 upregulated genes, which were not any longer than the unchanged genes. Overall these results show that, with the notable exception of ATM, most long genes (including BRCA1 and BRCA2) do not show dramatic downregulation in prostate cancer cells that have adapted to CDK12 loss. Furthermore, though CDK12-KO in the Skov3 ovarian cancer cells showed some preferential downregulation of long genes overall, BRCA1 and BRCA2 were not affected.

<u>*CDK12 loss does not affect RAD51 foci formation*</u>. A key early step in HR is BRCA2-mediated loading of RAD51 onto resected ssDNA. Loss of key HR genes, including BRCA1, BRCA2, or PALB2, all lead to loss of RAD51 loading and initiation of HR repair. Though CDK12-KO cells retain BRCA1 and BRCA2 protein expression, it is possible HR function could still be altered by other means. To test this possibility, LNCaP, 22Rv1, and Skov3 cells were engineered with Tet-inducible shBRCA2 or shCDK12 (**FIG 3**). Cells were irradiated (6Gy) and immunostained for γH2A.X and RAD51 at 3h post radiation. BRCA2 shRNA successfully reduced RAD51 foci formation, but shCDK12 had no effect in LNCaP, 22Rv1, and Skov3 cells. CDK12(-) models including the 22Rv1-KO clones and 189.4_CL all showed robust RAD51 induction following radiation, further supporting the idea that CDK12 deficient cells maintain functional HR induction.

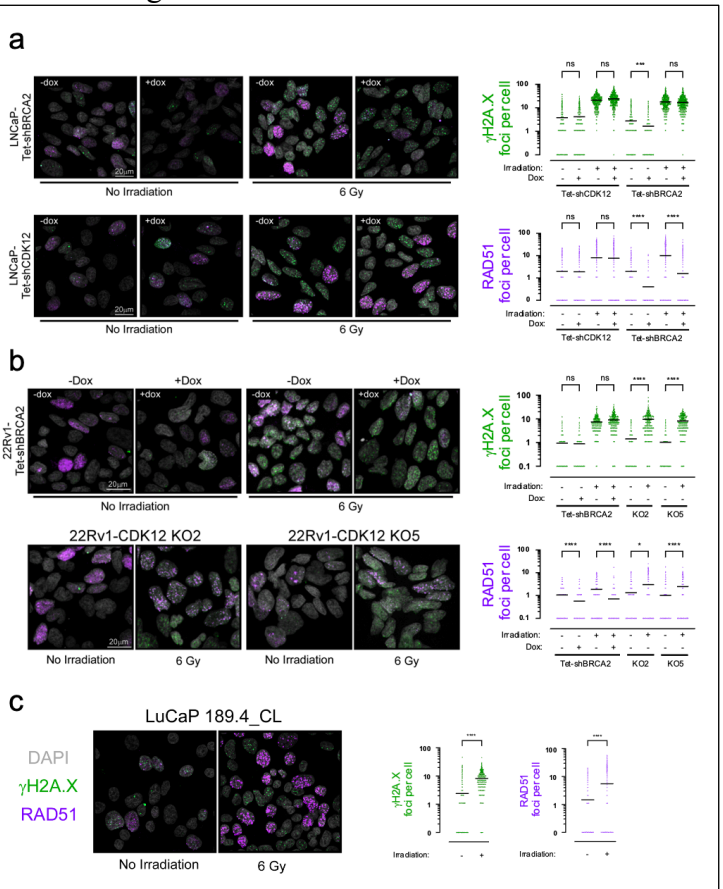

FIG 3. CDK12 loss does not affect RAD51 foci formation. (a) LNCaP cells with either Tet-inducible shBRCA2 or shCDK12 were induced with doxycycline (100ng/mL) for 4 days then exposed to 6Gy ionizing radiation and fixed at 3h post IR. Immunofluorescence staining was performed for γH2A.X and RAD51 and images were acquired by confocal microscopy. Left: representative images (white: DAPI, green: γH2A.X, purple: RAD51). Right: quantification of images (~200-500 cells analyzed per treatment). Line is at mean and significance was determined by one-way ANOVA (Kruskal-Wallis) with Dunn's multiple testing correction. (b) Same conditions using 22Rv1-Tet-shBRCA2 or CDK12-KO lines. (c) Same conditions using LuCaP 189.4_CL. Significance determined by paired, one-way t-test (Wilcoxon).

Overall, these experiments demonstrate that while acute CDK12 loss does confer HRD, cells adapted to chronic CDK12 loss recover HR proficiency and do not demonstrate HRD – thus these date suggest that PCs with CDK12 loss will not demonstrate enhanced sensitivity to PARPi or DNA damaging agents.

<u>AIM 3.</u> Identify pharmacological agents that promote HDR deficiency (HRD) and that enhance the effects of genotoxic drugs and PARPi.

<u>Overall Strategy</u>: The objectives for this Aim are to: (i) identify drug combinations that will act synergistically to eradicate all tumor cells that are HRD; and (ii) take advantage of 'conditional haploinsufficiency' to induce a full 'BRCAness' phenotype in tumors with partial attenuation of repair capacity. We utilize a well-characterized panel of PDX lines and engineered models (e.g. cell lines) to develop support for advancing promising combinations into the clinic. Following the functional studies detailed in Aim 2 regarding CDK12 and HRD, work during PY2 focused on determining whether or not prostate cancers with CDK12 loss would respond to PARPi or DNA damaging agents, and identifying drugs that would selectively eliminate tumors cells with CDK12 loss.

<u>CDK12 loss does not confer sensitivity to PARPi or PLAT</u>. Though adapted CDK12 loss does not appear to cause functional HRD, there is still a possibility that CDK12 loss could sensitize to PARPi via other mechanisms. Dose response curves were performed with carboplatin and olaparib using various lines, including BRCA1(-) UWB1.289 ovarian cancer cells (**FIG 4**). CDK12 KO in 22Rv1 did not increase sensitivity to carboplatin, as seen with the BRCA2 shRNA positive control. 22Rv1-CDK12-KO clones and LuCaP 189.4_CL showed mixed responses to PARPi. In a 12-day treatment, 22Rv1-CDK12-KO clones and 189.4_CL displayed some sensitivity to though not as great as the true BRCA1(-) UWB1.289 line olaparib. However, in an 8-day treatment, though UWB1.289 showed sensitivity to carboplatin and PARPi (olaparib, rucaparib, talazoparib), LuCaP 189.4_CL did not. In addition, a 14-day treatment of organoids harvested from PDX tumors found that 189.4 did not show particular sensitivity to olaparib or rucaparib. Skov3 CDK12 KO cells showed no difference in sensitivity to carboplatin or olaparib. Colony forming assays were performed with the 22Rv1 and Skov3 lines, with cells undergoing 12 days (Skov3) or 18 days (22Rv1) treatment with olaparib. UWB1.289 show reduced colony formation at 0.2uM and almost no colonies at 1uM. 22Rv1-KO5 showed reduced colonies at 0.2uM olaparib, but Skov3-CDK12-KO1 did not show clear decrease until 1 or 5 uM. These results show that CDK12 loss does not sensitize to carboplatin, but the effect on PARPi sensitivity is more complicated. Skov3-CDK12-KO1 do not seem to show strong sensitivity in any of the tested conditions. However, 189.4 and 22Rv1-CDK12-KO5 show sensitivity to olaparib under some conditions but not others.

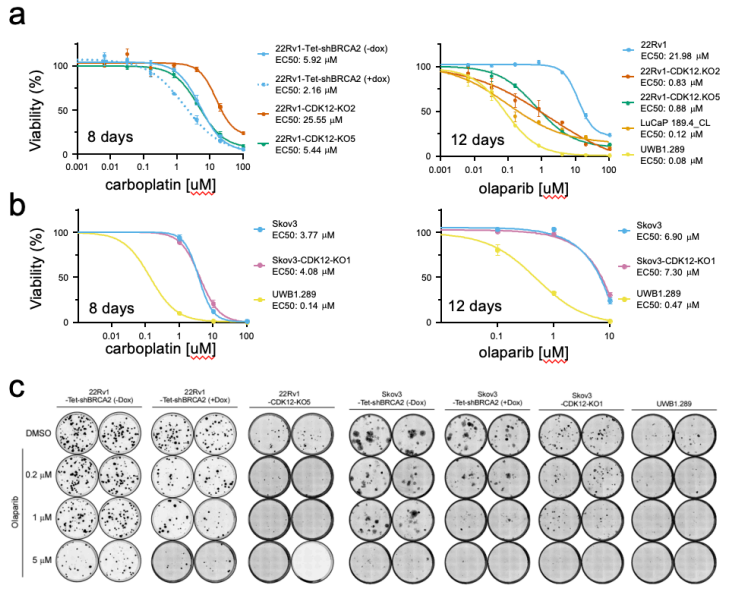

FIG 4. CDK12 Loss does not confer platinum or PARPi sensitivity. (a) Dose response curves for prostate lines treated with carboplatin or olaparib (n=3). (b) Dose response curves for ovarian cancer lines treated with carboplatin or olaparib (n=4). Note: UWB1.289 is a BRCA1(-) ovarian cancer line and bona fide HRd model. (c) Colony growth assay with 22Rv1 and Skov3 Tet-shBRCA2 and CDK12-KO lines seeded in 12-well plates and treated 12d (Skov3, UWB1.289) or 18d (22Rv1) with olaparib, replaced fresh weekly (n=2). Tet-inducible lines were treated -/+ 100ng/mL doxycycline. Plots show quantification of colony number.

<u>CDK13 is synthetic lethal in CDK12(-) cells</u>. Analysis of CRISPR screening data from DepMap shows that CDK13 KO is generally tolerated in most lines, while CDK12 sgRNAs are negatively selected (**FIG 5**). Moreover, cells are highly sensitive to CCNK/CyclinK KO, whose loss ablates both CDK12 and CDK13 activity. We performed whole genome CRISPR screening in the isogenic CDK12 models. Strikingly, the CDK12(-) lines showed more CDK13 sgRNA depletion, especially Skov3-CDK12-KO1. LuCaP 189.4-CDK12 cells showed correspondingly less depletion of CDK13 sgRNAs, though the gene still had a very negative score which may indicate that the low level of CDK12 re-expression could not sustain the cells. Infection with lentivirus containing sgCDK13 and Cas9 led to reduced growth in 22Rv1 and Skov3 CDK12-KO lines. LuCaP 189.4_CL were confirmed to show greatly reduced growth when infected with CDK13 sgRNAs.

Due to high protein conservation, all currently available inhibitors that target CDK12 also inhibit CDK13. We performed dose response curves with two different CDK12/13 inhibitors (SR4835, THZ531) and found that LuCaP 189.4_CL are one of the most sensitive of the lines tested. 22Rv1 CDK12 KO lines showed increased sensitivity to THZ531 but not SR4835 (**FIG 5**).

To confirm whether the CDK13 vulnerability could be used for *in vivo* treatment, we performed xenograft drug treatments in three LuCaP PDX lines (35, 136, and 189.4) treated 30 days with vehicle or SR4835 and found that the 189.4 showed a significant decrease in tumor volume and mass, with no significant tumor reduction in the CDK12-intact lines. Mouse body weights were not significantly different in treated vs control groups. These results support the hypothesis that cells lacking CDK12 become dependant on CDK13 for their CyclinK activity, thus presenting a potential targeted vulnerability with potential *in vivo* efficacy, even with dual CDK12/13 targeting compounds.

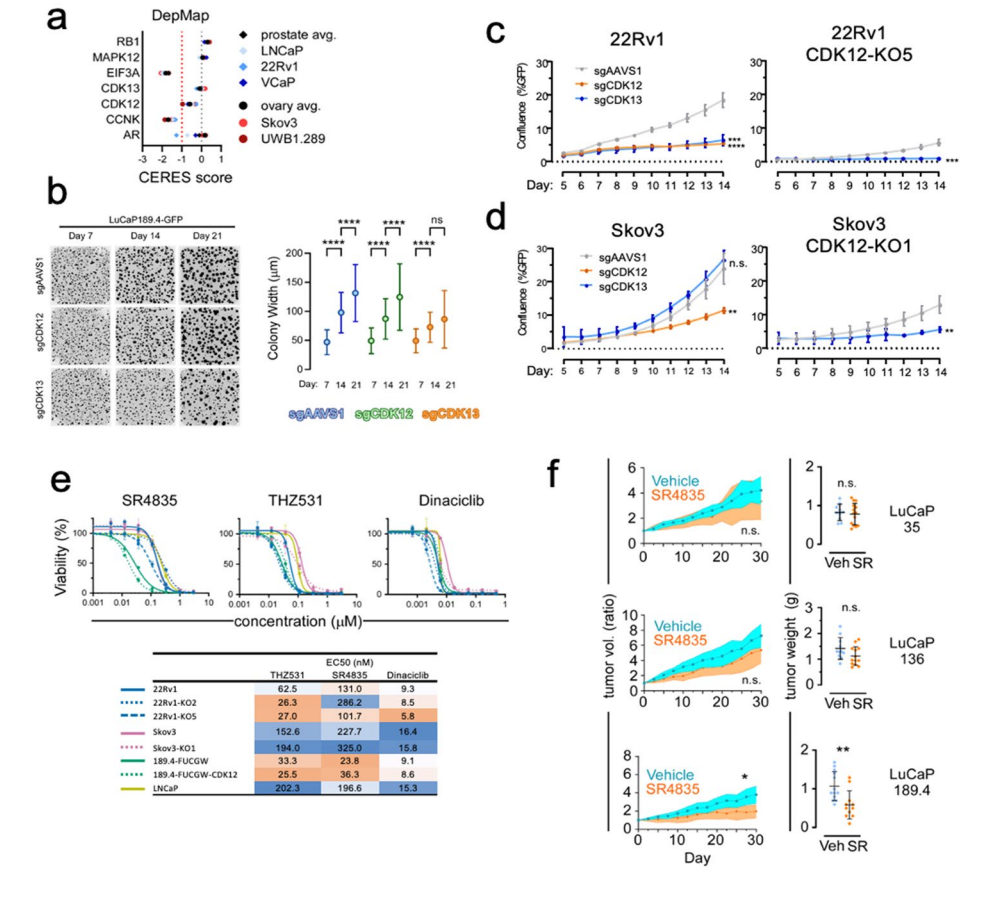

FIG 5. CDK12(-) cells are sensitive to CDK13 loss. (a) Selection of control and CDK12-related sgRNA fitness results from DepMap, including average score for all prostate or ovarian lines. *RB1* is a control for positive selection, *MAPK12* is a redundant gene (p38g) and control for neutral selection, and *EIF3A* is a pan-essential gene and control for negative selection. (b) GFP-tagged LuCaP 189.4 cells were transduced with CRISPR vectors containing dual sgRNAs against *AAVS1* (safe harbor cut site/neg. control), *CDK12*, or *CDK13* and growth was monitored by microscopy (n=5). Colonies were quantified and plot shows colony width over time as mean -/+stdev, with statistics determined by two-way ANOVA with Tukey multiple testing correction. (c,d) GFP-tagged lines were transduced with dual sgRNA vectors and growth was measured with daily GFP imaging (n=5). Plots show mean GFP % area -/+ stdev, with statistics determined by two way ANOVA with Dunnett correction. (e) Dose response curves with two selective CDK12/13 inhibitors (SR4835, THZ531) and dinaciclib, a less-selective transcription-associated CDK inhibitor. Cells were treated 6 days (n=3). The table shows EC50 values derived from the response curves. (f) *in vivo* PDX LuCaP tumors were treated 28 days with vehicle or SR4835 (20mg/kg, 3 days on, 2 days off). Data show tumor volume (left) and final tumor weight (right). n=LuCaP 35 (10 veh, 13 SR), LuCaP 136 (11 veh, 11 SR), LuCaP 189.4 (13 veh, 11 SR). Tumor volume shading = 95% confidence interval, with significance determined by Kolmogorov-Smirnov test. Tumor weight significance was determined with unpaired, two-tailed t-test.

CDK12 loss increases R-loop formation and sensitivity to some transcription-targeting drugs. The most studied function of CDK12 is to maintain RNA polymerase II processivity and proper splicing and polyadenylation. Though stable CDK12(-) cells don't show much APA usage (Fig. 3D,E) or decreased RNA-Pol II Ser2 phosphorylation, they may still have impaired transcription which could be targeted. CDK12 isogenic models were immunostained with the S9.6 antibody to detect the presence of DNA:RNA hybrid, marking sites of R-loop formations. Pladienolide B, an SF3BP1 splicesom inhibitor, served as a positive control for inducing R-loops. CDK12 negative cells showed an increase in the number of R-loops, as did treatment with SR4835. R-loops have been reported to cause PARPi sensitivity, so cells were engineered with Tet-RNASEH1 to see if degradation of R-loops could reverse the sensitivity. R-loops can form from impairments in transcription or replication. Dose response curves were generated with drugs targeting transcription and selective sensitivity was seen with a-amanitin, an RNA-Pol II poison (Fig. 6a) and showed that the 22Rv1 CDK12-KO clones showed increased sensitivity

(Fig. 6a), with a more subtle difference with Skov3-CDK12-KO1. Treatment with 5-fluorouracil (5-FU), which can impair transcription and translation, showed slight selectivity in the 22Rv1 CDK12-KO clones (Fig. 6b), but no sensitivity in Skov3-CDK12-KO1. Cells were also tested with 5-FU by colony forming assay, with 22Rv1-CDK12-KO5 showing far fewer colonies at 1uM than the parental line (Fig. 5c). On the other hand, Skov3-CDK12-KO1 did not show fewer colonies with 5-FU. These results suggest that even cells adapted to CDK12 loss struggle with increased transcriptional DNA damage, as indicated by R-loop formation, and that this might lead to a vulnerability targeting transcription.

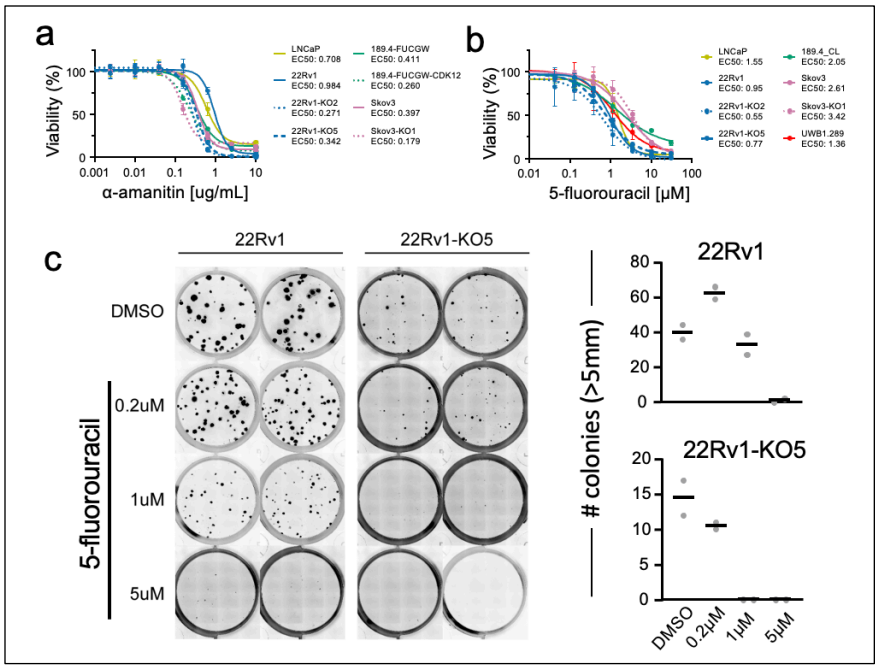

FIG 6. DK12(-) cells show increased transcription-linked DNA damage and sensitivity to transcription-targeting therapies. (a) CDK12(-) lines were stained for R-loops with the S9.6 antibody, which is selective for RNA:DNA hybrid sequences. (b) Dose response curve for cells treated 4 days with α-amanitin (n=3) and the calculated EC50 values. (c) dose response curves for cells treated 6 days with 5-fluorouracil (n=3)) and the calculated EC50 values. (d) Colony growth assay with 22Rv1 and CDK12-KO5 cells seeded in 12-well plates (n=2) and treated 18 days with 5-fluorouracil, replaced fresh on Days 6 and 12. Colonies were counted and quantified on plots, with line at mean.

4) other achievements

None to report.

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

The project has supported the training and professional development of Dr. Tony Chu (external support), a post-doctoral fellow, and Canan Dirican, a research technician who managed the PDX studies. The project also supported the career development of Ilsa Coleman, a bioinformatics specialist who received a Masters degree in bioinformatics for work related to this proposal. The project provided professional development for Dr. Nelson, who delivered several seminars relating to DNA repair and prostate cancer (see below in 'Products').

How were the results disseminated to communities of interest?

The study results have primarily been disseminated through peer-reviewed publications. The results have also been presented at scientific meetings through oral presentations (see below in 'Products').

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

(1) Next Steps for Analytical Validation of OncoPlexHRD: In the coming year we will continue to evaluate the performance of the modified OncoPlex*HRD* sequencing panel in accordance with metrics defined for the validation of laboratory developed clinical tests (CLIA/CAP), specifically, confirmation of basic performance metrics defined by the Clinical and Laboratory Standards Institute (sensitivity, analytic specificity, within and between run reproducibility/precision, limit of detection/limit of quantitation, analytic measurement range). We have set an initial threshold for calling 'LOH high' at 11% based on the optimal sensitivity and specificity to detected *BRCA1* and *BRCA2* mutation. Prospective clinical data is needed to refine this analysis.

In year 3 we will lock the analysis to evaluate the analytic sensitivity and specificity will be performed by 1) comparison to samples with HRD gene mutation status (*BRCA1, BRCA1, PALB2, RAD51B, RAD51C, RAD51D*) established by a clinically-validated method (UW-OncoPlex or BROCA, available to the Pritchard group). We will reflex selected samples with upper quartile LOH% but without detected HRD gene mutations to whole genome sequencing.

Our data to date supports an optimal prostate-specific LOH% threshold for positivity at 11%. Within- and between run reproducibility will be performed across at least 3 runs for 20 sample pairs. Lower limit of detection will be established by mixing studies using a low-positive and high-positive HRD tumor.

In PY3 we will continue to evaluate OncoPlexHRD using prostate cancer samples from prospective clinical trials of PARPi and PLAT for which we can obtain access to biological specimens and treatment outcomes. We have already obtained biospecimens from the ABCD clinical trial of platinum chemotherapy in men with metastatic prostate cancer and underling HRR gene mutations. This trial has completed and outcomes are available, and we have extracted detailed structured data for 17 patients to date. We will also focus on obtaining samples from the PLATIPARP, and POPCAP/VA Olaparib trials. We will follow REMARK biomarker criteria guidelines (PMC3362085). We will establish the clinical validity of OncoPlex*HRD* by ROC analysis for clinical trial populations based on the pre-determined primary endpoints of PARPi/PLAT responsiveness. We will perform Kaplan Meyer analysis for each trial using OncoPlex*HRD* vs. mutation-only analysis, with particular attention on HRD+ cases without detected HR gene mutations.

(2) Next steps for identifying combinations of DNA repair gene and metabolic parameters that confer functional homology directed DNA repair deficiency. We will continue to follow the plan outlined in the original SOW and develop isogenic models with heterozygous alterations in key genes involved in HR repair and identify combinations that confer HRD. We have completed the planned detailed analysis of CDK12 – and will now move forward with a focus on ATM as well as HR gene combinations. We will also focus on metabolic pathways with the potential to produce genotoxic events or downregulate HR repair mechanisms.

(3) Next steps for identifying pharmacological agents that promote HDR deficiency (HRD) and that enhance the effects of genotoxic drugs and PARPi. We will continue to follow the plan outlined in the initial SOW and continue to screen specific drugs and drug combinations that potentially induce HRD – including additional synthetic androgens (e.g. methylT). We will also evaluate drugs that alter metabolic pathways to produce products that alter HR repair, and that consequently have the capability to induce 'BRCAness' (e.g. aldehyde metabolizing agents). We anticipate that such agents would produce synthetic lethality with genotoxic drugs and PARPi.

4. IMPACT

What was the impact on the development of the principle disciplines of the project?

1) We have developed and tested a new assay, termed OncoplexHRD, that represents a read-out of functional HDR-D through LOH events. This assay is suitable for tissue based analyses or circulating tumor DNA (ctDNA);
2) We developed a composite assays for functional HRD that includes mutation signatures – termed iHRD, and demonstrated strong associations with responses to platinum chemotherapy and PARPi;
3) We determined that inherited mutations in DNA repair genes are rare in men with low risk prostate cancer;
4) We determined that TP53 is an inherited prostate cancer predisposition gene, and that TP53 can influence metrics usually associated with HDR-D such as LOH scores;
5) We determined that aggressive prostate cancers with BRCA2 loss exhibiting neuroendocrine features respond to PARPi, concordant with typical adenocarcinomas with HDR-D;
6) We confirmed that HR gene mutations ascertained in analyses of primary prostate cancers are generally concordant with events identified in metastatic biopsies or ctDNA – confirming that primary tumors can serve as a relevant source for ascertaining HDR-D status and allocating appropriate treatment;
7) We identified a pattern of structural DNA alterations that associate with HRD, adding an additional parameter for clinical testing for determining HDR-D in patients.
8) We determined that acute CDK12 loss confers HRD via loss of expression of several genes involved in HR.
9) We determined that chronic loss of CDK12 does not confer HRD – and that cells adapted to CDK12 loss do not show susceptibility to PARPi or DNA damaging agents.
10) We determined that prostate cancers with chronic loss of CDK12 show replication stress and the development of R loops that confer modest sensitivity to CDK13 loss and drugs targeting DNA synthesis.

What was the impact on other disciplines?
Nothing to report

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: Nothing to report.
Actual or anticipated problems or delays and actions or plans to resolve them: Nothing to report.

6. PRODUCTS:

Publications, conference papers, and presentations

Publications
Lozano R, Castro E, Lopez-Campos F, Thorne H, Ramirez-Backhaus M, Aragon IM, Cendón-Florez Y, Gutierrez-Pecharroman A, Salles DC, Romero-Laorden N, Lorente D, González-Peramato P, Calatrava A, Alonso C, Anido U, Arévalo-Lobera S, Balmaña J, Chirivella I, Juan-Fita MJ, Llort G, Y Cajal TR, Almagro E, Alameda D, López-Casas PP, Herrera B, Mateo J, **Pritchard CC**, Antonarakis ES, Lotan TL, Rubio-Briones J, Sandhu S, Olmos D. *Impact of concurrent tumour events on the prostate cancer outcomes of germline BRCA2 mutation carriers*. Eur J Cancer. 2023 May;185:105-118. PMID: 36972661; acknowledgement of federal support (yes).

Graham LS, Haffner MC, Sayar E, Gawne A, Schweizer MT, **Pritchard CC**, Coleman I, **Nelson PS**, Yu EY. *Clinical, pathologic, and molecular features of amphicrine prostate cancer.* Prostate. 2023 May;83(7):641-648. PMID: 36779357; acknowledgement of federal support (yes).

De Sarkar N, Patton RD, Doebley AL, Hanratty B, Adil M, Kreitzman AJ, Sarthy JF, Ko M, Brahma S, Meers MP, Janssens DH, Ang LS, Coleman IM, Bose A, Dumpit RF, Lucas JM, Nunez TA, Nguyen HM, McClure HM, **Pritchard CC**, Schweizer MT, Morrissey C, Choudhury AD, Baca SC, Berchuck JE, Freedman ML, Ahmad K, Haffner MC, Montgomery RB, Corey E, Henikoff S, **Nelson PS**, Ha G. *Nucleosome patterns in circulating tumor DNA reveal transcriptional regulation of advanced prostate cancer phenotypes*. Cancer Discov. 2023 Mar 1; 13(3): 632-653. PMCID: PMC9976992; acknowledgement of federal support (yes).

Cheng HH, Sokolova AO, Gulati R, Bowen D, Knerr SA, Klemfuss N, Grivas P, Hsieh A, Lee JK, Schweizer MT, Yezefski T, Zhou A, Yu EY, **Nelson PS**, Montgomery B. *Internet-Based Germline Genetic Testing for Men With Metastatic Prostate Cancer*. JCO Precis Oncol. 2023 Jan;7:e2200104. PMCID: PMC9928882; acknowledgement of federal support (yes).

Schweizer MT, Gulati R, Yezefski T, Cheng HH, Mostaghel E, Haffner MC, Patel RA, De Sarkar N, Ha G, Dumpit R, Woo B, Lin A, Panlasigui P, McDonald N, Lai M, Nega K, Hammond J, Grivas P, Hsieh A, Montgomery B, **Nelson PS**, Yu EY. *Bipolar androgen therapy plus olaparib in men with metastatic castration-resistant prostate cancer*. Prostate Cancer Prostatic Dis. 2023 Mar; 26(1):194-200. PMCID: PMC10286318; acknowledgement of federal support (yes).

Presentations
Exploiting Addiction: Understanding and Targeting the Requirement for Androgen Receptor Function in Advanced Prostate Cancer. Keynote – 3rd Nuclear Receptors Conference. Cancun, Mexico 5/2022.

Exploiting the AR "Goldilocks" Phenomenon for Prostate Cancer Therapy. Coffey-Holden Prostate Cancer Academy. UCLA. Los Angeles, CA 06/2022.

Hallmarks of Cancer – the Next Generation for the Next Generation. SURP Lecture Series, Fred Hutchinson Cancer Center, WA 8/2022

Anticipating and Targeting the Emergence of New Cancer 'Species' Under Treatment Pressure. Institute of Molecular Medicine at McGovern Medical School, Houston, TX. 11/2022

Anticipating, Tracking and Targeting New Prostate Cancer Lineages Driven by Treatment Pressures. AACR Special Conference "Advanced in Prostate Cancer" Denver, CO 3/2023

Intra and inter-tumor heterogeneity across cancer metastases: A reality check for targeted therapeutics and the utility of non-invasive biomarkers. AACR Annual Meeting. Orlando, FL 4/2023.

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?

Name:	**Peter S. Nelson, MD**
Project Role:	Principal Investigator
Nearest person month worked:	2
Contribution to Project:	Dr. Nelson provided oversight and direction for the entire project. His research effort was primarily devoted toward: (i) assisting Dr. Pritchard with biospecimen acquisition for OncoPlexHRD evaluation; (ii) interpreting results of OncoPlexHRD in the context of clinical outcomes to PARPi and PLAT; (iii) managing trainees and staff conducting the preclinical in vitro and in vivo studies of HDR deficiency; (iv) designing and interpreting experiments; (v) analyzing data; (vi) disseminating research findings through scientific presentations and manuscripts; (vii) managing the fiscal and regulatory components of the project. Dr. Nelson also works closely with collaborator Drs. Haffner and Etzioni.
Funding Support:	PC200608; PC200262 (this award); P01 CA163227; R01 CA271457; P50 CA097186; P30 CA015704; R01 CA234715; PC200608; PC180686; PC170350P1

Name:	**Michael Haffner, MD, PhD**
Project Role:	Other Significant Contributor
Nearest person month worked:	No effort
Contribution to Project:	Dr. Haffner has provided his expertise as a urologic pathologist who provides pathology review for the clinical samples.
Funding Support:	PC190137; PC200608; Doris Duke Foundation; PC210181; V

Foundation, Brotman Baty Institute; PC210181; PC210387; R01 CA266452; P50 CA097186; R01 CA271457; R01 CA234715; P30 CA015704

Name: **Ilsa Coleman**
Project Role: Research Scientist
Nearest person month worked: 1
Contribution to Project: Ms. Coleman assisted with experiments and procedures that involve preparing libraries for NextGen sequencing and primarily focus on analyzing sequencing data including gene expression levels (RNAseq), single cell RNAseq, pathway analyses, mutation assessments and CNV analysis. She also provided biostatistical support and worked closely with Dr. Etzioni.
Funding Support: PC200262 (this award); P01 CA163227; R01 CA266452; R01 CA271457; P50 CA097186; R01 CA234715; R01 CA2495285; PC180686; PC170350P1

Name: **Jared Lucas**
Project Role: Staff Scientist
Nearest person month worked: 4
Contribution to Project: Mr. Lucas assiststed with biostatistical work and analysis.
Funding Support: PC200262 (this award); P01 CA163227; R21 CA277368; P50 CA097186; R01 CA234715

Name: **Lisa Ang, PhD**
Project Role: Research Technician
Nearest person month worked: 2
Contribution to Project: Ms Ang assisted with animal work and experiments.
Funding Support: PC200262 (this award); P01 CA163227; R01 CA234715

Name: **Ruth Dumpit**
Project Role: Research Technician
Nearest person month worked: 4
Contribution to Project: Ms. Dumpit purified nucleic acids from tumors, assisted with confirmatory assays of molecular abberations in human metastases, isolated CTCs, isolated ctDNA and performed ARV7 assays.
Funding Support: PC200262 (this award); R21 CA277368; P50 CA097186; R01 CA234715

Name: **Marc Villanueva Martinez**
Project Role: Laboratory Assistant
Nearest person month worked: 3
Contribution to Project: Mr. Martinez assisted with cell cultures and DNA/RNA/protein purifications.
Funding Support: PC200262 (this award)

Name: **Reza Alizadeh Ghodsi**
Project Role: Post-Doctoral Fellow
Nearest person month worked: 4
Contribution to Project: Dr. Ghodsi provided expertise in measurements of gene expression by qRTPCR and immunoblotting in the preclinical models.
Funding Support: PC200262 (this award); PC220447

Name:	**Tony Lok Heng Chu**
Project Role:	Post-Doctoral Fellow
Nearest person month worked:	1
Contribution to Project:	Dr. Chu provided expertise in cell culture and modifications to cell lines/model systems. He also assisted with the generation of DNA repair gene mutations and measurements of gene expression in the preclinical models.
Funding Support:	PC200262 (this award); R01 CA266452; R01 CA234715

Name:	**Canan Dirican**
Project Role:	Research Technician
Nearest person month worked:	8
Contribution to Project:	Mr. Dirican assisted with animal work and experiments.
Funding Support:	PC200262 (this award); R01 CA271457

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
Multiple changes have occurred in active support for Dr. Nelson, for efficiency, updated PCPS has been attached to the appendices to illustrate changes.

What other organizations were involved as partners?
There is one other organization involved with this project, University of Washington, Award #W81XWH-21-1-0264-P1

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:
This is a collaborative award between Dr. Peter Nelson at the Fred Hutchinson Cancer Center and Dr. Colin Pritchard at the University of Washington. Dr. Pritchard will submit the same overall SOW and research results that reflect the research outcomes in Y2 for this collaborative award.

QUAD CHART
Attached

9. APPENDICES:
Previous, current, and pending support has been attached for Dr. Peter Nelson.

PREVIOUS / CURRENT / PENDING SUPPORT – NELSON, PETER S.

PREVIOUS SUPPORT

Title: *Resistance to Cancer Therapeutics Through Microenvironment Damage Responses*
Grant #: R01 CA165573
Time Commitments: 0.6 CM, 5% effort
Supporting Agency: NIH/NCI
Name and address of the funding agency's procuring Contracting/Grants Officer: Nicholas Mitrano, 9609 Medical Center Drive, Room 2W348, Rockville, MD 20850
Performance Period: 03/01/2012 - 01/31/2018
Level of funding (direct costs):
Brief description of the project's goals: To evaluate the concept that conventional cancer therapeutics modify the tumor microenvironment to enhance resistance to subsequent treatments, consequently promoting adverse cancer phenotypes.
Overlap with proposed research: None
List of specific aims:
Aim 1: Determine the ability of specific paracrine-acting DNA-damage Secretory Program (DDSP) proteins (e.g. SPINK1) to modulate adverse tumor cell behaviors (e.g. therapy resistance) and determine the mechanism(s) by which they do so.
Aim 2: Determine the intracellular signal transduction programs that differentially modulate subsets of effector proteins comprising DDSP.
Aim 3: Determine the consistency of the DDSP across different tumor types and establish the temporal and cell type-specific variability of damage response programs.

Title: *Androgen Receptor Action in Castration Resistant Prostate Cancer,* Core A: Administrative / Clinical / Biostatistics Core
Grant #: P01 CA163227
Time Commitments: 0.6 CM, 5% effort
Supporting Agency: Beth Israel Deaconess Medical Center – NIH/NCI
Name and address of the funding agency's procuring Contracting/Grants Officer: Linda M.S. Fritze, 330 Brookline Avenue, BR264, Boston, MA 02215-5491
Performance Period: 05/24/2013 - 04/30/2018
Level of funding (direct costs):
Brief description of the project's goals: Support is limited to Dr. Nelson's effort.
Overlap with proposed research: None
List of specific aims: Not applicable - inter-SPORE interactions.

Title: *Pacific Northwest Prostate Cancer SPORE,* Core A: Leadership and Administration
Grant #: P50 CA097186
Time Commitments: 0.91 CM, 7.6% effort
Supporting Agency: NIH/NCI
Name and address of the funding agency's procuring Contracting/Grants Officer: Renee Carruthers, 9000 Rockville Pike, Bethesda, MD 20892
Performance Period: 09/17/2013 - 08/31/2018
Level of funding: (Core A)
Brief description of the project's goals: The Leadership and Administrative Core (LAC) will serve to integrate and enhance the research conducted by the SPORE projects and cores, as well as the Career

Development faculty and investigators supported through the Developmental Research Program, through the application of general administrative support and the facilitation of communication and data dissemination.
Overlap with proposed research: None
List of specific aims:
Aim 1: To provide oversight of all SPORE-activities involving the independent research projects, the Career Development Program (CDP), the Developmental Research Program (DRP), the shared resource cores, and the parent institutions.
Aim 2: To provide the organizational structure, based on a group of interacting committees, for supporting and evaluating the key objectives of the PNW SPORE.
Aim 3: To organize and coordinate forums for interactions of the Executive Committee, Internal Advisory Board, and External Advisory Board.
Aim 4: To provide efficient and effective fiscal management of **Grant** funds.
Aim 5: To communicate and consult with the NCI Project Officer(s) and staff in the preparation of required progress reports, publications lists, and regulatory documents.
Aim 6: To develop and maintain virtual mechanisms that efficiently facilitates multi-institutional intra- and inter-SPORE interactions.

Title: *Pacific Northwest Prostate Cancer SPORE*
Project 4: Clinical Development of Therapeutic Strategies Targeting Damage Responses in the Prostate Tumor Microenvironment
Grant #: P50 CA097186
Time Commitments: 0.91 CM, 7.6% effort
Supporting Agency: NIH/NCI
Name and address of the funding agency's procuring Contracting/Grants Officer: Renee Carruthers, 9000 Rockville Pike, Bethesda, MD 20892
Performance Period: 09/17/2013 - 08/31/2018
Level of funding: (Project 4)
Brief description of the project's goals: To determine whether inhibiting components of the microenvironment-derived DNA damage secretory
program will enhance the responses of prostate tumors to commonly used genotoxic cancer treatments.
Overlap with proposed research: None
List of specific aims:
Aim 1: To evaluate the effects of inhibiting key regulators and effectors of the microenvironment DNA Damage Secretory Program on therapy responses in preclinical models of prostate cancer.
Aim 2: To conduct a Phase l-II trial evaluating the clinical effect of inhibiting master regulators and specific effectors of the DNA Damage Secretory Program in augmenting genotoxic chemotherapy in men with metastatic CRPC.

Title: *Developing PDX Models of Prostate Cancer for Use in Evaluating Novel Therapeutic Approaches*
Grant #: P50 CA097186-14S1
Time Commitments: 0.46 CM, 3.8% effort
Supporting Agency: NIH/NCI
Name and address of the funding agency's procuring Contracting/Grants Officer: Crystal Wolfrey, Office of **Grant**s Administration, NCI, 9609 Medical Center Drive, Room #2W472, Bethesda, MD 20892
Performance Period: 09/01/2016 - 08/31/2018
Level of funding (direct costs):
Brief description of the project's goals: This proposal is designed to address a major limitation in the development of effective therapeutics for advanced PC by generating PDX models representing the diverse genotypes and phenotypes found across the spectrum of advanced prostate cancer.
Overlap with proposed research: None

List of specific aims:
Aim 1: Develop and characterize new prostate cancer PDX models that can be used to test cancer therapies, including drug combinations and NCI-IND agents.
Aim 2: Test existing PDX models of prostate carcinoma against NCI-IND agents and agent combinations for tumor response, to integrate and analyze PDX molecular characteristics against response to therapeutic regimens, and to collaborate with NCI-funded investigators in the study of mechanisms of drug sensitivity and resistance.

Title: *Minimally-Invasive Assessments of Prostate Cancer Molecular Heterogeneity to Direct Precision Therapy*
Grant #: W81XWH-15-1-0430
Time Commitments: 0.91 CM, 7.6% effort
Supporting Agency: DOD/CDMRP
Name and address of the funding agency's procuring Contracting/Grants Officer: Joshua D. McKean, USAMRAA, 820 Chandler Street, Ft. Detrick, MD 21702
Performance Period: 09/01/2015 - 08/31/2018
Level of funding (direct costs):
Brief description of the project's goals: This application focuses on the developing an accurate and minimally-invasive approach for evaluating the molecular composition of metastatic castration resistant prostate cancer (mCRPC).
Overlap with proposed research: None
List of specific aims:
Aim 1: Determine the molecular identity and genomic diversity across the spectrum of metastasis found within individual men with CRPC. This aim will take advantage of a robust tissue acquisition necropsy (TAN) program where multiple metastasis are acquired from each patient.
Aim 2: Determine the genomic and gene expression states of individual circulating tumor cells (CTCs) acquired from the same men that will undergo a TAN procedure.
Aim 3: Compare the molecular alterations present in original untreated primary tumors with those alterations found in metastatic tumors and CTCs to assess how well an archival sample represents the spectrum of actionable targets found in metastatic prostate cancer.

Title: *Development of Precision Immunotherapy for Advanced Prostate Cancer*
Grant #: W81XWH-15-1-0562
Time Commitments: 0.91 CM, 7.6% effort
Supporting Agency: DOD/CDMRP
Name and address of the funding agency's procuring Contracting/Grants Officer: Joshua D. McKean, USAMRAA, 820 Chandler Street, Ft. Detrick, MD 21702
Performance Period: 09/30/2015 - 09/29/2018
Level of funding (direct costs):
Brief description of the project's goals: Long-term goal is to propel integration of the precision immunotherapy enabled by nanoparticle-mediated reprogramming T cells into the standard-of-care for the treatment of metastatic prostate cancer.
Overlap with proposed research: None
List of specific aims:
Aim 1: Establish that engineering T cells to express CARs recognizing multiple tumor subtypes improves their anticancer activity.
Aim 2: Measure the ability of injectable DNA nanocarriers to program CAR expression by host T cells.
Aim 3: Determine how some prostate cancer subtypes resist CAR-based T cell therapy, and identify novel targets expressed by them.

Title: *Non-Invasive Biomarkers for Diagnosing Clinically Significant Prostate Cancer*
Grant #: R01 CA181605
Time Commitments: 0.91 CM, 7.6% effort
Supporting Agency: NIH/NCI
Name and address of the funding agency's procuring Contracting/Grants Officer: Renee Carruthers, 9000 Rockville Pike, Bethesda, MD 20892
Performance Period: 01/01/2014 - 12/31/2018
Level of funding (direct costs):
Brief description of the project's goals: The goal of this proposal is to exploit distinct prostate cancer associated RNAs using quantitative urine assays and a multi-institutional longitudinal cohort of men managed by active surveillance with attendant biospecimens, pathological, and clinical data.
Overlap with proposed research: None
List of specific aims:
Aim 1: Determine if PCA3 and TMPRSS2:ERG mRNA concentrations in urine associate with the presence or development of clinically-significant prostate cancer using longitudinal repeat assessment in men on Active Surveillance.
Aim 2: Evaluate a panel of long non-coding RNAs in tissue and urine for the detection of significant prostate cancer in men on Active Surveillance.
Aim 3: Define and evaluate a panel of Gleason Pattern-associated RNAs in tissue and urine for the detection of significant prostate cancer in men on Active Surveillance.

Title: *Androgen Receptor Action in Castration Resistant Prostate Cancer,* Project 1: Steroid Metabolism in Castration Resistant Prostate Cancer
Grant #: P01 CA163227
Time Commitments: 0.46 CM, 3.8% effort
Supporting Agency: Beth Israel Deaconess Medical Center – NIH/NCI
Name and address of the funding agency's procuring Contracting/Grants Officer: Linda M.S. Fritze, 330 Brookline Avenue, BR264, Boston, MA 02215-5491
Performance Period: 05/24/2013 - 02/11/2019
Level of funding (direct costs):
Brief description of the project's goals: To determine whether identification and subsequent inhibition of key ligand-generating mechanisms would reduce AR signaling, with potential substantially improved responses, reduced morbidity, and prolonged survival.
Overlap with proposed research: None
List of specific aims:
Aim 1: Determine the contribution of steroid biosynthetic enzymes to the generation of intratumoral androgens and consequent tumor cell survival in CRPC.
Aim 2: Determine the mechanism(s) underlying the coordinate regulation of androgen biosynthetic enzymes and activation of the AR gene expression program in prostate cancers following androgen suppression.
Aim 3: Determine the therapeutic effectiveness of progressive inhibition of androgen metabolism and identify resistance mechanisms to metabolism-directed therapy in CRPC.

Title: *Determining and Exploiting Mechanisms of AR-Mediated Suppression of Cell Proliferation and Survival*
Grant #: R01 CA233863
Time Commitments: 1.2 CM, 10% effort
Supporting Agency: NIH/NCI
Name and address of the funding agency's procuring Contracting/Grants Officer: Crystal Wolfrey, 240-276-6277, wolfrey@mail.nih.gov
Performance Period: 09/01/2018 - 02/11/2023
Level of funding:

Brief description of the project's goals: The objectives of the current proposal are to define the molecular pathway(s) by which SPA represses PC growth and activates senescence/apoptotic programs, and to identify the genomic and epigenomic cellular states that rewire the AR cistrome to respond to – or resist – high androgen concentrations.
Overlap with proposed research: There was significant overlap with the currently-funded (renewed) P01. Thus, this award was relinquished prior to acceptance of the P01 (CA163227-06A1).
List of specific aims:
Aim 1: Determine the primary mechanism(s) by which SPA represses CRPC.
Aim 2: Define the AR cistrome in prostate cancers reprogrammed by SPA and identify cooperating genes and pathways that are essential or suppressive of SPA effects.
Aim 3: Identify drug combinations that synergize with SPA to repress tumor growth and optimize the effects of AR agonism based on a mechanistic understanding of SPA-mediated growth arrest.

Title: *Selective Androgen Receptor Modulators for the Treatment of Prostate Cancer*
Grant #: R21 CA230138
Time Commitments: 1.2 CM, 10% effort
Supporting Agency: NIH/NCI
Name and address of the funding agency's procuring Contracting/Grants Officer: Terri Jarosik, 301-443-3858, tjarosik@mail.nih.gov
Performance Period: 07/11/2018 – 06/30/2020
Level of funding:
Brief description of the project's goals: This proposal will evaluate the feasibility of using small molecule replacements for testosterone in the treatment of prostate cancer.
Overlap with proposed research: None
List of specific aims:
Aim 1: Determine effects of non-steroidal AR agonists on AR activity in prostate cancer cells.
Aim 2: Determine whether non-steroidal AR agonists recapitulate the physiological and molecular changes associated with exposure to supraphysiological testosterone (SPT).
Aim 3: Determine anti-tumor efficacy of AR agonists and explore dynamic dosing strategies.

Title: *Targeting the Mechanisms Driving Double-Negative Basal-Like Prostate Cancer*
Grant #: W81XWH-17-1-0415
Time Commitments: 0.91 CM, 7.6% effort
Supporting Agency: DOD/CDMRP
Name and address of the funding agency's procuring Contracting/Grants Officer: Joshua McKean, 301-619-4046, Joshua.d.mckean3.civ@mail.mil
Performance Period: 08/01/2017 – 07/31/2020
Level of funding:
Brief description of the project's goals: The objective of this proposal is to address the fundamental lack of knowledge concerning the biology underlying therapy-related DNPC in order to understand the key molecular mechanisms responsible for their genesis, behavior and response to treatment.
Overlap with proposed research: None
List of specific aims:
Aim 1: Identify survival and growth programs operative in DNPC though deep molecular profiling of metastatic tumors and PDX models.
Aim 2: Utilize engineered cell lines and PDX models to determine which specific molecular alterations observed in the human DNPC tumors cause AR-bypass and promote a DNPC phenotype.
Aim 3: Evaluate pharmacological agents targeting DNPC driver pathways that emerge following AR ablation to confirm anti-tumor effects and support further clinical evaluation.

Title: *Evaluating DNA Damage and DNA Repair Capacity as Biomarkers of Non-Indolent Prostate Cancer*
Grant #: PC171001
Time Commitments: 0.90 CM, 7.5% effort
Supporting Agency: DOD/CDMRP
Name and address of the funding agency's procuring Contracting/Grants Officer: DOD/CDMRP, help@ebrap.org; 301-682-5507
Performance Period: 08/01/2018 - 07/31/2021
Level of funding:
Brief description of the project's goals: This proposal will test the hypothesis that subtypes of prostate cancer – defined by mutational processes and DNA repair deficiency – associate strongly with adverse prostate cancer characteristics including extra-prostatic spread, biochemical recurrence, and metastasis. We further hypothesize that these molecular characteristics can assist in further stratifying patients for less intensive or no further active monitoring or conversely, for immediate curative therapy.
Overlap with proposed research: None
List of specific aims:
Aim 1: Determine if DNA repair signatures and metrics of DNA damage associate with adverse reclassification and adverse pathology in men on active surveillance.
Aim 2: Determine if genomic signatures of DNA mutational processes associate with prostate cancer characteristics indicative of non-indolent behavior.
Aim 3: Determine if specific defects in specific genes involved in DNA repair are predictive of adverse prostate cancer outcomes.

Title: *The MSKCC-UW/Fred Hutch Prostate Cancer Drug Resistance and Sensitivity Center*
Grant #: U54 CA224079-S1
Time Commitments: 0.12 CM, 1% effort
Supporting Agency: MSKCC - NIH/NCI
Name and address of the funding agency's procuring Contracting/Grants Officer: Vincent Madonia, madoniav@mskcc.org
Performance Period: 12/1/2020 – 08/31/2021
Level of funding: (total costs)
Brief description of the project's goals: In this supplement, the role of translational control in the context of resistance to AR pathway-directed therapeu-tics will be evaluated.
Overlap with proposed research: None
List of specific aims:
Aim 1: Determine how alterations in mRNA translation initiation promote resistance to AR pathway directed therapies.
Aim 2: Delineate the translational gene networks that promote resistance to AR signaling inhibitors.

Title: *Therapeutic Strategies to Target Treatment-Resistant Merkel Cell Carcinoma (MCC) and Neuroendocrine Prostate Cancer (NEPC)*
Grant #: 19CHAL02
Time Commitments: 0.12 CM, 1% effort
Supporting Agency: Prostate Cancer Foundation
Name and address of the funding agency's procuring Contracting/Grants Officer: Audrey Gardner, 1250 4th Street, Santa Monica, CA 90401 (audrey.gardner@pcf.org)
Performance Period: 01/01/2019 – 8/14/2021
Level of funding: year (total costs)
Brief description of the project's goals: This Challenge Award is designed to integrate the underlying biology of MCC and NEPC to identify or ex-pose tumor vulnerabilities to target the substantial number of treatment resistant MCCs and NEPC that contribute to high mortality rates for these aggressive cancers.

Overlap with proposed research: None
List of specific aims:
Aim 1: Identify mechanisms contributing to the resistance of MCC and NEPC to conventional and immune-based therapeutics.
Aim 2: Evaluate strategies to augment immune-based therapy of MCC and NEPC.
Aim 3: Identify combinations of therapeutics targeting lineage and survival pathways in MCC and NEPC that can be rapidly advanced to clinical trials.

Title: *Eradicating Metastatic Prostate Cancer through the Systematic Identification of Synergistic Drug Combinations*
Grant #: PC170431
Time Commitments: 0.12 CM, 1% effort
Supporting Agency: DOD/CDMRP
Name and address of the funding agency's procuring Contracting/Grants Officer: DOD/CDMRP, help@ebrap.org; 301-682-5507
Performance Period: 08/01/2018 – 12/31/2021
Level of funding: Total Costs for the Award Period (Nelson Program only)
Brief description of the project's goals: We will test the hypothesis that specific combinations of therapeutics can rapidly and completely eradicate subtypes of mPC, and that patient-derived xenografts (PDX) representing the diversity of molecular 'driver' aberrations found in human CRPC can be used to rapidly and systematically identify these potent drug combinations.
Overlap with proposed research: None
List of specific aims:
Aim 1: Conduct a systematic assessment of combination pharmacological therapy to eradicate CRPC using panels of PDX models that reflect the diversity of molecular aberrations found in mCRPC.
Aim 2: Identify molecular features (genotype/phenotype) of PDX responders to drug combination(s).
Aim 3: Establish an International consortium for evaluating and validating novel therapeutic combinations capable of eradicating CRPC.

Title: *Targeting the Subtype of Metastatic Prostate Cancer Deficient in DNA Repair Capacity*
Grant #: PC170503
Time Commitments: 0.12 CM, 1% effort
Supporting Agency: DOD/CDMRP
Name and address of the funding agency's procuring Contracting/Grants Officer: DOD/CDMRP, help@ebrap.org; 301-682-5507
Performance Period: 08/15/2018 – 11/30/2021
Level of funding: Total Costs for the Award Period
Brief description of the project's goals: This proposal will address the challenge of effectively treating mPC by exploiting specific tumor vulnera-bilities conferred by defects in HR DNA repair.
Overlap with proposed research: None
List of specific aims:
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 with homologous recombination repair deficiency.

Title: *2021 cfDNA Award*

Grant #: No Grant Number
Time Commitments: 0.0 CM, 0% effort
Supporting Agency: Brotman Baty Institute – University of Washington
Name and address of the funding agency's procuring Contracting/Grants Officer: Nola Klemfuss, klemfuss@uw.edu
Performance Period: 03/01/2021 – 02/28/2022
Level of funding: No Funding (award in-kind)
Brief description of the project's goals: We hypothesize a low pass whole-genome (LP_WGS) sequence data do is a substantial representation of high depth whole genome sequencing in retaining nucleosome and transcription factor binding genomic signatures. LP_WGS data can be reliably utilized in classifying epigenetic/phenotypic subtypes of prostate cancer. (DNPC, Amphicrine, NEPC and ARPC).
Overlap with proposed research: None
List of specific aims:
Aim 1: Determine whether ctDNA inserts aligning to actively transcribing genes are consistently different than the inserts aligning to non-transcribing or repressed genes.

Title: *The MSKCC-UW/Fred Hutch Prostate Cancer Drug Resistance and Sensitivity Center*
Project 3: Defining the Appropriate Context for Targeting Kinase Signaling in Combination with Androgen Receptor Blockade to Enhance Therapeutic Response in Metastatic Prostate Cancer
Grant #: U54 CA224079
Time Commitments: 0.6 CM, 5% effort
Supporting Agency: MSKCC – NIH/NCI
Name and address of the funding agency's procuring Contracting/Grants Officer: William Zurich, zuricihiw@mskc.org, 646-227-3374, MSKCC, 633 3rd Avenue, 3rd Floor, NYC, NY 10017
Performance Period: 09/30/2017 – 08/31/2022
Level of funding: Total Costs for the Award Period
Brief description of the project's goals: Our proposal aims to: 1) define biomarkers of intrinsic sensitivity and resistance to inform appropriate patient selection for combination therapy; 2) define the mechanisms of acquired resistance; 3) devise therapeutic strategies to overcome resistance; 4) optimize AR pathway targeting in the setting of PI3K pathway inhibition to maximize tumor response; and 5) explore the therapeutic role of FGFR in a novel subset of AR negative prostate cancers.
Overlap with proposed research: None
List of specific aims:
Aim 1: Identifying metastasis suppressors within skeletal muscle.
Aim 2: Reverse engineering growth resistance using rare tumor cells that successfully colonize skeletal muscle.
Aim 3: Delivering skeletal muscle derived factors to prevent colonization of metastasis prone organs.

Title: *Therapeutic Targeting of Neuroendocrine Prostate Cancer*
Grant #: PC170350P1
Time Commitments: 0.6 CM, 1% effort Y1; 0.0 CM, 0% effort Y2 (administrative effort only as subaward PI)
Supporting Agency: UW – DOD/CDMRP
Name and address of the funding agency's procuring Contracting/Grants Officer: Kelly Sales, 206-606-7595, ksales@seattlecca.org
Performance Period: 09/01/2020 – 08/31/2022
Level of funding:Total Costs for the Award Period
Brief description of the project's goals: Dr. Nelson's lab at the Fred Hutch Cancer Research Center (Fred Hutch) will provide bioinformatics expertise for the RNA-Seq data in Years 2 and 3 of the project. RNA will be isolated from patient-derived xenografts (PDX; n=75). Once the raw array data are acquired, Ms. Ilsa Coleman will use bioinformatic tools working with Drs. Lam and Morrissey to compare and assess treatment response and resistance to therapy in the PDX lines.

Overlap with proposed research: None
List of specific aims: Not applicable – subaward

Title: *3D Light-Sheet Microscopy: Identification and Molecular Characterization of Prostate Carcinoma with De Novo Resistant to Total Androgen Ablative Therapy*
Grant #: PC180686 / W81XWH-19-1-0589
Time Commitments: 0.12 CM, 1% effort
Supporting Agency: UW – DOD/CDMRP
Name and address of the funding agency's procuring Contracting/Grants Officer: Mirelle Aziz, Program Operations Specialist, maziz@uw.edu
Performance Period: 08/15/2020 – 08/14/2022
Level of funding: Total Costs for the Award Period
Brief description of the project's goals: Dr. Nelson will coordinate the RNA sequencing work to be performed by the Fred Hutch Genomics Core and participate in data analysis and interpretation. Additionally, he will oversee the work of Ms. Ilsa Coleman, Research Scientist, who will be designing and carrying out the activities outlined in all Aims 2 and 3 of the project.
Overlap with proposed research: None
List of specific aims:
Aim 1: To demonstrate improved sensitivity of OTLS-guided 3D microdissection, we will compare sequencing results using 3D microdissection versus slide-based methods.
Aim 2: To define the molecular landscape of PC response to neoadjuvant total AAT, we will sequence 3D microdissected residual carcinoma regions from RP specimens.
Aim 3: To better understand PC pathogenesis in response to neoadjuvant total AAT, we will sequence carcinoma regions from pre-treatment biopsies and post-treatment lymph node metastases.

Title: *Pacific Northwest Prostate Cancer SPORE,* Core A: Leadership and Administration
Grant #: P50 CA097186-21
Time Commitments: 1.2 CM, 10% effort
Supporting Agency: NIH/NCI
Name and address of the funding agency's procuring Contracting/Grants Officer: Renee Carruthers, 9000 Rockville Pike, Bethesda, MD 20892
Performance Period: 09/01/2018 - 08/31/2023
Level of funding:
Brief description of the project's goals: The Leadership and Administrative Core (LAC) will serve to integrate and enhance the research conducted by the Pacific Northwest Prostate SPORE Projects and Cores – as well as the faculty supported through both the career Enhancement and Developmental Research Programs – through the application of general administrative support and the facilitation of communication and data dissemination.
Overlap with proposed research: None
List of specific aims:
Aim 1: To provide the organizational structure, based on a group of interacting committees, for supporting and evaluating the key objectives of the PNW SPORE.
Aim 2: To provide oversight of all SPORE activities involving the independent research projects, the Career Enhancement Program (CEP), the Developmental Research Program (DRP), the shared resource cores, and the parent institutions.
Aim 3: To organize and coordinate forums for interactions of the Executive Committee, Internal Advisory Board, and External Advisory Board.
Aim 4: To provide efficient and effective fiscal management of SPORE **Grant** funds.
Aim 5: To communicate and consult with the NCI Project Officer(s) and staff in the preparation of required progress reports, publications lists, and regulatory documents.

Aim 6: To develop and maintain virtual mechanisms that efficiently facilitate multi-institutional, intra- and inter-SPORE interactions.
**This project was previously listed as CURRENT*

Title: *Pacific Northwest Prostate Cancer SPORE,* Project 4: Clinical Development of Therapeutic Strategies Targeting DNA Damage Repair
Grant #: P50 CA097186-21
Time Commitments: 1.2 CM, 10% effort
Supporting Agency: NIH/NCI
Name and address of the funding agency's procuring Contracting/Grants Officer: Renee Carruthers, 9000 Rockville Pike, Bethesda, MD 20892
Performance Period: 09/01/2018 - 08/31/2023
Level of funding:
Brief description of the project's goals:
Our studies will optimize SPT-based therapy, elucidate the mechanisms underlying responses to SPT, develop novel combinatorial SPT-based treatment strategies, and identify predictive biomarkers for response/resistance to SPT-based therapies.
Overlap with proposed research: None
List of specific aims:
Aim 1: Conduct in vitro and in vivo testing of combinatorial SPT-strategies to inhibit CRPC progression and evaluate mechanism of action.
Aim 2: Conduct a clinical trial to test alternative dosing schedules of SPT-based therapy and a trial to test combinatorial SPT-based therapy.
Aim 3: Identify mechanisms of SPT action, sensitivity and resistance, and biomarkers of responses.
**This project was previously listed as CURRENT*

CURRENT SUPPORT

Title: *Cancer Center Support Grant*
Grant #: P30 CA015704
Time Commitments: 0.6 CM, 5% effort
Supporting Agency: NIH/NCI
Name and address of the funding agency's procuring Contracting/Grants Officer: Michael A. Marino, PhD, 9609 Medical Center Drive, Room 2W204, Rockville, MD 20850
Performance Period: 01/01/1997 - 12/31/2024
Level of funding (direct costs): Total Costs for the Award Period (Nelson Program only)
Brief description of the project's goals: The Cancer Center Support Grant provides funding for the infrastructure, shared resources, and other activities which promote interdisciplinary cancer research conducted within the Fred Hutchinson/University of Washington Cancer Consortium. Dr. Nelson's support is limited to his charged effort.
Overlap with proposed research: None
List of specific aims: Not applicable

Title: *Androgen Receptor Action in Castration Resistant Prostate Cancer,* Project 1: Determining and Exploiting Mechanisms of AR-Mediated Suppression of Cell Proliferation & Survival
Grant #: P01 CA163227-07
Time Commitments: 1.5 CM, 12.5% effort
Supporting Agency: Beth Israel Deaconess Medical Center – NIH/NCI

Name and address of the funding agency's procuring Contracting/Grants Officer: Renee Carruthers, 9000 Rockville Pike, Bethesda, MD 20892
Performance Period: 02/12/2019 - 01/31/2024
Level of funding: Total Costs for the Award Period
Brief description of the project's goals: The objective of this project is to identify mechanisms contributing to the activation and activity of androgen metabolic enzymes in castration resistant prostate cancer.
Overlap with proposed research: None
List of specific aims:
Aim 1: Determine the primary mechanism(s) by which SPA represses CRPC.
Aim 2: Define the AR cistrome in prostate cancers reprogrammed by SPA and identify cooperating genes and pathways that are essential or suppressive of SPA effects.
Aim 3: Identify drug combinations that synergize with SPA to repress tumor growth and optimize the effects of AR agonism based on a mechanistic understanding of SPA-mediated growth arrest.

Title: *Defining and Targeting Lineage Transition Programs Operative in AR Pathway Independent Prostate Cancer*
Grant #: R01 CA234715-03
Time Commitments: 1.8 CM, 15% effort
Supporting Agency: NIH/NCI
Name and address of the funding agency's procuring Contracting/Grants Officer: Jason Gill, gilljas@mail.nih.gov
Performance Period: 08/15/2020 - 04/30/2025
Level of funding: Total Costs for the Award Period
Brief description of the project's goals: We will test the hypothesis that AR Pathway-Independent Prostate Cancers (APIPC) activate – and are dependent upon – a limited set of specific survival and growth regulatory pathways that are regulated via de-repressed feedback loops and/or genetic/epigenetic alterations.
Overlap with proposed research: None
List of specific aims:
Aim 1: Target prostate cancer vulnerabilities exposed by resistance to AR-directed therapy.
Aim 2: Determine alterations in the prostate cancer epigenome resulting from AR pathway signaling inhibition that regulate druggable signaling programs driving survival and growth.
Aim 3: Develop co-targeting strategies directed toward permissive epigenomic regulators and deterministic features associated with prostate cancer cell lineage.

Title: *Defining and Modulating BRCAness to Improve the Precision of Prostate Cancer Therapy*
Grant #: W81XWH-21-1-0264 / PC200262
Time Commitments: 1.2 CM, 10% effort
Supporting Agency: DOD/CDMRP
Name and address of the funding agency's procuring Contracting/Grants Officer: Joshua D. McKean, joshua.d.mckean3.civ@mail.mil
Performance Period: 08/01/2021 – 07/31/2024
Level of funding: Total Costs for the Award Period
Brief description of the project's goals: This proposal is designed to address two challenges: First, improve the accuracy of detecting prostate cancers with HDR defects for appropriate treatment allocation. Second, to increase the number of men with metastatic prostate cancer that could benefit from therapeutics that target DNA repair deficiency be converting partially sensitive tumors into fully-sensitive tumors; that is, generating a 'BRCAness'phenotype.
Overlap with proposed research: None
List of specific aims:

Aim 1: Develop and test clinical grade assays that define prostate cancers with functional homology directed DNA repair deficiency to improve sensitivity and specificity relative to HR gene mutations.
Aim 2: Identify specific combinations of DNA repair gene and metabolic parameters that confer functional homology directed DNA repair deficiency.
Aim 3: Identify pharmacological agents that promote HRR-D and that enhance the effects of genotoxic drugs and PARPi.
[THIS AWARD]

Title: *Defining and Targeting the DNA Hypomethylation Phenotype in Advanced Prostate Cancer*
Grant #: W81XWH-21-1-0229 / PC200608
Time Commitments: 0.12 CM, 1% effort
Supporting Agency: DOD/CDMRP
Name and address of the funding agency's procuring Contracting/Grants Officer: Joshua McKean, **Grant**s Officer, joshua.d.mckean3.civ@mail.mil
Performance Period: 07/01/2021 – 06/30/2024
Level of funding: Total Costs for the Award Period, Nelson Lab)
Brief description of the project's goals: Dr. Nelson will contribute next generation sequencing data of LuCaP patient derived xenografts and rapid autopsy samples. He will also share novel cell line models derived from LuCaP xenografts with Dr. Haffner. In addition, he will provide guidance as the project evolves and will meet with Dr. Haffner on a quarterly basis to discuss the results, assess progress and provide assistance when needed.
Overlap with proposed research: None
List of specific aims:
Aim 1: Establish the prevalence, clinical associations and patterns of DNA hypomethylation in PC.
Aim 2: Determine the biological consequences of global DNA hypomethylation in PC.
Aim 3: Define tumor cell intrinsic targetable vulnerabilities in DHMPC.

Title: *Targeting Vulnerabilities Exposed by Cancer Treatment-Induced Lineage Plasticity*
Grant #: R01 CA266452-01
Time Commitments: 1.8 CM, 15% effort
Supporting Agency: NIH/NCI
Name and address of the funding agency's procuring Contracting/Grants Officer: Jacquelyn Saval, savalj@mail.nih.gov, 240-276-6312
Performance Period: 07/01/2022 – 06/30/2027
Level of funding: Total Costs for the Award Period
Brief description of the project's goals: Dr. Nelson will provide oversight and direction for the research plan which involves defining mechanisms driving treatment-associated lineage switching and developing therapeutic strategies that can be rapidly translated for clinical evaluation. Dr. Nelson will also supervise the PDX studies, bioinformatics analyses and interactions with clinical colleagues involving access to novel therapeutics and clinical trial designs that exploit findings from the planned preclinical work.
Overlap with proposed research: None
List of specific aims:
Aim 1: Identify the key determinants and permissive factors that promote a lineage switch from conventional ARdriven.
prostate cancer to new phenotypes following AR-directed treatment.
Aim 2: Determine if modulating factors that drive or permit lineage specification can prevent, delay, or reverse resistance to AR pathway inhibition.
Aim 3: Determine if co-targeting characteristics of re-directed lineages that emerge in the context of lineage switching will prolong responses to AR pathway inhibition.

Title: *A Prostate Cancer Dependency Map to Identify Tumor Subtype-Specific Vulnerabilities*
Grant #: R21 CA277368-01
Time Commitments: 1.2 CM, 10% effort
Supporting Agency: NIH/NCI
Name and address of the funding agency's procuring Contracting/Grants Officer: To Be Determined
Performance Period: 12/01/2022 – 11/30/2024
Level of funding: Total Costs for the Award Period
Brief description of the project's goals: Dr. Nelson will provide oversight and direction for the research plan which involves defining mechanisms driving treatment-associated lineage switching and developing therapeutic strategies that can be rapidly translated for clinical evaluation. Dr. Nelson will also supervise the PDX studies, bioinformatics analyses and interactions with clinical colleagues involving access to novel therapeutics and clinical trial designs that exploit findings from the planned preclinical work.
Overlap with proposed research: None
List of specific aims:
Aim 1: Conduct genome-wide loss-of-function genetic screens in established and new models of mPC.
Aim 2: Conduct in vitro drug screens of approved agents and those in developmental pipelines in new and existing models of mPC and integrate responses with PC dependency map data.
Aim 3: Validate synthetic lethal/collateral lethal responses observed in the cell line screens using in vivo patient derived xenograft (PDX) models to confirm responses and support clinical advancement.

Note: This proposal is designed to produce a second generation dependency map and demonstrate feasibility for developing and utilizing novel PDX-derived cell lines for large-scale screens. The work provided the preliminary data for the present proposal designed to build a third generation map – with additional novel models, a larger screen base of genetic and drug libraries, and novel approaches to validate hits using dTAG and PROTAC methods. There is no scientific or effort overlap.
**This award has been added to Dr. Nelson's CURRENT support*

Title: *Pacific Northwest Prostate Cancer SPORE,* Core A: Leadership and Administration
Grant #: P50 CA097186-22
Time Commitments: 1.2 CM, 10% effort
Supporting Agency: NIH/NCI
Name and address of the funding agency's procuring Contracting/Grants Officer: Renee Carruthers, 9000 Rockville Pike, Bethesda, MD 20892
Performance Period: 09/08/2023 – 08/31/2028
Level of funding: total award
Brief description of the project's goals:
The Leadership and Administrative Core (LAC) will serve to integrate and enhance the research conducted by the Pacific Northwest Prostate SPORE Projects and Cores – as well as the Career Enhancement faculty, and investigators supported through the Developmental Research Programs – through the application of general administrative support and the facilitation of communication and data dissemination.
Overlap with proposed research: None
List of specific aims:
Aim 1: To provide the organizational structure, based on a group of interacting committees and working groups, for supporting and evaluating the key objectives of the PNW SPORE, which are to:

- Conduct innovative and high-impact translational science
- Provide opportunities for the development of novel early phase ideas or hypotheses
- Provide opportunities for career development in prostate cancer translational research
- Engage patient advocates in the promotion of prostate cancer research and treatment
- Educate scientists, clinicians, and the lay public.

Aim 2: To provide oversight of all SPORE activities involving the independent research projects, the Career Enhancement Program (CEP), the Developmental Research Program (DRP), the shared resource cores, and the four parent institutions: Fred Hutchinson Cancer Center (FHCC), the University of Washington (UW) (and

affiliated Veterans Affairs Puget Sound Health Care System (VAPSHCS)), the Oregon Health and Sciences University (OHSU), and the University of British Columbia / Prostate Centre at Vancouver General Hospital (UBC/VGH).
Aim 3: To organize and coordinate forums for interactions of the Executive Committee, Internal Advisory Board, and External Advisory Board.
Aim 4: To provide efficient and effective fiscal management of **Grant** funds, including **Grant** disbursements, record-keeping, and coordination of equipment and supply purchasing; and to provide rigorous record-keeping for and compliance with governmental, NIH, NCI, and institutional regulations and requirements, including Radiation Safety, Animal Care, and Protection of Human Subjects.
Aim 5: To communicate and consult with the NCI Project Officer(s) and staff in the preparation of required progress reports, publications lists, and regulatory documents.
Aim 6: To develop and maintain virtual mechanisms that facilitate efficient multi-institutional intra- and inter-SPORE interactions. These include the maintenance of a PNW Prostate Cancer SPORE website, and the organization of on-site and video 'face-to-face' meetings across the institutions of the SPORE investigators.
**This award has been added to Dr. Nelson's CURRENT support*

Title: *Pacific Northwest Prostate Cancer SPORE,* Project 4: Clinical Development of Therapeutic Strategies Targeting DNA Damage Repair
Grant #: P50 CA097186-22
Time Commitments: 1.2 CM, 10% effort
Supporting Agency: NIH/NCI
Name and address of the funding agency's procuring Contracting/Grants Officer: Renee Carruthers, 9000 Rockville Pike, Bethesda, MD 20892
Performance Period: 09/08/2023 – 08/31/2028
Level of funding: total award
Brief description of the project's goals: In this proposal, our objective is to integrate and leverage two key aspects of PC biology: AR activity and DNA damage/repair. Integrating AR signaling and HR repair has important treatment ramifications as a substantial body of preclinical and clinical work indicates that HR deficiency (HRd) result in vulnerabilities to at least two drug classes: platinum (PLAT) chemotherapy and PARP inhibitors (PARPi).
Overlap with proposed research: None
List of specific aims:
Aim 1: Conduct a Phase 2 clinical trial combining genotoxic therapeutics and supraphysiological androgen (SPA) in patients with mCRPC to determine response rates, identify resistance mechanisms, and establish biomarkers that associate with clinical responses.
Aim 2: Identify the mechanism(s) by which therapeutics overdriving AR activity induce DNA damage, regulate DNA repair processes, and enhance genotoxic chemotherapy.
Aim 3: Identify therapeutic drug combinations and dosing/administration strategies that optimize the therapeutic window resulting from AR expression and activity in mCRPC.
**This award has been added to Dr. Nelson's CURRENT support*

PENDING SUPPORT

Title: *Combinatorial Phenotype-Targeted Therapy to Overcome Mechanisms Driving Prostate Cancer Treatment Resistance*
Grant #: HT9425-23-PCRP-TSA / PC230582 (Nelson)
Time Commitments: 1.2 CM, 10%
Supporting Agency: Department of Defense, Prostate Cancer Research Program, Translational Science Award
Name and Address of the Funding Agency's Procuring Contracting/Grants Officer: Pending

Performance Period: 02/01/2024 – 01/31/2027
Level of funding:
Brief Description of the Project's Goals: In this proposal we will test the hypothesis that mPC can be effectively targeted and eliminated using a strategy of gated combinatorial drug delivery (GCDD).
Overlap with proposed research: None
List of Specific Aims:
AIM 1: Exploit the specificity of ADC therapy for eradicating metastatic prostate cancers of specific phenotypes using a gated drug delivery strategy to maximize the therapeutic window.
AIM 2: To determine the effectiveness of combinatorial ADC therapy in eradicating metastatic prostate cancers comprised of heterogeneous phenotypes.
AIM 3: To determine response and resistance mechanisms operative in vivo to ADC therapeutics.

Title: *Molecular features promoting sensitivity to LSD1i in castration-resistant prostate Cancer*
Grant #: R01 (PA-20-185)
Time Commitments: 0.36 CM, 3% effort
Supporting Agency: John Hopkins University, NIH/NCI
Name and address of the funding agency's procuring Contracting/Grants Officer: Pending
Performance Period: 9/1/2023-8/31/2028
Level of funding: (FHCC only)
Brief description of the project's goals: The overall goals of this proposal are to validate LSD1 as a target in NE+ and AR+ tumors, including heterogeneous mixed tumors; utilize pharmacogenomics pipelines to nominate predictive biomarkers of response through elucidating genetic and epigenetic molecular features that promote sensitivity to LSD1i, and identify synergistic drug combinations using molecularly and phenotypically diverse patient-derived models.
Overlap with proposed research: None

Title: *Augmenting PSMA expression to enhance PSMA directed therapeutic efficacy*
Grant #: R01 CA286450-01
Time Commitments: 0.12 CM, 1% effort
Supporting Agency: NIH
Name and address of the funding agency's procuring Contracting/Grants Officer: To Be Determined
Performance Period: 10/1/2023-9/30/2028
Level of funding:
Brief description of the project's goals: The proposed research will establish a novel epigenetic 'priming' strategy designed to enhance PSMA expression and in turn augment clinical activity of PSMA-directed therapies such as Lutetium-PSMA-617. In addition to testing the effect of vorinostat (HDAC inhibitor) in a clinical trial, we will also conduct preclinical studies evaluating other epigenetic therapies alone and in combination, which will inform iterative clinical trials. Finally, we will evaluate imaging, tissue and blood-based predictive biomarkers, which will lead to new molecular diagnostics that will improve the lives and outcomes for patients with prostate cancer.
Overlap with proposed research: None

Title: *Targeting the epigenetic regulator LSD1 in treatment-emergent neuroendocrine prostate cancer (NEPC) – clinical correlates and predictors of response*
Grant #: Pending
Time Commitments: 0.36 CM, 3% effort
Supporting Agency: Prostate Cancer Foundation
Name and address of the funding agency's procuring Contracting/Grants Officer: To Be Determined
Performance Period: 10/1/2023-9/30/2025

Level of funding: (FHCC only)
Brief description of the project's goals: FHCC will lead the sequencing efforts and methylation studies for all models. We will provide bioinformatics support to integrate datasets for a comprehensive analysis of LSD1 biology in the context of advanced prostate cancer, subtype selectivity, and therapeutic vulnerabilities.
Overlap with proposed research: None

Title: *Evaluating Prostate Cancer Phenotype and Genotype Classification from Circulating Tumor DNA as Biomarkers for Predicting Treatment Outcomes*
Grant #: R01 CA280056-01
Time Commitments: 1.2 CM, 10% effort
Supporting Agency: NIH/NCI
Name and address of the funding agency's procuring Contracting/Grants Officer: To Be Determined
Performance Period: 12/1/2023 – 11/30/2028
Level of funding:
Brief description of the project's goals: The overall objective is to test the hypothesis that nucleosome profiling of ctDNA can be used to classify mixed CRPC phenotypes by capturing intra-tumor or inter-tumor phenotype heterogeneity within mCPRC patients. The aims will also test the hypothesis that ctDNA nucleosome profiling can be used to predict treatment outcomes for patients receiving standard and new targeted therapies.
Overlap with proposed research: None
List of specific aims:
Aim 1: Develop ctDNA classifiers that distinguish prostate cancer phenotypes.
Aim 2: Evaluate ctDNA classifiers for assessing phenotype heterogeneity in men with mCRPC.
Aim 3: Determine the utility of ctDNA for predicting prostate cancer treatment outcomes.

OVERLAP
There exists neither scientific nor budgetary overlap. Should pending application be funded, effort will not exceed 100%.

CERTIFICATION
I certify this the current and pending support provided on the application and in this document are current, accurate, and complete:

Peter S. Nelson, MD

9/7/2023
Date