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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

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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	6-30	Nelson Team		70%

studies evaluating gene combinations and agents 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.

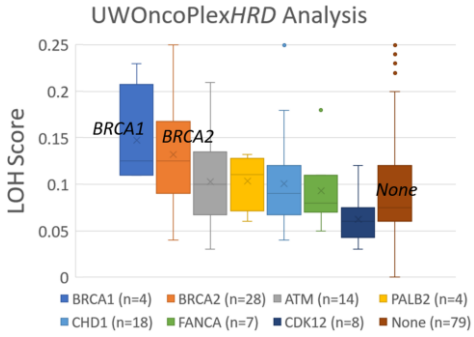

FIG 1. OncoPlexHRD results according to underling HRR gene mutation. Excluded from this box boxplot graph are cases with fewer than 4 samples (CHEK2 n=4, BRIP1 n=1, BAP1 n=1, RAD51B n=1, RAD51D n=1, FANCD2 n=2).

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.

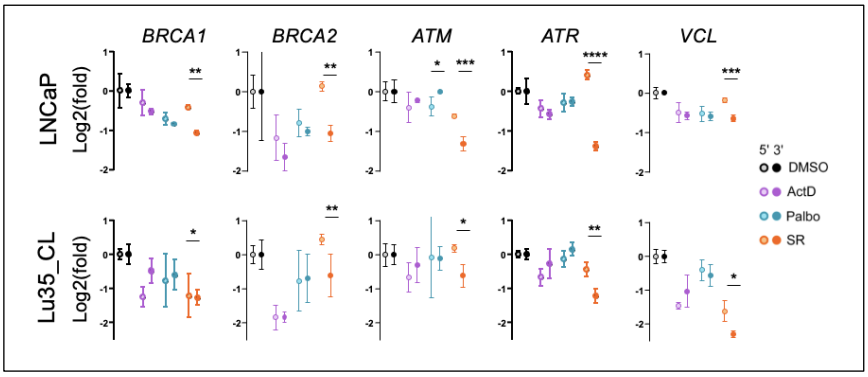

FIG 2. Acute CDK12 loss causes DNA repair gene downregulation. qPCR analysis with RNA samples from above using two sets of primers for each target to show selective loss of 3' transcripts. Vinculin (*VCL*) is a long gene not involved in DNA repair. Plots show mean Log2(fold) vs vehicle -/+stdev. Data 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.

CDK12 loss does not affect RAD51 foci formation. 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.

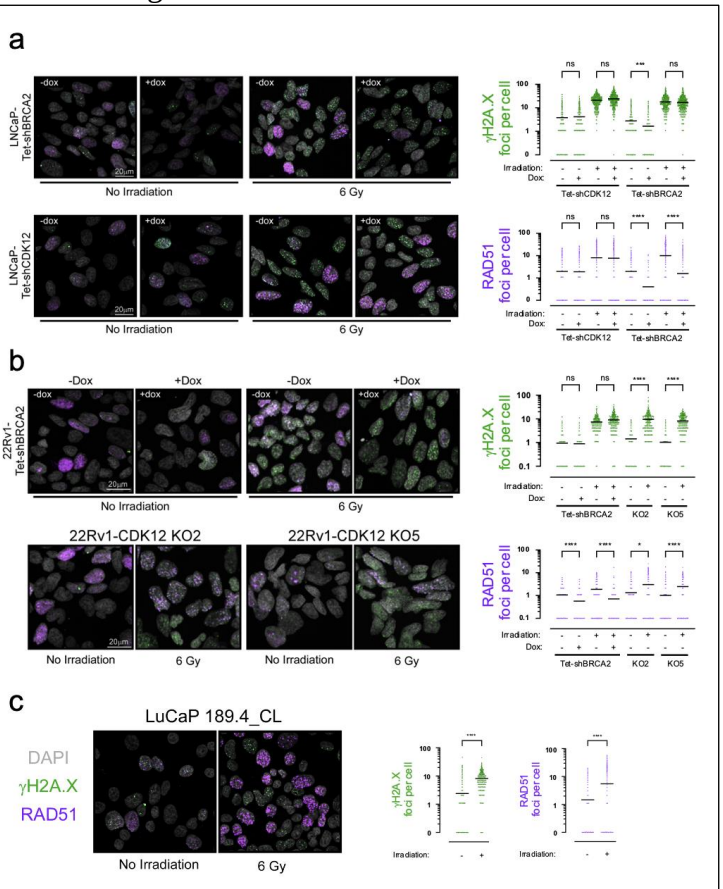

FIG 3. CDK12 loss does not affect RAD51 foci formation. (a) LNCaP cells with either Tet-inducible shBRCA2 or shCDK12 were induced with doxycycline (100ng/mL) for 4 days then exposed to 6Gy ionizing radiation and fixed at 3h post IR. Immunofluorescence staining was performed for γH2A.X and RAD51 and images 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.

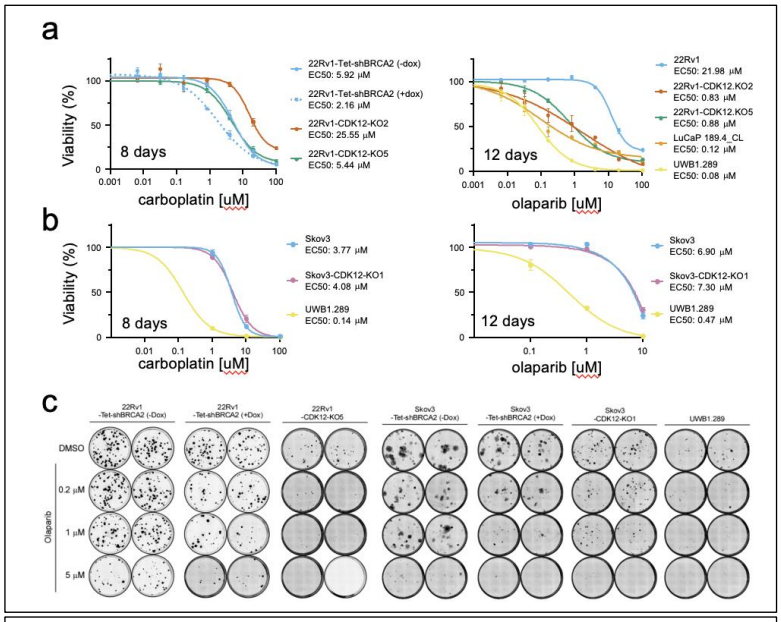

FIG 4. CDK12 Loss does not confer platinum or PARPi sensitivity. (a) Dose response curves for prostate lines treated with carboplatin or olaparib (n=3). (b) Dose response curves for ovarian cancer lines treated with carboplatin or olaparib (n=4). Note: UWB1.289 is a BRCA1(-) ovarian cancer line and bona 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.

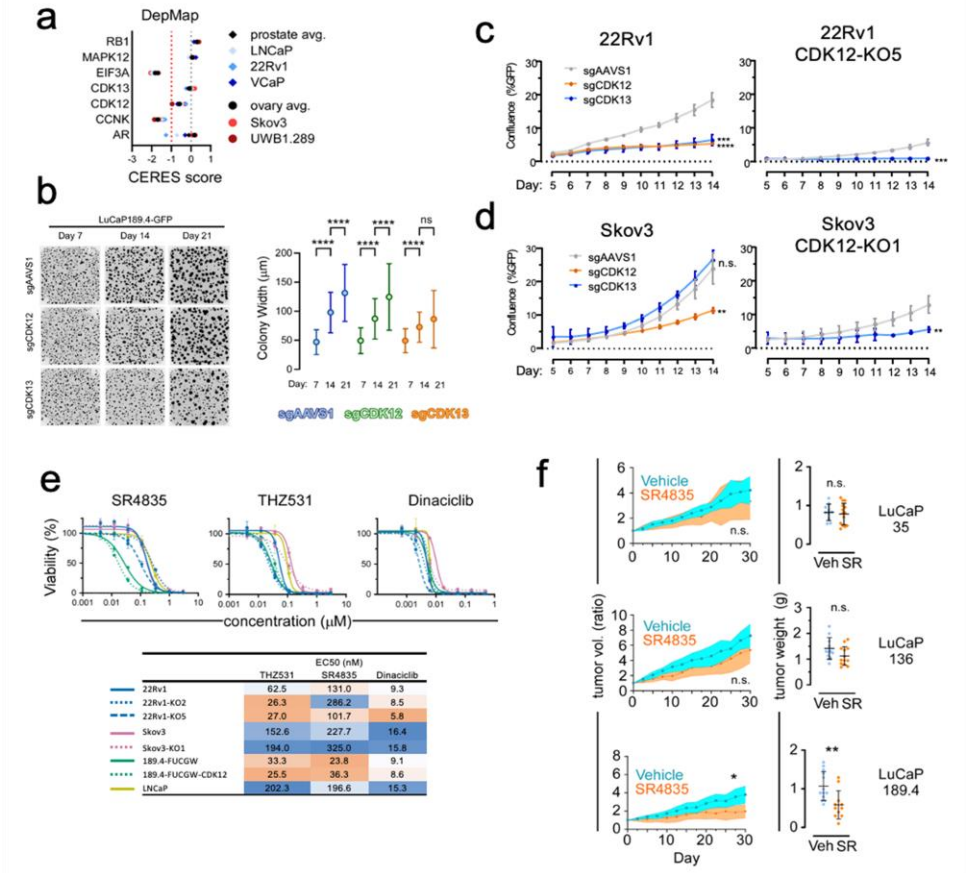

FIG 5. CDK12(-) cells are sensitive to CDK13 loss. (a) Selection of control and CDK12-related sgRNA fitness results from DepMap, including average score for all prostate or ovarian lines. *RB1* is a control for positive selection, *MAPK12* is a redundant gene (p38g) and control for neutral selection, 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.

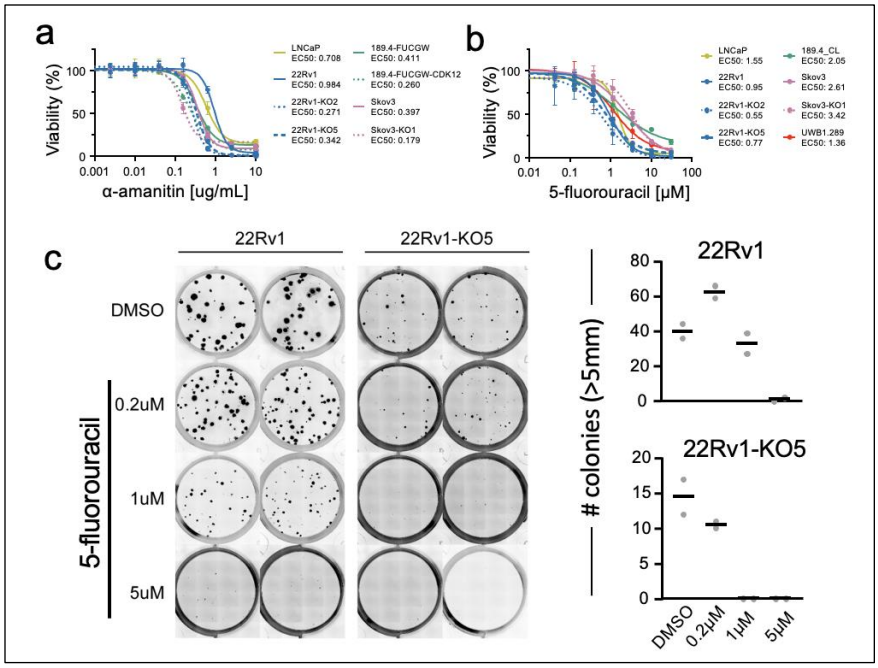

FIG 6. DK12(-) cells show increased transcription-linked DNA damage and sensitivity to transcription-targeting therapies. (a) CDK12(-) lines were stained for R-loops with the S9.6 antibody, which is selective for RNA:DNA hybrid sequences. (b) Dose response curve for cells treated 4 days with α-amanitin (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 185:105-118; 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 83: 641-648; 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.; 2022: CD-22-0692; 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. doi: 10.1200/PO.22.00104. PMID: 36623239 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. doi: 10.1038/s41391-022-00636-0. Epub 2022 Dec 23.PMID: 36564459 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:	Colin Pritchard, M.D. PhD
Project Role:	Co-Investigator
Nearest person month worked:	3
Contribution to Project:	Dr. Pritchard has managed and led 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.
Funding Support:	(list current effort award numbers) PC200262 (this award), P50 CA097186-21, MVP CC 8600, 0001140818 FHCC, 3OT2OD002748-01S4, W81XWH-18-1-0756, Institutional Support

OTHER SUPPORT
PRITCHARD, COLIN

<u>Current Funding</u>
Title: Returning clinically actionable results to MVP participants with metastatic prostate cancer: a pilot study (62-8454)
Time Commitments: 1.44 Calendar Months
Supporting Agency: VA Puget Sound Health Care System

Address: VA Puget Sound Health Care System,
Seattle Division, 1660 S. Columbian Way, Seattle, WA 98108
Contracting/Grants Officer: N/A
Performance Period: 6/1/2022 – 5/31/2024
Level of funding: Total Award
Project Goals: Dr. Pritchard will be molecular pathologist for Dr. Montgomery's Million Veteran Project (MVP CC 8600) entitled "Returning clinically actionable results to MVP participants with metastatic prostate cancer: a pilot study". His laboratory will perform the clinical retest for all participants in the study. He will interpret the results of the retest as to whether the results indicate the presence or absence of a pathogenic germline BRCA1, BRCA2, or PALB2 allele. He will assist in the interpretation of the final data addressing the objectives of the study
Overlap: None

Title: Clinical qualification of DNA repair defects as biomarkers in metastatic prostate cancer using integrated genomics and tissue-based functional assays (61-7639)
Time Commitments: 0.60 calendar
Supporting Agency: Department of Defense US Army; W81XWH-18-1-0756
Address: 820 Chandler ST, Fort Detrick, MD 21702-5000
Contracting/Grants Officer: Elena Howell
Performance period: 9/30/2018 – 9/30/2023
Level of funding: Total Award
Project Goals: The major goals we propose will provide physicians tools to develop more effective treatment strategies for men with mCRPC, by assessing DNA repair defects as predictive biomarkers of patient outcome to standard therapies. In the near term, developing and validating functional biomarkers of HR functionality would facilitate implementation of personalized treatment-decisions in mCRPC into clinical practice in the community and also provide valuable information to address mechanisms of drug resistances to PARP inhibitors and DNA damaging chemotherapy in this subclass of the disease. Eventually these data could be relevant for men with localized disease too, and help personalizing treatment to prevent progression to lethal disease.
Specific Aims: Aim 1: To correlate the presence or absence of somatic/germline alterations in DNA repair genes with overall survival from mCRPC, and specific response to taxanes, Abiraterone, Enzalutamide, and Ra-223, in samples from a prospective study.
Aim 2: To optimize tissue-based tests of HR functionality samples for CRPC samples, and study the correlation with genomic aberrations in HR genes.
Aim 3: To clinically qualify this HR functional test in a clinical trial of carboplatin in CRPC.
Overlap: N/A

Title: Project 1: Molecular Predictors of Prostate Cancer Progression and Mortality (66-5883; 63-7248)
Time Commitments: 1.32 calendar
Supporting Agency: Fred Hutchinson Cancer Center through NIH
Address: 1100 Fairview Ave N, Seattle, WA 98109
Contracting/Grants Officer: Mackenzie Krouse
Performance period: 09/18/2018 – 08/31/2023
Level of funding: Total Award
Project Goals: Prostate cancer (PCa) is the most common solid tumor in men and is a major cause of cancer-related morbidity and mortality. Prostate-specific antigen (PSA) testing is controversial, and current consideration of high risk men is inadequate. Also, clinicopathological criteria are insufficient to differentiate indolent vs aggressive disease. The recent discovery of high prevalence of high to moderate penetrance germline cancer risk mutations in metastatic PCa will lead to increased testing and cascade testing of unaffected male relatives, thus identifying men at high risk for developing aggressive PCa. Preliminary evidence suggests the need for refined cancer screening in this high risk group. The overall intent of this population sciences research is to find men at high genetic risk for aggressive prostate cancer and to conduct an early Pca detection study and incorporate novel

PCa biomarkers.
The proposed plan builds on our prior SPORE work, taking advantage of our experience to prospectively recruit a population-based PCa cohort with germline mutations (index cases) and their male first degree relatives (high risk cohort) with the goal of conducting a PCa early detection study that will incorporate germline DNA sequencing to characterize risk, novel PCa biomarkers, clinical and PCa-specific outcomes data. Univariate, stratified, and multivariate analyses will be completed to evaluate sensitivity and specificity of new biomarkers. The Cox proportional hazards model will be used to calculate hazard ratios, 95% CIs, and p-values to examine the association of individual and combinations of germline genetic biomarkers and with PCa outcomes. The overall goal is to identify and validate prognostic genetic-epigenetic biomarkers and begin to translate these findings into better patient management by investigating novel screening and detection approaches for men at high risk for aggressive PCa.
Specific Aims:
1) To ascertain and recruit men at high genetic risk for developing aggressive prostate cancer.
2) To test new approaches to early detection of prostate cancer in men with high genetic risk for aggressive prostate cancer.
3) To identify and evaluate new prostate cancer biomarkers in men with high genetic risk for aggressive prostate cancer.
Overlap: None

Title: Pacific NW Prostate Cancer SPORE (66-5269; 68-6188)
Time Commitments: 0.36 Calendar Months
Supporting Agency: Fred Hutch
Address: 1100 Fairview Ave N, Seattle WA 98109
Contracting/Grants Officer: Mackenzie Krouse
Performance Period: 9/1/2020 – 8/31/2023
Level of funding: Total Award
Project Goals: The University of Washington (PI: Dr. R. Bruce Montgomery, MD; Co-Investigator: Colin C. Pritchard, MD, PhD) will design and conduct the clinical trials described in this proposal that include platinum-based chemotherapy and the maintenance therapy with PARP inhibitors. Drs. Montgomery and Pritchard will work closely with Dr. Nelson to facilitate the molecular assays from biospecimens acquired on the clinical studies and will work closely with Biospecimen Core B for sample collection and Clinical Core D for trial management.
Specific Aims:
1) Conduct Phase 2 clinical trials of FDA-approved genotoxic therapeutics and PARPi in patients with mCRPC to determine response rates, identify resistance mechanisms, and establish associations between those specific genomic defects predicted to result in HRD and the depth and duration of clinical responses.
2) Systematically assess tumor responses to rational combinations of genetic and pharmacological targeting DNA repair pathways using Patient Derived Xenograft (PDX) models with inherent or engineered HRD aberrations.
3) Develop minimally-invasive biomarkers involving the capture and analysis of circulating tumor DNA capable of distinguishing patients for therapeutics targeting DNA repair pathways.
Overlap: None

Title: Northwest Genomics Center for All of Us (61-8385)
Time Commitments: 0.60 calendar
Supporting Agency: National Institutes of Health; 1 OT2 OD 002748-01
Address: 9000 Rockville Pike, Bethesda, Maryland 20892
Contracting/Grants Officer: Irene Haas
Performance period: 9/25/18 – 8/31/2023
Level of funding: Total Award
Project Goals: The goal of the proposal is to establish a Genome Center for the All of Us Research Program. The NWGC for All of Us will provide whole genome sequencing, genotyping and clinical validation of variants in the ACMG 59 genes.

Specific Aims: To advance the goals and objectives of the All of Us Research Program we will produce and interpret variants from genotyping arrays for up to 100,000 samples in year 1 and up to 200,000 samples in years 2 - 5. We will also produce and interpret variants on more than 10,000 samples by WGS in year 1; up to 100,000 samples in year 2; and up to 200,000 samples in years 3-5 using the Illumina NovaSeq platform. To accomplish this, we will:

1- Work with the All of Us program, the DRC, the Biobank, and other groups to deliver an efficient and effective process for evaluating and completing high-throughput genotyping and WGS, call variants, and interpret the impact of variants in the ACMG 59 genes and other genes as indicated by the program in a CLIA-certified environment.

2- Interact directly with the Biobank to carefully develop the logistics and methods for preparing and receiving samples.

3- Track all samples and data transfers for all samples at every stage of the process (from project initiation to data delivery using our secure, completely interactive, and integrated laboratory information management system (LIMS)) and provide reports to the program, the DRC, and other groups as required.

4- Provide genotype and WGS data of the highest quality, in formats required by the program such as IDAT files for genotyping and CRAMs and VCFs for WGS.

5- Provide a team of specialized personnel and staff versed in the workflow of a well-established high throughput CLIA-certified genome center. These include individuals specifically trained in DNA sample receipt, quality control, and large-scale bioinformatics analysis and variant interpretation.

6- Assist as needed with additional data interpretation (beyond the ACMG genes), with publications (i.e., materials and methods), and other activities as required for the program.

7- Provide secure backup of raw sequence data from the samples and all metadata associated with the project (i.e., sample tracking, storage, and QC information).

Overlap: None

Changes/Ended

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

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

Please find Previous/Current/Pending Support attached for Dr. Peter Nelson, Ruth Etzioni, and Michael Haffner.

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:
None