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Exploiting Recurrent Chromatin Modifier Mutations for Prostate Cancer Targeted Therapy

PRINCIPAL INVESTIGATOR:
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CONTRACTING ORGANIZATION:
Fred Hutchinson Cancer Research Center

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

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14. ABSTRACT

This research project is designed to test the hypothesis that mutations in different chromatin-modifying (CM) enzymes result in similar effects on the cell because they feed into the same biological processes and pathways that promote cell survival, cell growth, and resistance to treatment. A corollary of this hypothesis is that different tumors, with mutations in different chromatin-modifying enzymes, can be targeted by the same therapy designed to reverse or suppress the downstream effects of these mutations.

In Aim 1 of our proposal, we will identify the chromatin-modifying genes whose loss leads to therapy resistance and determine how those factors are related to each other. In Aim 2, we will investigate the biological basis of this resistance by measuring global changes to gene regulation. Lastly, in Aim 3 we will devise a therapeutic strategy to be used alongside androgen-blockade - specifically in PCs that harbor alterations in genes that modify or interact with chromatin.

15. SUBJECT TERMS

prostate cancer; enzalutamide; castration resistance; precision medicine; chromatin modifiers

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1. INTRODUCTION:

Prostate cancer (PC) is the second leading cause of cancer deaths in men. After an initial variable period of response to castration therapy, metastatic PC recurs/progresses to a clinical state termed castration-resistant prostate cancer (CRPC). Recent large-scale studies designed to determine the landscape of genomic and gene expression alterations that occur in CPRC have identified recurrent inactivating mutations in genes that modify chromatin. These include recurrent mutations in KDM6A/UTX, KMT2C/MLL3, KMT2D, and KMT2A. Currently, the role that inactivation of epigenetic regulators/chromatin modifiers (hereafter, CMs) play in the progression of PC is unknown.

This proposal is designed to test the hypothesis that the loss of specific chromatin modifier function (e.g. UTX/KDM6A), through mutation and/or copy loss, drives resistance to AR pathway antagonism (e.g. ENZ). We further hypothesize that, despite dissimilar protein functions, loss of UTX or other CMs in the same complex or pathway will produce convergent phenotypic and epigenetic changes in cell state. Consequently, I hypothesize that this change in cell state, which results in resistance to ENZ, will be reversed by pharmacological targeting of specific chromatin modifying and interacting proteins like EZH2, BET family proteins, and KDM6B.

2. KEYWORDS:

prostate cancer; enzalutamide; castration resistance; precision medicine; chromatin modifiers

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Specific Aim 1: Determine the role of chromatin modifier loss in mediating enzalutamide resistance.

Major Task 1: Characterize the selective benefit of CM loss under enzalutamide conditions.
Subtask 1: Clone sgRNAs into vectors and transduce cell lines Cell lines used: LNCaP, VCaP, 22PC-EP (1-6).
Subtask 2: Carry out competition assays on transduced cells (1-12).

Major Task 2: Define the combinatorial effects of CM loss.
Subtask 1: Carry out dual competition assays on transduced cells (6-12).

Major Task 3: Determine the phenotype of CM mutant PC.
Subtask 1: Generate CRISPR/CAS9 knockouts of UTX, CREBBP, and SETDB1 (1-12).
Subtask 2: Carry out qRT-PCR on phenotype genes (6-12).
Subtask 3: Western blots and cell cycle analyses (6-12).

Major Task 4: Identify protein complex interactions of CMs.
Subtask 1: Perform mass-spec on UTX pulldowns (1-6).
Subtask 2: Validate MS results using western blots (6-9).

Specific Aim 2: Determine the transcriptional and epigenetic alterations that occur due to loss of specific chromatin modifier function in prostate cancer.

Major Task 1: Characterize the transcriptional effects of UTX knockdown/knockout (KD/KO) under enzalutamide conditions.

Subtask 1: Engineer UTX-KO and EZH2 overexpression cell lines: lines used: LNCaP, VCaP (1-12).
Subtask 2: Perform RNA-seq analysis on engineered cell lines lines used: LNCaP, VCaP (6-18).

Major Task 2: Determine the effects of UTX KD/KO on chromatin.
Subtask 1: Determine global changes to chromatin with UTX-loss with western blots (6-9).
Subtask 2: Perform ChIP experiments for histoneH3K4me1, H3K4me3, H3K27ac, H3K27me1, H3K27me3 (6-24).
Subtask 3: Determine the effect of UTX-KO on the AR-cistrome using AR ChIP-seq (12-24).
Subtask 4: Determine the effect of UTX-KO on the E2F1-cistrome using E2F1 ChIP-seq (12-24).

Specific Aim 3: Evaluate pharmacological approaches to target prostate cancers with mutated/inactivated chromatin modifiers to reverse resistance to AR pathway inhibition.

Major Task 1: Determine the efficacy of epigenetic inhibitors in reversing ENZ resistance due to CM loss.
Subtask 1: Generate shRNA and sgRNA vectors and create cell lines (1-6).
Subtask 2: Perform dose-response assays to determine effective concentrations of drugs (1-6).
Subtask 3: Carry out competition assays (3-18).

Major Task 2: Establish the in vivo efficacy of combined ENZ and EZH2 or BET inhibition in UTX-KO PC models.
Subtask 1: Generate UTX-KO PC lines (1-3).
Subtask 2: Evaluate the efficacy of drug combinations in vivo (12-24).
Subtask 3: Perform IHC stains on UTX-KO xenograft tissue to define molecular changes resulting from combination epigenetic therapies (24-30).

Major Task 3: Prepare and publish manuscript (30-36).

What was accomplished under these goals?

Major Activities

Specific Aim 1: Determine the role of chromatin modifier loss in mediating enzalutamide resistance.

Major Task 1: Characterize the selective benefit of CM loss under enzalutamide conditions.
Subtask 1: *Clone sgRNAs into vectors and transduce cell lines:* 90% completed

Based on sgRNA screens for enzalutamide (ENZ) resistance and literature regarding chromatin modifier (CM) mutations in prostate cancer we've identified 36 tumor suppressors that have the potential to mediate ENZ resistance when lost. The factors are listed in **Table 1**.

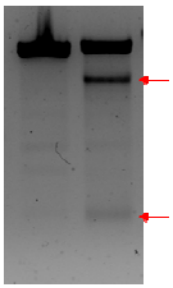
Table 1: Putative tumor suppressor chromatin modifiers

ARID1A	MBD1	EHMT1	SIRT1
ARID4A	MECP2	ERF	SIRT6
BRD9	MGA	KAT6A	SMARCA2
BRPF1	NCOR1	KDM4D	SMARCC1
BRWD1	NCOR2	KDM6A	SMARCD1
CHD1	NSD1	KMT2A	SPEN
CHD2	PHF2	KMT2B	TP53
CLOCK	SETD1A	KMT2C	UTY
CREBBP	SETD1B	KMT2D	WDR5

Each gene has 4 sgRNAs cloned into an expression vector for use in competition assays. With the exceptions of SMARCC1 and CHD2, this task has been accomplished for all targets. To limit the

experimental matrix, we decided to use only the best two sgRNAs in the competition assays. To test which sgRNAs are the most efficient at cutting their target sequences, we performed T7 assays. T7 assays detect DNA scars left by imperfect repair by causing a cleavage in a PCR amplicon of the gDNA that contains the sgRNA target site. **Figure 2** is an example T7 for KDM6A showing two cleavage products, indicating cutting. We have validated and found acceptable results for all genes except for SMARCC1 and CHD2. sgRNAs to these genes are being redone.

Figure 2: Example T7 for KDM6A/UTX

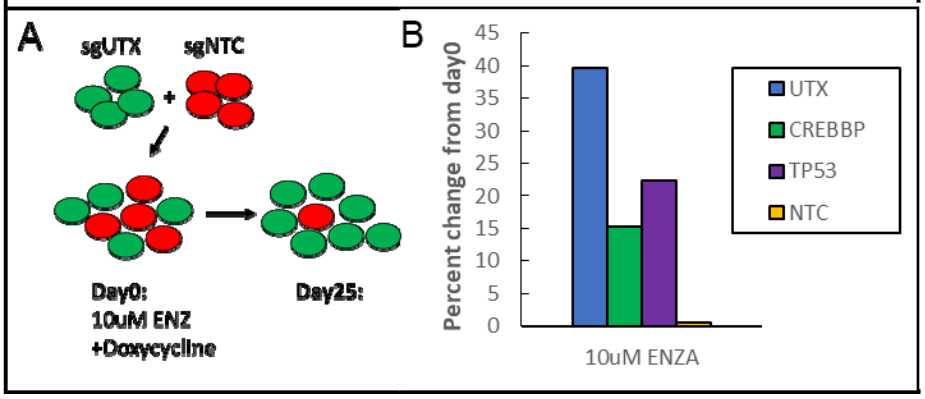


Most sgRNAs have been transduced into LNCaP cells. Our approach was to first test the efficacy of sgRNAs in LNCaP cells then proceed to the other lines with only those sgRNAs that mediate ENZ resistance.

Subtask 2: *Carry out competition assays on transduced cells (75% completed)*

We employed competition assays to examine the relative fitness of each CM loss under ENZ conditions. We started by performing the competition assays only in LNCaP then proceed to the other cell lines with only those sgRNAs that mediated resistance to ENZ. **Figure 3** shows a conceptual depiction of the competition assays as well as some preliminary data of competition assay results for UTX, CREBBP, and TP53 versus a non-targeting control. An increase in the percent of GFP+ cells represents a selective advantage of cells harboring that sgRNA under ENZ conditions.

Figure3: Competitive enrichment assays demonstrate selective benefit of CM loss with ENZ treatment: A) Experimental design of single-target competitive enrichment assays. B). Percent change from day 0 (%final - %day0) of the cells harboring sgRNAs to UTX, CREBBP, TP53, and a non-targeting control (NTC). A positive %change indicates that the loss of that factor is beneficial to the cell.

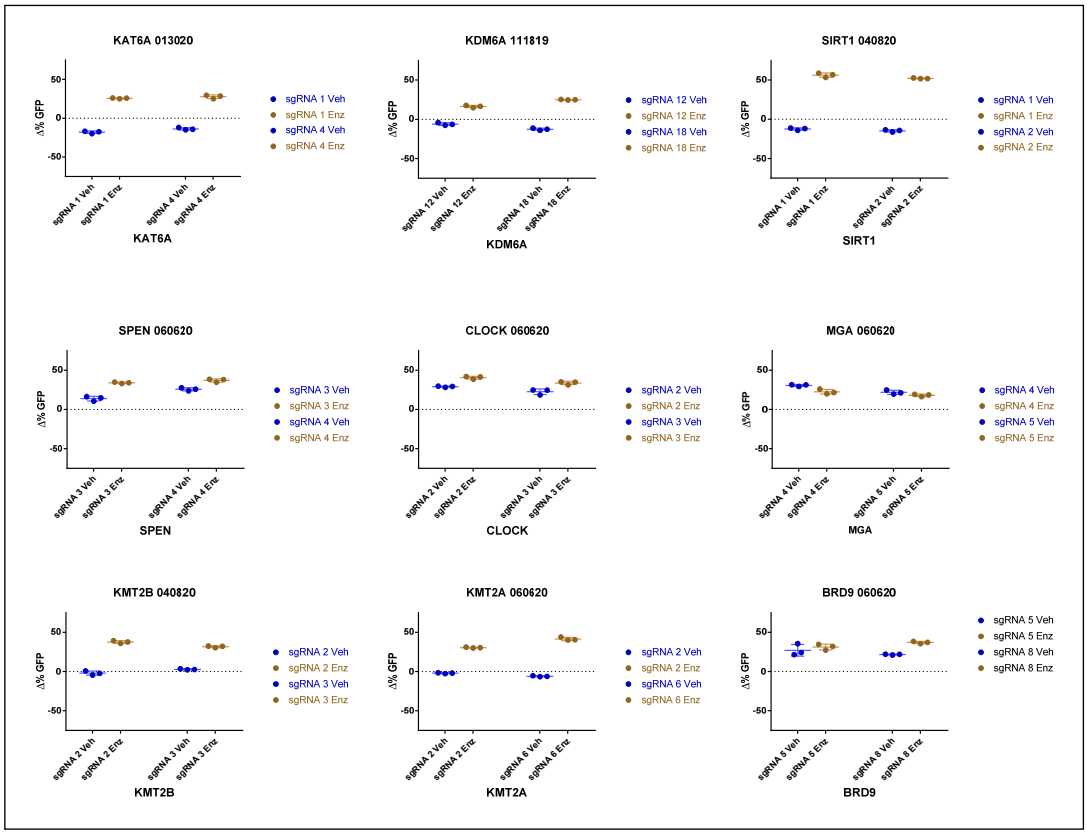


In this first project year we have screened all sgRNAs except CHD2 and SMARCC1 targets for AR. We have identified several tumor suppressors that mediated either a general growth advantage, meaning the percent of cells harboring the test sgRNA increased in both the ENZ and the vehicle control group, or a selective enrichment only in ENZ conditions **Figure 4.** All other sgRNAs either had no enrichment or were depleted. A few, including CREBBP, had discordant results with one sgRNA showing in enrichment and the other showing no enrichment. For these discordant results. We will clone several additional sgRNAs and retest to determine the true effect of gene knockdown.

Major Task 2: Define the combinatorial effects of CM loss.
Subtask 1: *Carry out dual competition assays on transduced cells (6-12).*

We are currently starting these and will have results for Y2.

Figure 4: LNCaP sgRNA competition assay validated targets

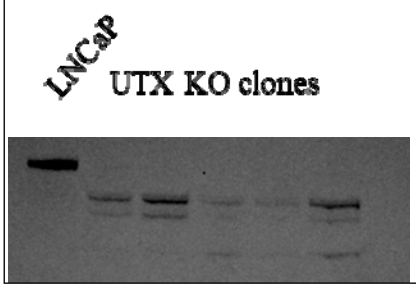


Major Task 3: Determine the phenotype of CM mutant PC.

Subtask 1: *Generate CRISPR/CAS9 knockouts of UTX, CREBBP, and SETDB1 (1-12). (75% completed)*

We currently have generated clonal LNCaP KO lines for UTX and CREBBP. **Figure 5** shows a western blot for UTX confirming loss of protein expression in the KO clones. In Figure 5 UTX is the top band in LNCaP cells. While SETDB1 did not validate in our competition assays. Other KOs such as KAT6A or KMT2A/B could be performed instead. To make more mechanistic progress, we will focus on UTX and CREBBP KO lines before proceeding to other genes. The combinatorial competition assays will further inform on the most relevant genetic models to make. For example, if KMT2A loss shares a redundant role with KDM6A loss in mediating ENZ resistance, creation of a KMT2A KO line would be of higher priority.

Figure 5: UTX KO clones



Subtask 2: *Carry out qRT-PCR on phenotype genes (6-12).*

This task will be accomplished in Y2.

Subtask 3: *Western blots and cell cycle analyses (6-12).*

This task will be accomplished in Y2.

Specific Aim 2: Determine the transcriptional and epigenetic alterations that occur due to loss of specific chromatin modifier function in prostate cancer.

Major Task 1: Characterize the transcriptional effects of UTX knockdown/knockout (KD/KO) under enzalutamide conditions.

Subtask 1: Engineer UTX-KO and EZH2 overexpression cell lines: lines used: LNCaP, VCaP (1-12).

Experiments pertaining to EZH2 overexpression will be accomplished for Y2.

Subtask 2: Perform RNA-seq analysis on engineered cell lines lines used: LNCaP, VCaP (6-18). (50% complete).

We are currently preparing to run RNA-seq analysis on CREBBP KO lines. We have performed RNA-seq on KDM6A knockout LNCaP cells. While we work to generate data on EZH2 overexpression and CREBBP-KO, we performed a preliminary analysis on the KDM6A-ko RNA-seq. Figure 6 depicts a heatmap of mean-centered log2(CPM) RNA-seq expression values. Red and blue color represents high and low relative expression respectively. As shown in **Figure 6,** many of the genes upregulated in the KDM6A loss clones (labeled by their numbers 12,14,27,30,34) were repressed by 10 nM R1881, a steroidal agonist to AR. This may indicate that KDM6A loss effects AR signaling and potentially interferes with AR-repressed target gene expression. We will compare these results to EZH2 overexpression and CREBBP loss to further investigate this phenomenon. Other genes, such as KLHL3 and HDX do not follow this pattern. Geneset and pathway analyses are planned for Y2 and are likely to yield further insights and clarify the mechanism by which KDM6A/UTX and CREBBP loss results in enhanced resistance to ENZ.

Figure 6: RNA-seq heatmap of KDM6A-KO cells

SYMBOL	WT1_DMSO	WT2_DMSO	WT2_ENZA_10uM	WT1_ENZA_10uM	WT1_R1881_10nM	WT2_R1881_10nM	KDM6A_12_DMSO	KDM6A_14_DMSO	KDM6A_27_DMSO	KDM6A_30_DMSO	KDM6A_34_DMSO	KDM6A_12_ENZA_10uM	KDM6A_14_ENZA_10uM	KDM6A_27_ENZA_10uM	KDM6A_30_ENZA_10uM	KDM6A_34_ENZA_10uM	KDM6A_12_R1881_10nM	KDM6A_14_R1881_10nM	KDM6A_27_R1881_10nM	KDM6A_30_R1881_10nM	KDM6A_34_R1881_10nM
DDX18	-1	-1	-1	-1	-1	-1	1	1	1	1	1	1	1	1	1	1	-1	-1	-1	-1	-1
KLHL3	-3	-4	-2	-2	-3	-2	1	1	1	1	1	2	1	2	2	2	1	1	1	1	1
HDX	-2	-2	-2	-3	-2	-2	1	1	1	1	1	1	1	1	1	1	1	0	1	1	0
ZFHX4	-2	-2	-1	-1	-3	-3	1	1	0	1	1	1	2	1	2	2	-0	-1	-1	-0	-0
CDH18	-2	-2	-4	-4	-3	-4	3	3	1	2	1	4	3	2	3	2	0	-0	-2	-1	-1
LINC01351	-1	-1	-2	-1	-3	-2	2	1	2	1	2	2	1	2	1	2	-1	-1	-1	-1	-2
USEL	0	0	-1	-1	-2	-2	1	2	1	2	2	1	1	1	1	2	-2	-2	-1	-1	-2
CCDC93	-1	-1	-0	-0	-1	-1	0	0	0	0	0	1	1	1	1	1	-0	-0	-0	-0	-1
NRXN1	-6	-2	-6	-3	-5	-6	2	2	3	2	4	3	2	4	3	4	-1	0	1	-1	1
FIGN	-2	-3	-2	-3	-2	-2	1	1	2	2	2	0	-0	1	1	2	0	0	0	1	1
NLGN1	-2	-2	-1	-1	-4	-3	2	2	1	1	1	2	2	1	1	2	0	1	-1	-0	-1
FAM174A	-1	-1	-1	-1	-0	-1	-0	0	0	-0	1	1	1	1	0	1	-0	-0	-0	-0	0
PIK3R1	-1	-1	-0	-0	-1	-2	0	0	1	1	0	1	1	1	1	1	-1	-1	-1	-0	-0
VTA1	-1	-1	-1	-1	-2	-2	1	1	1	1	1	2	1	1	1	1	0	-0	-1	-1	-2
QKI	-1	-1	-1	-1	-1	-2	0	1	0	0	1	0	0	1	1	1	-0	0	0	1	-0
MYOT	2	2	3	2	7	4	2	1	1	2	1	1	1	1	2	2	2	0	1	1	1
CITED2	-1	-1	-1	-1	-0	-0	-0	0	-0	-0	-0	1	1	0	0	0	1	1	-0	-0	1
PEX2	-1	-1	-1	-1	-1	-1	0	0	0	1	0	0	1	0	1	1	0	-0	-0	0	-0
SAMD5	-1	-1	-1	-1	-1	-2	0	1	1	1	1	0	1	1	1	1	-1	-0	-0	1	0
ATP10D	-2	-1	-1	-2	-1	-1	0	-0	0	1	-0	1	1	1	1	0	1	0	0	1	1
LINC01322	-0	-0	0	0	-5	-6	2	2	3	2	2	3	3	4	3	3	-6	-1	-2	-2	-6
EPHA6	-0	-0	-0	-0	-2	-2	1	0	0	0	1	1	1	1	1	1	-0	-1	-1	-1	-1
ST8SIA4	-3	-2	-2	-2	-4	-3	1	1	2	1	3	2	1	2	1	4	-0	-2	-1	-1	1
NAALAD2	-2	-2	-1	-1	-3	-3	2	-0	1	1	2	3	0	1	1	3	0	-1	-0	-0	-0

Major Task 2: Determine the effects of UTX KD/KO on chromatin.

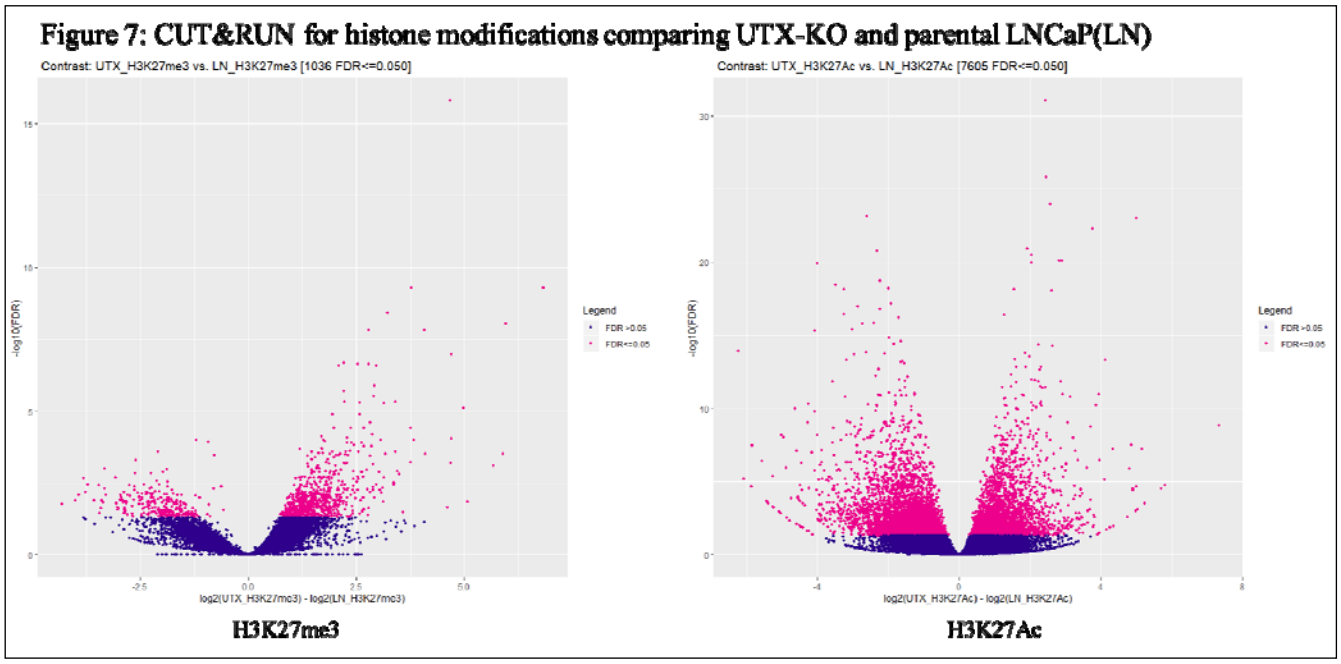
Subtask 1: Determine global changes to chromatin with UTX-loss with western blots (6-9).

These studies will be accomplished in Y2.

Subtask 2: *Perform ChIP experiments for histoneH3K4me1, H3K4me3, H3K27ac, H3K27me1, H3K27me3 (6-24).* (33% completed)

Currently we have performed CUT&RUN, a technique similar to ChIP-seq, for H3K27me3 and H3K27Ac on LNCaP, LNCaP-UTX-ko, and LNCaP-CREBBPko cell lines. While analysis is still ongoing, there appear to be global changes in KDM6A-KO cells. **Figure 7** shows volcano plots for differentially enriched H3K27Me3 (left) and H3K27Ac (right) peaks in the UTX KO cell lines. Pink dots represent significantly altered sites comparing LNCaP and UTX-KO cells. Sites gained with UTX loss are on the right arm. As expected, H3K27me3 sites are gained when there is loss of the demethylase. Acetylation changes do not seem to have a clear overall gain or loss pattern. Determining the significance of these sites require further comparisons to CREBBP knockout and EZH2 overexpression lines, which are ongoing.

Figure 7: CUT&RUN for histone modifications comparing UTX-KO and parental LNCaP(LN)



Subtask 3: *Determine the effect of UTX-KO on the AR-cistrome using AR ChIP-seq (12-24).*

We will perform this subtask in Y2.

Subtask 4: *Determine the effect of UTX-KO on the E2F1-cistrome using E2F1 ChIP-seq (12-24).*

We will perform this subtask in Y2.

Specific Aim 3: **Evaluate pharmacological approaches to target prostate cancers with mutated/inactivated chromatin modifiers to reverse resistance to AR pathway inhibition.**

Major Task 1: Determine the efficacy of epigenetic inhibitors in reversing ENZ resistance due to CM loss.

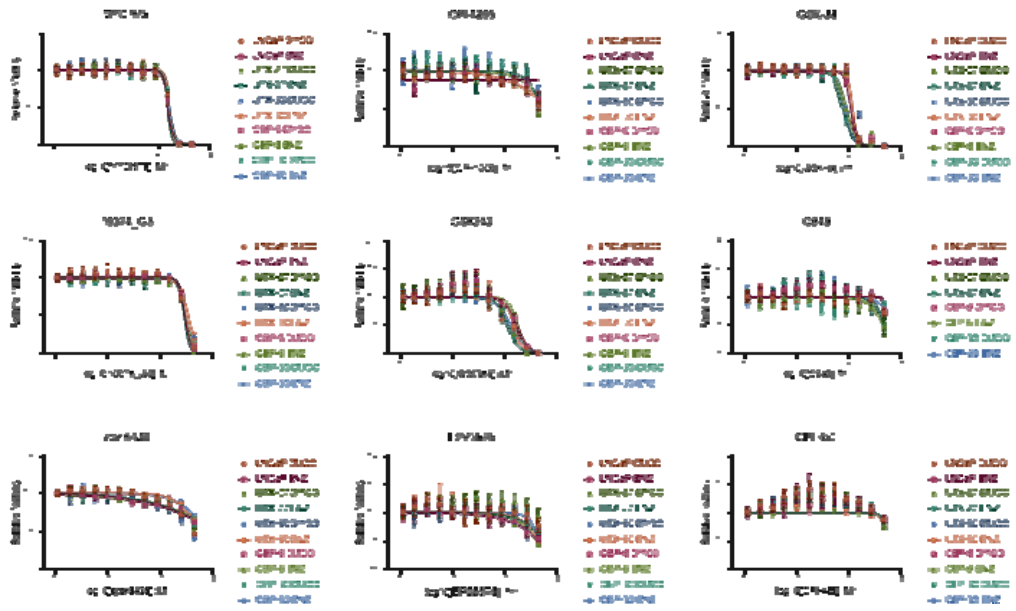
Subtask 1: *Generate shRNA and sgRNA vectors and create cell lines (1-6).*

This task was achieved as described by the earlier aims.

Subtask 2: *Perform dose-response assays to determine effective concentrations of drugs (1-6). (50% completed).*

We performed dose-response assays in UTX and CREBBP knockout cell lines with or without 10uM ENZ. Cells were grown over a period of 5 days in the drugs and the readout is viability as measured by celltiter-glo. The drugs used were chosen based on prior literature and/or the ability to target KDM6A, CREBBP/p300, MYC, BRD4, EZH2, as well as other targets supported by literature. 13 drugs were tested in total. **Figure 8** shows a subset of the drugs tested for vehicle and ENZ conditions.

Figure 8: Dose response assays to test for synthetic lethal responses



To date no drug was found to be selectively effective against any KO genotype. However, the results of these assays gave information on which compounds can kill or suppress the growth of these cells and the doses at which they do so. This information will be critical for the design of competition assays that use these drugs to be performed in Y2.

Dose-response assays are not well suited for determining whether a drug can reverse ENZ resistance since the growth effects of these knockouts may require two or more weeks to become apparent due to the subtly of the growth resistance. For this reason, in Y2, we will use competition assays to the determine if the selective advantage of a sgRNA can be mitigated or neutralized by a drug treatment.

Subtask 3: *Carry out competition assays (3-18).*

This task will be accomplished for Y2.

Major Task 2: Establish the in vivo efficacy of combined ENZ and EZH2 or BET inhibition in UTX-KO PC models.

Subtask 1: *Generate UTX-KO PC lines (1-3). (100%)*

The knockouts that will be used for PDX experiments have been generated.

Subtask 2: *Evaluate the efficacy of drug combinations in vivo (12-24).*

These studies will be performed in Y2-3.

Subtask 3: *Perform IHC stains on UTX-KO xenograft tissue to define molecular changes resulting from combination epigenetic therapies (24-30).*

These studies will be performed in Y3.

Other Achievements

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

How were the results disseminated to communities of interest?
Nothing to Report.

What do you plan to do during the next reporting period to accomplish the goals?
Due to disruptions of the flow cytometry and high-throughput sequencing core caused by center-wide shutdowns in response to COVID-19. We will focus on achieving the rate-limiting components of the project.

The first main area that we will focus on is defining the complex responsible for modulating sensitivity to ENZ. At this point, our data points towards the COMPASS complex as being particularly important. We will apply additional focus on this by performing pulldowns to confirm interaction in prostate and that these interactions are disrupted by KDM6A, CREBBP, and possibly KMT2A/B loss. We will also perform combinatorial competition assays with KDM6A and CREBBP knockout cells to determine whether further LOF events like KMT2A/B loss are redundant or synergistic in terms of ENZ resistance. This will allow us to focus on how specific enzymatic events control chromatin states and establish a molecular signature of CM loss that could be used to guide prognosis or therapy decisions.

The second main area will be to perform KDM6A and CREBBP competition assays in the presence of a drug to determine which drug candidates reverse the effects of KDM6A or CREBBP loss on ENZ resistance. To add confidence and robustness to the results we also will develop orthogonal assays to measure the directed progression of prostate cancer cell lines to ENZ resistance. In particular, assays can be read on the incubator + plate reader - BioSpa Live Cell Analysis System – will be the main focus. This system will allow us to examine absolute growth, clonogenic potential, and migration of our engineered clones.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?
Nothing to Report.

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:
As stated earlier, the main problem we encountered was COVID related shutdowns that limited availability of Flow Cytometry or High-throughput sequencing.

To ensure that work can continue we will develop assays that we can analyze without a core facility in case of another shutdown/slowdown. These assays will include completion assays that could be readout on the BioSpa Live Cell Analysis System rather than flow cytometry. We also plan on accelerating RNA-seq and CUT&RUN studies to take advantage of the current window of opportunity and obtain those datasets to be analyzed at a later point.

Changes that had a significant impact on expenditures:
Nothing to Report.

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

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

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

6. PRODUCTS:

Publications, conference papers, and presentations:
Journal publications:
Nothing to Report.

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

Other publications, conference papers and presentations:
Nothing to Report.

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:	Michael D. Nyquist, PhD
Project Role:	Principal Investigator
Nearest person month worked:	3
Contribution to Project:	Direction of research efforts; ChIP-seq / CUT&RUN studies; analysis of RNAseq data
Funding Support:	See attached Other Support document.

Name:	Yong Tao, PhD
Project Role:	Postdoctoral Fellow
Nearest person month worked:	2
Contribution to Project:	Chromatin-modifier studies; will perform PDX work in Y2/Y3
Funding Support:	PC180550; PCF Challenge Award; PC170431; PC160622P1; U54 CA224079

Name:	Ilsa Coleman
Project Role:	Research Scientist
Nearest person month worked:	2
Contribution to Project:	RNA sequencing; analysis of RNAseq and ChIP-seq data
Funding Support:	P50 CA097186; P01 CA163227; PC170503; PCF Challenge; PC171001; PC170431; PC180550; Plymate DOD

Name:	Hannah Meade
Project Role:	Research Technician
Nearest person month worked:	8
Contribution to Project:	AR assays and assistance in general lab experiment setup/work
Funding Support:	PC180550;

Name:	Ruth Dumpit
Project Role:	Research Technician
Nearest person month worked:	1
Contribution to Project:	Will perform PDX studies and perform / assist with AR studies
Funding Support:	PC180550; P01 CA163227; P50 CA097186; PC171001; PC170431; PC170431; R21 CA230138; PCF Challenge

Name:	Talina Nunez
Project Role:	Research Technician
Nearest person month worked:	3

Contribution to Project:	IHC and cell culture work; assist with PDX studies in Y2
Funding Support:	PC180550; P01 CA163227

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
See attached Updated / Existing / Pending Support document for the PD/PI.

What other organizations were involved as partners?
None.

8. SPECIAL REPORTING REQUIREMENTS:

Collaborative Awards:
Not applicable.

Quad Charts:
See appended Quad Chart.

9. APPENDICES:

None.

PREVIOUS, CURRENT AND PENDING SUPPORT – NYQUIST, MICHAEL

PREVIOUS SUPPORT

Title: *Targeting Androgen Receptor-Bypass Mechanisms to Enhance Prostate Cancer Therapy*
Time commitments: 100%
Supporting agency: DOD/CDMRP
Name and address of the funding agency's procuring Contracting/Grants Officer:
Juan A. Rodriguez, Grants Administration Office; juan.a.rodriguez236.civ@mail.mil; 301-619-2367
Performance Period: 08/01/2016 - 07/31/2018
Level of funding: $125,000
Brief description of the project's goals:
We will evaluate the hypothesis that SARMs and non-steroidal pure AR agonists can recapitulate or exceed the efficacy of Supra-Physiological Testosterone (SPT) in suppressing PC growthy.
Overlap with proposed research: None
List of specific aims:
Aim 1: Identify cell survival and growth-promoting mechanisms that are active in human castration-resistant 'double negative' prostate cancers devoid of AR activity.
Aim 2: Utilize genome-wide screening approaches to define signaling pathways capable of sustaining AR-null prostate cancer growth.
Aim 3: Determine whether genetic and pharmacological inhibition of AR-bypass pathways can induce apoptotic responses and/or suppress proliferation and growth of DNPC *in vitro* and *in vivo*.

Title: *Selective Androgen Receptor Modulators for the Treatment of Prostate Cancer*
Time commitments: 50%
Supporting agency: NIH/NCI
Name and address of the funding agency's procuring Contracting/Grants Officer:
Sarah M. Lee, Grants Management Specialist
Performance Period: 07/11/2018 - 06/30/2020
Level of funding: $108,750
Brief description of the project's goals:
The proposed studies are designed to test the hypothesis that APIPC, particularly DNPC, arise by activating mechanisms capable of substituting for the cell survival and growth functions normally carried out by the AR. I further hypothesize that these 'AR-bypass pathways' can be targeted to suppress DNPC growth and also co-targeted earlier in the disease course to more effectively treat castration-sensitive 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.

CURRENT SUPPORT

Title:
Exploiting Recurrent Chromatin Modifier Mutations for Prostate Cancer Targeted Therapy
Time commitments: 25%
Supporting agency: DOD/CDMRP
Name and address of the funding agency's procuring Contracting/Grants Officer:
Joshua D. McKean, Grants Officer

USAMRAA
820 Chandler Street
Ft. Detrick, MD 21702
Performance Period: 07/01/2019 – 06/30/2022
Level of funding: $150,811
Brief description of the project's goals:
This proposal is designed to test the hypothesis that the loss of specific chromatin modifier function (e.g. U*TX/KDM6A)*, through mutation and/or copy loss, drives resistance to AR pathway antagonism (e.g. enzalutamide). We further hypothesize that, despite dissimilar protein functions, loss of *UTX* or other CMs in the same complex or pathway will produce convergent phenotypic and epigenetic changes in cell state. Furthermore, this change in cell state, which results in resistance to ENZ, will be reversed by pharmacological targeting of specific chromatin modifying and interacting proteins like EZH2, BET-family proteins, and KDM6B.
Overlap with proposed research: None
List of specific aims:
Aim 1: Determine the role of chromatin modifier loss in mediating resistance to AR pathway inhibition.
Aim 2: Determine the transcriptional and epigenetic alterations that occur due to loss of specific chromatin modifier function in prostate cancer.
Aim 3: Evaluate pharmacological approaches to target prostate cancers with mutated/inactivated chromatin modifiers to reverse resistance to AR pathway inhibition.

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: 15% 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: $208,511
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.

PENDING SUPPORT

None.

Exploiting Recurrent Chromatin Modifier Mutations for Prostate Cancer Targeted Therapy

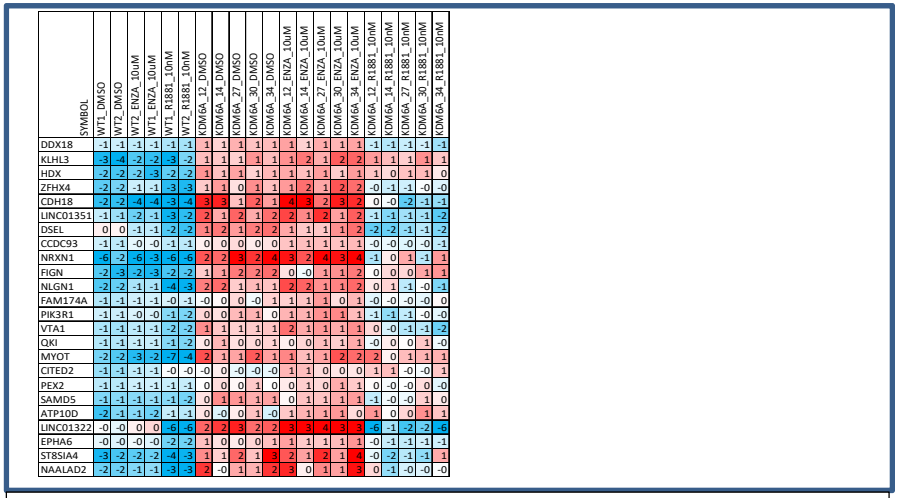
PC180550 Annual Report

W81XWH-19-1-0383

PI: Michael D. Nyquist, PhD **Org:** Fred Hutchinson Cancer Research Center **Award Amount:** $1,055,795.00

Specific Aim(s): Aim 1: Determine the role of chromatin modifier loss in mediating resistance to AR pathway inhibition. Aim 2: Determine the transcriptional and epigenetic alterations that occur due to loss of specific chromatin modifier function in prostate cancer. Aim 3: Evaluate pharmacological approaches to target prostate cancers with mutated/inactivated chromatin modifiers to reverse resistance to AR pathway inhibition.

Approach: Aim 1: Identify CM knockouts that mediate ENZ resistance in human PC models using competitive enrichment assays. Aim 2: Establish the mechanism of ENZ resistance using RNA-seq and ChIP-seq based approaches to determine the transcriptional and epigenetic consequences of CM loss in PC cell lines. Aim 3: Determine whether the effects of CM-KOs can be targeted and reversed pharmacologically by testing inhibitors to downstream and/or interacting pathways.



SYMBOL	WT1_DMSO	WT2_DMSO	WT2_ENZA_10uM	WT1_ENZA_10uM	WT1_R1881_10nM	WT2_R1881_10nM	KDM6A_12_DMSO	KDM6A_34_DMSO	KDM6A_27_DMSO	KDM6A_30_DMSO	KDM6A_34_DMSO	KDM6A_12_ENZA_10uM	KDM6A_34_ENZA_10uM	KDM6A_27_ENZA_10uM	KDM6A_30_ENZA_10uM	KDM6A_34_ENZA_10uM	KDM6A_12_R1881_10nM	KDM6A_34_R1881_10nM	KDM6A_27_R1881_10nM	KDM6A_30_R1881_10nM	KDM6A_34_R1881_10nM
DDX18	-1	-1	-1	-1	-1	-1	1	1	1	1	1	1	1	1	1	1	-1	-1	-1	-1	-1
KLHL3	-3	-4	-2	-2	-3	-2	1	1	1	1	1	1	2	1	2	2	1	1	1	1	1
HDX	-2	-2	-2	-3	-2	-2	1	1	1	1	1	1	1	1	1	1	0	1	1	0	0
ZFHX4	-2	-2	-1	-1	-3	-3	1	1	0	1	1	1	2	1	2	2	-0	-1	-1	-0	-0
CDH18	-2	-2	-4	-4	-3	-4	-3	-3	1	-2	1	-4	-3	-2	-3	-2	0	-0	-2	-1	-1
LINC01351	-1	-1	-2	-1	-3	-2	2	1	2	1	2	2	1	2	1	2	-1	-1	-1	-1	-2
DSEL	0	0	-1	-1	-2	-2	1	2	1	2	2	1	1	1	1	2	-2	-2	-1	-1	-2
CCDC93	-1	-1	-0	-0	-1	-1	0	0	0	0	0	1	1	1	1	1	-0	-0	-0	-0	-1
NRXN1	-6	-2	-6	-3	-6	-6	2	2	3	2	4	3	2	4	3	4	-1	0	1	-1	1
FIGN	-2	-3	-2	-3	-2	-2	1	1	2	2	2	0	-0	1	1	2	0	0	0	1	1
NLGN1	-2	-2	-1	-1	-4	-3	2	2	1	1	1	2	2	1	1	2	0	1	-1	-0	-1
FAM174A	-1	-1	-1	-1	-0	-1	-0	0	0	-0	1	1	1	1	0	1	-0	-0	-0	-0	0
PIK3R1	-1	-1	-0	-0	-1	-2	0	0	1	1	0	1	1	1	1	1	-1	-1	-1	-0	-0
VTA1	-1	-1	-1	-1	-2	-2	1	1	1	1	1	2	1	1	1	1	0	-0	-1	-1	-2
QKI	-1	-1	-1	-1	-1	-2	0	1	0	0	1	0	0	1	1	1	-0	0	0	1	-0
MYOT	-2	-2	-3	-2	-7	-4	2	1	1	2	1	1	1	1	2	2	2	0	1	1	1
CITED2	-1	-1	-1	-1	-0	-0	-0	0	-0	-0	-0	1	1	0	0	0	1	1	-0	-0	1
PEX2	-1	-1	-1	-1	-1	-1	0	0	0	1	0	0	1	0	1	1	0	-0	-0	0	-0
SAMD5	-1	-1	-1	-1	-1	-2	0	1	1	1	1	0	1	1	1	1	-1	-0	-0	1	0
ATP10D	-2	-1	-2	-2	-1	-1	0	-0	0	1	-0	1	1	1	1	0	1	0	0	1	-1
LINC01322	-0	-0	0	0	-6	-6	2	2	3	2	2	3	3	4	3	3	-6	-1	-2	-2	-6
EPHA6	-0	-0	-0	-0	-2	-2	1	0	0	0	1	1	1	1	1	1	-0	-1	-1	-1	-1
ST8SIA4	-3	-2	-2	-2	-4	-3	1	1	2	1	3	2	1	2	1	4	-0	-2	-1	-1	1
NAALAD2	-2	-2	-1	-1	-3	-3	3	-0	1	1	2	3	0	1	1	3	0	-1	-0	-0	-0

Accomplishment: We have created KDM6A and CREBBP knockout prostate cancer cell lines to direct progression to enzalutamide resistance and have begun to analyze the molecular changes that results. Shown above is RNA-seq gene expression heatmap of UTX loss

Timeline and Cost

Activities / Year	1	2	3
Specific Aim 1			
Specific Aim 2			
Specific Aim 3			
Estimated Budget ($K)	**$161**	**$242**	**$197**

Updated: 07/31/2020

Goals/Milestones (Example)

Year 1 Goal(s) – Model creation and preliminary evaluation

☑ Generate and validate KDM6A and CREBBP knockout cell lines

☑ Clone and test sgRNA constructs to determine the genetic interactions relevant to enzalutamide resistance.

Year 2 Goal(s) – Model characterization and drug testing

☐Characterize global gene expression and molecular changes

☐Evaluate drugs that target cells with CM mutations

Year 3 Goal(s) – In vivo evaluation

☐ Use PDX and xenografts harboring CM mutation to test drug suseptablities.

Comments/Challenges/Issues/Concerns:

- COVID-19 related shutdowns have challenged the portions of our work that rely on core facilities that provide high-throughput sequencing and flow cytometry.

Budget Expenditure to Date:

Projected Expenditure: $198,435

Actual Expenditure: $160,890