AWARD NUMBER:
W81XWH-19-1-0383

TITLE:
Exploiting Recurrent Chromatin Modifier Mutations for Prostate Cancer Targeted Therapy

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REPORT DATE:
October 2022

TYPE OF REPORT:
Final

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

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
October 2022	Final	01Jul2019 – 30Jun2022

4. TITLE AND SUBTITLE

Exploiting Recurrent Chromatin Modifier Mutations for Prostate Cancer Targeted Therapy

5a. CONTRACT NUMBER
W81XWH-19-1-0383

5b. GRANT NUMBER
PC180550

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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8. PERFORMING ORGANIZATION REPORT

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

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

10. SPONSOR/MONITOR'S ACRONYM(S)

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

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Purpose: This proposal is focused on exploiting the role of genes/proteins that regulate the structure of chromatin – termed chromatin modifiers (CMs) in the context of advanced prostate cancer (PC) – particularly involving resistance to therapeutics designed to inhibit androgen receptor (AR) signaling.
Scope: This proposal was designed to test the hypothesis that the loss of specific chromatin modifiers (e.g., KDM6A and CREBBP) drive resistance to AR pathway antagonism (e.g. enzalutamide/ENZ). The aims were further designed to test the hypothesis 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 that result in resistance to ENZ. Furthermore, these changes can be reversed by pharmacological targeting of specific CMs and epigenetic regulatory factors like EZH2, BRD4, DOT1L, and KDM6B. Three Specific Aims were proposed: 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 CM mutant prostate cancer and reverse resistance to AR pathway inhibition.
Major Findings: (1) Using genetic/genomic loss of function/knockout screens, we identified multiple CMs that promoted the resistance of PCs to AR antagonism by ENZ; (2) We identified gene expression program alterations resulting from loss of UTX/KDM6A and CBP – a key pathway upregulated in this context involved E2F1/cell cycle regulation; (3) Despite a rationally designed drug screen, we did not identify a particular drug capable of differentially targeting PCs with UTX or CBP loss in the context of ENZ treatment; (5) We identified additional factors capable of altering the epigenome and gene expression that contribute to resistance to AR pathway modulation (e.g. RBL1/2); (5) We identified two epigenetic drugs targeting CBP/p300 and HDACs, respectively, produced robust growth repression phenotypes across PCs with and without AR activity.
Conclusion/Impact: These findings further support the advancement of therapeutics targeting CM proteins in AR active and AR inactive prostate cancers.

15. SUBJECT TERMS
prostate cancer; enzalutamide; castration resistance; precision medicine; chromatin modifiers

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON USAMRDC
a. REPORT	b. ABSTRACT	c. THIS PAGE	Unclassified	22	19b. PHONE NUMBER *(include area code)*
Unclassified	Unclassified	Unclassified			

TABLE OF CONTENTS

	Page
1. Introduction	**5**
2. Keywords	**5**
3. Accomplishments	**5**
4. Impact	**15**
5. Changes/Problems	**15**
6. Products	**16**
7. Participants & Other Collaborating Organizations	**17**
8. Special Reporting Requirements	**17**
9. Appendices	**17**

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 was 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. enzalutamide). The proposal was further designed to test the hypothesis that despite dissimilar protein functions, loss of *UTX* or other CMs in the same complex or pathway would produce convergent phenotypic and epigenetic changes in cell states. Furthermore, this change in cell state, which results in resistance to ENZ, would 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, epigenetics, therapeutics, androgen receptor

3. ACCOMPLISHMENTS:

Major Goals.

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 ENZ conditions. **(100% Complete)**

Major Task 2: Define the combinatorial effects of CM loss. **(100% Complete)**

Major Task 3: Determine the phenotype of CM mutant PC. **(100% Complete)**

Major Task 4: Identify protein complex interactions of CMs. **(100% Complete)**

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 ENZ conditions. **(100% Complete)**

Major Task 2: Determine the effects of UTX KD/KO on chromatin. **(100% Complete)**

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. **(100% Complete)**

Major Task 2: Establish the in vivo efficacy of combined ENZ and EZH2 or BET inhibition in UTX-KO PC models. **(100% Complete).**

What was accomplished under these goals?

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: **100% complete**

Significant Results: As described in the Y1 and Y2 progress reports, we designed and completed CRISPR-based gene deletion/inactivation screens against prostate cancer (PC) cells grown in standard basal media and with PC cells grown in the presence of the AR antagonist enzalutamide (ENZ). The focus of the project centered on those genes that are involved in modifying chromatin, which we designated as chromatin modifiers (CM). We specifically investigated a subset of CMs that have been identified to be altered in the context of advanced prostate cancer and that potentially function as tumor suppressors. This list centered in 36 genes/proteins with these characteristics. In addition to the primary whole genome libraries, we constructed separate CM CRISPR libraries. During this year of support, we repeated the CRISPR screens in replicate cell lines to ensure rigor and reproducibility. This tertiary CRISPR screen confirmed our prior results demonstrating a key role for CMs to regulate ENZ resistance (**Figure 1**). We also carried out an 'over-expression' screen to identify genes that when over-expressed, would induce ENZ resistance. This screen identified several CMs, including CREB5 and MECP2, as drivers of ENZ resistance (**Figure 2**).

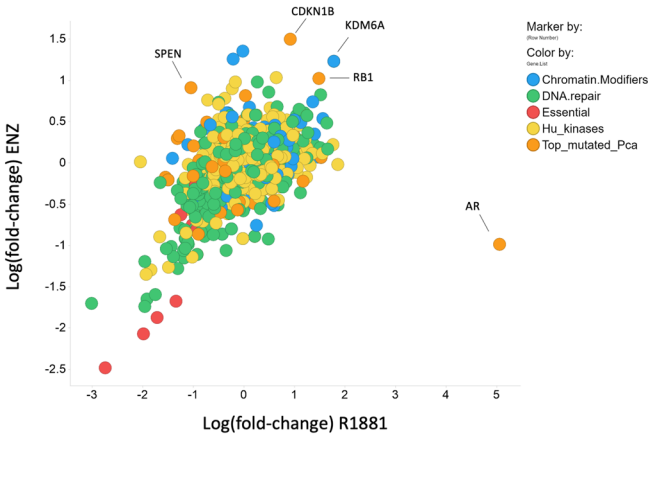


Figure 1. CRISPR validation screen for modulators of ENZ resistance. Note CMs – KDM6A and RB1.

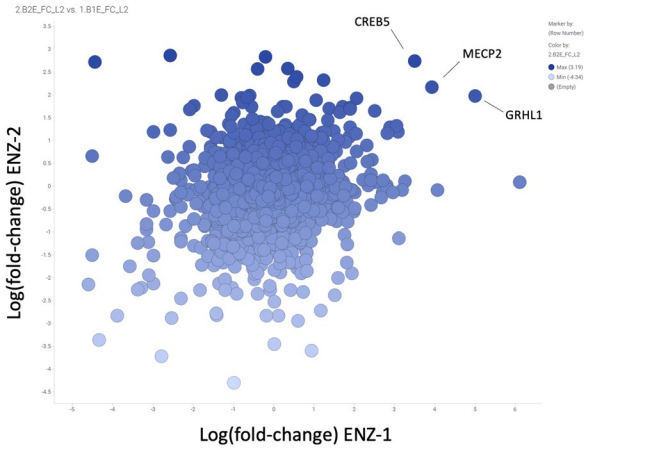


Figure 2. Open Reading Frame (ORF) overexpression screen for modulators of ENZ resistance. Note CMs – CREB5 and MECP2.

Subtask 2: Carry out competition assays on transduced cells **(100% completed)**

Significant Results: As detailed in the Y1 and Y2 progress reports, we employed competition assays to examine the relative fitness resulting from the targeted loss of each CM under ENZ conditions. We confirmed that UTX, CREBBP, and TP53 promoted ENZ resistance – clones with sgRNAs targeting these genes were

enriched in the clone populations, compared to control guide sgRNAs.

In Y1 of the project we screened all sgRNAs except CHD2 and SMARCC1 targets for their influence on AR antagonism (e.g. enrichment/depletion with the AR antagonist enzalutamide -ENZ).. We 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. In Y2 we repeated the screens with selected sgRNAs targeting selected CMs**.** All other sgRNAs either had no enrichment or were depleted. A few, including CREBBP, had discordant results with one sgRNA showing enrichment and the other showing no enrichment. We re-cloned and re-evaluated the discordant CREBBP sgRNAs (see AIM1 Major Task 2 results below). In the present year of support (Y3), we also determined that several other CMs were enriched in the context of ENZ: these included KAT6A, KMT2B, KMT2A, 'CLOCK' and CHD1. However, not all CMs modulated ENZ resistance as we found no enrichment for KMT2D or MGA.

When combining our iterative screen results and re-analysis of response/resistance assays to ENZ, we also found evidence supporting the role of MYC, RB1, and the pocket proteins RBL1 and RBL2. Each of these factors has defined roles in modulating chromatin and regulating gene expression. We sought to further assess their contributions as potential PC chromatin modifying tumor suppressors with particular roles in modulating AR activity (response/resistance to AR pathway targeted drugs) – see below in 'Major Task 2 and 3' results.

***Major Task 2*: Define the combinatorial effects of CM loss.**

Subtask 1: Carry out dual competition assays on transduced cells. **(100% completed)**

Significant Results: As described in the Y1 and Y2 progress reports, we used the method of 'dual competition assays' to determine synthetic lethal or synergistic LOF mutations between two key CMs: UTX and CREBBP. This involved flow cytometry assays with cell lines isogenic for alterations in UTX, CREBBP, TP53, RB1, and TP53/RB1. Surprisingly, we found that UTX sgRNAs were negatively selected in UTX knockout cell lines. We also found that CREBBP loss was only lethal in cells with concurrent loss of TP53 in the context of ENZ treatment. This important result next requires clinical validation: evaluating specific responses to ENZ in men with TP53-/-; CREBBP-/- tumors. We have alerted our clinical colleagues to evaluate such patients for 'exceptional' responses to ENZ.

As noted above, we have further assessed the combinatorial effects of alterations in the RB1, TP53 and MYC genes with respect to chromatin modification read out as alterations in gene expression – particularly relating to AR activity. We also evaluated alterations in these genes with respect to response to ENZ, as well as a new therapeutic strategy that involves 'overdriving' the AR program with supraphysiological levels of androgens/testosterone – termed SPA.

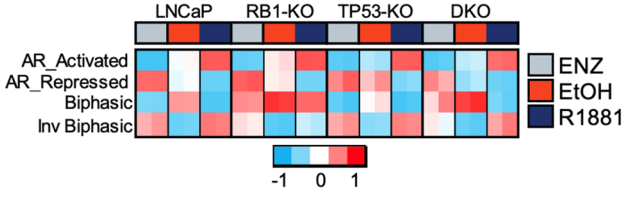


Figure 3. Analysis of AR program activity in the context of loss of the key chromatin modifiers RB1, TP53 and the combination of RB1/TP53 loss (DKO). Treatment with ENZ, EtOH control, or R1881 which is equivalent to supraphysiological androgen (SPA).

Overall, loss of RB1, loss of TP53, and loss of the combination of RB1 and TP53 (DKO) modestly diminished the activity of the AR program, with RB1 loss maintaining the activity of the Biphasic component of AR activity in the context of SPA/R1881 therapy (**Figure 3**). Loss of

RB1 and TP53 also attenuated the ENZ-mediated repression of AR activity (**Figure 3**).

We also engineered PC cells to include knockdown of the RB pocket proteins RBL1 and RBL2 (designed as 4xKO). While wild-type and DKO cells could be growth repressed by ENZ and by SPA/R1881, cells with combinatorial loss of all 4 modifiers (RB1/TP53/RBL1/RBL2) were not effectively repressed by SPA (or ENZ) (**Figure 4**).

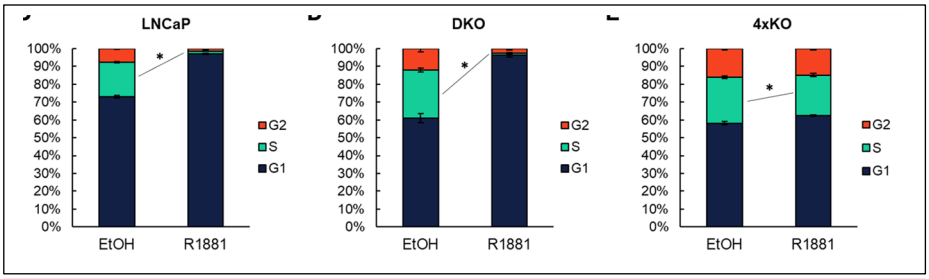


Figure 4. Analysis of the effects of loss of chromatin modifiers on response (cell cycle arrest) to SPA given as R1881 treatment. RB1-/-;TP53-/- is DKO. DKO cells also with loss of RBL1 and RBL2 is 4xKO.

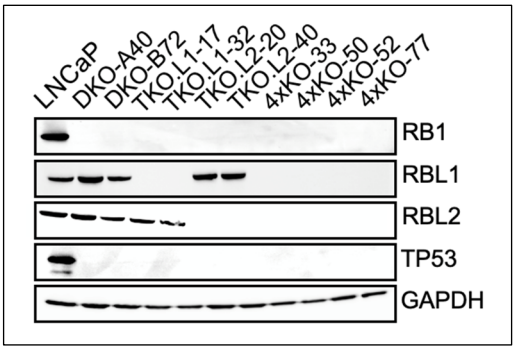


Figure 5. Western analysis of manipulated CM genes including RB1, RBL1, RBL2 and TP53 – multiple clones of each.

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

Subtask 1: *Generate CRISPR/CAS9 knockouts of UTX and CREBBP.* **(100% completed)**

Significant Results: As described in the Y1 and Y2 progress reports, we generated clonal LNCaP KO lines for UTX and CREBBP (multiple independent clones for each line to ensure rigor and reproducibility). In Y3, we also generated clonal PC lines with KO of TP53, RB1, RBL1, RBL2 and overexpression of MYC.

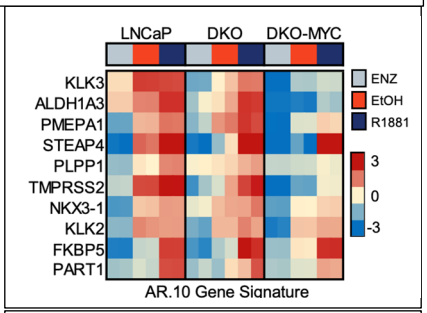


Figure 6. AR program gene expression in the context of TP53/RB1 knockout (DKO) with

Subtask 2: *Carry out qRT-PCR on phenotype genes.* **(100% complete)**

Significant Results: We completed these studies that confirmed loss or overexpression of each gene under study, respectively (data not shown).

Subtask 3: *Western blots and cell cycle analyses*. **(100% complete)**

Significant Results: We completed Western/immunoblot analyses and cell cycle analyses (e.g. via flow cytometry) for the cell lines with alterations in CMs (representative examples shown in **Figure 4** and **Figure 5**).

As noted above, we found that the phenotype of PCs with RB1/TP53 loss was a modest attenuation of AR signaling (**Figure 3, 6**). However, AR signaling could be rescued/amplified with

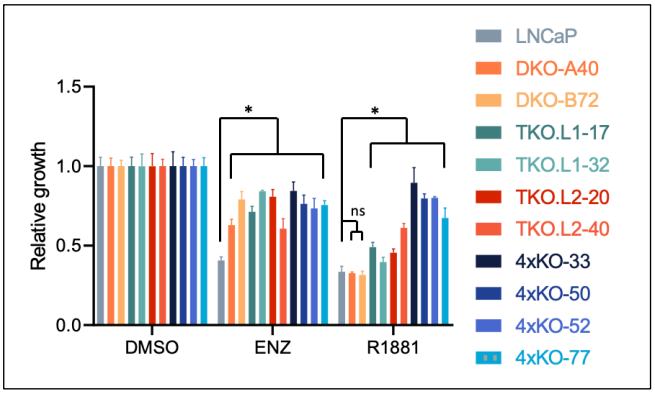


Figure 7. Effects of CM loss on response and resistance of PC cells to ENZ and SPA (R1881). DKO is RB1/TP53 loss; TKO is DKO plus RBL1 loss, 4xKO is TKO plus RBL2 loss.

SPA/R1881 treatment. Notably, overexpression of MYC markedly repressed the AR program, indicating either chromatin modification of AR binding sites, or a reduction in AR itself in the context of high MYC activity (**Figure 6**). We also determined that loss of these CMs (RB1, TP53, RBL2, RBL1) collectively promoted the resistance of PC to both ENZ treatment and SPA (**Figure 7**).

Major Task 4: ***Identify protein complex interactions of CMs.***

Subtask 1: Identify protein complex interactions of CMs. **(100% complete)**

Significant Results: The major approach taken for this subtask involved Rapid Immunoprecipitation Mass spectrometry of Endogenous proteins (RIME) analysis. Antibodies suitable for RIME analysis of UTX and CBP were not identified. We opted to perform RIME for LIN37, a core member of the DREAM/MuvB core complex that is involved in chromatin modification and the regulation of gene expression – particularly genes involved in cell cycle regulation in the DREAM complex. All known members of the DREAM complexes were detected by RIME and were significantly enriched over the IgG control (**Figure 8**). The 10 most enriched proteins by LIN37 RIME included members of the DREAM complex LIN9, LIN54, RBL1 and RBL2 (**Figure 8b**). Chromatin modifiers E2F4 and RBL2 were enriched 2.3-fold (FDR = 0.1, p = 0.003) and 2.2-fold (FDR = 0.07, p = 0.002) respectively, following SPA treatment.

In addition to established DREAM complex members, LIN37 RIME also identified interactions with components of the SIN3A chromatin repressive complex (**Figure 8b,c**). The entire SIN3A complex was significantly enriched over the IgG control for both treatment groups (**Figure 8c**). Both the DREAM and SIN3A complexes contain RBBP4, which could facilitate the interaction between the DREAM complex and the SIN3A histone deacetylase complex to effect chromatin silencing (**Figure 8d**). Several

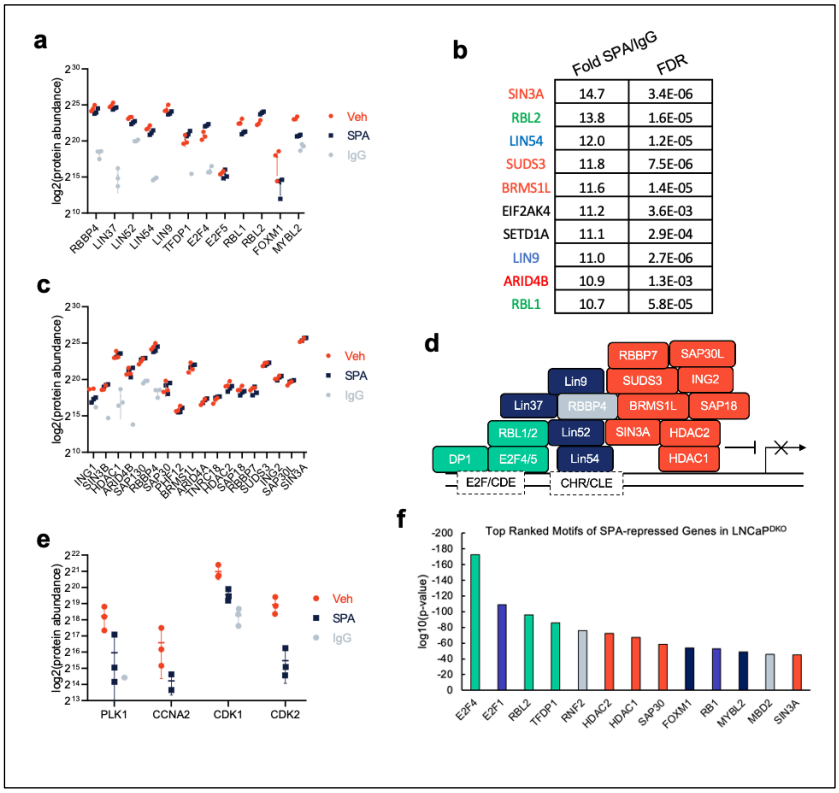


Figure 8. CM protein complex interactions. (a) Log2-protein abundance scores of proteins enriched with CM LIN37-bound chromatin in LNCaP^{DKO} cells treated with 10nM R1881 (SPA) or EtOH (Veh) control for the DREAM and MuvB-FOXM1/MYBL2 complexes; **(b)** Top 10 enriched proteins by LIN37-bound chromatin precipitation of LNCaP^{DKO} cells. DREAM complex members are indicated by green - MuvB core by blue, and SIN3A complex members by red text; **(c)** Log2-protein abundance scores of proteins enriched with LIN37-bound chromatin in LNCaP^{DKO} cells treated with 10nM R1881 or EtOH control for the SIN3A complex. All proteins shown were significantly enriched (FDR < 0.05) over the IgG pulldown control; **(d)** Diagram of DREAM and SIN3A repression complex members precipitated with LIN37-bound chromatin; **(e)** Log2-protein abundance scores of proteins enriched with LIN37-bound chromatin in LNCaP^{DKO} cells treated with 10nM R1881 or EtOH control for DREAM destabilizing factors. All proteins shown were significantly enriched (FDR < 0.05) over the IgG pulldown control; **(f)** Top motifs of HDA-repressed genes in LNCaP^{DKO} RNA-seq datasets as predicted by LISA analysis. MuvB core = dark blue; Repressive DREAM components = green SIN3A complex members = orange; E2F1/RB1 = Light blue; other = grey.

cell-cycle progression proteins were also reduced in SPA-treated cells versus controls, including PLK1, CCNA2, CDK1, and CDK2 (**Figure 8e**). Chromatin modifying proteins that regulate promoter and enhancer activation were also significantly enriched in LIN37 complexes including the RBBP5, SETD1A, and ASHL2 histone methyltransferase complex as well as SWI/SNF members SMARCA4, BRD2.

We next used Lisa (epigenetic Landscape In Silico deletion Analysis and the second descendent of MARGE) analysis as an orthogonal approach to predict transcription factors that regulate AR-repressed genes identified by RNAseq in the LNCaP^{DKO} cells. The set of transcription factors predicted to regulate genes repressed by SPA largely mirrored the RIME results, with DREAM/MuVB, SIN3A, and E2F/RB1 members as the most significant contributors (**Figure 8f**). In contrast, AR-activated genes were predicted to be regulated by AR and AR-cofactors

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. **(100% complete)**
Significant Results: In the Y1 and Y2 progress reports we detailed the construction of PC lines that were engineered to knockdown/delete KDM6A and CREBBP. Cells with knockout of additional CMs were generated in Y3 as described above (e.g. RB1, TP53, RBL1, RBL2). EZH2 overexpressing cells were generated in Y2 as well as additional lines overexpressing cMYC (see **Figure 6** above).

Subtask 2: Perform RNA-seq analysis on engineered cell lines. **(100% complete)**
Significant Results: In the Y2 progress report we detailed the results of RNAseq analyses of PC cells with and without KDM6A and CREBBP deletion. KDM6A and CREBBP RNA-seq analysis yielded insights into the mechanism underlying the selective benefit of their loss. As noted, the most striking results were increased GSVA scores for three gene sets in the ENZ groups for both the CBP and UTX knockouts: (1) cell cycle proliferation (2) "Biphasic genes" – these are AR-modulated genes normally repressed by ENZ as well as growth repressive 'SPA' doses of the steroid R1881; (3) LNCAP.RBKO.UP.50 – these are gene that are upregulated when RB1 is mutated. Collectively, these results indicate enhanced E2F signaling, results that are

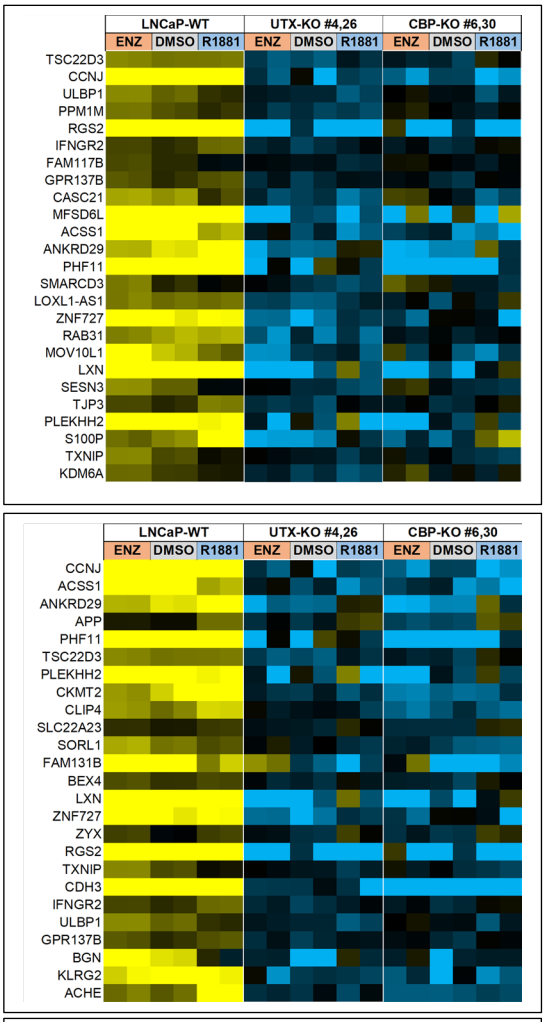


Figure 9. Transcript alterations determined by RNAseq in PC cells with UTX and CBP deletions. Top downregulated genes in UTX-loss (Top) and CBP-loss (Bottom).

similar to the reported effects of high EZH2 activity.

In Y3 we further analyzed the consistent expression alterations resulting from UTX and CBP loss (**Figure 9**). There were strong similarities in the transcriptional alterations pointing to commonalities in gene regulation that may influence ENZ resistance. For example, the expression of the stem cell regulatory gene Latexin (LXN) is markedly downregulated in the context of UTX and CBP loss. LTX has been shown to function as a tumor suppressor and modulator of drug responses.

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. **(100% complete)**

Significant Results: As detailed in the Y2 progress report, we opted to use a new method for the global analysis of protein/chromatin associations termed CUT&RUN, rather than Western/immunoblot analyses. The technique is similar to ChIP-seq. We analyzed alterations in histone marks including H3K4me1, H3K4me3, H3K27Ac, and H3K27me3 and compared these marks in the context of intact UTX and CBP vs loss of UTX and CBP, with and without exposure to ENZ. Overall, we identified a large number of gained H3K27me3 sites in the UTX-KO and CBP-KO when compared to isogenic control LNCaP cells. Notably, these sites were enriched for genes involved in cell cycle/cell proliferation (see Y2 progress report).

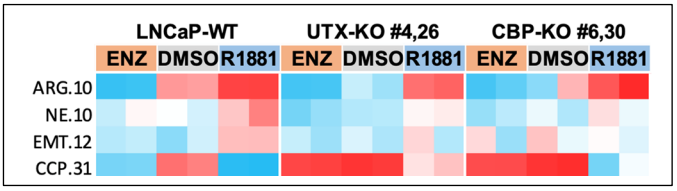


Figure 10. Pathway analysis of PC cells with and without UTX or CBP deletion. Note no substantial alterations in AR activity (ARG10) but increases in proliferation CCP.31).

Subtask 3: Determine the effect of UTX-KO on the AR-cistrome using AR ChIP-seq **(100% complete)**

Significant Results: Further analysis of the RNA-seq data did not reveal substantial changes to AR signaling in the context of UTX or CBP loss. Due to this we deprioritized these experiments as AR ChIP would be unlikely to identify changes to AR binding in the absence of any substantial alterations in the expression of AR target genes.

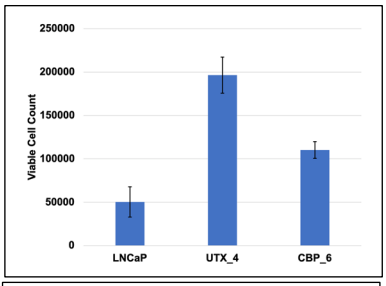


Figure 11. PC cell viability under treatment with ENZ. UTX and CBP deletions enhance growth.

We opted to proceed with an alternative strategy. First, using multiple UTX-KO clones, we confirmed that major changes in gene expression programs involved enhancement of cell cycle (e.g. CCP 31 gene scores) and did not alter AR activity (panel of AR target genes) (**Figure 10**). Second, we confirmed that UTX (and CBP) knockdown promoted the growth/survival of PC cells in the context of ENZ exposure (**Figure 11**). These results reaffirmed a focus on major regulators of cell cycle that could bypass AR effects and consequently supported proceeding with 'Subtask 4' involving the analysis of E2F1.

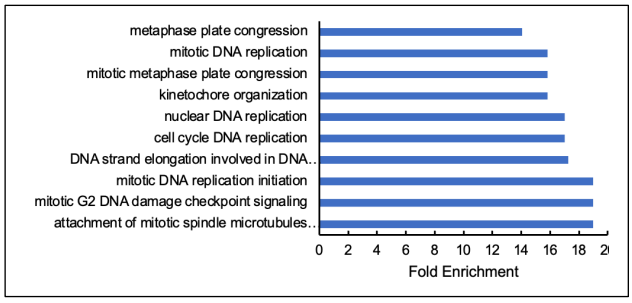


Figure 12. E2F1 ChIP-seq identifies enrichment in genes/pathways regulating mitosis/cell cycle.

Subtask 4: Determine the effect of UTX-KO on the

E2F1-cistrome using E2F1 ChIP-seq **(100% complete)**

Significant Results: As UTX loss did not directly influence AR activity, we considered alternative mechanisms for how UTX (and CBP) may influence ENZ resistance. We next evaluated E2F1 as a driver of cell proliferation, potentially uncoupling the role of AR in regulating cell cycle progression. We performed E2F1 ChIPseq in the LNCaP-DKO cells. Motif analysis identified significant enrichment for genes/pathways involved in DNA replication, mitosis, and cell cycle (**Figure 12**).

In support of these findings, we re-analyzed RNAseq data comparing the effects of EZH2 over-expression against UTX-knockout. We found consistent enrichment of key pathways involved in driving cell proliferation: (i) E2F targets; (ii) G2M checkpoint; (iii) MYC targets (**Figure 13**).

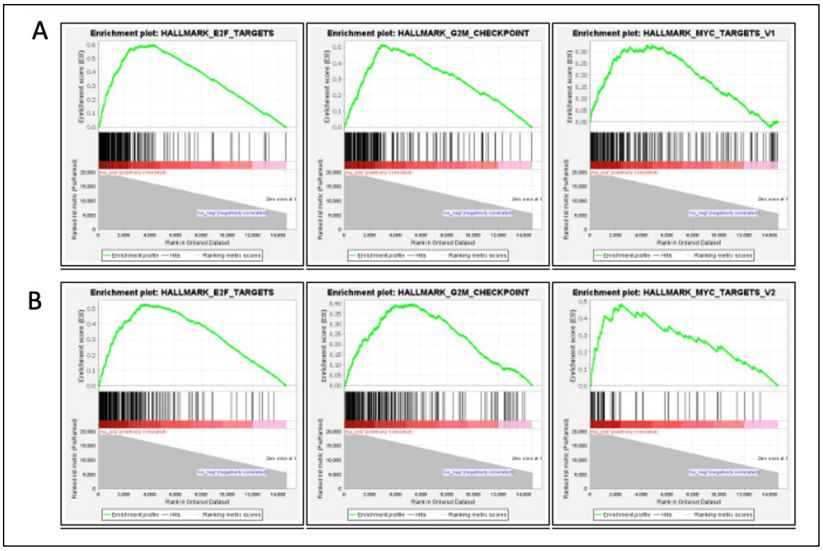


Figure 13. Gene Set Enrichment Analysis of RNAseq data from PC cells overexpressing EZH2 (A) or with UTX deletion (B).

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. **(100% completed)**

Significant Results: Isogenic cell lines for various chromatin modifiers (e.g. UTX knockout) were detailed in the Y1 and Y2 progress reports and for additional CMs in Y3 (see Figure 5).

Subtask 2: Perform dose-response assays to determine effective concentrations of drugs. **(100% completed).**

Significant Results: As detailed in the Y2 progress report, we performed dose-response assays in UTX and CREBBP (CBP) knockout cell lines with or without ENZ. The drugs used were chosen based on prior literature and/or the ability to target UTX/KDM6A, CREBBP/p300, MYC, BRD4, EZH2. In total, 13 drugs were

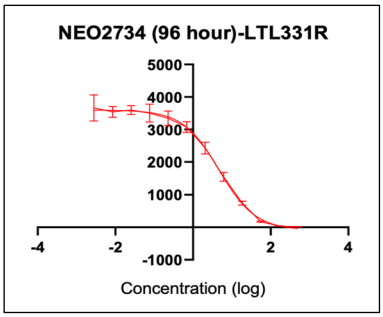


Figure 14. Growth inhibition effects of NEO2734 against the AR-null neuroendocrine PC line LTL331R with IC50 of ~0.1 uM.

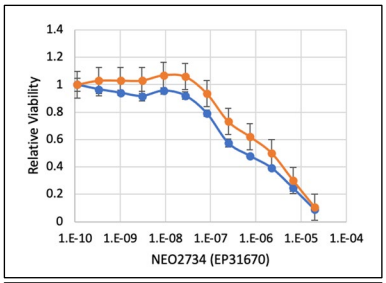


Figure 15. Growth inhibition effects of NEO2734 against the PC cells overexpressing MYC with (orange) or without (blue) androgen treatment.

tested using a 5 day readout for activity. In this initial rational screen, no drug was found to be selectively effective against any KO genotype. We note that for a subset of drugs that modify the epigenome, dose-response assays may not be 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 modest influence on growth resistance and a requirement for chromatin alterations.

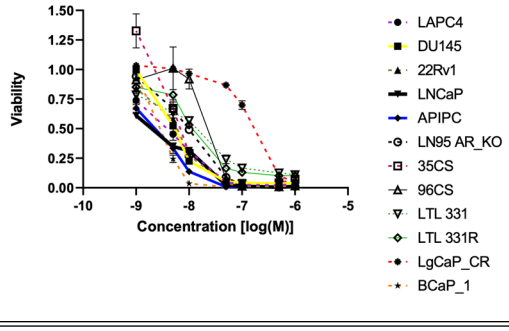


Figure 16. Cell viability assay evaluating the effects of NEO2734 across PC lines. NEO2734 was effective against all lines except LAPC4.

During Y3 of the proposal, we evaluated two additional epigenetic altering drugs. We tested a CBP/p300 inhibitor (NEO2734/EP31670), and also a dual HDAC/PI3K inhibitor, Fimepinostat. We determined that NEO2734 had potent activity toward both AR active and AR-low/null PC lines (**Figure 14**). Further, NEO2734 also effectively inhibited the growth of PC cells overexpressing MYC, an oncogene with potent chromatin modifying effects (**Figure 15**).

With respect to the HDAC/PI3K inhibitor Fimepinostat, a broad screen across a panel of PC lines demonstrated generally strong activity in both AR active lines and AR inactive lines – supporting the interruption of chromatin organization via histone deacetylation as a meaningful clinical target (**Figure 16**).

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

<u>Subtask 1</u>: Generate UTX-KO PC lines (1-3). **<u>(100% completed)</u>**
<u>Significant Results</u>: Isogenic cell lines with UTX deleted have been generated.

<u>Subtask 2</u>: Evaluate the efficacy of an epigenetic modifying drug *in vivo* **(100% Complete).**
<u>Significant Results:</u> Our in vitro studies did not identify a benefit in combining ENZ with EZH2i or BETi. Consequently, we further evaluated alternative epigenetic modifiers. Based on the in vitro efficacy of Fimepinostat, we elected to test the efficacy of this agent towards PC models *in vivo*. For these studies, we collaborated with Dr. John Lee, Dr. Colm Morrissey and Dr. Peter Nelson. As determined in vivo, we found that Fimepinostat exhibited efficacy in vivo against both ARPC lines (e.g. LuCaP35) (**Figure 17**) and AR-null PC (e.g. LuCaP173.2)(**Figure 18**). These data indicate the potent effects of the epigenetic drug Fimepinostat and support clinical evaluation. Further studies are required to understand and confirm the mechanism(s) by which Fimepinostat alters HDAC activity, chromatin organization and gene expression.

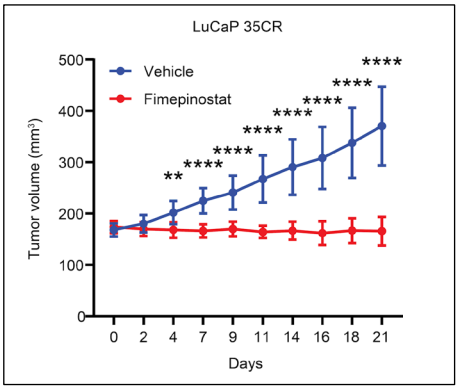


Figure 17. Growth inhibitory effects of Fimepinostat toward the ARPC LuCaP35CR PDX line in vivo.

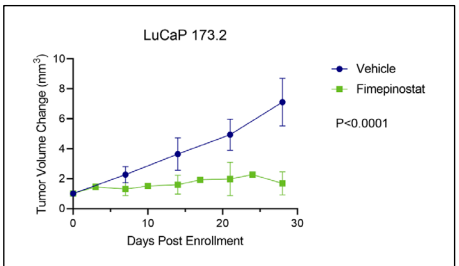


Figure 18. Growth inhibitory effects of Fimepinostat toward the DNPC AR-null LuCaP173.2 PDX line in vivo.

What opportunities for training and professional development has the project provided?
This project provided opportunities for the PI, Michael Nyquist, to present data and interact with collaborative scientists and peers at other leading institutions. Research presentations were delivered during monthly AR focused meetings involving Dr. Steve Balk at Beth Israel in Boston, Myles Brown at the Dana Farber Cancer Institute in Boston, Peter Nelson at FredHutch in Seattle, and Steve Plymate at the University of Washington in Seattle, and their research groups. Feedback was provided for professional development.

M.Nyquist. *The role of RBL1/2 in RB/TP53-null Prostate Cancer* FredHutch/DFCI AR Program Project Grant Meeting. 3.07.22

M.Nyquist. *AR Reactivators* FredHutch/DFCI AR Program Project Grant Meeting 2.3.2020

How were the results disseminated to communities of interest?
To date, the results were disseminated to the research groups denoted above and also to local investigators at the Fred Hutchinson Cancer Center and the University of Washington through oral interactive discussions during laboratory/research meetings.

What do you plan to do during the next reporting period to accomplish the goals?
Nothing to Report

4. IMPACT: What was the impact on the development of the principal discipline(s) of the project?
(1) Using genetic/genomic loss of function/knockout screens, we identified multiple CMs that promoted the resistance of PCs to AR antagonism by ENZ;
(2) We identified gene expression program alterations resulting from loss of UTX/KDM6A and CBP
(3) a key pathway upregulated in this context involved E2F1/cell cycle regulation;

(3) Despite a rationally designed drug screen, we did not identify a particular drug capable of differentially targeting PCs with UTX or CBP loss in the context of ENZ treatment;
(4) We identified additional factors capable of altering the epigenome and gene expression that contribute to resistance to AR pathway modulation (e.g. RBL1/2);
(5) We identified two epigenetic drugs targeting CBP/p300 and HDACs, respectively, produced robust growth repression phenotypes across PCs with and without AR activity.

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. Beyond the typical slight redirections based on scientific results and obvious next steps in following the acquired/new data, there were no substantive changes to the overall research direction, goals and objectives as detailed in the original Statement of Work.

Actual or anticipated problems or delays and actions or plans to resolve them

As noted in prior progress reports for Y1 and Y2, this project was impacted by the COVID pandemic with delays due to employee work prohibitions, challenges conducting animal experiments, and delays in obtaining relevant supplies due to supply chain disruptions. However, we were able to complete the stated objectives.

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

Nothing to Report.

Significant changes in use or care of human subjects

Nothing to Report.

Significant changes in use or care of vertebrate animals

Nothing to Report.

Significant changes in use of biohazards and/or select agents

Nothing to Report.

6. PRODUCTS:

- **Publications, conference papers, and presentations**.

  Journal publications

  The influence of chromatin modifying factors in modulating prostate cancer resistance to AR-directed therapeutics. H.Meade, L.Ang, A.Bose, J.Lucas, R.Dumpit, M.Haffner, JK Lee, C.Morrissey, PS Nelson, and M.Nyquist. In Preparation. CDMRP support acknowledged

  Supraphysiological Androgens Promote the Tumor Suppressive Activity of the Androgen Receptor Through cMYC Repression and Recruitment of the DREAM Complex. MD Nyquist, IM Coleman, B Hanratty, H Meade, EA Mostaghel, SR Plymate, E Corey, MC Haffner, and PS Nelson. Submitted to Cancer Research. CDMRP support acknowledged.

  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:	10
Contribution to Project:	Direction of research efforts; ChIP-seq / CUT&RUN studies; analysis of RNAseq data
Funding Support:	See attached Other Support document.

Name:	Helen Akushie
Project Role:	Research Technician
Nearest person month worked:	3
Contribution to Project:	Assisted with nucleic acid extractions and cell line propagation.
Funding Support:	PC180550; R01 CA234715; P01 CA163227; 19YOUN08

Name:	Lisa Ang
Project Role:	Research Technician
Nearest person month worked:	2
Contribution to Project:	Assisted with PDX studies/analyses.
Funding Support:	PC170431; PC170503; PC180550; PC200262; R01 CA234715; P01 CA163227; 19CHAL02

Name:	Tony Chu
Project Role:	Postdoc
Nearest person month worked:	1
Contribution to Project:	Assisted with nucleic acid extractions and cell line propagation.
Funding Support:	PC171001; PC180550; PC200262; P50 CA097186; P01 CA163227; R01 CA234715; BD527719; C21868065

Name:	Ruth Dumpit
Project Role:	Research Technician
Nearest person month worked:	3
Contribution to Project:	Assisted with nucleic acid extractions and cell line manipulation.
Funding Support:	PC170431; PC171011; PC170503; PC180550; PC200262; P50 CA097186; R01 CA234715; 19CHAL02

Name:	Jared Lucas
Project Role:	Staff Scientist
Nearest person month worked:	2
Contribution to Project:	Assisted with drug studies.
Funding Support:	PC170431; PC171001; PC170503; PC180550; PC200262; P50 CA097186; P01 CA163227; R01 CA234715

Name:	Hannah Meade
Project Role:	Research Technician
Nearest person month worked:	12
Contribution to Project:	Performed AR assays and assistance in general lab experiment setup/work including tissue culture and flow cytometry.
Funding Support:	PC180550; P01 CA163227; R01 CA234715

Name:	Talina Nunez
Project Role:	Research Technician
Nearest person month worked:	1
Contribution to Project:	Assisted with drug studies.
Funding Support:	PC180550; PC200262; 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 other support document for Dr. Michael Nyquist

What other organizations were involved as partners?
Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: N/A

QUAD CHARTS: Attached.

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; **Performance Period:** 08/01/2016 - 07/31/2018
Level of funding:
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
sarah.lee@nih.gov;
Performance Period: 07/11/2018 - 06/30/2020
Level of funding:
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.

Title: *Eradicating Metastatic Prostate Cancer through the Systematic Identification of Synergistic Drug Combinations*
Grant #: PC170431
Time commitments: 10% effort
Supporting agency: DOD/CDMRP

Name and address of the funding agency's procuring Contracting/Grants Officer:
DOD/CDMRP, help@ebrap.org;
Performance Period: 08/01/2018 – 07/31/2021
Level of funding:
Brief description of the project's goals:
We will test the hypothesis that specific combinations of therapeutics can rapidly and completely eradicate subtypes of mPC, and that patient-derived xenografts (PDX) representing the diversity of molecular 'driver' aberrations found in human CRPC can be used to rapidly and systematically identify these potent drug combinations.
Overlap with proposed research: None
List of specific aims:
Aim 1: Conduct a systematic assessment of combination pharmacological therapy to eradicate CRPC using panels of PDX models that reflect the diversity of molecular aberrations found in mCRPC.
Aim 2: Identify molecular features (genotype/phenotype) of PDX responders to drug combination(s).
Aim 3: Establish an International consortium for evaluating and validating novel therapeutic combinations capable of eradicating CRPC.

Title: *The MSKCC-UW/Fred Hutch Prostate Cancer Drug Resistance and Sensitivity Center*
Project 3: Defining the Appropriate Context for Targeting Kinase Signaling in Combination with Androgen Receptor Blockade to Enhance Therapeutic Response in Metastatic Prostate Cancer
Grant #: U54 CA224079
Time commitments: 50% effort
Supporting agency: MSKCC – NIH/NCI
Name and address of the funding agency's procuring Contracting/Grants Officer:
William Zurich
zuricihiw@mskc.org

MSKCC
633 3rd Avenue, 3rd Floor
NYC, NY 10017
Performance Period: 09/30/2017 – 08/31/2022
Level of funding:
Brief description of the project's goals:
Our proposal aims to: 1) define biomarkers of intrinsic sensitivity and resistance to inform appropriate patient selection for combination therapy; 2) define the mechanisms of acquired resistance; 3) devise therapeutic strategies to overcome resistance; 4) optimize AR pathway targeting in the setting of PI3K pathway inhibition to maximize tumor response; and 5) explore the therapeutic role of FGFR in a novel subset of AR negative prostate cancers.
Overlap with proposed research: None
List of specific aims:
Aim 1: Identifying metastasis suppressors within skeletal muscle.
Aim 2: Reverse engineering growth resistance using rare tumor cells that successfully colonize skeletal muscle.
Aim 3: Delivering skeletal muscle derived factors to prevent colonization of metastasis prone organs.

Title: *Exploiting Recurrent Chromatin Modifier Mutations for Prostate Cancer Targeted Therapy*
Grant #: PC180550
Time commitments: 40%
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:
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.

CURRENT SUPPORT

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:
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

OVERLAP

There is no scientific, budgetary, or commitment overlap in the above projects. At no time will Dr. Nyquist's total committed effort exceed 12 calendar months.

CERTIFICATION

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

Michael Nyquist, PhD

Digitally signed by Michael Nyquist, PhD
Date: 2022.12.23 10:19:28 -08'00'

Michael Nyquist	12/23/2022
	Date

Exploiting Recurrent Chromatin Modifier Mutations for Prostate Cancer Targeted Therapy

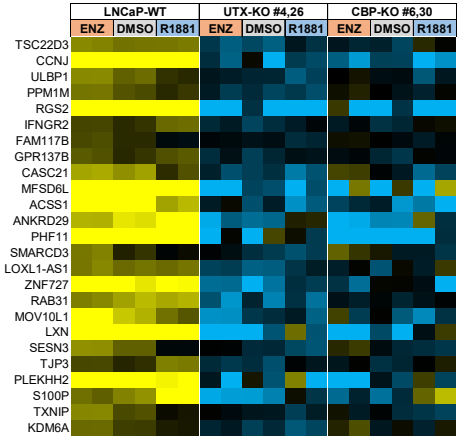
PC180550 Final Report

W81XWH-19-1-0383

PI: Michael D. Nyquist, PhD **Org:** Fred Hutchinson Cancer Center **Award Amount:** $1,055,795

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.



Accomplishment: We have quantitated gene expression in KDM6A/UTX Prostate Cancer cells using genome-wide RNAseq analysis. These studies included multiple replicate lines with UTX knockout and treatments with the AR antagonist enzalutamide (ENZ) and hyperactivation of the AR with R1881. The findings were highly concordant with deletion of CBP/p300.

Timeline and Cost

Activities / Year	1	2	3
Specific Aim 1			
Specific Aim 2			
Specific Aim 3			
Estimated Budget ($K)	**$198**	**$204**	**$197**

Updated: 6/30/2022

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

☑ Evaluate drugs that target cells with CM mutations

☑ Characterize global gene expression and molecular changes

Year 3 Goal(s) – In vivo evaluation and drug treatments of novel CMs

☑ Use PDX and xenografts harboring CM mutation to test drug susceptibilities.

☑ Evaluate CMs for modulation of AR therapeutics (SPA; AR antagonists)

Comments/Challenges/Issues/Concerns:

- COVID-19 related shutdowns challenged portions of our work that relied on core facilities that provide high-throughput sequencing and flow cytometry as well as access to sterile animal housing. These issues were resolved

Budget Expenditure to Date:

Projected Expenditure: $1,055,795

Actual Expenditure: $1,055,795