AWARD NUMBER: W81XWH-16-1-0532

TITLE: Epigenetic Machinery Regulates Alternative Splicing of Androgen Receptor (AR) Gene in Castration-Resistant Prostate Cancer (CRPC)

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REPORT DATE: SEPTEMBER 2019

TYPE OF REPORT: Annual

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

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
SEPTEMBER 2019	Annual	09/01/2018-08/31/2019

4. TITLE AND SUBTITLE
Epigenetic machinery regulates alternative splicing of androgen receptor gene in castration-resistant prostate cancer

5a. CONTRACT NUMBER
W81XWH-16-1-0532

5b. GRANT NUMBER
PC150152P1

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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

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8. PERFORMING ORGANIZATION REPORT NUMBER

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

U.S. Army Medical Research and Materiel 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

Androgen deprivation therapy (ADT) is the primary treatment for metastatic prostate cancer (PCa) since PCa depends on androgen for growth. Although initially responsive, most tumors progress into androgen-independent/castration-resistant PCa (CRPC). No curative therapy is available. One of the reasons for the resistance to ADT and newer anti-androgen drugs is the emergence of constitutively active AR variants (AR-Vs) such as AR-V7 that are induced under ADT conditions. Our research goal is to test the hypothesis that the epigenetic regulator KDM4B, a histone lysine demethylase, promotes AR-V7 via alternative splicing, leading to CPRC. A multi-disciplinary approach including molecular biology, tumor biology, cell biology, and biochemical method is used to test this hypothesis. In collaboration with a partnering principal investigator we are also testing the efficacy of our newly identified KDM4B inhibitor(s) as a monotherapy or combined with approved anti-androgen agents in AR-V7-expressing CRPC in preclinical mouse models.

15. SUBJECT TERMS

NONE LISTED

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	8	19b. TELEPHONE NUMBER *(include area code)*

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

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

The subject of our research is to identify the molecular mechanism of the drug resistance in castration-resistant prostate cancer (CRPC). Our preliminary data suggest that one of the mechanisms of the resistance is the emergence of constitutively active androgen-receptor variants such as AR-V7. Our goals are to demonstrate that histone lysine demethylase KDM4B regulates AR-V7 via alternative splicing and to test the efficacy of our newly identified KDM4B inhibitor(s) as a monotherapy or combined with approved anti-androgen agents in AR-V7-expressing CRPC in pre-clinical animal models of CRPC.

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

Histone lysine demethylase, castration-resistant prostate cancer, alternative splicing, AR-V7, KDM4B, small molecule inhibitors.

3. **ACCOMPLISHMENTS:**

What were the major goals of the project?

List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

There are two specific aims in this proposal. We have made significant progresses for both aims in the past year. One manuscript for publication is written and is currently under review.

Aim 1. To establish that KDM4B promotes AR-V7 expression and identify the regulatory mechanisms.

Major Task 1: Determine the role of KDM4B in promoting AR-V7 expression in various PCa cell lines, including those resistant to enzalutamide. — **completed (6/30/2017).**

Major Task 2: Determine how KDM4B binds to the spliceosome associated with pre-mRNA. — **completed (6/30/2017).**

Milestone #1: *Co-author manuscript on KDM4B-RNA interaction. — We have met this milestone. The manuscript was submitted to Nuclear Acid Research and is currently under review (9/15/2019).*

Major Task 3: Map RNAPII, H3K9/K36me3 occupancy around AR locus using ChIP-qPCR in several CRPC cells. — **completed (9/15/2019).**

Major Task 4: To identify potential KDM4B-regulated alternative splice gene(s) using RNA-seq and map KDM4B-RNA interactions with CLIP-seq.—in progress, 50% completed.

Milestone #2: *Co-author manuscript on mechanism by which KDm4B regulates AR-V7 at chromatin level--- We have met this milestone. The manuscript is under 3rd revision for Nuclear Acid Research (9/15/2019).*

Aim 2. To evaluate the clinical application of KDM4B inhibitors on CRPC tumors expressing AR-Vs.

Major Task 5: Identify two lead compounds using CPRC cell lines and optimizing their dosage and schedule in xenograft models.

What was accomplished under these goals?

1) **Major activities;**

Aim 2, Major task 5: Based on the previous study, 50 mg/kg of B3 (daily for 5 days per week) exhibit a significant tumor inhibition after 10 days after treatment. In order to characterize the in vivo effect of B3 on 22RV1 tumors, we performed immunohistochemical staining (IHC) using several antibodies against AR, AR-V7, KDM4B and H3K9me3. As shown in Figure 1, B3-treated tumors exhibit significant reduction of AR and AR-V7 levels. As we expected, B3 is able to reduce its target KDM4B expression in treated tumors. These data confirm the in vivo target validation of B3 and support the efficacy of B3 treatment for recurrent CRPC-expressing AR-V7.

Furthermore, we compared the therapeutic efficacy of B3 (50 mg/kg), Enzalutamide (50 mg/kg, daily orally administration for 1 week) and combination therapy using VCAP subcutaneous tumor model. VCAP is a CRPC cell line-expressing AR-V7. As shown in Figure 2A, either B3 or Enzalutamide is ablr to suppress

tumor growth, which could be due to its inhibitory effect on both AR and AR-V7 protein expression (Figure 2B). However, combination treatment exhibits an antagonistic effect on tumor growth (Figure 2A) and AR and AR-V7 protein expression (Figure 2B), implying both agents may have the similar mechanism of action. We are currently investigating this issue.

Figure 1. **The target validation of the in vivo effect of B3.** Representative IHC staining of AR, AR-V7, and KDM4B in tumors treated with B3 compound.

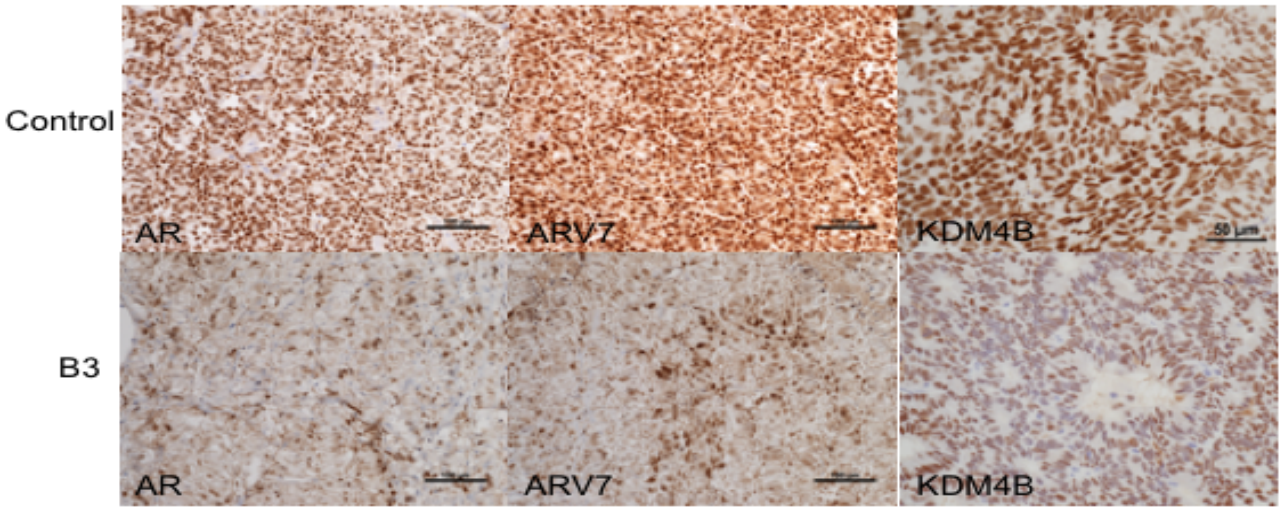
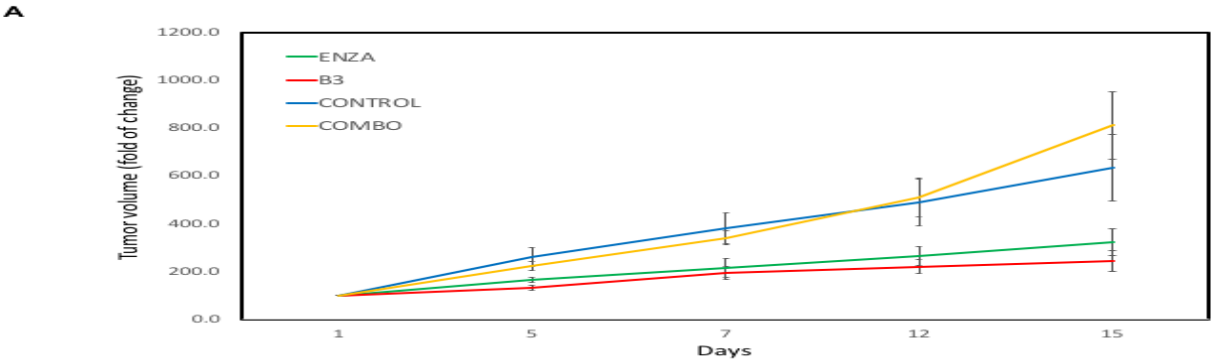
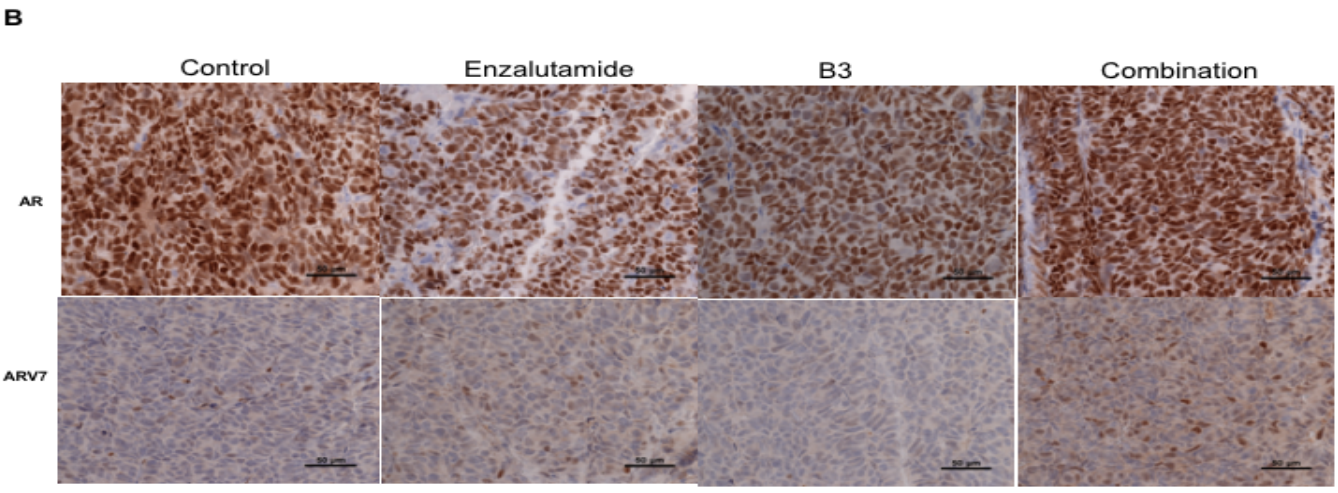


Figure 2. **The therapeutic efficacy of B3 or Enzalutamide or combination on VCAP growth in vivo.** **(**A) Tumor growth curves of VCAP-xenografts treated with control (n=6), Enzalutamide (n=7), or B3 (n=6) or combination (n=5). Mean ± SEM, *, *p<0.05*. (B) IHC of AR and AR-V7 in tumors.





2) **Specific objective;**
Based on these data, we are characterizing pharmacologic properties of B3 in order to explore its clinical application.

3) **Significant results or key outcomes;**
Significant results Several xenograft animal models provide strong evidence that KDM4B is a critical target of CRPC.
Key outcomes B3, a specific KDM4B small molecule inhibitor, can be a new therapeutic agent for CRPC therapy.

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

How were the results disseminated to communities of interest?
We have submitted a manuscript to Cancer cell under review right now.

What do you plan to do during the next reporting period to accomplish the goals?
We will follow the proposed plan in SOW to complete our experimental therapy of using KDM4B small molecule inhibitor (B3). We will expand the xenograft model to use other CRPC cell lines to ensure the tumor heterogeneity will not alter the drug potency of B3. In addition, we will examine any synergistic effect of B3 by combining Enzalutamide.

4. **IMPACT:** Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

What was the impact on the development of the principal discipline(s) of the project? What was the impact on other disciplines?
Alternative splicing not only increases protein variety but the resultant gene products might have considerably different functions. In fact, more than 90% of genes are subjected to alternative splicing in humans. Obviously, alternative splicing greatly contributes to biological complexity, alternation in splicing machinery has been correlated with oncogenesis and drug resistance. Thus targeting this machinery represents a new avenue of cancer therapy. Since splicing is ubiquitously present in every cell, agents directly targeting this machinery are often very toxic. Thus, designing agents targeting regulator of gene splicing machinery could circumvent this problem. Here, we identified a novel mechanistic link between epigenetic factor (i.e., histone lysine demethylase KDM4B) and alternative splicing of AR gene. KDM4B is overexpressed in prostate cancer and many other cancer types; we have shown the potential efficacy of KDM4B small molecule inhibitor in prostate cancer. The outcome of this project is expected to impact on therapeutic strategy of prostate cancer but also other cancer types as well.

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:** The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:

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

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:** List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."

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

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Name:	*Zhi-Ping Liu*
Project Role:	*PI*
	No change
Name:	*LingLing Duan*
Project Role:	*Research associate*
	No change
Name:	*Qing-Jun Zhang*
Project Role:	*Research Associate*
	No change

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

Nothing to Report

What other organizations were involved as partners?
Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: please see Initiating PI Zhi-Ping Liu's progress report

9. Appendix

N/A