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TITLE: Investigating the Molecular Mechanisms of Acquired Resistance to BET Bromodomain Inhibitors in Castration-Resistant Prostate Cancer

PRINCIPAL INVESTIGATOR: Dr. Irfan Asangani

CONTRACTING ORGANIZATION: Trustees of the University of Pennsylvania

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Investigating the Molecular Mechanisms of Acquired Resistance to BET Bromodomain Inhibitors in Castration-Resistant Prostate Cancer

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

Trustees of the University of
Pennsylvania
3451 Walnut St,
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U.S. Army Medical Research and Materiel Command
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Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

BRD4 plays a major role in the transcription networks orchestrated by androgen receptor (AR) in castration resistant prostate cancer (CRPC). Several BET inhibitors (BETi) that displace BRD4 from chromatin are being evaluated in clinical trials for CRPC. Here we describe mechanisms of acquired resistance to BETi that are amenable to targeted therapies in CRPC. BETi-resistant CRPC cells displayed cross-resistance to a variety of BETi in the absence of gatekeeper mutations, exhibited reduced chromatin-bound BRD4, and were less sensitive to BRD4 degraders/knockdown, suggesting a BRD4-independent transcription program. Transcriptomic analysis revealed reactivation of AR-signaling due to CDK9-mediated phosphorylation of AR, resulting in sensitivity to CDK9 inhibitors and enzalutamide. Additionally, increased DNA damage associated with PRC2-mediated transcriptional silencing of DDR genes was observed leading to PARP inhibitor sensitivity. Collectively, our results identify the therapeutic limitation of BETi as a monotherapy, however; our BETi-resistant data suggests unique opportunities for combination therapies in treating CRPC.

15. SUBJECT TERMS
CRPC, Chromatin readers, BRD4, AR, BET bromodomain inhibitors, Transcriptional plasticity, PROTAC, Synthetic lethal, CDK9, PARP, DNA damage, Olaparib, PRC2 complex, EZH2.

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
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Final Technical Report and Closeout Documents Due for DoD Award W81XWH-17-1-0404, PC160610

FRONT COVER

The report type (e.g., grant, contract, SBIR, MIPR, etc.) and the distribution statement required will determine which Cover is appropriate for the report.

REPORT OUTLINE

The text of the report must include all sections addressed in the table of contents to include the following. **DO** include the bolded section headings, but **DO NOT** include the *italicized* descriptions of section contents in your submitted reports.

1. INTRODUCTION:

There is a critical need to better understand the mechanism of acquired resistance to targeted therapies in castration resistant prostate cancer. Our proposal represents the first step towards understanding the mechanism of acquired resistance to BET inhibitors that has shown immense promise in preclinical cancer models and early results from clinical trials have also been encouraging with durable patient responses in CRPC patients. This proposed work has important translational relevance and high potential clinical impact. The underlying hypothesis of this proposal is that resistance to BET inhibitor therapy leads to reactivation of AR signaling pathway due to widespread re-orchestration of the chromatin landscape; however with increased DNA damage and modulated PRC2 activity, BETi resistant cells are sensitive to anti-androgens, PARP inhibitors and EZH2 inhibitors.

2. KEYWORDS:

CRPC, Chromatin readers, BRD4, AR, BET bromodomain inhibitors, Transcriptional plasticity, PROTAC, Synthetic lethal, CDK9, PARP, DNA damage, Olaparib, PRC2 complex, EZH2.

3. ACCOMPLISHMENTS:

What were the major goals of the project?

- **Specific Aim 1: To elucidate the mechanism of transcriptional plasticity causing acquired resistance to BETi therapy by integrative genomic, epigenomic and transcriptomic profiling.**
- Task 1: To investigate genomic alteration in BETi resistant cells (75% completed)
- Task 2: To investigate changes in the chromatin landscape (50% completed)
- Task 3: To investigate enhancer deregulation and its effect on AR chromatin recruitment in BETi resistant cells (80% completed)
- **Specific Aim 2: To elucidate the mechanism of AR signaling reactivation and the role of PRC2 in acquired resistant to BETi and to screen for novel epigenetic regulators of a BETi resistant state.**
- Task 1: To investigate the role of PARP in the activation of AR signaling pathway in prostate cancer cells with acquired resistance to BETi (85% completed).
- Task 2: To elucidate the role of PRC2 in acquired resistance to BETi in prostate cancer cells (70% completed).
- Task 3: To identify novel epigenetic and transcriptional mediators that alleviate BET inhibitor resistance. (50% completed).

- **Specific Aim 3: To evaluate BETi drug combinations focusing on Enzalutamide/PARPi/EHZ2i combinations in vivo.**
- Task 1: Mouse xenograft therapeutic studies with BETi sensitive and acquired resistant prostate cancer models.
- Task 2: To utilize physiologically relevant prostate cancer models to test enzalutamide/PARPi/EZH2i with BET inhibitor therapy.

What was accomplished under these goals?

1) major activities: Significant majority of the goals were achieved that resulted in the high impact publication titled "Resistance to BET Inhibitor Leads to Alternative Therapeutic Vulnerabilities in Castration-Resistant Prostate Cancer" in the journal Cell Reports (Feb. 2018; PMID: 29490263). For complete description of the data and conclusion, please refer to the published manuscript attached.

2) Specific objectives: The major objective of the proposal was to understand the molecular mechanisms of BET inhibitor resistance in prostate cancer cells.

3) Significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or: Please see the enclosed Pawar et. al. 2018 Cell Reports manuscript for the detailed description of the major findings.

4) Other achievements. Include a discussion of stated goals not met.

- Emanating from this proposal, we recently published a new study titled "CDK7 inhibition suppresses Castration-Resistant Prostate Cancer through MED1 inactivation" in the journal Cancer Discovery (August 2019; PMID: 31466944).

- Provisional Patent Filed 4.24.2019. Serial # serial number 62/838,271 on "TREATMENT OF CANCER WITH CDK INHIBITORS" where I am the Senior-inventor.

- Received R01 grant on a project that originally emanated from this DOD Idea Development award.

- Received funding from Concern Foundation of America to work on a project that originally emanated from this DoD Idea Development award.

- Have applied for two DoD application for the year 2020, one of which is the invited PCRP Idea Expansion Award.

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

- I participated and presented a poster in the AACR annual meeting 2018 held in Chicago.
- I participated and presented a poster in the AACR annual meeting 2019 held in Chicago.
- I presented our work on COVID-19 as an invited speaker in the American Association for Cancer Research (AACR) Virtual Meeting: COVID-19 and Cancer, July 20-22, 2020.
- Aishwarya Pawara – a research technician who worked on the project presented a poster on the work at AACR annual meeting 2018 held in Chicago.

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?

"Nothing to Report."

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

- We hope that the published results as a manuscript in the journal “Cell Reports” will help in the rationale design of a combination therapy for refractory castration resistant prostate cancer patients. Further, the results described will help us appreciate the diverse cellular pathways a cancer cell takes to overcome the BET inhibitor action.
- Our manuscript in Cell Reports has already garnered a total citation of 38 in the short span of 30 months since publication suggesting its high impact on scientific reporting.

What was the impact on other disciplines?

- Our published results will help explain the BET inhibitors resistance in diverse cancer type including BRD4 dependent cancers such as cancer of breast, thyroid, sarcoma etc.
-

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:
"Nothing to Report."

Changes in approach and reasons for change:
"Nothing to Report."

Actual or anticipated problems or delays and actions or plans to resolve them: The progress for the 2019-2020 particularly the proposed in vivo mouse experiments was severely impacted by research activity lockdown due to the COVID-19 pandemic.

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:

Journal publications.

- Journal: Cell Reports (published in February 2018)
- Title: Resistance to BET Inhibitor Leads to Alternative Therapeutic Vulnerabilities in Castration-Resistant Prostate Cancer
- Authors: Pawar A, Gollavilli PN, Wang S, Asangani IA.
- https://www.sciencedirect.com/science/article/pii/S2211124718301797?via%3Dihub
- Acknowledgement of federal support: YES

Books or other non-periodical, one-time publications.

- Journal: **Adv Exp Med Biol** (published in December 2019)
- Title: Epigenetic regulation of chromatin in prostate cancer
- Authors: Natesan R., Aras S., Sander Effron S., Asangani I.A.
- https://link.springer.com/chapter/10.1007%2F978-3-030-32656-2_17
- Acknowledgement of federal support: YES

Other publication, conference papers, and presentation.

- **Journal publications**: Cancer Discovery (published in August 2019)
- Title: CDK7 inhibition suppresses Castration-Resistant Prostate Cancer through MED1 inactivation
- Authors: Rasool RU, Natesan R, Deng Q, Aras S, Lal P, Sander Effron S, Mitchell-Velasquez E, Posimo JM, Carskadon S, Baca SC, Pomerantz MM, Siddiqui J, Schwartz LE, Lee DJ, Palanisamy N, Narla G, Den RB, Freedman ML, Brady DC, Asangani IA.
- https://cancerdiscovery.aacrjournals.org/content/early/2019/08/27/2159-8290.CD-19-0189.long
- Acknowledgement of federal support: YES

- **Journal publications**: Cancer Discovery (published in June 2020).
- Title: Global regulation of the histone mark H3K36me2 underlies epithelial plasticity and metastatic progression.
- Authors: Yuan S., Natesan R., Sanchez-Rivers F.J., Li J., Bhanu N.V., Yamazoe T., Lin J.H., Merrell A.J., Sela Y., Thomas S.K., Jiang Y., Plesset J.B., Miller E.M., Shi J., Garcia B.A., Lowe S.W., Asangani I.A., Stanger B.Z.
- https://cancerdiscovery.aacrjournals.org/content/10/6/854.long
- Acknowledgement of federal support: YES

- **Journal publications**: JBC (published in December 2020).
- Title: Using Biochemistry and Biophysics to Extinguish Androgen Receptor Signalling in Prostate Cancer.
- Authors: Asangani IA, Blair I, Van Duyne G, Hilser VJ, Moiseenkova-Bell V, Plymate S, Sprenger C, Wand AJ, Penning TM.
- https://www.jbc.org/content/early/2020/12/31/jbc.REV120.012411.long
- Acknowledgement of federal support: YES

- **Conference papers**: AACR Annual Meeting 2018.
- Abstract 1394: Resistance to BET inhibitor leads to new therapeutic vulnerabilities in castration resistant prostate cancer
- Authors: Pawar A, Asangani IA.
- Acknowledgement of federal support: YES

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
BET inhibitor resistant LNCaP and 22RV1 prostate cancer cells have been developed. We are open to share those lines to any interested researcher upon request.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS:

What individuals have worked on the project?

Name:	Irfan Asangani
Project Role:	Principal Investigator
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	3
Contribution to Project:	Dr. Asangani supervised the study
Funding Support:	Zenith Epigenetics, NIH, DoD, Institutional Funding,

Name:	Ramakrishnan Natesan
Project Role:	Research Associate
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	2
Contribution to Project:	Mr. Natesan performed work related to development and characterization of BETi resistant prostate cancer cells.

Funding Support:	DoD, Institutional Funding, *Zenith Epigenetics*

Name:	Reyaz Ur Rasool
Project Role:	Post Doctoral Researcher
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	2
Contribution to Project:	Dr. Rasool has performed work related to development and characterization of BETi resistant prostate cancer cells.
Funding Support:	DoD, Institutional Funding, *Zenith Epigenetics*

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

o COLLABORATIVE AWARDS

- **QUAD CHARTS**

9. APPENDICES

ADDITIONAL NOTES

MARKING OF PROPRIETARY INFORMATION

REPORTS NOT PROPERLY MARKED FOR LIMITATION WILL BE DISTRIBUTED AS APPROVED FOR PUBLIC RELEASE