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TITLE: Combinational Targeting EZH2 and PARP1 in Prostate Cancer

PRINCIPAL INVESTIGATOR: Qi Cao

CONTRACTING ORGANIZATION: Methodist Hospital
Houston, TX 77030

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6. AUTHOR(S)
Qi Cao

E-Mail: qi.cao@northwestern.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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

METHODIST HOSPITAL RESEARCH INSTITUTE
6565 Fannin St.
Houston, TX 77030

8. PERFORMING ORGANIZATION REPORT NUMBER

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

U.S. Army Medical Research and Materiel Command
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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

Most advanced prostate cancer cells have higher levels of EZH2 and PARP1 proteins compared to that in early stage prostate cancer cells, suggesting the importance of these proteins in prostate cancer progression. We found that PARP1 directly interacts with EZH2. In the proposed project, we will identify precisely how EZH2 and PARP1 interact and how these two proteins regulate each other in prostate cancer. Next, we will study how EZH2 and PARP1 work together to decrease the expression of tumor suppressors (genes/proteins that inhibit tumor growth) and increase genetic instability in advanced prostate cancer. Understanding these mechanisms will lead to the future design of new inhibitors of EZH2 and PARP1. Furthermore, our preliminary data strongly suggest that PARP inhibition-resistant tumors have higher levels of EZH2 compared to PARP inhibition-sensitive tumors and that inhibiting EZH2 alone enhances the enzymatic activities of PARPs; thus, overcoming the therapeutic effectiveness of PARP inhibition. Therefore, our work provides a novel rationale to target both PARPs and EZH2, and we predict that the inhibition of both PARPs and EZH2 will kill more cancer cells than inhibiting either PARPs or EZH2 alone. We anticipate that this combination therapy will overcome therapeutic resistance and will substantially benefit the majority of prostate cancer patients, regardless of any DNA repair defects.

15. SUBJECT TERMS

NONE LISTED

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
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TABLE OF CONTENTS

1. INTRODUCTION:

Most advanced prostate cancer cells have higher levels of EZH2 and PARP1 proteins compared to that in early stage prostate cancer cells, suggesting the importance of these proteins in prostate cancer progression. We found that PARP1 directly interacts with EZH2. In the proposed project, we will identify precisely how EZH2 and PARP1 interact and how these two proteins regulate each other in prostate cancer. Next, we will study how EZH2 and PARP1 work together to decrease the expression of tumor suppressors (genes/proteins that inhibit tumor growth) and increase genetic instability in advanced prostate cancer. Understanding these mechanisms will lead to the future design of new inhibitors of EZH2 and PARP1. Furthermore, our preliminary data strongly suggest that PARP inhibition-resistant tumors have higher levels of EZH2 compared to PARP inhibition-sensitive tumors and that inhibiting EZH2 alone enhances the enzymatic activities of PARPs; thus, overcoming the therapeutic effectiveness of PARP inhibition. Therefore, our work provides a novel rationale to target both PARPs and EZH2, and we predict that the inhibition of both PARPs and EZH2 will kill more cancer cells than inhibiting either PARPs or EZH2 alone. Although pharmacological inhibitors of EZH2 and PARP have been clinically proven to be safe, the combination of these drugs has never been tested and does pose some risks. To decrease the risk to patients, we will preclinically test, in this proposal, the safety and efficacy of this combination therapy. We anticipate that this combination therapy will overcome therapeutic resistance and will substantially benefit the majority of prostate cancer patients, regardless of any DNA repair defects.

2. KEYWORDS:

Polycomb, EZH2, PARP1, protein methylation, PRC2, DNA damage repaire, castration-resistant prostate cancer

3. ACCOMPLISHMENTS:

The 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.

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.

Major Task 1: To characterize the methylation of PARP1 mediated by EZH2 in PCa 30%
Major Task 2: To characterize the unique and common downstream targets of PRC2 and PARP1 in PCa 20%
Major Task 3: To evaluate the combination effect of EZH2 inhibitor and PARP inhibitor, and the new EZH2 inhibitor in cell lines and CRPC xenograft models 50%
Major Task 4: To evaluate the synergistic efficacy of EZH2 and PARP inhibition in PCa bone metastasis xenograft models 10%

What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

1. **Construct PARP1 truncated mutants**

PAPR1 has 3 functional domains, N-terminal DNA-Binding domain (DBD), central Auto-modification domain (AD) and C-terminal Catalytic domain (CD), and we generated 3 his-tagged PARP1 truncated mutants containing these 3 domains respectively, as well as full-length PARP1 (FL) (**Fig. 1A**). As shown in **Fig. 1B,** when we overexpressed these 3 his-tagged PARP1 mutants or full-length PARP1 in HEK-293T cells, we did observe correctly expressed PARP1 full-length and truncated mutants.

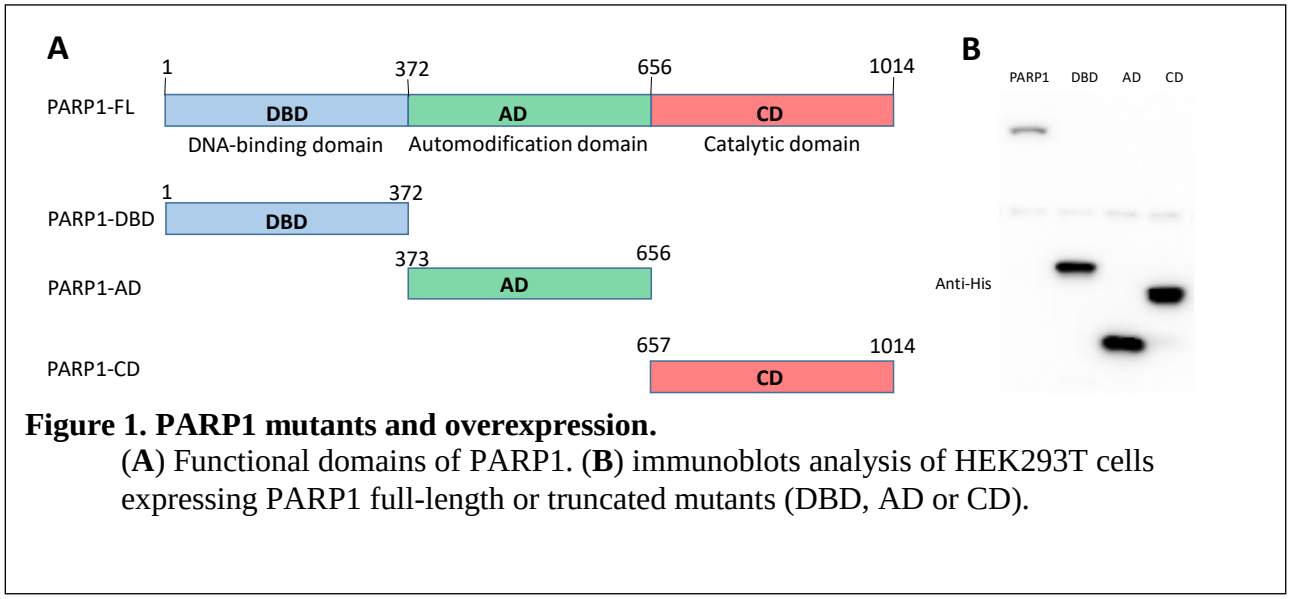

Figure 1. PARP1 mutants and overexpression.

(**A**) Functional domains of PARP1. (**B**) immunoblots analysis of HEK293T cells expressing PARP1 full-length or truncated mutants (DBD, AD or CD).

2. Construct EZH2 truncated mutants

EZH2 has 2 SANT protein-protein interaction domains, a cysteine-rich domain and c-terminal SET (enzymatic domain) functional domains. We generated the myc-tagged EZH2 truncated mutants deleted these domains respectively (ΔSANT1, ΔSANT1-2, ΔSANT2, ΔCys-rich and ΔSET) , as well as full-length EZH2 (FL) (**Fig. 2A**). As shown in **Fig. 2B,** when we overexpressed these myc-tagged EZH2 mutants or full-length in HEK-293T cells, we did observe correctly expressed EZH2 full-length and domain-deleted mutants.

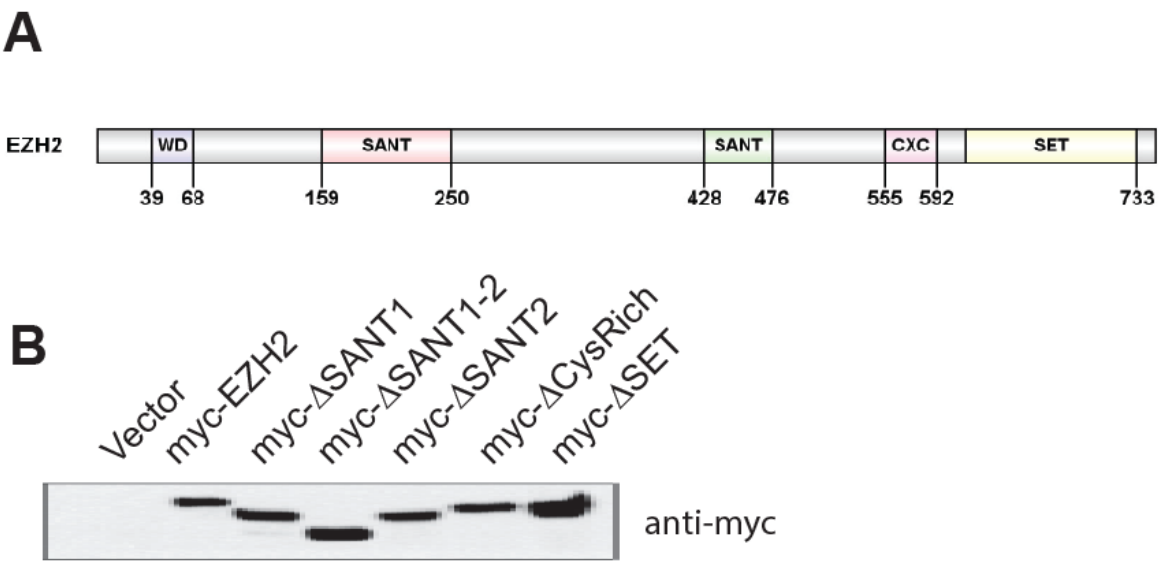

Figure 2. EZH2 mutants and overexpression.
(**A**) Functional domains of EZH2. (**B**) immunoblots analysis of HEK293T cells expressing EZH2 full-length or truncated mutants.

3. EZH2 is critical for the DNA damage response. We induced DNA damage in DU145 cells by etoposide treatment, and then performed comet assays. Inhibiting EZH2 by EPZ5687 or DZNep attenuated cancer cell response to etoposide-induced comet assays, with decreased tail moments and decreased amounts of DNA in the tails (**Fig. 3A, B**).

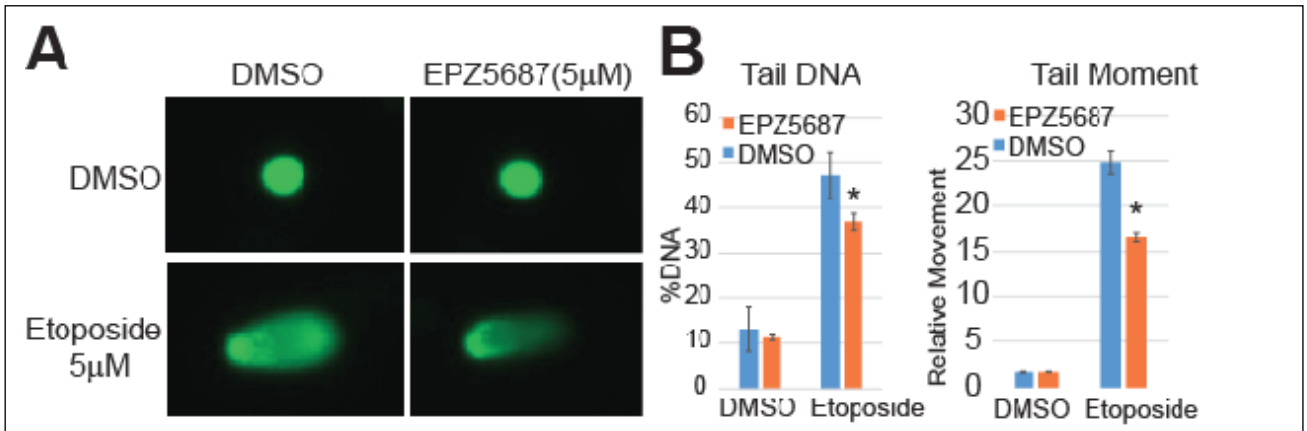

Figure 3. Inhibiting EZH2 decreases DNA damage response.
DU145 cells were treated with EPZ5687, etoposide, or combination at the indicated doses for 24 hours and analyzed by neutral comet assays. Representative images from 3 independent experiments indicated significantly less tail moment in EPZ5687 treated cells (**A**). (**B**) The graphs show the mean percent of DNA in tails and mean tail moments for 100 cells for each condition. *p<0.001.DU145

4. **To evaluate the synergistic efficacy of EZH2 and PARP inhibition in TNBC cell line murine xenograft and PDX models.**
We test the new EZH2 inhibitor EZi in C4-2 xenograft and observed that targeting EZH2 and PARP in combination significantly reduced tumor growth compared to vehicle control or single inhibitors (Fig. 4A and 4B).

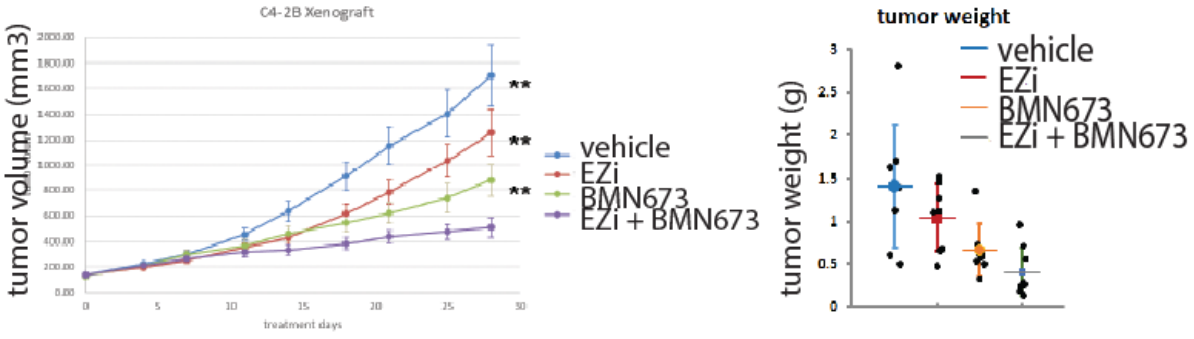

Figure 4. Combinational targeting EZH2 decreases PCa tumor growth.
DU145 cells were treated with EPZ5687, etoposide, or combination at the indicated doses for 24 hours and analyzed by neutral comet assays. Representative images from 3 independent experiments indicated significantly less tail moment in EPZ5687 treated cells (**A**). (**B**) The graphs show the mean percent of DNA in tails and mean tail moments for 100 cells for each condition. *p<0.001.

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Award/grant

2017	Houston Methodist Research Institute Career Cornerstone Award
2017	Houston Methodist Research Institute NIH Competitiveness Award
2018	Houston Methodist Research Institute Award for Excellence in Peer-Reviewed Publication

Promotion and new position

5/1/2018-6/30/2018	Associate Professor, Houston Methodist Research Institute, Houston, TX
7/1/2018-	Associate Professor, Department of Urology and Robert H. Lurie Comprehensive Cancer Center, Northwestern University Feinberg School of Medicine, Chicago, IL

National/International conferences attended

Oct 5-8, 2017	Prostate Cancer Foundation Scientific Retreat, Washington DC
April 14-18, 2018	AACR Annual Meeting, Chicago, IL

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Nothing to Report

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

We are continuously working on this project and pursue the aims. Because we just moved to a new institute, we will obtain the local IACUC and ACURO approval first and then perform the proposed animal work.

4. **IMPACT:** D*escribe 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?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

Nothing to Report

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to Report

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to Report

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

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:*

We just moved to a new institute (Department of Urology and Robert H. Lurie Comprehensive Cancer Center, Northwestern University Feinberg University. We are working on the grant transfer and getting the local IACUC and ACURO approval.

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

Nothing to Report

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

We just moved to a new institute (Department of Urology and Robert H. Lurie Comprehensive Cancer Center, Northwestern University Feinberg University. We are working on the grant transfer, hiring new post-doc and getting the local IACUC and ACURO approval.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Nothing to report

Significant changes in use or care of vertebrate animals

We just moved to a new institute (Department of Urology and Robert H. Lurie Comprehensive Cancer Center, Northwestern University Feinberg University. We are working on the grant transfer, hiring new post-doc and getting the local IACUC and ACURO approval.

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**

Report only the major publication(s) resulting from the work under this award.

Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

1. Zhu S, Zhao D, Yan L, Jiang W, Kim JS, Gu B, Liu Q, Wang R, Xia B, Zhao JC, Song G, Mi W, Wang RF, Shi X, Lam HM, Dong X, Yu J, Chen K, Cao Q. BMI1 regulates androgen receptor in prostate cancer independently of the polycomb repressive complex 1.Nat Commun. 2018 Feb 5;9(1):500. doi: 10.1038/s41467-018-02863-3. PubMed PMID: 29402932; PubMed Central PMCID: PMC5799368.
   acknowledgement of federal support (yes)
2. Liu Q, Li Q, Zhu S, Yi Y, Cao Q. B lymphoma Moloney murine leukemia virus insertion region 1: An oncogenic mediator in prostate cancer. Asian J Androl. 2018 Jun 1. doi: 10.4103/aja.aja_38_18. [Epub ahead of print] Review. PubMed PMID: 29862993.
   acknowledgement of federal support (yes)

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

no

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

no

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

N/A

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

N/A

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

N/A

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance,

or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

- *data or databases;*
- *physical collections;*
- *audio or video products;*
- *software;*
- *models;*
- *educational aids or curricula;*
- *instruments or equipment;*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other.*

N/A

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".

Example:

Name:	*Mary Smith*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	*1234567*
Nearest person month worked:	*5*
Contribution to Project:	*Ms. Smith has performed work in the area of combined error-control and constrained coding.*
Funding Support:	*The Ford Foundation (Complete only if the funding support is provided from other than this award.)*

Name:	*Qi Cao*
Project Role:	*PI*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	2.5
Contribution to Project:	*Conceive the idea, lead the project, design experiments and analyze the data*
Funding Support:	*DoD PCRP IDA, Prostate Cancer Foundation, American Cancer Society, Start-up*

Name:	*Qingshu Meng*
Project Role:	*Post-Doctoral*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	10
Contribution to Project:	*Perform major experiments and analyze the data*
Funding Support:	*DoD PCRP IDA*

Name:	*Kaifu Chen*
Project Role:	*Co-investigator*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	1
Contribution to Project:	*analyze the data*
Funding Support:	*DoD PCRP IDA, NIH, Start-up*

Name:	*Haifa Shen*
Project Role:	*Co-investigator*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	0.5

Contribution to Project:	*Help animal work*
Funding Support:	*DoD PCRP IDA, NIH, Start-up*

Name:	*Dongyu Zhao*
Project Role:	*Post-doc*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	2.5
Contribution to Project:	*analyze the data*
Funding Support:	*DoD PCRP IDA, NIH, Start-up*

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Nothing to Report

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
<u>Organization Name:</u>
<u>Location of Organization: (if foreign location list country)</u>
<u>Partner's contribution to the project</u> (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *N/A*

QUAD CHARTS: *N/A*

9. APPENDICES: *N/A.*