**AWARD NUMBER:**

CDMRPL-16-0-DM160510

**TITLE:**

Defining Mutations of DNA Repair Genes in Prostate Cancer Patients Towards Enhancing Treatment

**PRINCIPAL INVESTIGATOR:**

Dr. Shiv Srivastava, PhD

**CONTRACTING ORGANIZATION:**

Uniformed Services University of the Health Sciences (USUHS)
Bethesda, MD 20814

**REPORT DATE: May 2018**

**TYPE OF REPORT: Annual**

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

# REPORT DOCUMENTATION PAGE

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| May 2018 | Annual | 1 May 2017 - 30 Apr 2018 |

**4. TITLE AND SUBTITLE**

Defining Mutations of DNA Repair Genes in Prostate Cancer Patients Towards Enhancing Treatment

**5a. CONTRACT NUMBER**

**5b. GRANT NUMBER**
CDMRPL-16-0-DM160510

**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)**

Henry M. Jackson Foundation
CPDR, USU
1530 E. Jefferson Street
Rockville, MD 20852

**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**

DNA damage repair genes (DDRGs) are critical for protecting genome integrity and have been implicated in several cancer types. Recent genomic studies of metastatic castration resistant prostate cancer (mCRPC) highlight the contributions from mutations or copy number changes in DDRG alterations, including *BRCA1, BRCA1, ATM, CHEK2,* and *MSH2*. It has also been shown that prostate cancer (CaP) patients harboring inherited mutations in DDRGs may benefit from early targeted PARP inhibitor therapy. However, the association of germline mutations of DDRGs with earlier stage high risk CaP patients remains to be defined. Accumulating evidence suggest for increased association of *BRCA2* mutations with more aggressive CaP. Our recent data show an association of increased frequency of *BRCA2* gene mutations in CaP patients with African ancestry with elevated risk of developing metastasis. We hypothesize that AA CaP patients have an increased frequency of mutated DDRGs. We aim to assess blood derived germline DNAs of AA (N=300) and CA (N=300) CaP patients archived in USU-CPDR, Center of Excellence, for the association frequency all known DDRGs genomic alterations with disease aggressiveness (based on pathologic grade, pathologic stage, time to recurrence/ metastasis, family history and African ancestry). The results will be assessed to refine patient stratification for specific targeted therapy. Longer term implication of this project will impact early targeted therapy to reduce of racial disparity in CaP, given a higher anticipated rate of DDRG mutations in AA CaP patients. Within the DOD context, outcome of this research strategy will be valuable for developing approaches to reduce mutagenic exposures of affected service members, and have a broader impact on other inherited cancers.

**15. SUBJECT TERMS**

| 16. SECURITY CLASSIFICATION OF: | | | 17. LIMITATION OF ABSTRACT | 18. NUMBER OF PAGES | 19a. NAME OF RESPONSIBLE PERSON |
|---|---|---|---|---|---|
| a. REPORT | b. ABSTRACT | c. THIS PAGE | | | 19b. TELEPHONE NUMBER *(include area code)* |
| Unclassified | Unclassified | Unclassified | Unclassified | 12 | |

## TABLE OF CONTENTS

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

   This study, the first of its kind, will provide ground breaking data on inherited defects of genome associated with aggressive prostate cancer (CaP) with focus on comparative status of DNA damage repair genes (DDRGs) in African American (AA) and Caucasian American (CA) CaP patients treated under equal access MHS.

   DDRGs play a critical role in protecting genome integrity and have been implicated in several cancer types. Recent studies demonstrate that PARP inhibitors (inhibiting single strand DNA break repair), such as Olaparib, can slow progression-free survival and extend overall survival in patients with BRCA1/2 mutations.

   The goal of this proposal is to identify and evaluate all DDRG germline mutations, along with other germline cancer driver mutations, in AA CaP patients, which may provide critical information for treatment stratification and targeted therapy of this disparately affected patient population.

   A) Perform an in depth evaluation of germline mutations in all DDRGs (over 100 genes), and other cancer driver genes, in a large DOD cohort of AA (N=300) and CA (N=300) CaP patients
   B) Assess DDRG mutation data, with emphasis on understudied AA CaP, for association with clinical and pathological data, including disease progression, and evaluate how this information can refine patient stratification for specific targeted therapeutic options

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

   Prostate cancer, DNA damage repair genes (DDRG), germline mutations, African American, racial disparity, therapeutic stratification, PARP inhibitors, disease progression, military healthcare system

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: Perform an in depth evaluation of germline mutations in all DRGs, and other cancer driver genes, in a large DOD cohort of AA and CA CaP patients

   Subtasks: Evaluation of germline mutations in all DDRGs from multiple repair pathways, and other cancer driver genes, will be performed by whole genome/exome sequence analysis of archived blood DNA samples from AA (N=300), and control CA (N=300), CaP patients who underwent primary treatment at Walter Reed National Military Medical Center (WRNMMC) over the past 20 years (total N=600).

   Cases that are positive for germline mutations in any DDRGs will be further evaluated for somatic aberrations in the same genes using genomic DNA from matched prostate tumor specimens that are also archived at CPDR.

Major Task 2: Assess DRG mutation data, with emphasis on understudied AA CaP, for association with clinical and pathological data including disease progression, and evaluate how this information can refine patient stratification for specific targeted therapeutic options

Subtasks: All DDRG mutations will be evaluated for association with clinical and pathological data in both AA and CA CaP patients. Correlation with pathological grade and stage, as well as progression to recurrence and metastasis will be assessed. As an embedded additional focus, we will also analyze the DDRG mutation data to develop a metastasis predictive signature.

**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.*

The first task under the SOW was the IRB protocol submission and approval process. In the first quarter we wrote and submitted the IRB protocol. It went through two sets of stipulations and the protocol was approved by WRNMMC IRB on November 7, 2017. As the approval letter states: “This is an IRB approval only. You may not begin your research until you have received a Command Start Letter”. At the time of this approval, a new functionality within the electronic IRB system was made live – “multi-site”. Guidance on how to use this new functionality did not become available until January 2018. Based on this guidance, an amendment was submitted to convert the protocol into a “multi-site” so that USUHS could view the WRNMMC approved documents and provide their HRPO level review. During the same time the CRADA, which is a condition of receiving WRNMMC's Command Start Letter, was also under negotiation.
Most importantly, based on the protocol approvals at WRNMMC and USUHS, and the CRADA approval, we received the Command Start Letter on March 5, 2018.
The Postdoctoral Fellow was recruited in April and the work immediately started following the steps described in the SOW. Brief summary of the main steps:

- IRB protocol approval (March, 2018)
- Postdoctoral Fellow recruited (April, 2018)
- Selection of the AA (N=300) and CA (N=300) patient cohorts (Epidemiologist)
- Identification of the archived blood genomic DNA specimens (N=600)
- Processing of the specimens by 200 cases at a time (100 AA and 100 CA)
- QC of the specimens by Qubit (quantity) and Bioanalyzer (quality) assays
- Over 400 of the 600 specimens have been QC-d by Qubit to date
- Diluting and aliquoting of the DNA samples for sequence analysis is also in progress. Plates of the final QC-d DNA samples with the required concentration and amount will be submitted for WGS to TAGC.

**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.*

Although the goal of this project is not to provide training, the Postdoctoral Fellow is being provided with both training and other professional development opportunities.

Training: one-on-one laboratory work with the mentor and senior laboratory personnel

Professional development: local seminars and individual study

**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*

**What do you plan to do during the next reporting period to accomplish the goals?**
*If this is the final report, state "Nothing to Report."*
*Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.*

During the next reporting period we will continue to closely follow our SOW tasks and timeline.

Main goals for the next quarter:

- Complete the selection of the AA (N=300) and CA (N=300) patient cohorts and the identification of blood genomic DNA specimens archived in our specimen inventory
- Process, QC and aliquot the specimens by 200 at a time (100 AA and 100 CA)
- Submit the aliquots for WGS analysis
- Start the WGS analysis process

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?** *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 at this early point of the process*

**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:

*Nothing to Report*

**Changes in approach and reasons for change**
*Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.*

*Nothing to Report*

**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.*

*Nothing to Report*

**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**
**Significant changes in use or care of vertebrate animals**
**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).*

  **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).*

*Nothing to Report*

**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.*

*Nothing to Report*

- **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.*

*Nothing to Report*

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

*Nothing to Report*

- **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.*

*Nothing to Report*

- **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.*

*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: Dr. Shiv Srivastava
Project Role: Principal Investigator
Contribution to Project: *Dr. Srivastava has overseen the administrative aspect of the project initially focusing on IRB protocol and approval*.

Name: Dr. Gyorgy Petrovics
Project Role: Co-Investigator
Contribution to Project: *Dr. Petrovics has been working on the coordination of the IRB protocol writing, review and approval process*.

Name: Dr. Jennifer Cullen
Project Role: Co-Investigator
Contribution to Project: *Dr. Cullen developed the patient/specimen cohort selection for this research project.*

Name: Dr. Kevin Babcock
Project Role: Postdoctoral Fellow
Contribution to Project: *Dr. Babkock has been working on the QC and aliquoting of the blood DNA specimens.*

Name: Ms. Lakshmi Ravindranath
Project Role: Senior Research Assistant
Contribution to Project: *Ms. Ravindranath has been working on the QC and aliquoting of the blood DNA specimens*.

**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:*

*Organization Name:* The American Genome Center (TAGC) at USU
*Location of Organization:* Bethesda, MD
*Partner's contribution to the project (identify one or more)*

- **Collaboration** *(e.g., partner's staff work with project staff on the project);*

All sequencing (WGS) and sequence data analysis is performed at TAGC. DDRG mutation data is transferred to CPDR for further analysis and validation.

## 8. SPECIAL REPORTING REQUIREMENTS

**COLLABORATIVE AWARDS:** For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to **https://ers.amedd.army.mil** for each unique award.

**QUAD CHARTS:** If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.

**9. APPENDICES:** Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.

**Defining mutations of DNA repair genes in prostate cancer patients towards enhancing treatment**
**LOG#** DM160510

**PI: Shiv Srivastava, Ph.D.** **Org:** Uniformed Services University **Award Amount:** $741,632

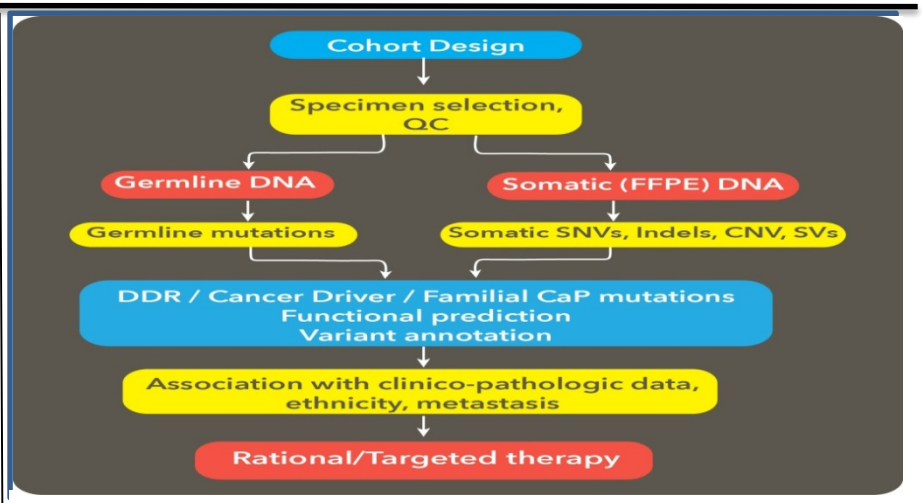
## Study/Product Aim(s)

**Define and evaluate mutation spectrum of all known DNA damage repair genes (DDRGs) in archived specimens from African American (AA) and Caucasian American (CA) prostate cancer (CaP) patients for association with disease aggressiveness, and assess how this information can refine patient stratification for specific targeted therapeutic options.**

## Approach

Number of total subjects: N=600

- Task 1) Perform an in depth evaluation of germline mutations in all DDRGs in a large DOD cohort of AA and CA CaP patients
- Task 2) Assess DDRG mutation data, with emphasis on understudied AA CaP, for association with clinical and pathological data including disease progression, and evaluate how this information can refine patient stratification for specific targeted therapy




Accomplishment: IRB protocol has been approved (March 2018), cohorts and specimens have been selected, specimen QC ongoing.

## Timeline and Cost

| Activities CY | 17 | 18 | 19 | Total |
|---|---|---|---|---|
| Task 1 | | | | |
| Task 2 | | | | |
| | | | | |
| | | | | |
| **Estimated Budget ($K)** | **$242K** | **$247K** | **$252K** | **$741K** |

**Updated:** (5/30/2018)

**Goals/Milestones (Example)**

**CY17 Goals**

❑ Complete DNA QC on all 600 cases (ongoing, 400 of 600 completed)

**CY18 Goals**

☐ Complete DNA sequencing on 50% of cases
☐ Complete data analyses on 50% of cases
☐ Complete DNA sequencing on all remaining cases

**CY19 Goals**

☐ Complete data analyses and validations on all remaining cases
☐ Publish conclusions

**Comments/Challenges/Issues/Concerns**

- Due to lengthy process of IRB protocol approval (March 2018), we are completing 2017 and 2018 goals together in 2018

**Budget Expenditure to Date**

Projected Expenditure: $741K
Actual Expenditure: $43K