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TITLE: Long-Read DNA-Sequencing and Targeted RNA-Seq to Identify Previously Undetectable Classes of Mutations in Families with Lethal Prostate Cancer

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CONTRACTING ORGANIZATION: University of Washington

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Long-Read DNA-Sequencing and Targeted RNA-Seq to Identify Previously Undetectable Classes of Mutations in Families with Lethal Prostate Cancer

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Prostate cancer has a significant heritable component, with 10-15% of patients with advanced disease harboring pathogenic germline mutations in DNA repair genes. It is critical for these patients to know their genotypes, because mutations in *BRCA2* and *PALB2*, and to a more modest degree *BRCA1* and *ATM*, predict favorable response to poly-ADP ribose polymerase inhibitors (PARPi) and platinum chemotherapy. In May 2020, the FDA approved two PARPi drugs for men with metastatic-castration resistant prostate cancer and mutations in these genes. However, many patients with metastatic prostate cancer and severe family histories remain without a detected germline pathogenic mutation. These patients cannot benefit from molecularly-directed therapies, because these drugs are useful only to patients with damaging mutations in DNA repair genes, and hence are targeted only to them. Our proposal aims to identify previously undetectable classes of pathogenic mutations in 450 patients for whom no pathogenic mutation has been found by gene-panel sequencing. We will use two newly developed genomic techniques: 1) CRISPR excision and long-read sequencing to identify complex structural mutations and 2) targeted RNA-Seq to evaluate patient RNA for cryptic regulatory and splice variants.

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**			USAMRDC
U	U	U	UU	16	**19b. TELEPHONE NUMBER** *(include area code)*

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

Our research aims to identify, in 450 patients with metastatic prostate cancer and their families, classes of mutations in BRCA1, BRCA2, and other DNA-repair genes that have been previously undetectable, even by the best current sequencing approaches. We directly address the Overarching Challenge of reducing lethal prostate cancer for men who are genetically predisposed to the disease and would potentially benefit from targeted therapy, but precluded from these therapies because no causal mutation has been identified for them. We will use two newly developed genomic techniques: 1) CRISPR excision and long-read sequencing to identify complex structural mutations and 2) targeted RNASeq to evaluate patient RNA for cryptic regulatory and splice variants.

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

Metastatic prostate cancer, genetic testing, sequencing, DNA repair gene, mutation

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.

Specific Aim 1.
Major Task 1: Obtain regulatory approval. Milestone Achieved. 100% complete.
Major Task 2: Enroll study participants and their informative relatives.
Subtask 1 (Months 4-30): 450 patients have been recruited and returned their consent documents and blood kits. Milestone achieved. 100% complete.
Subtask 2 (Months 4-30): Lymphoblast cell lines have successfully been established 450 participants. Milestone achieved. 100% complete.

Specific Aim 2.
Major Task 1: Prepare, sequence SMRT-CATCH libraries.
Subtask 1, 2, 3 (Months 5-32): We have performed SMRT-CATCH on 240 participants so far. 53% complete.
Major Task 2: Identify structural variants Subtask 1, 2, 3 (Months 8-36): We have analyzed the SMRT-CATCH on 240 participants so far. 53% complete.

Specific Aim 3.
Major Task 1: Perform targeted RNA-Seq.
Subtask 1, 2, 3 (Months 5-36): We have performed RNA-Seq on 96 participants so far. 21% complete.

What was accomplished under these goals?

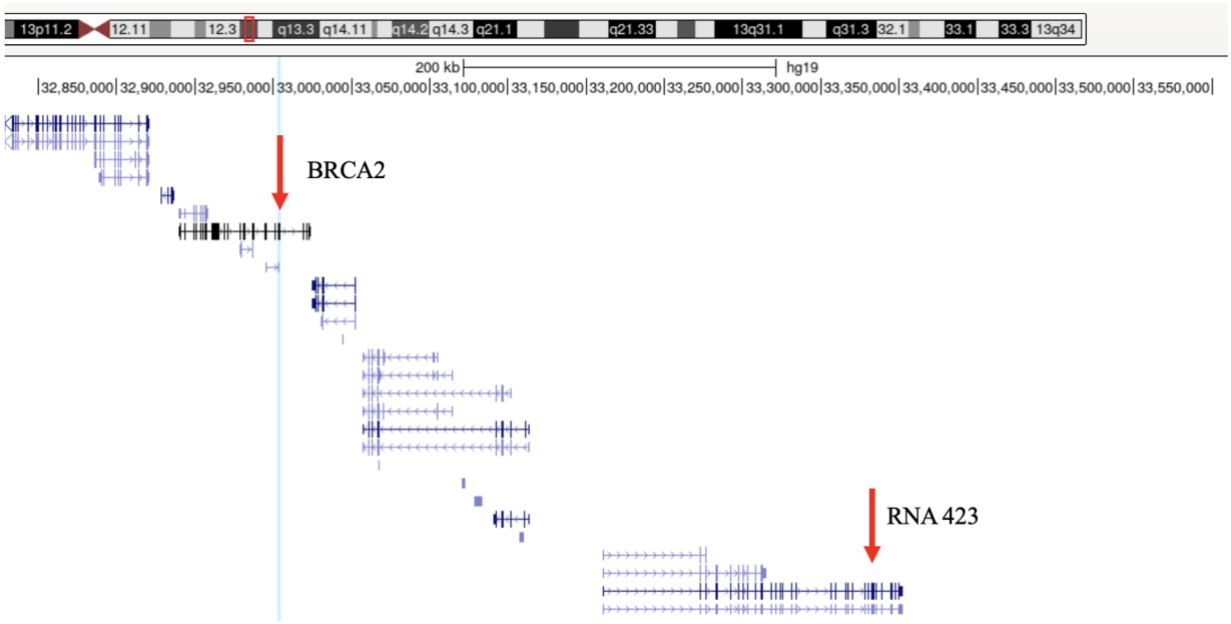
For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include 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. Major activities: We have completed patient recruitment and established lymphoblast cell lines on all participants.

2. Specific Objectives: The major tasks in Specific Aims 1 and 2 are progressing well with 53% and 21% completion, respectively.

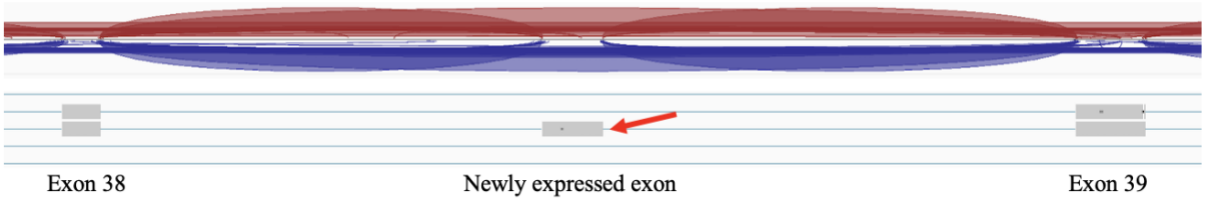
3. Significant results in this reporting period include:

a) Using long read genomic DNA sequencing we identified a novel BRCA2 structural variant. The event is a genomic inversion that flips the orientation of 532kb of normal DNA sequence in this patient. The breakpoints are in intron 9 of BRCA2 intron 4 of a non-coding protein RNA 423.
As far as we are aware this structural variant has not been described in the literature or deposited in any publicly available databases of clinical variants. We hypothesize that this variant disrupts normal function of BRCA2 and will conduct experiments to determine its consequence. Below is a UCSC Genome Browser view showing the location of the inversion (chr13:32,905,362-33,437,583).



Upper panel. Ideogram of Chromosome 13, scale bar and genomic coordinates. Lower panel shows genes located in this region of Chromosome 13 and red arrows indicate the locations of the inversion breakpoints in BRCA2 and RNA 423.

b) From our RNA data we have discovered two different deep intronic variants in ATM that lead to new exons being transcribed in the mRNA. Both new exons disrupt normal ATM gene function based on our RNA data. Representative RNA reads showing one of the new exon events, located at chr11:108,179,696-108,179,832: are shown below.



Upper panel. Sashimi plot of splicing events in ATM in the research participant, as defined by our RNA sequencing data. Lower panel. Grey bars indicate normally expressed exons and the newly expressed exon caused by an inherited point mutation (at the location of the red arrow).

We continue to evaluate newly developed bioinformatic tools to discover structural variants and deep intronic variants from our data. At this stage of the project, we feel it is premature to describe potentially negative data.

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.

Nothing to report

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.

For the next reporting period we will continue with the experiments outlined in Specific Aims 2 and 3 of the SOW.

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).

We have identified three new pathogenic mutations, one in BRCA2 and two in ATM in three different participants.

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:*

Changes in approach and reasons for change

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

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**
  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).*

  Nothing to report

  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".

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.)*

No changes from the personnel Tasks outlined in the approved SOW.

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*