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TITLE: Prostate Tissue Gene Expression Patterns Predict Prostate Tissue Inflammation, Aggressive Prostate Cancer, and a Poorer Prognosis Among Black and White Men

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CONTRACTING ORGANIZATION:
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Prostate Tissue Gene Expression Patterns Predict Prostate Tissue Inflammation, Aggressive Prostate Cancer, and a Poorer Prognosis Among Black and White Men

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5b. GRANT NUMBER

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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

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12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Black men have a 63% higher prostate cancer (PC) risk than white men, and a more than 2-fold higher mortality. Our objective is to determine the connection between immune cell infiltration and gene expression of pro- and anti-inflammatory messengers in prostate tissue in black and white men with PC. We also will determine the link between differential gene expression on aggressive vs. non-aggressive PC and clinical progression following treatment in black and white men with PC. We hypothesize increased immune cell invasion believed to be pro-carcinogenic in the prostate will increase the expression of genes involved with immune system signaling and cell cycle regulation in the prostate, leading to an aggressive PC. This effect may be stronger in black men who in general have greater systemic inflammation.

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	13	**19b. TELEPHONE NUMBER** *(include area code)*

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1. INTRODUCTION:

This proposal asks what aspect of the immune system alters gene expression in prostate tissue that results in greater prostate cancer in black or white men. We are thoroughly characterizing systemic inflammatory markers and prostate tissue inflammatory markers in 150 black and 150 white men. We will investigate the race-specific link between specific immune cell patterns and the expression of inflammatory regulatory gene expression in prostate tissue. The immune system is complex, and therefore we include a broad spectrum of immune cells to investigate pro- and anti-inflammatory immune cell reactions that may affect prostate cancer outcomes and that differ with race. This analysis will help us identify those components of inflammation most relevant to the diagnosis of aggressive prostate cancer, or that affect the response to prostate cancer treatment, and the similarity or differences in these relationships between black and white men.

2. KEYWORDS:

Prostate cancer, race, inflammation, biomarker, microenvironment, prognosis, risk

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Accomplishments are consistent with implementation of the protocol. Financial subcontracts with our partnering institution are in place. IRB protocols were submitted and approved. We have hired 3 research staff to support the implementation of study protocols. As these processes evolved, obstacles faced included challenges in negotiating RNA-Seq contracts with outside vendors and coordinating immune cell scoring with the RNA-Seq protocol. Solutions to these challenges are discussed below, and RNA-Seq and immune cell scoring are underway.

Project Run-In (Specific Aims 1 &2)	Month	Site 1	Site 2
• Complete institutional sub-contracts	1-2	X	X
• Complete material use transfer agreements	1-2	X	X
• Maintain all IRB applications and consent forms	1-36	X	X

• Train Research Staff in all research protocols	1-3	X	X
• Create, revise, and distribute manual of operations of research protocols	2-3	X	
• DoD HRPO review	3-4	X	X
Specific Aim 1	Month	Site 1	Site 2
Stage 1: Subject Identification and Study Data			
• Identify prostate cancer patients with data and biospecimens for analysis through our on-going case-control studies at Cedars and Vanderbilt	5-6	X	X
• Create analytic datasets from medical records for health, pathology, genetic African ancestry, and screening data, as per proposal	7-9	X	X
• Assess data consistency and harmonization	10-11	X	X
• Create analytic datasets of immune cell infiltration	12-24		X
Stage 2: RNA Extraction and Sequencing			
• Access tissue repositories for prostate biopsy blocks	7-10	X	X
• Send blocks to institutional path lab core for slice preparation	7-11	X	X
• Send slices to Hudson Alpha for RNA extraction and quantification	12	X	X
• RNA is returned to University of Tennessee Health Science Center	18	X	
• Conduct RNA sequencing and quality control protocols as stated	19-28	X	
• Perform alignment, quantify reads	29-30	X	
Stage 3: Statistical Analysis			
• Generate analytic data sets from each institution	30-34	X	
• Perform necessary data transformations to meet statistical assumptions	30-34	X	
• Test study of inflammatory pathways, cell cycle regulation, and immune cell invasion associated with prostate cancer aggressiveness	30-36	X	
• Evaluate functional significance and pathways	30-36	X	
• Interpretation of Results, create figures and data tables.	30-36	X	X
Product: Manuscript for investigation of gene expression profiles linked with immune cell infiltration			
Specific Aim 2	Month	Site 1	Site 2
Stage1: Study Data			
• Update medical chart review and create analytic data sets from medical chart review and patient questionnaires during follow-up	6-10	X	X
• Assess follow-up, determine vital status	11-18	X	X
• Determine outcomes and generate final datasets	19-24	X	X

Stage 2: Statistical Analysis			
• Perform exploratory analysis and test modeling assumptions	30-36	X	
• Test study hypotheses	30-36	X	
• Conduct post-doc data analysis	30-36	X	
• Data interpretation	30-36	X	X
Product: Manuscript of prospective investigation: magnesium, prostate cancer outcomes and race.			

What was accomplished under these goals?

Toward specific aim 1, we have identified prostate cancer patients suitable for analysis from our consortium protocol. Tissue has been acquired from our biorepository and labeled for RNA sequencing. Dataset templates have been created. Laboratories were identified to conduct sequencing assays. RNA is being extracted, leading to sequencing. Toward specific aim 2, we have created analytic datasets for medical chart data, and are preparing to conduct a follow-up assessment of selected patients for clinical outcomes. Protocol for immune cell scoring was revised and scoring is in progress.

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

Nothing to report. Not intended for training purposes.

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?

Finish tissue scoring and initiate statistical analysis toward manuscripts.

4. **IMPACT:**

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

The present stage in this protocol, the study has limited impact on our knowledge of inflammation and prostate cancer. The next steps in the research program will determine gene expression response to tissue inflammation in black vs. white men, and the role of these inflammatory markers in prostate tissue.

What was the impact on other disciplines?

Nothing to report

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:

Changes in approach and reasons for change

The specific aims and overall approach of this project have not changed.

Actual or anticipated problems or delays and actions or plans to resolve them

The COVID-19 shut down led to a substantial delay in progress during this year, as investigators were unable to access datasets or coordinate work with staff. Administrative and staffing efforts have taken longer than expected. A problem was that a MTA could not be reached between samples from the VA (Cedars-Sinai) and Hudson Alpha. Negotiations proceeded for several months without a satisfying resolution. As a solution, RNA-Seq will be performed at the core lab at Cedars-Sinai, where negotiated arrangements between the VA and Cedars have been in place for some time. The Year 3 subcontract with Cedars was adjusted upward to accommodate the additional costs associated with RNA-Seq.

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

Not applicable

Significant changes in use of biohazards and/or select agents

Not applicable.

6. PRODUCTS:

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

  Journal publications.

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?

Stephen Freedland; Co-Investigator; ORCID: 0000-0002-8104-6419; Effort: No change
Yunhee Choi-Kuaea; Clinical Research Specialist I: Person months: 1.44

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

no changes
no conflicts

What other organizations were involved as partners?

Cedars-Sinai Medical Center
8700 Beverly Blvd.
Los Angeles, CA 90048
Partnering PI: Dr. Stephen J. Freedland, MD

Collaboration on project

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

QUAD CHARTS:

9. APPENDICES: