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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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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.						
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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.

Keywords: Prostate cancer, race, magnesium, calcium, inflammation, biomarker, microenvironment, prognosis, risk

Accomplishments in context to statement of work

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.

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.

Project Run-In (Specific Aims 1 &2)	Month	Site 1	Site 2
<ul style="list-style-type: none"> • Complete institutional sub-contracts 	1-2	X	X
<ul style="list-style-type: none"> • Complete material use transfer agreements 	1-2	X	X
<ul style="list-style-type: none"> • Maintain all IRB applications and consent forms 	1-36	X	X
<ul style="list-style-type: none"> • Train Research Staff in all research protocols 	1-3	X	X
<ul style="list-style-type: none"> • Create, revise, and distribute manual of operations of research protocols 	2-3	X	
<ul style="list-style-type: none"> • DoD HRPO review 	3-4	X	X
Specific Aim 1			
Stage 1: Subject Identification and Study Data	Month	Site 1	Site 2
<ul style="list-style-type: none"> • 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
<ul style="list-style-type: none"> • Create analytic datasets from medical records for health, pathology, genetic African ancestry, and screening data, as per proposal 	7-9	X	X
<ul style="list-style-type: none"> • Assess data consistency and harmonization 	10-11	X	X
<ul style="list-style-type: none"> • Create analytic datasets of immune cell infiltration 	12-24		X
Stage 2: RNA Extraction and Sequencing			
<ul style="list-style-type: none"> • Access tissue repositories for prostate biopsy blocks 	7-10	X	X
<ul style="list-style-type: none"> • Send blocks to institutional path lab core for slice preparation 	7-11	X	X

<ul style="list-style-type: none"> Send slices to Hudson Alpha for RNA extraction and quantification RNA is returned to University of Tennessee Health Science Center Conduct RNA sequencing and quality control protocols as stated Perform alignment, quantify reads 	12 18 19-28 29-30	X X X X	X
Stage 3: Statistical Analysis			
<ul style="list-style-type: none"> Generate analytic data sets from each institution Perform necessary data transformations to meet statistical assumptions Test study of inflammatory pathways, cell cycle regulation, and immune cell invasion associated with prostate cancer aggressiveness Evaluate functional significance and pathways Interpretation of Results, create figures and data tables. 	30-34 30-34 30-36 30-36 30-36	X X X X X	X
Product: Manuscript for investigation of gene expression profiles linked with immune cell infiltration			
Specific Aim 2			
Stage 1: Study Data			
<ul style="list-style-type: none"> Update medical chart review and create analytic data sets from medical chart review and patient questionnaires during follow-up Assess follow-up, determine vital status Determine outcomes and generate final datasets 	6-10 11-18 19-24	X X X	X X X
Stage 2: Statistical Analysis			
<ul style="list-style-type: none"> Perform exploratory analysis and test modeling assumptions Test study hypotheses Conduct post-doc data analysis Data interpretation 	30-36 30-36 30-36 30-36	X X X X	X
Product: Manuscript of prospective investigation: magnesium, prostate cancer outcomes and race.			

Impact: 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.

Changes/Problems: The specific aims and overall approach of this project have not changed. 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.

Products: Nothing to Report

Participants and other Collaborating Organizations: There are no changes in the individuals involved in the study. Collaborating organizations include:

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Site 2: Cedars-Sinai Medical Center
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Special Reporting Requirements: We submit this report from UTHSC and Cedars Sinai