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14. ABSTRACT

In 2022, an estimated 268,490 men will be newly diagnosed with prostate cancer and 34,500 men will die from this disease in the United States (1). The burden of disease is particularly heavy on Black/African American men, who have a 1.5-fold incidence rate and 2.5-fold mortality rate, as compared to White/Caucasian Americans (1). However, data on determinants of prostate disease aggressiveness at time of initial cancer detection are sorely lacking. **Predictive models that simultaneously incorporate demographic, clinical, social determinants of health (SDOH), and biological data are urgently needed to identify men at earlier time points, who are fated for aggressive disease, especially for Black men, to inform treatment stratification and informed treatment decision-making.** Such models could help avoid overtreatment of clinically indolent disease, as well as spare men unnecessary repeat biopsies, which are costly in terms of dollars but also in terms of reductions in patient quality of life, and to ensure proper treatment intensity for men whose disease may be fated to metastasize. By addressing biological underpinnings in the form of protein expression of indolent versus aggressive PCa at diagnosis, as well as comparing protein expression in men who remain under suspicion for PCa but do not develop cancer, this study could have immediate relevance for informing timing, type, and intensity of treatment for men undergoing biopsy due to suspicion for PCa. Data on early markers of detection for PCa specific to African American men are very limited, potentially leading to sub-optimal care. **The overarching study objective** is to identify and validate prostate biopsy tissue-derived proteomics biomarkers of disease aggressiveness at time of initial prostate cancer detection, with careful examination of racial variation in markers of aggressiveness, as well as the independent and joint roles of SDOHs together with proteomics profiles, on prostate disease aggressiveness at time of biopsy for Black and White men. This study builds on previously published work led by multiple co-authors ***This proposal will focus on biological factors that may contribute to racial disparities in PCa incidence and outcomes.*** Identifying new markers of cancer aggressiveness for each racial group will allow doctors to better tailor disease management in African American patients, and identify patients who are likely to benefit from earlier, timely treatment interventions, which will ultimately improve prostate cancer outcomes and quality of life among African American patients.

15. SUBJECT TERMS

Prostate cancer, racial disparity, metastasis, African American/Black men, proteomics

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Introduction

In 2022, an estimated 268,490 men will be newly diagnosed with prostate cancer and 34,500 men will die from this disease in the United States (*American Cancer Society, Facts and Figures, 2022*). The burden of disease is particularly heavy on Black/African American men, who have a 1.5-fold incidence rate and 2.5-fold mortality rate, as compared to White/Caucasian Americans (1). However, data on determinants of prostate disease aggressiveness at time of initial cancer detection are sorely lacking. **Predictive models that simultaneously incorporate demographic, clinical, social determinants of health (SDOH), and biological data are urgently needed to identify men at earlier time points, who are fated for aggressive disease, especially for Black men, to inform treatment stratification and informed treatment decision-making.** Such models could help avoid overtreatment of clinically indolent disease, as well as spare men unnecessary repeat biopsies, which are costly in terms of dollars but also in terms of reductions in patient quality of life, and to ensure proper treatment intensity for men whose disease may be fated to metastasize. By addressing biological underpinnings in the form of protein expression of indolent versus aggressive PCa at diagnosis, as well as comparing protein expression in men who remain under suspicion for PCa but do not develop cancer, this study could have immediate relevance for informing timing, type, and intensity of treatment for men undergoing biopsy due to suspicion for PCa.

Reasons for such racial disparities have been extensively researched and may be a function of both biological and social determinants of cancer development and aggressiveness. Early detection, when cancer is still confined to the prostate gland, greatly improves the disease-specific survival probability. However, very little is known about the molecular determinants of prostate cancer (PCa) that contribute to metastatic potential at time of initial cancer detection. Generating race-specific information about the molecular changes that occur during the PCa disease course is key to understanding the addressing the racial disparities in PCa outcomes. Predictive models that incorporate both clinical and biological expression data are urgently needed to identify men at earlier times points in life, who are fated for aggressive disease early in the cancer care continuum, especially for AA men. ***This study specifically addresses biological factors that may contribute to racial disparities in PCa incidence and outcomes.***

There is also an unmet clinical need to identify predictors of aggressive PCa, to avoid overtreatment of clinically indolent disease but ensure proper treatment intensity for men whose disease may be fated to metastasize. Use of mass spectrometry-based **quantitative proteomics** to analyze biopsy tissue is a critical and growing area in cancer research. These advanced analytic approaches will be used to generate and verify protein biomarker candidates and develop models to predict prostate cancer aggressiveness in a racially diverse cohort of military health care beneficiaries.

The overarching study objective is to identify and validate prostate biopsy tissue-derived proteomics biomarkers of disease aggressiveness at time of initial prostate cancer detection, with careful examination of racial variation in markers of aggressiveness, as well as the independent and joint roles of SDOHs together with proteomics profiles, on prostate disease aggressiveness at time of biopsy for Black and White men. This study builds on previously published work led by multiple co-authors.

Keywords: Prostate cancer, racial disparity, metastasis, African American, proteomics

Accomplishments for Year 2 reporting period (09/01/2021-08/31/2022):

(Listed per Aim, Major Task, and Subtask from SOW):

Under Specific Aim 1:

Major Task 1: Regulatory and Personnel
Status: Ongoing IRB monitoring and compliance, maintenance of required training and credentialing for investigative team, per institutional requirements.

Major Task 2: Data Procurement
Subtask 1: Conduct database query to determine eligible subjects with formalin-fixed, paraffin-embedded (FFPE) biopsy specimens needed for proteomics analyses.
Status: Completed for 284 eligible subjects; in Year 3, the final 16 subjects will be identified. Metastatic cases have been the most difficult to identify, but the final

Subtask 2: Extract all relevant data points and perform QA/QC steps to ensure highest data quality and completeness.
Status: Completed for 284 eligible subjects; in Year 3, all data for the final 16 subjects will be identified.

Subtask 3: Lock clinical dataset and prepare/ship biospecimen data to the PNNL investigators who will be blinded to patient race and cancer status.
Status: Locking of clinical dataset not yet completed—remaining 16 subjects to be identified in Year 3. All biospecimen data shipped to PNNL to date have been balanced across race and clinical group, with the PNNL investigative team blinded to the patient characteristics prior to experimental work.

Under Specific Aim 2:
Major Task 3: Biospecimen processing & proteomics analysis *for all patient specimens*
Subtask 1: Isobaric TMT-11 labeling based in-depth discovery analysis of a subset of FFPE biopsy samples and associated statistical analysis for selecting candidate protein biomarkers.
Status: This subtask was completed in Year 2, with the full discovery proteomics dataset delivered to CWRU team in July 2022.

Subtask 2a: Develop high-performance PRISM-SRM assays for the biomarker candidates as described in Aim 1 for 60 subject samples to inform the markers to be selected for the larger cohort
Status: This subtask was initiated in Year 2, but us still underway in Year 3. Final decisions as to which new assays will be needed to examine new biomarker candidates was made at end of Year 2 (August 2022). The development of those assays was initiated at the start of Year 3 (Sept-Oct) and will continue into the middle of Year 3. It is anticipated that a 1-year no-cost extension will be needed to complete the proteomics data expression for the verification cohort. This is a result of the significant delays in executing inter-institutional agreements in Year 1. Every effort is underway to complete this subtask in the first half of Year 3, to allow for the merge of final clinical data to the final proteomics data, and subsequent analytic modeling, in the second half of Year 3.

Subtask 2b: Expand the experimental work to 250 subject samples, based on findings from Subtask 2a
Status: This subtask was not initiated in Year 2; it is underway in Year 3.

Subtask 3: Quantification of the biomarker candidates in a large cohort of biopsy samples using PRISM-SRM and heavy isotope-labeled internal standards.
Status: PNNL generated the Discovery cohort proteome dataset for N=60 men which was delivered to CWRU in July 2022. These data were presented at the AACR Science of Health Disparities annual meeting in Philadelphia, PA on September 19, 2022. These findings have informed the efforts for this subtask which was not initiated in Year 2 but is underway in Year 3.

Major Task 4: Prediction Modeling

Subtask 1: Merge proteomics data with locked clinical dataset; perform data diagnostics and transformation of variables, as needed, for modeling
Status: Ongoing data diagnostics and preliminary modeling was conducted throughout Year 2 of the award, allowing for summary and presentation of early findings at a national conference.

Subtask 2: Perform all statistical analysis (descriptive, unadjusted, multivariable, bioinformatics)
Status: Early statistical analysis was ongoing throughout Year 2 of the award. Final models will be created in Year 3 to support all manuscripts.

Major Task 5: Publications & Presentations

Subtask 1: Prepare manuscripts for peer review
Status: There were no submitted manuscripts submitted in Year 2 though there were significant efforts with respect to data analyses and literature review to begin to prepare the 1st of 3 manuscripts planned in Year 3

Subtask 2: Present findings at national scientific conferences
Status: Highlights of findings from the Discovery cohort were presented at the AACR Science of Health Disparities annual meeting in Philadelphia, PA on September 19, 2022.

Monthly summary of accomplishments for Year 2 (9/1/2021 – 8/31/2022):

September 1-30, 2021: All final agreements (inter-institutional contract, MTA) were executed; HRPO approval was granted.

September 1, 2021-August 31, 2022: Monthly standing calls with CWRU (Jennifer Cullen) and PNNL (Tao Liu) to discuss study progress.

October 1, 2021 – August 31, 2022:

i. Identification of eligible patients who meet all study criteria at the University Hospitals (UH) main campus location using the Electronic Medical Record (EMR);

ii. Creation of IRB-compliant REDCap database for storing IRB protocol-approved clinical, demographic and disease-specific data elements.

iii. Conduct database query to determine eligible subjects with formalin-fixed, paraffin-embedded (FFPE) biopsy specimens needed for proteomics analyses; EMR used to locate biopsy tissue blocks for each patient, reviewed by senior Pathologist and histo-technician and development of processes for pulling specimen blocks from Iron Mountain, cutting new slides from selected blocks, and preparing all biospecimen materials for safe shipment to PNNL.

iv. Shipping log developed and maintained for all slide shipments; encrypted ID created to send to PNNL so investigators maintain blinding to patient race and clinical group (ID match list maintained at UH).

January 2022: First shipment of biopsy slides sent to PNNL for QA/QC; decision to alter the thickness and quantity of slides was made, based on protein generation results.

February 1, 2022 – August 31, 2022:

i. Preparation and shipment of slides (in batches of 10-15 patient materials per batch, balanced on race and clinical group) sent to PNNL; currently, we have completed the identification and slide shipment for 273 or the 300 men in the target study cohort.

ii. Completion of detailed data abstraction from UH EMR on 273 of 300 patients. Final 27 patient will be identified before end of calendar year (2022). Extraction of all relevant data points was conducted and QA/QC steps were employed to ensure highest data quality and completeness.

iii. Completion of the global deep discovery analysis and **generation of raw dataset** for the Aim 1 Discovery cohort (N=60); dataset delivered to CWRU (J.Cullen) by PNNL (T. Liu) in July 2022. Over 10,000 proteins were identified on each of the 60 men. Generation of the entire observable proteome is now completed. This included Isobaric TMT-11 labeling based in-depth discovery analysis of a subset of 60 FFPE biopsy samples and development to the high-performance PRISM-SRM assays for the biomarker candidates as described in Aim 1 for 60 subject samples.

August 1-31, 2022: Data modeling initiated for Discovery cohort; poster prepared for American Association of Cancer Research (AACR), Science of Health Disparities annual meeting to be presented in Philadelphia, PA on September 19, 2022.

Impact:

One in six American men will be diagnosed with prostate cancer (PCa) during his life-time. The burden of this disease disproportionately impacts African Americans (AA) who demonstrate 1.5-times the incidence rate and 2.5 times the mortality rate compared to Caucasian American men. If PCa is detected early, patient outcomes are greatly improved. But there is an unmet clinical need to identify early predictors of CaP aggressiveness. Such markers could help avoid overtreatment of clinically indolent disease, which is costly in terms of dollars but also in terms of reductions in patient quality of life, and to ensure proper treatment intensity for men whose disease may be fated to metastasize.

A unique aspect of the study cohort that will be leveraged in this proposal is its large subset of men who were under *suspicion for prostate cancer* but who have undergone multiple, negative transrectal ultrasound (TRUS)-guided biopsies and persistently show no evidence of cancer. Such biopsy negative patients serve as an ideal comparison group to men detected with prostate cancer since both groups have been subjected to the same rigor of cancer ascertainment.

Short term study impact:
By addressing biological underpinnings in the form of protein expression of indolent versus aggressive PCa at diagnosis, as well as comparing protein expression in men who remain under suspicion for PCa but do not develop cancer, this study could have immediate relevance for informing timing, type, and intensity of treatment for men undergoing biopsy due to suspicion for PCa. While findings will need to be confirmed in other health care settings, a key advantage to studying the proposed aims in this study cohort will be the potential impact on improving treatment decision making in African American men.

Long-term study impact:
The findings of this project will also provide comprehensive race-specific data for the prostate disease course—for those under suspicion for PCa, to those with indolent versus aggressive disease. This patient cohort is racially diverse which makes this study setting optimal for examining cancer health disparities. The markers examined could elucidate who can be spared repeat biopsy in the future, as well as how to optimize the cancer care of African American men.

Changes/Problems: *None to report*

Products:

1. Acceptance of American Association of Cancer Research (AACR), Science of Health Disparities **Abstract submission** *(work presented in September 2022 outside this reporting time window and will be discussed in APR for Year 3).*

2. Completion of mass-spectrometry analysis and **generation of raw dataset** for the Aim 1 Discovery cohort (N=60); dataset was delivered to CWRU (J.Cullen) by PNNL (T. Liu) in July 2022. Over 10,000 proteins were identified on each of the 60 men and are currently being prepared for data modeling. The generation of the entire observable proteome is now completed. This phase of the study will help inform the Verification Phase.

Participants & Other Collaborating Organizations: *NA*

Special Reporting Requirements: *NA*

Appendices: *None*