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14. ABSTRACT

Objective: Systemic low-grade inflammation is a prostate cancer risk factor in men of African descent, and correlates with West African ancestry, genetic susceptibility, a distinct tumor biology, and aggressive disease. Our overall objective is to understand these relationships with a view to informing prevention and therapeutic strategies.

Impact: Our study will be the first to explore the relationship between systemic/chronic inflammation, ancestry, and tumor biology as a cause of disease progression in men of African descent. Creating an understanding of how the interaction between chronic inflammation and tumor biology affects prostate cancer progression in a high-risk population, like African-American men, offers the opportunity to the develop improved prevention and therapeutic strategies using anti-inflammatory drugs and immune modulators to decrease the disease burden among all men

15. SUBJECT TERMS
Cancer, Prostate Cancer, Oncology

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1. INTRODUCTION:

Men of African descent experience a disproportionately high prostate cancer mortality. We and others have shown that prostate tumors in African Americans harbor a distinct immune-inflammation signature. Low-grade inflammation has been described as a prostate cancer risk factor that is associated with aggressive disease. We also reported that regular aspirin use reduces the risk of aggressive prostate cancer and disease recurrence in these men. We now validated this finding in the prospective Southern Community Cohort Study by showing that aspirin use at time of recruitment associates with a reduced prostate cancer mortality on follow-up among the African American men in this cohort (Tang…Ambs, CEBP, 2021; PMID: 33293340). Together, the observations suggest that a systemic low-grade chronic inflammation related to ancestral factors and tumor biology could be a driver of prostate cancer mortality in men with African ancestry. We therefore proposed to examine whether a systemic low-grade inflammation is a prostate cancer risk factor in men of African descent and correlates with West African ancestry, genetic susceptibility, a distinct tumor biology, and aggressive disease. Our research aims included the analysis of a unique immune-inflammation signature in men of African ancestry that relates to prostate cancer. In this context, we proposed to analyze circulating immune-oncology markers and fatty acid levels and urinary markers of pro-inflammatory cyclooxygenase signaling in a large cohort of Ghanaian, African American, and European American men with and without prostate cancer. We also proposed to assess the genetic and ancestral basis of prostate cancer-associated inflammation using a genome-wide association approach. Recent developments will also allow us to investigate the impact of the neighborhood environment on the profiles of these markers. Lastly, in partnership with our Co-PI, Dr. Clayton Yates at Tuskegee University, we will determine the prevalence and origin of an immune-inflammation signature in prostate tumors of Nigerian men and compare with African American and European American men from the NCI-Maryland cohort.

2. KEYWORDS:

African-American, Africa, ancestry, biomarker, blood, case control study, chromatin, cyclooxygenase, disease progression, DNA, genetic variation, genomics, immunity, inflammation, mutation, RNA, risk factor, fatty acid, tumor biology, transcriptome, urine, whole exome sequencing.

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Major goals for the review period are as follows:

Aim 1. Determine if there is a unique immune-inflammation signature in men of African ancestry that relates to prostate cancer.

Goal: Preparation of manuscripts and revisions

Aim 2. Assess the genetic and ancestral basis of prostate cancer-associated inflammation using a genome-wide association approach.

Goal 1: Statistical analysis

Assess whether germline genetic variants are associated with immune-inflammation markers and prostate cancer. Perform a combined analysis of the NCI-Maryland and NCI-Ghana Prostate studies. (also not outlined in the SOW, we are also investigating the association of these genetic variants with 24 circulating fatty acid levels).

Goal 2: Preparation of manuscript and revisions

Aim 3. Determine the prevalence and origin of an immune-inflammation signature in tumors of men of African and European ancestry.

Goal 1: Statistical analysis of transcriptomics and genomics data

Goal 2: Perform immunohistochemistry (IHC) for immune markers such as PD-1, PD-L1/2, CD45, CD68, CD80, CD86, CD163, and CD206.

Goal 3: Preparation of manuscript and revisions

The project is managed as a collaborative research study that involves two lead investigators, Dr. Stefan Ambs at the National Cancer Institute/NIH, and Dr. Clayton Yates who recently moved from Tuskegee University to his new position as a Professor of Pathology/Oncology at Johns Hopkins University. Dr. Ambs' group has the lead on the projects described under Aims 1 & 2, whereas Dr. Yates' group has the lead on the projects described under Aim 3.

The experimental parts for Aims 1 & 2, as described in the SOW, have been completed. For Aim 1, the remaining task is the publication of a submitted manuscript. For Aim 2, the remaining tasks are data analysis, manuscript preparation, and submission for acceptance in a peer-reviewed journal.

The experimental parts for Aim 3 have almost been completed and currently involve spatial analysis of immune marker expression in prostate tumors (goal 2). The genomics data have been generated and are currently analyzed for manuscript preparation and submission (goals 1 &3).

What was accomplished under these goals?

The assembly of databases, data analysis, and preparation of manuscripts have been the major tasks in the Ambs laboratory in the reporting period. All tasks related to **Specific Aim 1** have been completed. The final manuscript related to **Specific Aim 1,** reporting on circulating fatty acids and their association with circulating immune-oncology markers and prostate cancer outcomes is under review with *Nature Communications*. Additional data analysis and preparation of manuscripts will be the only remaining task in the Ambs laboratory moving forward. Here, we will complete the tasks specified under **Specific Aim 2**. The Yates laboratory at Tuskegee University has the lead on research and tasks specified under **Specific Aim 3**. However, all tasks under this aim are collaborative, involving efforts by both the Yates and Ambs laboratories. For **Specific Aim 3**, we had previously reported major delays in completing Major Task 1 & 2 (RNA-seq and whole exome sequencing - WES) due to COVID-19. Most issues were resolved. We could proceed with the sequencing tasks using the company, HudsonAlpha, as the service provider. While HudsonAlpha delivered good quality RNA-seq data for 167 tumors, we were still struggling in obtaining good quality whole exome sequencing data for a similar number of tumors. Nevertheless, a team effort has now been able to complete a WES analysis for a subset of Nigerian prostate tumors and published those data very recently in *Cancer Research Communications*, completing an important milestone under **Specific Aim 3**.

Completed tasks and progress in the reporting period.

During the past 48 months, our group addressed the Major Tasks for **Specific Aims 1 & 2**, as outlined in the Statement of Work for the grant. At this time, all tasks for **Specific Aim 1** have been completed.

Specific Aim 1: Measure 97 markers in plasma/serum or urine and examine their association with prostate cancer (PCa), genetic ancestry, family history, and lifestyle factors.	**Timeline**	**Site 1** NCI	**Site 2** TU
Major Task 1: Measurement of 92 immune-inflammation markers, lipopolysaccharide, and Omega-3 fatty acids, respectively, in plasma/serum, and three metabolites of cyclooxygenases - PGE-M, thromboxane B2 and prostacyclin - in urine.	Months		
Subtask 1: Prepare plasma/serum and urine samples for shipment • Obtain IRB approval and MTAs covering the NCI-Maryland and NCI-Ghana Prostate studies and the two study sites, NCI and University of Tuskegee. • Receive (Ambs) and aliquot plasma/serum samples from 1650 cases (150 samples from Nigerian PCa cases) and 1650 controls (150 samples from Nigerian men) and ship to Olink (Watertown MA), Leidos Biomedical Research, Inc., Frederick National Laboratory for Cancer Research (Frederick, MD), and OmegaQuant LLC (Sioux Falls, SD). Also, aliquot urine samples from the NCI-Maryland Prostate Cancer Case-Control study (n = 1800) and ship to the Eicosanoid Core Laboratory at Vanderbilt University (Nashville, TN).	1-8	Ambs, Cook, Dorsey, Minas	Yates
Subtask 2: Measure plasma/serum and urine markers and build a database • Measurement of 92 inflammation-related and immune-modulatory analytes at Olink; lipopolysaccharide at Leidos Biomedical Research Inc.; three metabolites of cyclooxygenases, PGE-M, thromboxane B2 and prostacyclin at Eicosanoid Core Laboratory, Vanderbilt University; and Omega-3 fatty acids at OmegaQuant LLC. Obtain measurement data and create a database for analysis with a statistical software.	8-16(24)	Ambs, Dorsey, Minas	

For **Specific Aim 1**, under Major Task 1, we described the preparation of serum and urine samples and their shipment to labs to measure immune-oncology markers (n = 92), omega-3 fatty acid levels (24 different fatty acids, not all are omega-3 fatty acids), lipopolysaccharide (LPS), and urinary metabolites of the cyclooxygenase signaling pathway (5 metabolites were measured). These tasks were completed ahead of schedule.

Major Task 1 describes as subtask 2 the measurements of these markers/metabolites at Olink (immune-oncology markers), at OmegaQuant (omega-3 and other fatty acids), at Leidos (LPS), and at the Eicosanoid Core

Laboratory at Vanderbilt University (urinary metabolites). This task had a timeline of 16 months and was completed as proposed. Regarding the measurements of the 5 urinary metabolites, we had observed a larger variation in the data for blinded duplicates than expected – as outlined in the 2019 report. This problem was resolved when the creatinine measurements, serving as reference, were repeated.

Serum markers were measured in blood samples of 1520 prostate cancer cases and 1518 controls from the NCI-Maryland and NCI-Ghana studies. In total, about 3190 measurements (including blinded duplicates) were performed for each assay type. For the 92 immune-inflammation markers, measurements of duplicates showed very small sample-to-sample variation, indicating a generally very solid platform that was developed by Olink. We could detect 61 of the analytes in all samples and 78 in 50% of the samples. Missing values mostly indicated that the abundance of these markers was below the detection limit in a subset of the samples. However, for ten of the immune-inflammation markers, the Olink multiplex assay may not have worked well, leading to a failure of detecting these markers in almost all samples (e.g., TNFα, IFNγ). We reported this experience back to the company. Because the markers in question could not be re-measured, we excluded them from the analysis. Hence, all analyses were performed with 82 immune-inflammation markers - but having high quality measurements for all of them.

As to the measurements of the omega-3 and other fatty acids, all assays performed very well, and the 24 fatty acids were measured in all samples. The estimated CV of 8.7% indicates very good performance of the assays, which is expected from a CLIA-certified assay that is applied to measure fatty acid contents in clinical samples as a routine task by OmegaQuant. Lastly, our LPS assay detected LPS, also called endotoxin, in about 14% of the samples. We did not expect to detect LPS in many samples, as it indicates an ongoing infection with gram-negative bacteria. We will use the LPS readings to examine if immune-inflammation marker measurements are affected by infections.

With the data for the blood and urine markers in hand, Maeve Bailey-Whyte and Tsion Minas, two postdoctoral fellows, began to analyze them starting in the second half of 2019. Results from the urine marker analysis were published first and discussed in the previous progress report. Findings related to thromboxane A2 were most interesting and were published in *JNCI* (PMID: 34264335), showing that increased formation of thromboxane A2, a candidate pro-metastatic metabolite produced by platelets, is significantly associated with development of metastatic and lethal prostate cancer among African American men. We also showed that aspirin use inhibits thromboxane A2 formation, suggesting that regular aspirin use may protect against lethal prostate cancer by inhibiting inflammation-related thromboxane A2 production. In contrast, we did not find that prostaglandin E2 formation associates with prostate cancer when measuring its surrogate, urinary PGE-M. These negative data were published by us in the peer-reviewed journal, *Cancers*, in 2021 (PMID: 34439226).

Moreover, the analysis of the 82 immune-oncology markers has been completed and the findings were published in *Nature Communications* in 2022 (PMID: 35365620). A summary of key findings reported in our paper **"Serum proteomics links suppression of tumor immunity to ancestry and**

lethal prostate cancer" is provided on the next five pages.

Cytokines associated with suppression of tumor immunity and chemotaxis are upregulated in men of African ancestry. We analyzed 82 immune-oncology markers and found that a subset of them showed a marked association with ancestry. To better define the functional implications of these population group differences, we grouped the 82 proteins into six biological processes according to Olink guidelines: apoptosis/cell killing, autophagy/metabolism, chemotaxis/trafficking to tumor, suppression of tumor immunity (Th2 response, tolerogenic), promotion of tumor immunity (Th1 responses), or vasculature and tissue remodeling (**Figure 1**). To gain insight on how activation of these processes/pathways may differ by population group, we compared process/pathway sum scores between Ghanaian, African American, and European American men without prostate cancer using multi-comparison-adjusted significance testing. Of these pathways, chemotaxis, promotion of tumor immunity, and suppression of tumor immunity were significantly different in their predicted activity between African American and European American men (**Figure 1**). African American men had significantly higher scores for chemotaxis and suppression of tumor immunity when compared to European American men, indicating higher activity in African American men, but a lower score for promotion of tumor immunity. Ghanaian men had even higher scores for chemotaxis and suppression of tumor immunity than both African American and European-American men (**Figure 1C and E**), indicating a possible association with West African ancestry. The latter was corroborated with our finding that the chemotaxis and suppression of tumor immunity scores positively correlated with the proportion of West African ancestry within the NCI-Maryland cohort, even after holding the other variables constant (i.e. age, BMI, education, aspirin use, diabetes, and smoking history) in the regression analysis (for chemotaxis score: regression coefficient = 5.12 (3.75, 6.49), P <0.0001; for suppression of immunity score: regression coefficient = 4.02 (2.01, 6.04), P <0.0001). Even though apoptosis and vasculature-associated cytokines were not significantly different between European American and African American men, we found both processes to be elevated in the Ghanaian men.

Suppression of tumor immunity is associated with reduced survival of prostate cancer patients. Next, we examined the clinical implication of our findings and assessed the association of pathway activity with survival of prostate cancer cases or controls in the NCI-Maryland study. As of the end of 2018, out of the 819 cases, there have been 202 deaths in our case population, of whom 103 (51%) had a cancer diagnosis as the recorded primary cause of death, and 28% of all deaths (n = 57) were directly attributed to prostate cancer. On the other hand, 99 of the 828 population controls had died by the end of 2018. Median survival follow-up for cases and controls were 8.6 and 6.7 years, respectively. With these data, we built a multivariable Cox regression model with all biological processes/pathways and adjustment for other covariables including disease status (see Methods). Among the six defined pathways, only suppression of tumor immunity showed independent association with survival of cases (**Figure 2**). Prostate cancer patients with an increased activity of this pathway had the highest risk of death from all causes (**Figure 2A**). In contrast, suppression of tumor immunity was not associated with all-cause mortality of population controls, suggesting that the association with all-cause mortality among cases might be prostate cancer related. Prostate cancer patients with elevated suppression of tumor immunity at diagnosis had also the highest risk of a prostate cancer-specific mortality (**Figure 2B**). Lastly, prostate cancer

patients with increased suppression of tumor immunity were also significantly more likely to die from any cancer (prostate cancer or secondary cancer) following the prostate cancer diagnosis (**Figure 2C**), indicating a more general predisposition to cancer in patients with a high suppression of tumor immunity score.

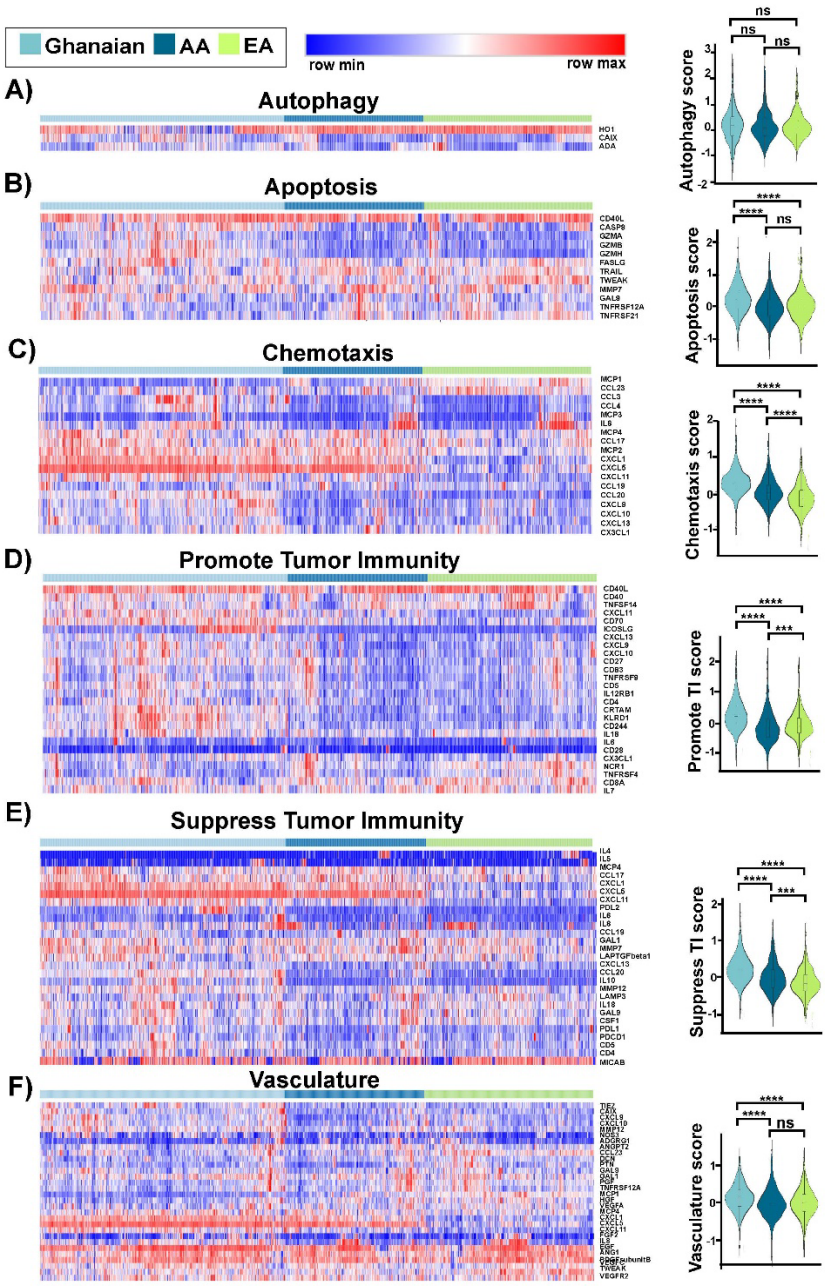


Figure 1. Population differences in the abundance of proteins driving (A) autophagy, (B) apoptosis, (C) chemotaxis, (D) promotion of tumor immunity, (E) suppression of tumor immunity, and (F) vasculature. Heatmaps showing levels of process/pathway-associated proteins in relationship to population group (Ghanaian, AA, EA). Shown to the right are the mean score differences for these processes/pathways among the three population groups. Profiles for Ghanaian (n=654), AA (n=374), and EA (n=454) men without prostate cancer. The process/pathway scores are derived from the average z-scores of all the associated proteins. These scores are shown as violin plots. TI = tumor immunity. FDR-adjusted *P* value significance was coded as <0.0001 (****), <0.001 (***), and =/> 0.05 (non-significant).

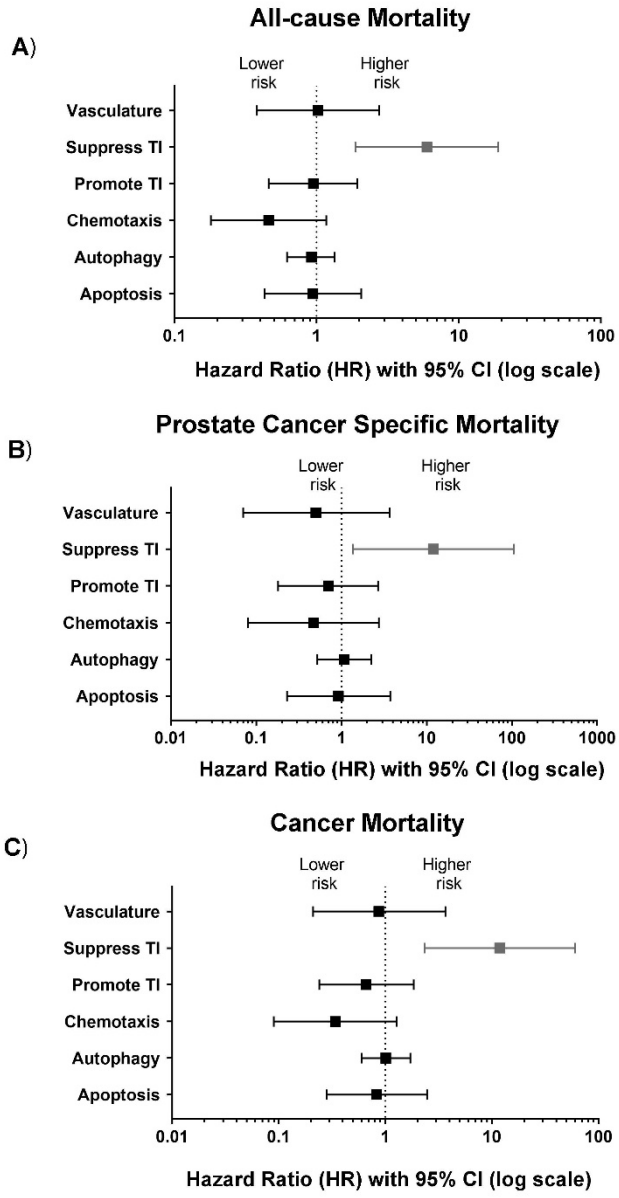


Figure 2. Suppression of the tumor immunity pathway associates with lethal prostate cancer. We assessed the association of the six pathways defined by the 82 immune-oncology markers with all-cause mortality, prostate cancer-specific mortality or mortality due to any cancer after a prostate cancer diagnosis. The pathway scores were evaluated as continuous predictor variables. Suppression of tumor immunity pathway was distinctively associated with all-cause mortality **(A)**, prostate cancer-specific mortality **(B)**, or a mortality due to any cancer after a prostate cancer diagnosis **(C)**. Multivariable Cox regression analyses were used to assess if the pathways were independently associated with survival of prostate cancer patients in the NCI-Maryland study. HRs were adjusted for NCCN risk score in addition to age at study entry, BMI, self-reported race, education, income, smoking history, diabetes, aspirin use, and treatment. The HRs indicate the change in risk of dying when the biological process z-score value increases by 1 while holding all the other biological processes' z-scores and covariates constant.

Blood levels of TNFRSF9/CD137/4-1BB and pleiotrophin predict lethal prostate cancer among AA men. To identify potential drivers of the relationship between immune-oncology markers and lethal prostate cancer, we applied a cross-validated, regularized Cox regression model using eNetXplorer. Included in this model were the 82 immune-oncology markers and 6 patient feature covariates (age, education, BMI, smoking history, aspirin use, and diabetes). Of those patient features, education as a surrogate for socioeconomic status and health care access, BMI, smoking status, and aspirin use have been associated with the risk of lethal prostate cancer whereas the association of diabetes with prostate cancer outcomes is less certain. Lasso regression (alpha=1) was selected based on overall performance. Utilizing this method, we could not identify a robust predictive signature of lethal prostate cancer for European American patients. However, for African American patients, a signature primarily driven by TNFRSF9 and pleiotrophin (PTN), both positively associated with the risk of lethal disease, and regular aspirin use (negatively associated with risk) were the top predictors ($P < 0.05$) based on two selection criteria: the feature frequency (**Figure 3A**) and the weight of the features' contribution to the prediction (**Figure 3B**). These three features combined predicted prostate cancer-specific mortality with an accuracy of 83.7% (SE=3.8%). Our finding that regular aspirin use was a predictor of improved survival among African American men is consistent with previously published data from this case-control study and SCCS. The combination of the two proteins alone predicted prostate cancer-specific mortality with 78.2% (SE=4.2%) accuracy. To gain additional insight of how the prediction of lethal prostate cancer by these two protein markers might be influenced by a patient's disease status, we added the National Comprehensive Cancer Network (NCCN) risk score to our model. Consistent with the baseline model that did not contain the NCCN risk score, lasso (alpha=1) remained the most predictive model. The NCCN risk score was the top predictor of the lethal disease. The two analytes PTN and TNFRSF9 remained the most predictive features besides the NCCN risk score, and the three features combined predicted prostate cancer-specific mortality with 90% accuracy. Additionally, African American prostate cancer patients with high levels (> median) of both TNFRSF9 and PTN in their blood at diagnosis had the worst prostate cancer-specific survival (**Figure 3C**). By 10 years, 33% of cases with high levels of both TNFRSF9 and PTN died of prostate cancer compared to only 5% of cases with low levels of both or either of these proteins (**Figure 3C**), highlighting the utility of these blood markers for risk stratification of African American prostate cancer patients.

To summarize, the serum immune-oncological profile in men of African ancestry may develop in an environment of systemic chronic inflammation and promote metastasis. It is a key finding of our study that suppression of tumor immunity was increased in Ghanaian and African American men, when compared to European American men, and associates with lethal prostate cancer. The current study is the first with a large representation of men of African descent who were profiled for their immune-oncological proteome. With the advent of increasing immunotherapies in the drug development pipeline, studies like ours may inform clinical research on population differences in the immune landscape that need to be considered when designing therapies that exploit the immune response. The elevated peripheral suppression of tumor immunity may have

important implication for guidance of cancer therapy which could particularly benefit African American patients.

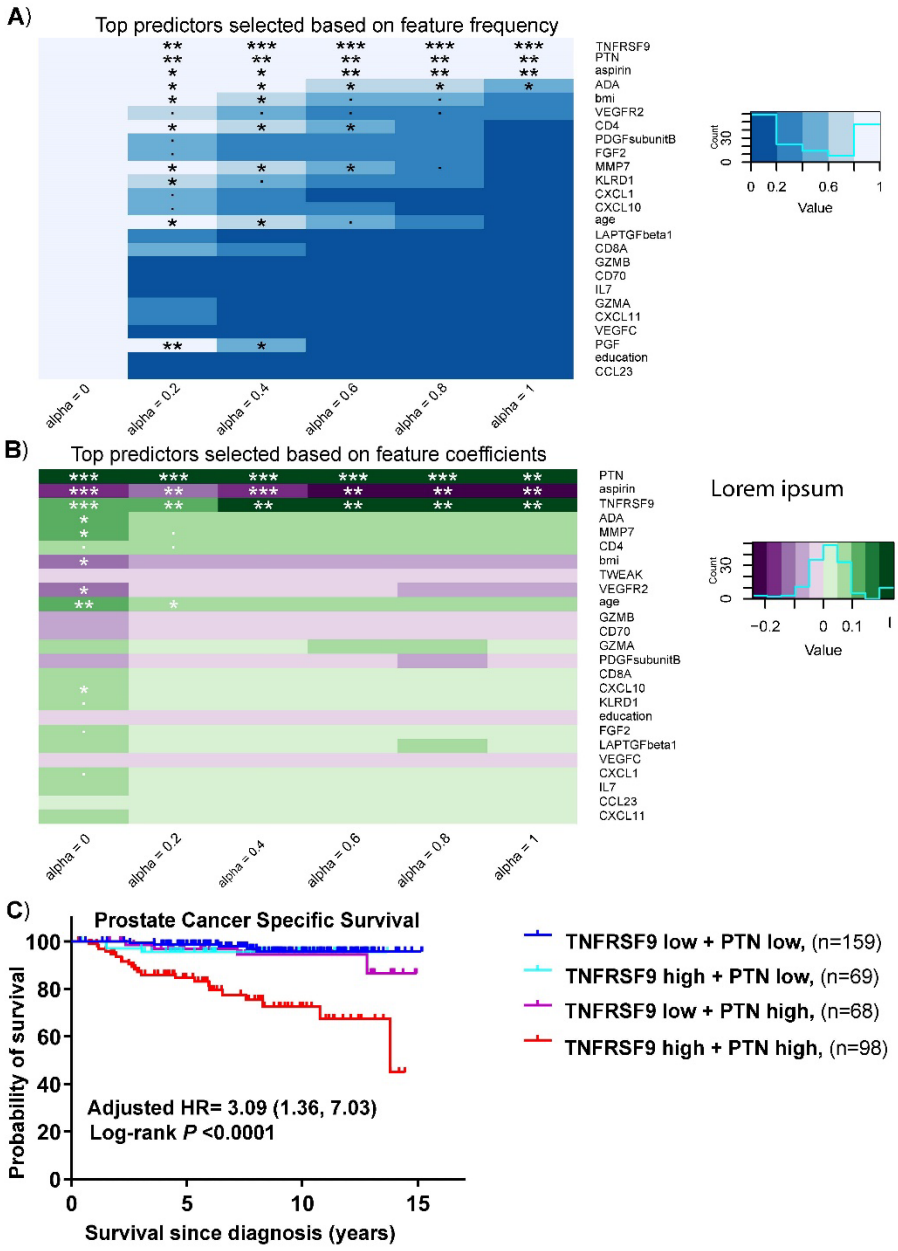


Figure 3. A signature of two serum markers is predictive of lethal prostate cancer in AA patients. Cross-validated, regularized Cox regression models with different elastic net mixture parameters from ridge (alpha=0) to lasso (alpha=1) were implemented to identify a predictive proteomic signature. **(A)** Heatmaps of feature frequencies across alpha. Features were ranked by *P* value for alpha=1. **(B)** Heatmaps of feature coefficients across alpha. Features were ranked by *P* value for alpha=1. **(C)** Kaplan-Meier plot comparing prostate cancer-specific mortality of AA cases with high levels (> median) of both TNFRSF9/CD137/4-1BB and pleiotrophin (PTN) vs. low levels of either or both proteins. Log-rank test was used to determine if there were statistically significant survival differences. Adjusted hazard ratio (HR) compares the risk of prostate cancer mortality for those with high levels of both TNFRSF9 and PTN vs. the remaining AA cases. HR estimates were adjusted for potential confounding factors: age, BMI, education, income, smoking history, diabetes status, aspirin use, treatment, and NCCN risk score. In **A** & **B,** *P* value significance was coded as <0.001 (***), <0.01 (**), <0.05 (*), and <0.1 (.).

Circulating fatty acids and prostate cancer in Ghanaian, African American, and European American men

Besides the analysis of the 82 immune-oncology markers and urinary arachidonic acid metabolites that are the product of COX signaling, **Specific Aim 1** further includes - as a third project - measurements of 24 circulating fatty acids. We proposed to analyze their association with the immune-oncology markers and prostate cancer risks and to investigate if there are differences in these associations between Ghanaian, African American, and European American men. For this reporting period, we have completed drafting a manuscript and submitted it to *Nature Communications* where it is currently under external review. Tsion Minas and Brittany Lord equally contributed to the manuscript. They characterized circulating fatty acid levels in sera from Ghanaian, African American, and European American men. Fatty acids were analyzed individually and grouped by type including total, saturated, *trans*, *cis*-monounsaturated, omega-6, omega-3 fatty acids, and the omega 6:3 fatty acid ratio to explore disease associations by population group.

A summary of the findings in the manuscript **"Circulating fatty acids and prostate cancer in Ghanaian, African American, and European American men"** is provided next seven pages.

Manuscript abstract: High fatty acid intake is thought to increase cancer risk. However, studies of prostate cancer did not reveal consistent associations between circulating fatty acids and the disease. Thus, we examined 24 circulating fatty acids in sera from 2,934 participants, including 1,431 prostate cancer cases and 1,503 population controls from Ghana and the United States using CLIA-certified mass spectrometry-based assays. Participants encompassed cases [658 Ghanaian, 381 African American, 464 European American] and controls (585 Ghanaian, 407 African American, 439 European American) from the NCI-Ghana and NCI-Maryland Case-Control studies. We assessed the association of individual and structurally grouped fatty acids with population groups, socio-demographic and clinical characteristics, diet, the *FADS1/2* gene locus, and prostate cancer. We found that circulating fatty acid levels are different between Ghanaian men and African American and European American men from the United States, particularly *trans*, omega-3 and omega-6 fatty acids. We did not find the same differences when comparing African American with European American men. Yet, *trans* and *cis* monounsaturated fatty acids tended to be higher in European American than African American men among both controls and cases. *FADS1/2* germline genetics and lifestyle explained some of the variation in fatty acid levels, with the *FADS1/2* locus showing population-specific associations. *Trans* fatty acids, namely elaidic, palmitelaidic, and linoelaidic acids, associated with prostate cancer in Ghanaian, African American, and European American men. In summary, we describe population differences in circulating fatty acid levels. *Trans* fatty acids, whose levels were higher in populations from the United States compared to Ghanaian men, associated with increased odds of prostate cancer.

Serum fatty acid levels are different between Ghanaian men and men from the United States
It was the aim of our study to gain knowledge whether serum fatty acid profiles and their association with prostate cancer are different between Ghanaian, African American, and European American men. We utilized two case-control studies with an overrepresentation of men of African ancestry: the NCI-Ghana and NCI-Maryland Prostate Cancer Case-Control Studies. To uncover

global differences in circulating fatty acid profiles between the three groups of men in our study, we applied unsupervised hierarchical clustering to examine how the levels of the 24 fatty acids may associate with population groups (**Figure 4**). We performed this analysis separately for controls (**Figure 4A**) and cases (**Figure 4B**). Among the controls, fatty acid levels tended to cluster by population group, with marked differences between Ghanaian, African American, and European American participants. A similar pattern was observed among cases but the separation into Ghanaian men as one group and the African American and European American men as the other group was not as robust. In another approach to characterize dissimilarities in circulating fatty acids between these groups of men, we investigated differences in fatty acid levels after grouping them into five structurally distinct classes: saturated, *trans*, *cis*-monounsaturated, omega-3, and omega-6 fatty acids. We then compared one group of men to another (i.e., Ghanaian vs. African American, Ghanaian vs. European American men, or African American vs. European American men) and calculated fatty acid level ratios from these comparisons (**Figure 4C)**. The analysis was performed for both controls and cases and particularly highlights the significantly higher concentrations of circulating omega-3 fatty acids in Ghanaian men, among both controls and cases, when compared to European American and African American men (**Figures 4C & D**). In contrast to the omega-3 fatty acid observations, *trans* and omega-6 fatty acid levels were consistently higher in European American and African American men, in both controls and cases, when compared to Ghanaian men (**Figures 4C & D**). We did not find these stark differences in an analysis that compared European American with African American men (**Figure 4E**). However, *trans* and *cis* monounsaturated fatty acids tended to be higher in European American compared to African American men among both controls and cases. Lastly, due to the elevated concentration of circulating omega-3 fatty acids in Ghanaian men, together with their rather low serum concentrations of omega-6 fatty acids, these men had the lowest omega 6:3 fatty acid ratio. Notable, the omega 6:3 fatty acid ratio may have implication for prostate cancer. A low ratio has been associated with a delay in prostate cancer progression by others.

Association of socio-demographic and clinical characteristics with circulating fatty acids

We investigated the association between various socio-demographic and clinical characteristics (age, BMI, education, smoking, diabetes, and aspirin use) with serum levels of circulating fatty acids using a multivariable linear regression model with adjustment for multicomparison analysis (**Figure 5**). Aspirin use and education level had few relationships with circulating fatty acids. Levels of saturated fatty acids tended to be negatively associated with age among European American men while omega-6 fatty acids were negatively associated with age in both African American and European American men (**Figure 5**). Yet, there was no relationship with age among the Ghanaian men. In contrast, BMI strongly associated with most of the circulating fatty acids among these men, but less so among US men. Lastly, smoking showed positive associations with two omega-6 fatty acids, namely docosapentaenoic-n6 and docosatetraenoic acids, among Ghanaian, African American, and European American men.

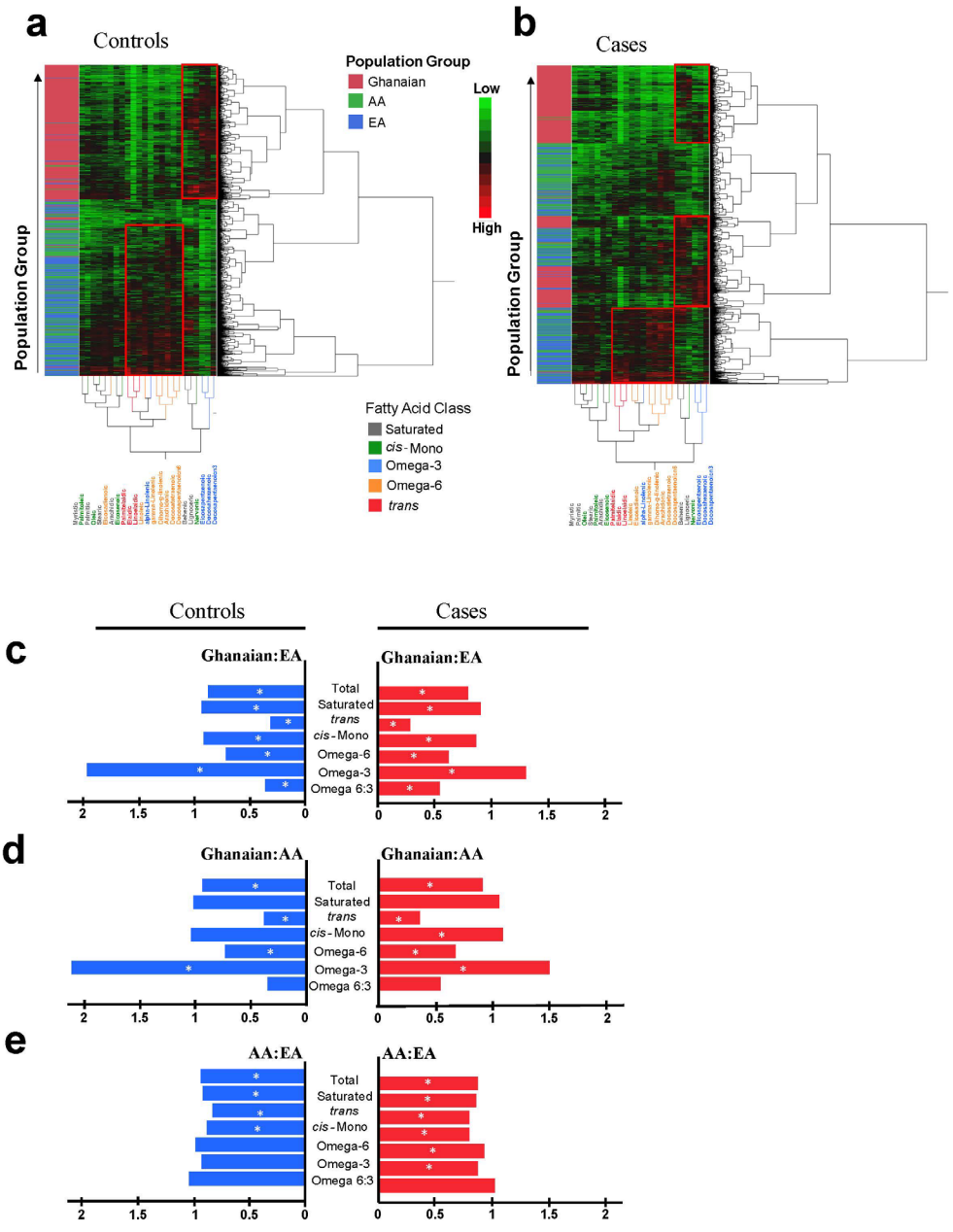


Figure 4. Circulating fatty acid levels by fatty acid class and population group in the NCI-Maryland and NCI-Ghana cohorts. Heatmaps depicting unsupervised hierarchical clustering using absolute (mean, µg/mL) concentrations from individual fatty acids in **(a)** controls (n=1,503) and **(b)** cases (n=1,431) with categorization into three population groups. Green indicates lower concentrations and red indicates higher concentrations for each participant. Samples were labeled by population group and includes Ghanaian (red, n=1,243), AA (green, n=788), and EA (blue, n=903) men. Individual fatty acids were color-coded by fatty acid class. Red boxes indicate areas of fatty acid concentrations that significantly differ by population group; **(c-e)** show ratios of mean concentrations by fatty acid class for controls (blue) and cases (red) comparing **(c)** Ghanaian vs. EA, **(d)** Ghanaian vs. AA, and **(e)** AA vs. EA men. Student's t-test was used for significance testing of differences in fatty acid levels (by class) between population groups (see Supplemental Table 5 for Bonferroni adjustment). *, significantly different. **AA = African American, EA = European American.**

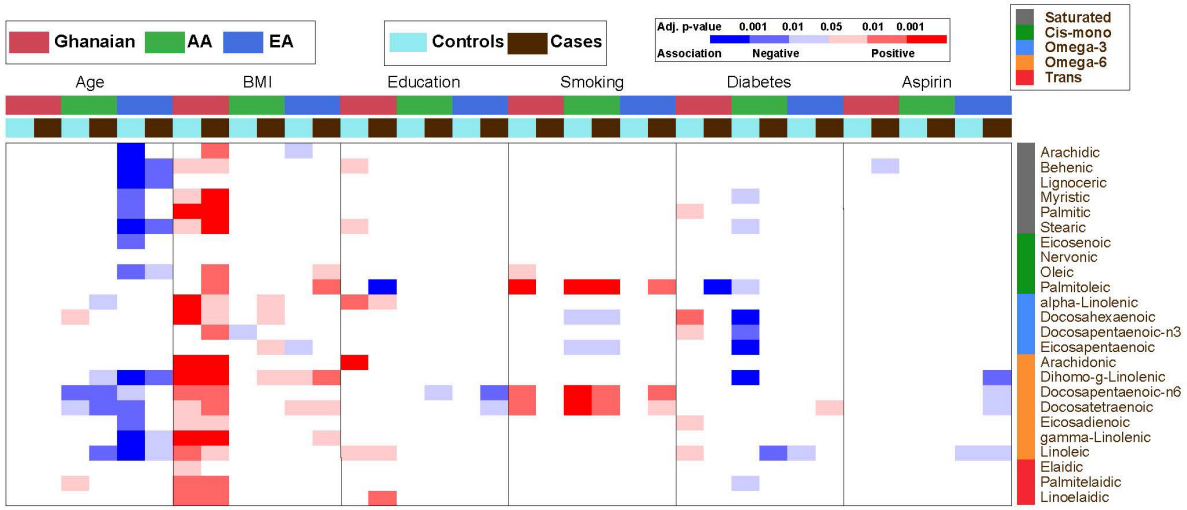


Figure 5. Association of socio-demographic and clinical characteristics with circulating fatty acids in Ghanaian, AA, and EA men with and without prostate cancer. The association of the 24 fatty acids (as continuous variables) with age, BMI, education, smoking, diabetes, and aspirin use was assessed in prostate cancer cases [Ghanaian (n=585), AA (n=407), and EA (n=439)] and controls [Ghanaian (n=658), AA (n=381), and EA (n=464)] using a multivariable linear regression test. *P* values were adjusted for multiple comparisons. An analyte was considered significantly associated with socio-demographic and clinical covariables if the multivariable model yielded a false discovery rate (FDR)-adjusted $P < 0.05$ on the F-statistic. Blue represents a negative association while red represents a positive association. The significance level (FDR-adjusted two-sided *P* value-based) for each association is color-coded. **AA = African American, EA = European American.**

Association of fatty acid desaturase 1 and 2 (*FADS1/2*) locus with circulating fatty acid levels

It has been shown that the levels of circulating fatty acids are partly under genetic control. Thus, we examined how germline genetic factors may relate to fatty acid concentrations in our diverse cohorts focusing on key examples from the published literature. We concentrated our efforts on single nucleotide polymorphisms in the *FADS1/2* locus that have been found to influence fatty acid levels in humans. In European descent individuals, this gene cluster has been shown to have the strongest effect than any genetic loci on the levels of certain fatty acids, namely omega-3 and omega-6 polyunsaturated fatty acids. We selected three SNPs covering the *FADS1/2* locus. We then tested if the levels of each of the 24 fatty acids in blood circulation are influenced by the selected SNPs in our cohort. We found that the SNPs had significant associations with the levels of several omega-6 fatty acids including arachidonic, dihomo-gamma-linolenic, docosatetraenoic (or adrenic), and γ-linolenic acids in European American men, consistent with the literature, explaining up to 19% of their variance in this population. However, with the exception of rs174556 SNP in *FADS1* gene explaining a small fraciton of the variance (i.e. 4.7%) in circulating arachidonic acid level among African American cases, these SNPs did not influence the levels of omega-6 fatty acids in African American or Ghanaian men (**Figure 6**). The observation suggests population differences in the genetic control of circulating fatty acids.

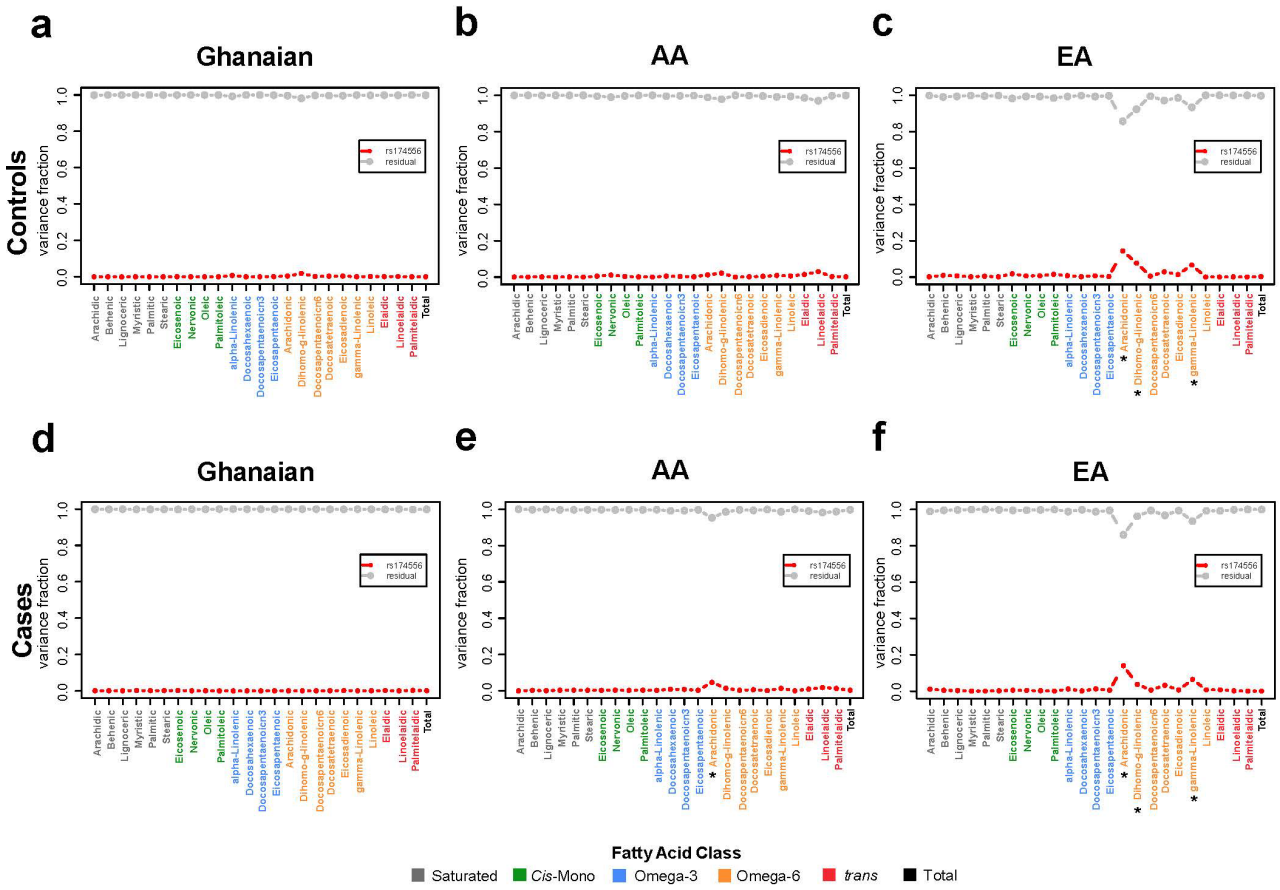


Figure 6. Influence of a single nucleotide polymorphism (SNP) in the *FADS1* gene on circulating levels of fatty acids. Variance analysis for the levels of each of the 24 fatty acids are assessed as a function of a SNP (rs174556) in *FADS1* among **(a)** Ghanaian controls (n=602) and cases (n=511), **(b)** AA controls (n=350) and cases (n=344), and **(c)** EA controls (n=394) and cases (n=362). The red plot represents the proportion of variance in the levels of the fatty acids that can be explained by rs174556 while the grey plot represents the residual variance that remains to be explained by other factors. Asterisks (*) indicate variances in fatty acid levels that are significantly explained by the SNP. Bonferroni-adjusted significance threshold of $P = 0.002$ is used to account for multiple testing. The fatty acids are color-coded to indicate their fatty acid class. **AA = African American, EA = European American.**

Association of fatty acid concentrations with prostate cancer across population groups

We next assessed the association of individual fatty acids and fatty acid classes with prostate cancer in all men and stratified by population group. In all men combined and across the three population groups, only *trans* fatty acids, including elaidic, palmitelaidic, and linoelaidic acids, were consistently associated with increased odds of having prostate cancer. This key finding and a few other associations are summarized in **Figure 7**, emphasizing how the three *trans* fatty acids – elaidic, palmitelaidic, and linoelaidic acids - associate with prostate cancer across

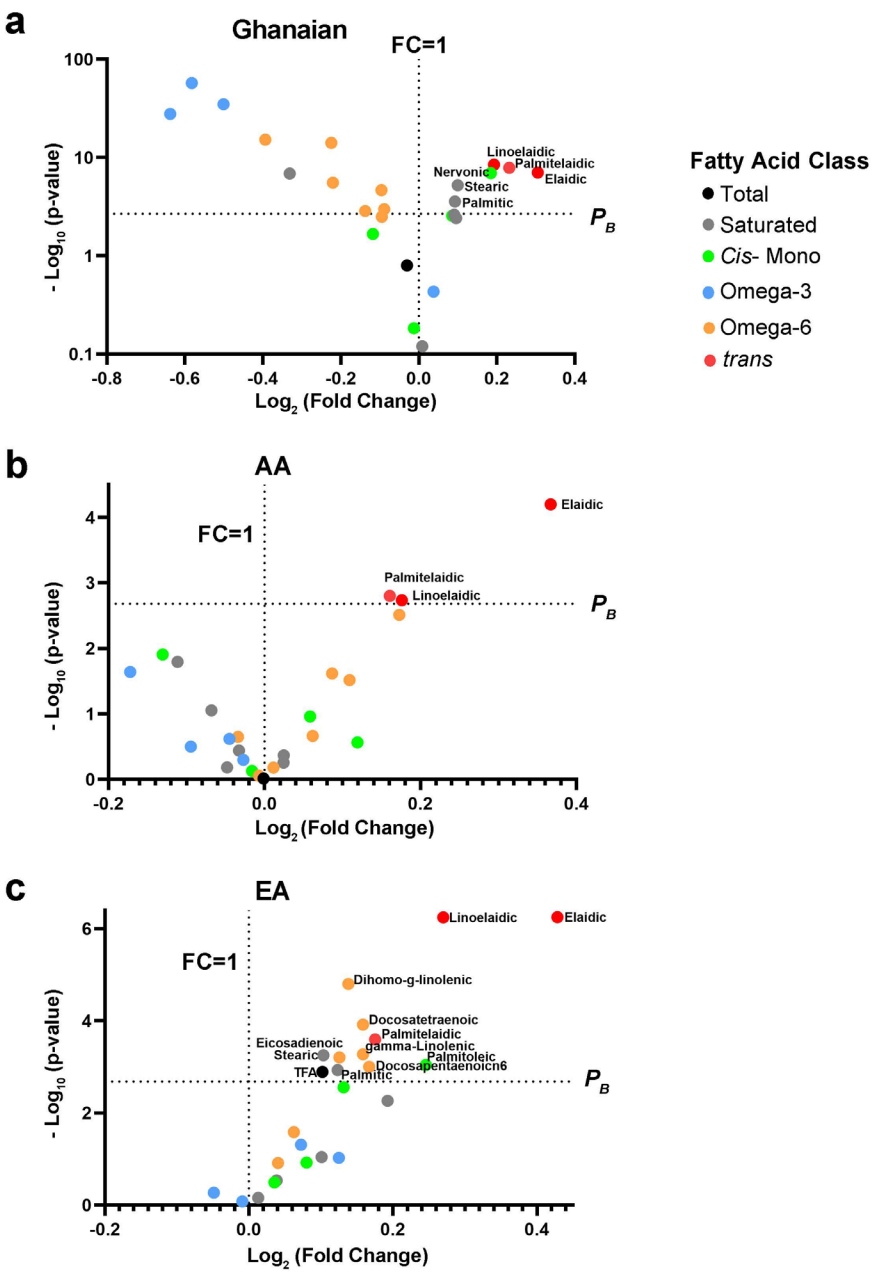


Figure 7. Volcano plot highlighting circulating fatty acids with the most significant differences in their serum levels comparing cases with controls. Shown are Volcano plots defined by fold difference between cases and controls (log2 [fold change]) (x-axis) and the *P* value from a student's t-test (-log10 [p-value]) (y-axis) assessing differences for **(a)** Ghanaian (n=1,243), **(b)** AA (n=788), and **(c)** EA (n=903) men. The horizontal dashed line represents a Bonferroni-adjusted *P* value (P_B) of 0.002 with differences above this line being significant after adjustment for multiple comparisons. The vertical dashed line represents a fold change of 1, meaning the fatty acid levels are equal in cases and controls. Fatty acids found to the right of the vertical dashed line are elevated in the cases whereas those to the left are lower among them when compared to the controls. Only the names of the fatty acids that are significantly elevated in cases are shown. FC= Fold Change, **AA = African American, EA = European American.**

Ghanaian, African American, and European American men. The link between *trans* fatty acids and prostate cancer was further investigated using multivariable logistic regression analyses with adjustments for potential confounders. Here, we divided each *trans* fatty acid concentration into tertiles, termed low, intermediate, and high, and related them to the odds of having prostate cancer across the three population groups (**Table 1**). The approach revealed a significant dose-dependent increase in the odds of having prostate cancer with increasing elaidic, palmitelaidic, and linoelaidic fatty acid concentrations in all three population groups. Notably, although Ghanaian men were found to have the lowest mean *trans* fatty acid concentration when compared to African American and European American men, they still experienced significantly increased odds of developing prostate cancer with increasing *trans* fatty acid levels.

Table 1. Association of *trans* fatty acids (elaidic acid, palmitelaidic acid, and linoelaidic acid) with prostate cancer among Ghanaian, AA, and EA men

	Ghanaian				**AA**				**EA**			
	Control *N* (%)	**Case *N* (%)**	**Multivariable[a] OR (95% CI)**	***P* value**	**Control *N* (%)**	**Case *N* (%)**	**Multivariable[a] OR (95% CI)**	***P* value**	**Control *N* (%)**	**Case *N* (%)**	**Multivariable[a] OR (95% CI)**	***P* value**
Elaidic acid (serum concentration)												
Low	219 (33)	143 (24)	Ref.		154 (40)	99 (24)	Ref.		128 (28)	70 (16)	Ref.	
Intermediate	219 (33)	169 (29)	**1.55 (1.03, 2.35)**	**0.036**	122 (32)	128 (31)	**1.83 (1.25, 2.68)**	**0.002**	160 (34)	134 (31)	1.38 (0.94, 2.03)	0.102
High	220 (33)	273 (47)	**1.92 (1.30, 2.84)**	**0.001**	105 (28)	180 (44)	**2.98 (2.04, 4.34)**	**<0.001**	176 (38)	235 (54)	**2.17 (1.51, 3.12)**	**<0.001**
Trend				**0.001**				**<0.001**				**<0.001**
Palmitelaidic acid												
Low	220 (33)	105 (18)	Ref.		172 (45)	140 (34)	Ref.		109 (23)	75 (17)	Ref.	
Intermediate	218 (33)	178 (30)	1.51 (0.98, 2.32)	0.062	130 (34)	135 (33)	**1.43 (1.00, 2.05)**	**0.047**	153 (33)	139 (32)	1.31 (0.89, 1.93)	0.165
High	220 (33)	302 (52)	**2.26 (1.50, 3.39)**	**<0.001**	79 (21)	132 (32)	**2.55 (1.73, 3.77)**	**<0.001**	202 (44)	225 (51)	**1.49 (1.04, 2.14)**	**0.031**
Trend				**<0.001**				**<0.001**				**0.035**
Linoelaidic acid												
Low	219 (33)	153 (26)	Ref.		178 (47)	125 (31)	Ref.		104 (22)	61 (14)	Ref.	
Intermediate	220 (33)	148 (25)	0.97 (0.64, 1.46)	0.868	117 (31)	158 (39)	**2.17 (1.52, 3.10)**	**<0.001**	165 (36)	129 (29)	1.26 (0.84, 1.88)	0.265
High	219 (33)	284 (49)	**1.52 (1.03, 2.23)**	**0.033**	86 (23)	124 (30)	**2.34 (1.59, 3.45)**	**<0.001**	195 (42)	249 (57)	**1.93 (1.32, 2.82)**	**0.001**
Trend				**0.025**				**<0.001**				**<0.001**

[a]Logistic regression with serum concentrations grouped into tertiles and adjusted for age at study entry, BMI, education, diabetes, smoking history, and aspirin use
AA = African American, EA = European American, OR = Odds Ratio, CI = Confidence Interval
Elaidic acid tertile cutoffs for NCI-Maryland cohort were 13.51 and 22.69 ug/ml
Elaidic acid tertile cutoffs for NCI-Ghana cohort were 3.47 and 4.98 ug/ml
Palmitelaidic acid tertile cutoffs for NCI-Maryland cohort were 3.70 and 5.51 ug/ml
Palmitelaidic acid tertile cutoffs for NCI-Ghana cohort were 2.21 and 3.25 ug/ml
Linoelaidic acid tertile cutoffs for NCI-Maryland cohort were 7.37 and 10.84 ug/ml
Linoelaidic acid tertile cutoffs for NCI-Ghana cohort were 4.09 and 5.34 ug/ml
Bolded data indicate significant associations in the multivariable logistic regression analysis

In summary, our findings point to a population-specific fatty acid profile that may impact prostate cancer development. Elevated serum *trans* fatty acid levels associated with increased odds of prostate cancer in the Ghanaian, African American, and European American men, pointing to *trans* fatty acids as potential prostate cancer risk factors, independent of ancestry or geographic location. Although the extent to which fatty acids are prostate cancer risk factors remains controversial, the relationship between fatty acids, immune function, and prostate cancer, specifically in men of African descent, merits further exploration to determine causal relationships.

Association of Neighborhood Deprivation with Prostate Cancer and Immune Markers in African American and European American Men

Margaret Pichardo analyzed the association of neighborhood deprivation with prostate cancer and immune markers in African American and European American men of the NCI-Maryland cohort – these data do not exist for the NCI-Ghana study. A manuscript with the findings have been submitted to *JAMA Network Open*. This project was not part of the SOW but is closely related to the research objectives under the DoD grant and analyzed the potential influence of neighborhood deprivation on the circulating immune oncology markers that were measured as part of the SOW, supplementing our approach to understand how genetic markers may influence the circulating levels of the 82 immune oncology markers.

Manuscript abstract: African American (AA) men more commonly develop lethal prostate cancer than European American (EA) men. The neighborhood socioeconomic environment may partly explain this disparity but remains understudied.
Objective: Examine associations between neighborhood deprivation, circulating immune-oncology markers, and prostate cancer among diverse men.
Design: Case-control study with up to 15 years of follow up.
Setting: Between 2005-2015, cases were recruited at the Baltimore Veterans Affairs and University of Maryland Medical Centers; controls were identified through the Maryland Department of Motor Vehicle Administration database.
Participants: Cases (n = 976, 489 AA, 487 EA men) and age and race-frequency matched population controls (n = 1,034, 486 AA, and 548 EA men). The median survival follow up was 8.4 years with 246 deaths among cases.
Exposure: 2000 Census-tract neighborhood deprivation index as a standardized score.
Main Outcomes and Measures: Primary outcomes included prostate cancer and all-cause and prostate-specific mortality. Secondary outcomes included the national comprehensive cancer network risk score and serum proteomes for 82 immune-oncology markers examined as proteome-defined pathways.
Results: In the multivariable logistic regression analysis [odds ratio (OR), 95% confidence interval (CI)] with individual socioeconomic status (SES) adjustment, neighborhood deprivation was associated with 46% increased odds of prostate cancer among AA men (1.46, 1.25-1.71), but did not associate with the disease among EA men (0.96, 0.74-1.25). Residing in the most deprived neighborhoods corresponded to 86% higher disease odds (1.86, 1.30-2.71, quintile (Q) 5 vs. Q1) among all men but to a 3-fold increase among AA men (3.00, 1.42-6.35), and no association among EA men (0.72, 0.40-1.28). In Cox regression analyses (hazard ratio (HR), 95% CI), SES-adjusted neighborhood deprivation was associated with an increased all-cause mortality only among AA men (1.27, 1.06-1.54) whereas it was associated with metastatic disease and a 47% increased hazard of a prostate cancer-specific death among all men (1.47, 1.05-2.07). In an analysis restricted to population controls, neighborhood deprivation was associated with increased activity scores of serum proteome-defined chemotaxis, inflammation, and tumor immunity suppression.
Conclusion and Relevance: A deprived neighborhood may predispose AA men to prostate cancer, potentially through its effects on systemic immune function and inflammation.

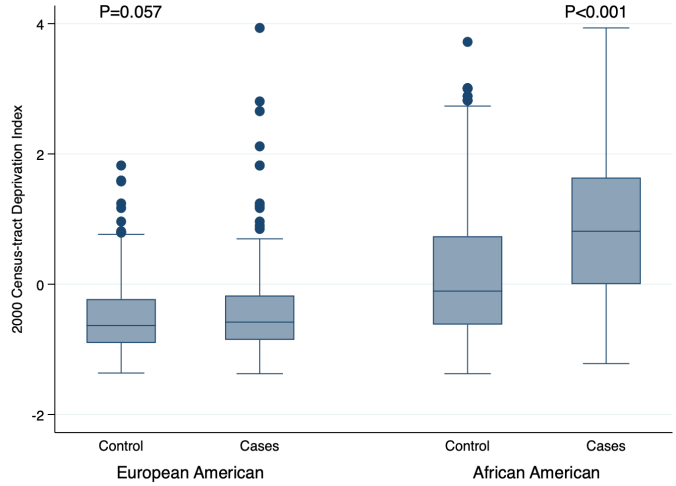
Higher neighborhood deprivation is associated with prostate cancer among African American men

An initial analysis of the relationship between neighborhood deprivation and prostate cancer showed that the deprivation scores were higher for African American than European American men (controls and cases), and highest among African American men who had developed prostate cancer (**Figure 8A**). In multivariable models that combined African American and European American men, neighborhood deprivation was associated with 65% higher odds of prostate cancer (continuous score OR, 1.65, 95% CI, 1.46-1.86) when not adjusted for SES. The association was robust and remained significant after an adjustment by individual SES (model 2; continuous score OR, 1.38, 95% CI, 1.21-1.57), suggesting that neighborhood factors independent of SES increase prostate cancer risk. Further stratification by race/ethnicity revealed a statistically significant association only for African American men (continuous score $OR_{model\ 1}$, 1.94, 95% CI 1.69-2.23; $OR_{model\ 2}$, 1.55, 95% CI, 1.33-1.81), but not for European American men (continuous score $OR_{mode\ 1}$, 1.26, 95% CI 0.99-1.59; $OR_{model\ 2}$, 0.99, 95% CI, 0.77-1.30). When dichotomized at the median, residence in neighborhoods with above median levels of deprivation was associated with higher odds of prostate cancer for all men and African American men, but not European American men, consistent with the findings with the continuous score model. When neighborhood deprivation was examined as quintiles (Q), only residence in the most deprived areas (Q4-5) was significantly associated with increased odds of prostate cancer ($OR_{model\ 1}$, 3.14, 95% CI 2.22-4.43; $OR_{model\ 2}$, 1.88, 95% CI, 1.30-2.75, for Q5 versus Q1). Consistent with the other models, stratification by race/ethnicity revealed an association only among African American men, suggesting a substantially increased cancer risk in the most deprived areas ($OR_{model\ 1}$, 7.39, 95% CI 3.73-14.65; $OR_{model\ 2}$, 3.58, 95% CI, 1.72-7.45).

Higher neighborhood deprivation associates with metastatic prostate cancer

NCCN risk scores are commonly used in clinical practice for risk assessment of prostate cancer. We assigned NCCN scores to all cases and evaluated whether neighborhood deprivation associates with them. The data showed that prostate cancer patients who had developed regional or distant metastatic disease resided in neighborhoods with significantly higher deprivation scores than men with a localized disease (**Figure 8B**). In the multivariable analysis, neighborhood deprivation as a continuous score was associated with elevated odds of regional or metastatic disease, independent of individual SES, for all men ($OR_{model\ 1}$, 1.69, 95% CI, 1.15-2.48; $OR_{model\ 2}$, 1.52, 95% CI, 1.01-2.29), African American men ($OR_{model\ 1}$, 1.64, 95% CI, 1.03-2.62; $OR_{model\ 2}$, 1.65, 95% CI, 0.99-2.74) and European American men ($OR_{model\ 1}$, 2.06, 95% CI, 1.04-4.06; $OR_{model\ 2}$, 1.28, 95% CI, 0.59-2.77). When we performed the same analysis but with dichotomized deprivation scores, a residence in neighborhoods with deprivation above the median was associated with regional or metastatic disease among all men ($OR_{model\ 1}$, 4.27, 95% CI, 1.42-12.82). Yet, this association became attenuated when further adjusted by SES.

A



B

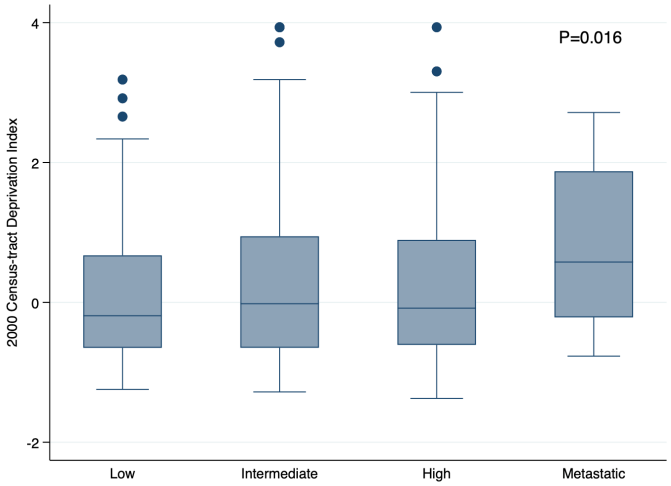


Figure 8. Associations between neighborhood socioeconomic deprivation and prostate cancer and National Comprehensive Cancer Network (NCCN) risk score categories. **A)** Neighborhood deprivation index scores shown as continuous data for controls and cases stratified into European American and African American men. **B)** Neighborhood deprivation Index scores shown as continuous data across 4 NCCN risk score categories defining the clinical presentation of prostate cancer. The error bars in the graphs represent the 95% confidence interval. Mann Whitney U (**A**) and Kruskal Wallis (**B**) tests were applied for significance testing.

Relationship between neighborhood deprivation and serum proteomics-defined biological pathways

We recently reported that immune-oncology marker profiles differ between men of African and European descent, which was partly explained by ancestry (*Minas et al.*, PMID: 35365620). To evaluate if immune-oncology marker profiles might be influenced by the neighborhood

environment, we examined potential associations with six serum proteomics-defined biological pathways based on measurement of 82 immune-oncology markers. We restricted this examination to the control population to exclude a confounding effect of prostate cancer. Applying multivariate ANOVA models, neighborhood deprivation was associated with these pathways, explaining 3.3% to 12.0% of the shared variance in the model (Wilk's lambda = 0.441, F statistic = 10.33, *P* <0.001). These findings were robust to inclusion of individual-level SES variables (Wilk's lambda = 0.395, F statistic = 6.74, *P* <0.001). Additional univariate ANOVA estimates suggested that chemotaxis (R-sq=12.0%, *P* =0.005), inflammation (R-sq=11.9%, *P* = 0.031), and tumor immunity suppression (R-sq=11.12%, *P* =0.018) were the key driving pathways of this association; however, these observations were sensitive to inclusion of SES variables as only chemotaxis remained significantly associated with the neighborhood deprivation index independent of individual-level SES (R-sq=13.9%, *P* =0.008).

Association between neighborhood deprivation and the variance of 82 serum proteins

To further define the effect of neighborhood deprivation on circulating proteins, we assessed the relationship of neighborhood deprivation with serum levels of the 82 individual immune-oncology markers among population controls. In multivariate ANOVA regression, we found evidence of statistically significant associations between neighborhood deprivation and the circulating levels of these serum proteins (Wilk's lambda=0.0092, F statistic=4.47, *P* <0.001). The association remained robust after in inclusion of SES factors (Wilk's lambda=0.0033, F statistic=2.97, *P* <0.001). We examined more comprehensively those proteins for which neighborhood explained >10% of the variance by applying a series of stepwise linear regressions. In a fully adjusted model that included West African ancestry, neighborhood deprivation associated with 53.0%, 52.2%, and 42.7% of the variance for PTN, CXCL5, and CXCL1, respectively, and between 20.4% and 27.1% for LAMP3, CD27, DCN, ADGRG1, MMP12, MMP7, and CXCL9.

Higher neighborhood deprivation associates with mortality after a prostate cancer diagnosis

Lastly, we investigated if neighborhood deprivation may predict the occurrence of lethal prostate cancer, using an analysis of all-cause and prostate cancer-specific mortality. In the model with continuous deprivation scores, higher deprivation was associated with an SES-adjusted 26-28% increased hazard of death from all causes for all men with prostate cancer ($HR_{model\ 1}$: 1.34, 1.16-1.55; $HR_{model\ 2}$: 1.26 1.08-1.47) and for African American men ($HR_{model\ 1}$: 1.31, 1.11-1.54; $HR_{model\ 2}$: 1.28 1.08-1.53), but not for European American men ($HR_{model\ 1}$: 1.37, 1.02-1.84; $HR_{model\ 2}$: 1.16 0.84-1.61). We did not observe this association when deprivation was coded as a dichotomized exposure. Furthermore, neighborhood deprivation as a continuous score was associated with a SES-adjusted 41% increased risk of death from prostate cancer among all men ($CSHR_{model\ 1}$: 1.41, 1.08-0.84; $CSHR_{model\ 2}$: 1.34, 1.01-1.79). A residence in neighborhoods with above median deprivation versus below median deprivation was associated with regional or metastatic disease, versus localized disease, among all men ($CSHR_{model\ 1}$: 2.31, 1.13-4.74; $CSHR_{model\ 2}$: 2.21, 1.00-4.87) (**Table 2**). Fine and Gray regressions estimated similar magnitudes of cause-specific hazard ratios, but the estimates from this secondary analysis did not reach statistical significance (**Table 2**).

Table 2. Association of Neighborhood Socioeconomic Deprivation with Disease Mortality Among Combined African American and European American Men with Prostate Cancer

	Fine and Gray Regression, SHR (95%CI)		Cox Regression CSHR (95% CI)	
Neighborhood Deprivation Index Operationalization	Model 1	Model 2	Model 1	Model 2
Continuous score	1.30 (0.98, 1.74)	1.28 (0.94,1.76)	**1.41 (1.08, 1.84)**	**1.34 (1.01, 1.79)**
Dichotomized				
Below Median	1.00	1.00	1.00	1.00
Above Median	1.95 (0.97, 3.92)	2.09 (0.96, 4.54)	**2.31 (1.13, 4.74)**	**2.21 (1.00, 4.87)**

Notes. SHR, Subdistribution Hazard Ratio; CSHR, Cause-Specific Hazard Ratio, CI, Confidence Interval
Bolded data indicated significant associations in multivariate regression analysis. Competing events in Fine and Gray models were defined as other cancers or death.
Neighborhood deprivation index was derived from principal components analysis using 2000 Census tract for four dimensions of socioeconomic standing: education, employment, occupation, and poverty, standardized to have mean 0 and standard deviation of 1. The index was operationalized as continuous (where higher scores indication greater deprivation) and dichotomized at the median based on control population cut offs (≤ median vs. >median).
Model 1 adjusted for age at study entry (continuous), aspirin use (yes/no), family history of prostate cancer (first-degree relatives, yes/no), diabetes history (yes/no), body mass index at study entry (continuous), self-reported race (not included in stratified analyses, African American, European American), smoking status (current, former, never), treatment (none, surgery, radiation, hormone, combination), and National Comprehensive Cancer Network risk score categories (low, intermediate, high/very high, regional/metastatic). **Model 2** additionally adjusted for education (high school or less, some college, college, professional school, missing), individual income (< $10,000, $10,000–$30,000, $30,000–$60,000, $60,000–$90,000, >$90,000).

In summary, we report that neighborhood socioeconomic deprivation is associated with lethal prostate cancer, independent of individual SES factors. This association was more robust among African American men. We obtained evidence that this relationship might be partly mediated through the effects of poverty on systemic immune-oncology protein expression and activation of signaling pathways related to systemic immune function and inflammation.

Progress with **Specific Aim 2**

For Specific Aim2, Major Task 1 describes as subtask 1 the preparation of DNA samples and shipment to the genotyping facility at Cancer Genomics Research Laboratory, NCI. We completed this subtask within the projected time frame of 8 months. We encountered a problem with the amount and quality of germline DNA that we sent to this genotyping core facility – as mentioned in 2019 annual reports. This problem has been resolved - after we re-extracted germline DNA from previously collected blood monocytes and buccal cells (done by Tsion Minas, Tiffany Dorsey, Anuoluwapo Ajao). The NCI Cancer Genomics Research Laboratory then performed genome-wide genotyping with the Infinium HumanOmni5-Quad BeadChip for a total of 1806 subjects in the study (subtask 2), corresponding to the numbers of 900 cases and 900 controls that we proposed to genotype per our Statement of Work for the award. The raw genotyping data were then moved to the analysis team at the Cancer Genomics Research Laboratory for quality control including "data cleaning". The subsequent inspection of the data by the team indicated that the raw data were not of optimal quality for a significant subset of samples. Some of them could be re-genotyped, yielding finalized and high-quality genotyping data for 1450 men (694 African American, 756 European American). This number of genotyped men is lower than reported in our last annual report because the previous number contained QC duplicates – which was unknown to us. The genotyping data for these 1450 men have now been deposited in the public database, dbGaP, under submission number phs002939.v1.p1. We submitted the data together with

Specific Aim 2: Assess whether germline genetic variants are associated with immune-inflammation markers and PCa using a genome-wide association approach (GWAS).		NCI
Major Task 1: Perform GWAS genotyping with Infinium HumanOmni5-Quad BeadChip	Months	
Subtask 1: Prepare DNA samples for shipment • Obtain IRB approval covering the NCI-Maryland Prostate study • Aliquot DNA samples from 900 cases and 900 controls, perform quality control, and ship to Cancer Genomics Research Laboratory, DCEG/NCI	1-8	Ambs, Minas
Subtask 2: Genotyping with Infinium HumanOmni5-Quad BeadChip, covering more than 4 million SNPs • Perform genotyping and preliminary data analysis at Cancer Genomics Research Laboratory. Receive data and add to database.	8-14	Ambs, Minas, Tang
Major Task 2: Statistical analysis		
Assess whether germline genetic variants are associated with immune-inflammation markers and PCa. Perform a combined analysis of the NCI-Maryland and NCI-Ghana Prostate studies. Genotyping data for the NCI-Ghana Prostate study exist already.	14-24	Ambs, Cook, Kelly, Minas, Tang

descriptors of the study population. The release date of the data is set for February 2023. This task completes the laboratory-based tasks for **Specific Aim 2**.

Specific Aim 2, Major Task 2.

After completion of Major Task 1, we continued to work with the raw genotyping data from the NCI-Maryland study (694 African American with 350 controls and 344 cases; 756 European American with 394 controls and 362 cases) and 1113 men from the NCI-Ghana study, with 4,426,323 SNPs passing study specific quality control for the NCI-Maryland study and 2,786,417 SNPs for NCI-Ghana study. Genotyping data for the NCI-Ghana study have previously been generated using another funding source. SNP coverage from the Infinium HumanOmni5-Quad BeadChip SNP genotyping array was increased through imputation using the TOPMED Imputation Reference Panel using the Michigan Imputation Server (https://topmed.nhlbi.nih.gov/), resulting in 46,919,584 assigned variants for NCI-Maryland and 307,510,644 variants for NCI-Ghana studies. 38,317,621 variants in the NCI-Maryland study and 298,483,657 variants in the NCI-Ghana study were removed due to falling below the minor allele threshold of 0.05, resulting in a total of 8,601,963 variants contained within the NCI-Maryland dataset and 9,026,987 variants contained within the NCI-Ghana dataset.

After Amy Zhang left our group for medical school, Hunter Schaumloffel took over the future work with the generated datasets and will conduct the quantitative trait analysis linking individual genotypes to the 82 immune-oncology markers, with guidance by our new data scientist, Huaitian Liu, and help from Wei Tang who has experience with this type of analysis. Initially, Hunter will generate Manhattan and Circos plots for high priority protein markers and for assigned pathways based on grouping of proteins into functional groups defining these pathways (e.g., chemotaxis, activation/inhibition of tumor immunity). He will generate the plots separately for Ghanaian, African American, and European American men to assess if levels of individual proteins or pathway activities are significantly associated with germline markers at the $P < 5 \times 10^{-8}$ threshold level among them, and whether there are differences between the population groups. After initially focusing on high priority protein markers like pleiotrophin and TNFSR9, the analysis will be extended to all 82 immune-oncology markers to fully assess the germline genetic control of the abundance of these markers in serum of men with and without prostate cancer. Following the statistical analysis, a literature review will be needed to gain an understanding of the observed relationships. In addition, the functional context of associated SNPs must be studied at the genome level to gain an understanding why a SNP may regulate the expression of an immune-oncology marker. We are hopeful that these analyses can be completed by mid-2023 and then packaged into one or two research papers. Hunter will also help completing a study started by Amy that investigated the germline genetic control of circulating fatty acid levels. This study found robust differences in the genetic control between African ancestry and European ancestry men that have not been reported by others. There are also plans to incorporate the genotyping data into a study of the viral infection history of men and how this history may relate to prostate cancer risks and germline genetics. This study includes the application of VirScan, which was developed by Stephen Elledge of Harvard Medical. It is a powerful technology to determine the viral exposure history of an individual. It uses a human virome peptide library displayed by bacteriophages to

screen for antivirome antibodies in blood samples. Theoretically, the method should allow for detection of any viral infection in a subject that triggered an immune response in the past. The VirScan technology has been established in our laboratory. We are applying this technology to the NCI-Maryland and NCI-Ghana studies and will investigate how germline genetic variants are associated with the infection history of men with and without prostate cancer.

Progress with **Specific Aim 3**

For Specific Aim 3, management of Major Tasks 1 & 2 primarily falls under the responsibility of Dr. Clayton Yates, the co-investigator at Tuskegee University. Yet, the Tuskegee and NCI research teams have been working on these tasks together, in close collaboration, involving Tiffany Dorsey, Wei Tang, and now Huaitian Liu from the Ambs group.

Specific Aim 3: Determine the prevalence of an immune-inflammation signature in prostate tumors of men of European and African ancestry, and evaluate how this signature relates to other gene expression patterns, genomic alterations, and chromatin structure in these tumors, and to patient characteristics.		NCI	TU
Major Task 1: Perform RNA sequencing (RNA-seq), whole exome sequencing (exome-seq; WES), and Assay for Transposase-Accessible Chromatin with high throughput sequencing (ATAQ-seq) for 250 tumors	Months		
Subtask 1: Prepare RNA and DNA for sequencing • Obtain IRB approval and MTAs covering the two study sites, NCI and University of Tuskegee. • Receive tumors from NCI (50 African-American and 50 European-American patients). Isolate RNA and DNA from NCI tumors and tumors from 150 Nigerian patients. Process all tumor tissues, including macro- and microdissection of tumor epithelium as needed. • Perform quality control of RNA and DNA	1-8(10)	Ambs	Grizzle, Wang, Yates
• Ship RNA and DNA samples to the sequencing facility at Leidos Biomedical Research, Inc., Frederick National Laboratory for Cancer Research. Facility will perform RNA-seq, exome-seq, and ATAQ-seq. Obtain raw output data together with quality control assessment data. Perform initial quality control analysis of datasets.	8(10)-14	Ambs, Tang	White, Yates

As previously reported, to obtain cores of tumor and adjacent non-cancerous tissue from FFPE tumor blocks for RNAseq and WES, as specified in the SOW, we asked the University of Maryland Department of Pathology for help with this task. RNAseq is a technology used to investigate the transcriptome whereas WES is used for mutational analysis of tumors. Under a contract, the

Department of Pathology assigned a pathologist who identified areas in the tissue blocks that could be processed into cores of tumor tissue and paired adjacent non-cancerous tissue for RNA and DNA extraction. Nevertheless, many of the tumor tissues from the Nigerian and University of Maryland prostate cancer cases did not present with enough tumor tissue so that cores could be obtain. This is a well-known problem with human prostate tumor biospecimens where tumor tissue is often less than 20%. When cores could be taken, they were then obtained by an experienced laboratory technician and sent to the NCI-Leidos Molecular Histopathology Laboratory. This laboratory extracted total RNA and DNA using a previously established protocol that allows further processing of the RNA for RNA sequencing and DNA for whole exome sequencing. A total of 399 cores (101 Nigerian tumors and 61 adjacent non-cancerous tissues; 62 African American tumors and 58 adjacent non-cancerous tissues; 60 European American tumors and 57 adjacent non-cancerous tissues) were processed. The RNA and DNA samples were then sent to the service provider, HudsonAlpha Institute for Biotechnology, a leader in applied genomics technologies. However, HudsonAlpha could not work with the isolated RNA and DNA samples as those failed its QC requirements. The company has a proprietary RNA extraction protocol for FFPE tissues and proceeded with this protocol that isolates RNA and DNA in one approach. Using it, we indeed obtained good quality RNAseq data for 77 prostate tumors from Nigerian men, 50 from African-American men, and 40 from European-American men. However, to our surprise, the extracted DNA failed quality control measures. The poor performance of DNA in the WES pipeline at HudsonAlpha was unexpected and may have something to do with the approach by this provider – optimizing RNA-seq results at the expense of WES results. To summarize, although HudsonAlpha delivered good quality RNA-seq data for 167 prostate tumors, we are struggling in obtaining good quality WES data for a similar number of tumors. Thanks to a second approach using NCI-Leidos for WES, previously initiated with NCI financial support, the Yates group and collaborators obtained good WES data for a cohort of 45 advanced stage, treatment-naïve Nigerian primary prostate cancer tumors. The WES data were then analyzed by Jason White, with Jason being the lead investigator. Jason had access to the NIH Biowulf computing cluster for his computational work and regularly consulted with Wei Tang from the Ambs group about his analysis approach. Findings from this study were recently published in the peer-reviewed AACR journal, *Cancer Research Communications*. We provide a copy of this Online First publication under Appendices. The publication is a milestone achievement under **Specific Aim 3.** Below is the manuscript abstract with the key findings. This is the first study utilizing whole-exome sequencing to characterize prostate tumors from Nigerian men.

Manuscript abstract: In this study, we used whole-exome sequencing of a cohort of 45 advanced stage, treatment-naïve Nigerian (NG) primary prostate cancer tumors and 11 unmatched nontumor tissues to compare genomic mutations with African American (AA) and European American (EA) in The Cancer Genome Atlas (TCGA) prostate cancer database. NG samples were collected from six sites in central and southwest Nigeria. After whole-exome sequencing, samples were processed using GATK best practices. *BRCA1*(100%), *BARD1* (45%), *BRCA2* (27%), and *PMS2* (18%) had germline alterations in at least two NG nontumor samples. Across 111 germline variants, the AA cohort reflected a pattern *[BRCA1* (68%), *BARD1* (34%), *BRCA2* (28%), and *PMS2* (16%)] similar to NG samples. Of the most frequently mutated genes, *BRCA1* showed a statistically

($P \leq 0.05$) higher germline mutation frequency in men of African ancestry and increasing variant frequency with increased African ancestry. Disaggregating gene level mutation frequencies by variants revealed both ancestry-linked and NG-specific germline variant patterns. Driven by rs799917 (T>C), *BRCA1* showed an increasing mutation frequency as African ancestry increased. BRCA2_rs11571831 was present only in MAA, and BRCA2_rs766173 was elevated in NG men. A total of 133 somatic variants were present in 26 prostate cancer–associated genes within the NG tumor cohort. *BRCA2* (27%), *APC* (20%), *ATM* (20%), *BRCA1* (13%), *DNAJC6* (13%), *EGFR* (13%), *MAD1L1* (13%), *MLH1* (11%), and *PMS2* (11%) showed mutation frequencies > 10%. Compared with TCGA cohorts, NG tumors showed statistically significant elevated frequencies of *BRCA2, APC*, and *BRCA1*. The NG cohort variant pattern shared similarities (cosign similarities $\geq$0.734) with Catalogue of Somatic Mutations in Cancer signatures 5 and 6, and mutated genes showed significant ($q < 0.001$) gene ontology (GO) and functional enrichment in mismatch repair and non-homologous repair deficiency pathways. Here, we showed that mutations in DNA damage response genes were higher in NG prostate cancer samples and that a portion of those mutations correlate with African ancestry. Moreover, we identified variants of unknown significance that may contribute to population-specific routes of tumorigenesis and treatment. These results present the most comprehensive characterization of the NG prostate cancer exome to date and highlight the need to increase diversity of study populations.
Significance: Men of African ancestry have higher rates of prostate cancer incidence and mortality, however, are severely underrepresented in genomic studies. This is the first study utilizing whole-exome sequencing in NG men to identify West African ancestry-linked variant patterns that impact DNA damage repair pathways.

Future efforts under **Specific Aim 3** will focus on the analysis of the existing RNAseq data from the 177 prostate tumors. This analysis should yield a second manuscript in 2023. The lead person for this analysis will be Isra Elhussin. Huaitian Liu in the Ambs group will join her with this task.

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.

In the past 12 months, the award provided training opportunities to the following fellows: Jason White and Isra Elhussin (both Tuskegee University and mentored by Clayton Yates); Tsion Minas, Maeve Bailey-Whyte, Brittany Lord, Amy Zhang and Margaret Pichardo (all NCI-associated and

mentored by Stefan Ambs). A new fellow, Hunter Schaumloffel, joined our team very recently. He is a volunteer working on a 6-month research project being the thesis project to obtain a bachelor's degree.

Jason White and Isra Elhussin are both PhD students at Tuskegee University and on track to graduate in November of 2022. The DoD grant provided the basis for their graduate work and thesis projects. Both obtained extensive training in data science and have gained the experience to independently perform advanced data analysis using various software tools including R programming. For them, the award was critically important in advancing their analysis skills and career development towards earning a PhD degree. Their research was further enabled because they had access to the NIH Biowulf Cluster for high-performance computing, as part of the collaboration between the Ambs and Yates laboratories. Both were co-mentored by two staff scientists and data science experts in the Ambs group, namely Wei Tang and Huaitian Liu. Here, one can certainly say that the award has made a significant impact in advancing the career of two junior scientists at Tuskegee University. I had the opportunity of discussing with them their career options and provided career advice, in addition to Dr. Yates' mentorship. Both expressed their desire in pursuing careers as scientists with Jason White expressing interest in coming to the NIH as a postdoctoral researcher. Isra Elhussin will pursue a postdoctoral research opportunity with Dr. Yates at Johns Hopkins University. She is a physician scientist with a minority background who wants to stay in biomedical research focusing on cancer. Her acquired skill set, being a physician with data science skills, should set her apart from others and should provide her with many opportunities for an academic research career.

Tsion Minas, Maeve Bailey-Whyte, and Amy Zhang completed their training in the Ambs laboratory and have moved into new career opportunities. Tsion Minas, a postdoctoral fellow, departed the Ambs lab at the beginning of 2022 and is now a Fulbright U.S. Scholar in Ethiopia, studying why human papillomavirus infection is spontaneously cleared in most women but persists in others, increasing their risk for cervical cancer. Her success was highlighted by the NCI (https://ccr.cancer.gov/news/article/ccr-postdoctoral-researcher-awarded-fulbright-to-study-hpv-and-cervical-cancer-in-africa). Tsion is the second recipient of a Fulbright grant among the trainees supported by this DoD award after Anuoluwapo Ajao, a postbaccalaureate fellow mentored by Tsion, received a Fulbright Research Fellowship in 2021, to study risk factors for breast cancer in Nigeria. It is Tsion's plan to become an independent researcher with a focus on cancer in Africa. I have no doubt that she will be very competitive in obtaining a tenure-track faculty position in the United States. She has been very productive as a postdoctoral fellow with her research studying prostate cancer in men of African descent. This research was largely funded through the award. Here again, the DoD award was instrumental to a trainee's success. Maeve Bailey-Whyte, a NCI Cancer Prevention Fellow, returned to Ireland in 2021 where she is competing for a lecturer position in academia. She is continuing her prostate cancer work and is applying for grant funding. She is currently in a transitional position. Her work showing that thromboxane A2 formation is a risk factor for lethal prostate cancer among African American men, a research project that was entirely funded by the DoD award and published in *JNCI* (PMID: 34264335), was covered as a 2021-22 research highlight as part of the NCI's Center for Cancer Research Milestones publication (https://ccr.cancer.gov/news/milestones-2022/a-new-application-for-an-old-medicine), a great

achievement. Amy Zhang was a postbaccalaureate fellow who performed data analysis with the genome-wide genotyping dataset that was generated with funding from this award, linking germline variants to circulating levels of fatty acids and immune-oncology markers to study their control by germline genetics. Although Amy could not finish all parts of the analysis, she generated a finalized dataset with imputed genotypes that was submitted to dbGaP for curation in a public database. This dataset covering the NCI-Maryland prostate cancer study and the genotyping dataset previously generated for the NCI-Ghana prostate cancer study are now in the hand of a new fellow, Hunter Schaumloffel. His contributions are aimed to get the data analysis into the stage of being manuscript-ready. Amy's work familiarized her with the principles of a genome-wide association study and quantitative trait analysis and provided her with new skills in using the R software. Like Anuoluwapo Ajao, Amy successfully competed for acceptance into a MD/PhD program and has joined the University of North Carolina Medical School. I would argue that the experience that Amy gained by being involved with research sponsored by the award contributed to this success.

Brittany Lord is a NCI Cancer Prevention fellow who partnered with Tsion Minas and Amy Zhang in the investigation of circulating fatty acids and their relationship with germline genetics and prostate cancer in Ghanaian, African-American, European-American men, making great progress. Findings from this work have now been submitted to *Nature Communications*, with the manuscript being sent out to external reviewers. Brittany is working on a second manuscript that examines the relationship of germline variants in the *DARC* locus with prostate cancer among men of African ancestry. The very productive partnership with the other fellows gives Brittany the opportunity to strengthen her analysis skills as she has only recently completed her epidemiology training as part of her earning a Master's in Public Health degree in 2019. In their research, Brittany and Tsion were learning to use new methods for data analysis and display. Brittany presented a virtual poster of their findings at the 2021 AACR conference on the Science of Cancer Health Disparities entitled "Determining the association between circulating fatty acids, immune oncological markers, and prostate cancer risk in a diverse cohort". Brittany received an AACR Scholar-in-Training-Award for this submission. She also received NCI Fare and WSA Scholars awards. In addition, she received the Eddie Méndez Scholar Award from the Fred Hutchinson Cancer Research Center. The award recognizes outstanding postdoctoral fellows who are conducting cancer research and are from backgrounds that are underrepresented in science. I predict that Brittany will move into a tenure-track faculty position within the next 12 months.

Lastly, Margaret Pichardo began to be involved with research related to this award, starting in 2020. She has been instrumental in establishing a research project that collected geospatial neighborhood data for the NCI-Maryland Prostate Cancer Study. She obtained census tract data for 1990, 2000, and 2010 and developed a neighborhood deprivation index. The linkage of census tract data to study participants in the NCI-Maryland Prostate Cancer study became a multi-year effort. Her research explores the relationship of the neighborhood deprivation index with prostate cancer risk and outcomes among African American men, and how this index may influence immune-oncology marker expression and the immune response related to prostate cancer. Her research has been very productive, and within less of a year she was able to package the findings into a research manuscript that is currently under review with *JAMA Network Open*. Margaret is a

rising star. She completed her PhD in epidemiology at Yale in 2021 and is now a resident at the University of Pennsylvania after finishing medical school at Howard University. It is Margaret's career plan to become a physician scientist. Her current research project, partly made possible by the award, is aimed to help her achieving this goal. Margaret has received several awards including the AACR Minority Scholar in Cancer Research award.

How were the results disseminated to communities of interest?

Tsion Minas gave her first data presentation at the 2019 AORTIC Cancer in Africa conference in Maputo, Mozambique, based on findings from this grant. She was selected for a Lightning Talk entitled: "Distinct circulating immune-oncological markers in men of African descent". She was also selected to be on the African Cancer Leadership Institute associated with this international cancer conference and received a grant to participate at this conference. In addition, Tsion presented more of her findings as a speaker at an NCI Interlaboratory Seminar and more recently at LHC seminars. In 2021, Tsion gave 10 minutes invited presentations at the 3rd NCI Symposium on Cancer Health Disparities and in the Hot Topic session at the 2021 AACR virtual conference on the Science of Cancer Health Disparities entitled "Blood levels of TNFRSF9 and PTN predict lethal prostate cancer among African-American men". She also gave a short presentation on this subject at the 2021 AORTIC virtual conference in November. Maeve Bailey-Whyte presented a poster at the AACR conference on the Science of Cancer Health Disparities, October 2-4, 2020. She presented findings from the urinary eicosanoid metabolites study. Her abstract was entitled "High urinary thromboxane B2 associates with aggressive prostate cancer and inversely correlates with aspirin use". She had an additional poster presentation at the 2021 AACR virtual conference on the Science of Cancer Health Disparities entitled "Association of urinary PGE-M with all-cause mortality in men with prostate cancer is influenced by aspirin use", for which she received an AACR Student and Early Career Investigator Scholarship. Maeve had two additional presentations: an oral abstract at the 2021 AORTIC virtual conference and an invited speaker presentation at the 7th Biennial Science of Global Prostate Cancer Disparities in Black men Conference, November, 2021. Brittany Lord presented a poster at the 2021 AACR virtual conference on the Science of Cancer Health Disparities and gave a short presentation on the same subject ("Exploring the relationship of circulating fatty acids and immune-oncological markers with prostate cancer in a diverse cohort of men") at the AORTIC 2021 virtual conference. Stefan Ambs was an invited speaker at the 6th Biennial Science of Global Prostate Cancer Disparities in Black men Conference in 2020 and at the 3rd NCI Symposium on Cancer Health Disparities in 2021, both virtual meetings. The presentations focused on results from this grant ("An investigation of how inflammation may underlie the excessive burden of prostate cancer in men of African ancestry"). In addition, Dr. Ambs presented work related to this grant in invited talks at the NCI Laboratory of Genitourinary Cancer Pathogenesis seminar series, the NIH Academy, and the NCI TRACO lecture series, to

support health disparity research. He was also invited to Grand Rounds at the University of Maryland (Frontiers in Oncology) where he presented the prostate cancer research related to this grant. More recently, he presented the newest findings at the 2022 CCR-DCEG Cancer Health Disparities workshop and at the Schoodic Scientific Workshop (2022 Redox and Metabolism in Inflammation and Disease Workshop, October 2-6) in Maine and has been invited to give a presentation at the Association of Health Care Journalists Fellowship meeting. The lecture will focus on findings related to the grant. Lastly, our research was highlighted by the NCI at https://ccr.cancer.gov/news/milestones-2022/a-new-application-for-an-old-medicine, NIH at https://irp.nih.gov/blog/post/2022/07/african-ancestry-may-influence-immune-response-to-prostate-cancer, and by The Geneva Foundation on their website at https://genevausa.org/news/story/reducing-lethal-prostate-cancer-in-men-with-african-descent/ As one can see from the list of presentations, the fellows have been highly successful in disseminating the research findings from this grant at various national and international meetings, giving many invited talks and receiving awards for their contribution.

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

The main objective for the last year of the grant will be writing up and submitting manuscripts to peer-reviewed scientific journals for publication. It is the key metric for fellows to advance their scientific career and for principal investigators to be successful with the scientific review of their achievements and to secure future funding.

4. IMPACT:

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

Our prostate cancer studies address the key challenge to develop strategies to prevent lethal cancer. This research will also provide new knowledge about differences in levels of inflammation-related and immune-modulatory factors between African American, African, and European American men, and how these differences can be explained by either ancestral factors or the environment, and how these factors contribute to lethal prostate cancer. These novel studies address DoD's mission to reduce health disparities that affect a race/ethnic group. Furthermore, our efforts to establish a rationale for the use of anti-inflammatory drugs and immune modulators as therapeutics to reduce lethal disease with a focus on African American men aligns with the challenge to identify novel targets and pathways for treatment to reduce lethal prostate cancer.

Research interest in the distinct immune-inflammation signature that is prevalent in prostate tumors of African American men (Wallace et al. 2008; PMID: 18245496) has surged with the recent finding by Sartor and colleagues that the cancer vaccine, Sipuleucel T, may specially improve survival of African American men with metastatic castration-resistant prostate cancer (PMID: 32111923). The presence of an embedded interferon signature in these tumors that our group first described (Wallace et al. 2008; Tang et al. 2018, PMID: 18245496,30012562) would suggest that African American prostate cancer patients may respond better to cancer vaccines than European American men because of this signature. We believe that the research questions that we pursue under this award could have important implications for immune therapy in African American men with otherwise lethal prostate cancer. Furthermore, tumors with an interferon-stimulated gene signature are susceptible to inhibition of adenosine deaminase acting on RNA (ADAR1), as was recently discovered (PMID: 30575730). These inhibitors are being developed for clinical use by pharmaceutical industry and may have therapeutic activity particularly in African American men with prostate cancer as their tumors frequently harbor an interferon signature.

What was the impact on other disciplines?

We are not specifically aware of the impacts that our research may have had on other disciplines. Our description of an inflammation signature in African American men with prostate cancer has reinforced the hypothesis that an environmentally-induced systemic inflammation may affect the African American community more so than other communities. Inducers of this inflammation may include neighborhood factors and persistent exposure to racism. This inflammation signature may well be a cause of other acute and age-related diseases that are more prevalent in the African American community than other US communities. With our publication by *Minas et al.* in *Nature Communications* (PMID: 35365620), we made our immune-oncology marker data publicly available. As was recognized by the reviewers, this could be an important resource for others, in disciplines beyond prostate cancer, as it covers 82 circulating proteins measured in almost 3000 Ghanaian, African American, and European American men. Lastly, we deposited the genome-wide genotyping data for the NCI-Maryland prostate cancer study in dbGaP (phs002939.v1.p1). The release date of the data is set for February 2023. This large-scale germline genetic dataset, covering 4 million SNPs in 1450 men, together with the measurements of immune-oncology markers and circulating fatty acids can be a rich resource for many researchers, beyond prostate cancer.

What was the impact on technology transfer?

Nothing to Report.

What was the impact on society beyond science and technology?

The main impact on society is making it aware of the existence of persistent cancer health disparities in the United States, what causes them, and how we can reduce them and their detrimental impact on minoritized and underserved communities. It is my experience that the research funded by this award achieves this goal. As one example, I have been invited to give a lecture at the Association of Health Care Journalists Fellowship meeting. The lecture will focus on findings related to the grant and will emphasize the excessive burden of cancer that is experienced by African Americans and other underserved communities. Furthermore, I have been invited to a data science and health disparities panel discussion on the Federal Insights Exchange (https://web.cvent.com/event/d97e56a7-c5f9-4f3e-8dc0-988cdf1ba618/summary), discussing how data science can help overcome health disparities and how partners from industry can support these efforts.

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

Nothing to report for the past 12 months. In the upcoming 12 months, we should be able to wrap up the remaining analysis parts and submit the resulting findings to peer-reviewed scientific journals.

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

Nothing to report for the past 12 months. Any delays have previously been reported.

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

Nothing to Report

6. PRODUCTS:

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

  Journal publications.

Nine publications in PubMed:

Minas, T.Z., Candia, J., Dorsey, T.H., Baker, F., Tang, W., Kiely, M., Smith, C.J., Zhang, A.L., Jordan, S.V., Obadi, O.M., Ajao, A, Tettey, Y., Biritwum, R.B., Adjei, A.A., Mensah, J.E., Hoover, R.N., Jenkins, F.J., Kittles, R., Hs.ing, A.W., Wang, X.W., Loffredo, C.A., Yates, C., Cook, M.B., and Ambs, S. Serum proteomics links suppression of tumor immunity to ancestry and lethal prostate cancer. Nat Commun., 13: 1759, 2022. PMID: 35365620

Jenkins, F.J., Minas, T.Z., Tang, W., Dorsey, T.H., and Ambs S. Human herpesvirus 8 infection is associated with prostate cancer among IFNL4-ΔG carriers. Prostate Cancer Prostatic Dis. 2022 Apr 25. doi: 10.1038/s41391-022-00546-1. Online ahead of print. PMID: 35468990

Kiely, M., Lord, B., and Ambs, S. Immune response and inflammation in cancer health disparities. Trends in Cancer, 8: 316-327, 2022. PMID: 34965905

Kiely, M., Milne, G.L., Minas, T.Z., Dorsey, T.H., Tang, W., Smith, C.J., Baker, F., Loffredo, C.A., Yates, C., Cook, M.B., and Ambs, S. Urinary Thromboxane B2 and lethal prostate cancer in African American Men. J Natl Cancer Inst., 114: 123-129, 2022. PMID: 34264335

Kiely, M., Milne, G.L., Minas, T.Z., Dorsey, T.H., Tang, W., Smith, C.J., Baker, F., Loffredo, C.A., Yates, C., Cook, M.B., and Ambs, S. Urinary PGE-M in men with prostate cancer. Cancers, 13: 4073, 2021. PMID: 34439226

Kiely, M., and Ambs, S. Immune inflammation pathways as therapeutic targets to reduce lethal prostate cancer in African American men. Cancers, 13: 2874, 2021. PMID: 34207505

Minas, T.Z., Kiely, M., Ajao, A., and Ambs, S. 40th Anniversary Review Article: An overview of cancer health disparities: new approaches and insights and why they matter. Carcinogenesis, 42:2-13, 2021. PMID: 33185680

Tang W, Fowke JH, Hurwitz LM, Steinwandel M, Blot WJ, Ambs S. Aspirin Use and Prostate Cancer among African-American Men in the Southern Community Cohort Study. Cancer Epidemiol Biomarkers Prev., 30: 539-544, 2021. PMID: 33293340

Online First published:

Jason A. White, Ernest T. Kaninjing, Kayode A. Adeniji, Paul Jibrin, John O. Obafunwa, Chidiebere N. Ogo, Faruk Mohammed, Ademola Popoola, Omolara A. Fatiregun, Olabode P. Oluwole, Balasubramanyam Karanam, Isra Elhussin, Stefan Ambs, Wei Tang, Melissa Davis, Paz Polak, Moray J. Campbell, Kathryn R. Brignole, Solomon Rotimi, Windy Dean-Colomb, Folake T. Odedina, Damali N. Martin, and Clayton Yates. Whole-exome Sequencing of Nigerian Prostate Tumors from the Prostate Cancer Transatlantic Consortium (CaPTC) Reveals DNA Repair Genes Associated with African Ancestry. Cancer Research Communications, 2: 9, 2022

Submitted manuscripts:

Margaret S. Pichardo, Tsion Zewdu Minas, Catherine M. Pichardo, Maeve Bailey Whyte, Wei Tang, Tiffany Dorsey, William Wooten, Brid M. Ryan, Christopher A. Loffredo, and Stefan Ambs. Association of Neighborhood Deprivation with Prostate Cancer and Immune Markers in African American and European American Men. JAMA Network Open (under review)

Tsion Zewdu Minas, Brittany D. Jenkins, Amy L. Zhang, Julián Candia, Tiffany H. Dorsey, Francine Baker, Wei Tang, Maeve Kiely, Cheryl J. Smith, Obadi M. Obadi, Anuoluwapo Ajao, Symone V. Jordan, Yao Tettey, Richard B. Biritwum, Andrew A. Adjei, James E. Mensah, Robert N. Hoover, Ann W. Hsing, Jia Liu, Christopher A. Loffredo, Clayton Yates, Michael B. Cook, and Stefan Ambs. Circulating fatty acids and prostate cancer in Ghanaian, African American, and European American men. Nature Communications (under review)

Books or other non-periodical, one-time publications.

Nothing to Report

Other publications, conference papers and presentations.

Nothing to Report

- **Technologies or techniques**

Nothing to Report

- **Inventions, patent applications, and/or licenses**

Nothing to Report

Other Products

Databases: dbGaP submission phs002939.v1.p1; preview at https://www.ncbi.nlm.nih.gov/projects/gapprev/gap/cgi-bin/preview1.cgi?GAP_phs_code=E78nwI8ib0BMjFZB

Description in dbGaP: The study seeks to examine the genetic and ancestral basis of prostate cancer-associated inflammation using a genome-wide association approach. The study population are the participants of the NCI Maryland Prostate Cancer Case-Control study. This study recruited both African American and European American men, and investigated the causes for the excessive burden of prostate cancer among African American men in more general terms. We measured 82 blood-based immune-oncology markers, and genotyped the participants using the HumanOmni5-Quad BeadChip, which provides a significant genome coverage for genetically-admixed and diverse populations (about 4 Million SNPs) , to evaluate the genetic basis and evidence for causality of any immune-inflammation marker that is associated with prostate cancer risk. We want to assess how germline genetics influence the abundance levels of these 82 markers in African American and European American men, which may contribute to differences between them.

- Study Design:
  - Case-Control
- Study Type:
  - Case-Control
- dbGaP estimated ancestry using GRAF-pop
- Total number of consented subjects: 1450

Data: Minas et al., Serum proteomics links suppression of tumor immunity to ancestry and lethal prostate cancer, Nature Communications 2022, 13: 1759 (PMID: 35365620)

Data availability discription as part of the publication:

Data availability of measured 82 serum immune-oncology marker measurements in almost 3000 men with annotations: Clinical, demographic and molecular data used for this study (i.e., self-reported race, degree of West African ancestry, age, BMI, education, income, aspirin use, diabetes use, smoking status, NCCN risk score, PSA, treatment type, proteomics data, and survival data) are

deposited at the Open Science Framework at https://osf.io/327ha (https://doi.org/10.17605/OSF.IO/327HA)75 and as a public GitHub repository at https://github.com/juliancandia/ProstateCancerProteomics (https://doi.org/10.5281/zenodo.5815262)76.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Assembly of databases, data analysis, and preparation of manuscripts have been the major tasks in the Ambs laboratory in the reporting period. All tasks related to **Specific Aim 1** have been completed. The final manuscript related to **Specific Aim 1** is under review with Nature Communications. Data analysis and preparation of manuscripts will be the only tasks in the Ambs laboratory moving forward to complete the tasks specified under **Specific Aim 2**. The Yates lab at Tuskegee University has the lead on research and tasks specified under **Specific Aim 3**. However, all tasks under this aim are collaborative, involving efforts by both the Yates and Ambs laboratories.

The following individuals have worked on the described tasks in the past 12 months. They are either members of the Ambs laboratory or with the co-PI (Yates). There are additional time commitments by the Yates laboratory and their collaborators in Nigeria, as it relates to tasks under **Specific Aim 3**, that are not captured here. Other time commitments included learning of new methods for data analysis, preparation of abstracts, posters, and talks, and mentoring using Zoom calls. The Ambs and Yates labs continued their collaborative interactions related to project management, data analysis, and mentorship.

The following staff and fellows have left the team during the reporting period: Michael Cook (PI, DCEG-NCI), returned to Great Britain; Wei Tang (Staff Scientist), now with the company, MedImmune; Tsion Minas, now a Fulbright Scholar in Ethiopia; Maeve Bailey-Whyte, now in a transitional faculty position at the University of Limerick; Amy Zhang, now MD/PhD program at the University of North Carolina. Two new members joined the team: Huaitian Liu (Staff Scientist), replacing Wei Tang; Hunter Schaumloffel, a volunteer from Colgate University, replacing Amy Zhang. Wei Tang, Tsion Minas, Maeve Bailey-Whyte, and Amy Zhang remain being affiliated with the Ambs laboratory, each in a volunteer status with the NCI. PI Michael Cook left the team and will not be replaced.

Staff and contribution

Name	Tsion Minas
Project Role	Postdoctoral Fellow
Researcher Identifier	
Nearest person month worked	3
Contribution to Project	Project manager for the immune-oncology marker and GWAS studies; data analysis for research in the immune-oncology marker and fatty acid studies and preparation of manuscript(s); preparation of abstracts, posters, and talks
Funding support	NCI intramural program; now Fulbright Scholar in Addis Ababa, Ethiopia, and a volunteer with NCI

Name	Tiffany Dorsey
Project Role	Laboratory Manager/Microbiologist
Researcher Identifier	
Nearest person month worked	1
Contribution to Project	Key person for all biospecimen-related tasks; prepares data summaries and databases for all projects; communication with service providers; shipment of samples from repository to laboratory and from laboratory to service providers
Funding support	NCI intramural program

Name	Maeve Bailey-Whyte
Project Role	NCI Cancer Prevention Fellow
Researcher Identifier	
Nearest person month worked	3
Contribution to Project	Project manager for the urine metabolite study; data analysis for research in the urinary eicosanoid marker study and preparation of manuscript(s) including two reviews; preparation of abstracts, posters, and talks
Funding support	NCI intramural program; now at University of Limerick, Ireland, and a volunteer with NCI

Name	Brittany Lord
Project Role	NCI Cancer Prevention Fellow
Researcher Identifier	
Nearest person month worked	2
Contribution to Project	Analysis of the existing fatty acid data in partnership with Tsion Minas; preparation of manuscript(s), abstracts, posters, and talks
Funding support	NCI intramural program

Name	Margaret Pichardo
Project Role	Volunteer
Researcher Identifier	
Nearest person month worked	3

Contribution to Project	She has the lead for the study with geospatial neighborhood data; obtained census tract data for 1990, 2000, and 2010 and developed neighborhood deprivation index under guidance by Drs. Brid Ryan and Stefan Ambs (both CCR/NCI) and mentors at Yale University. Linkage of census tract data to study participants in the NCI-Maryland study; establishment of database with neighborhood deprivation index for all study participants. Analyzed data and prepared and submitted a research manuscript.
Funding support	NCI intramural program and scholarship at Yale; now a resident at University of Pennsylvania and a volunteer with the Ambs laboratory

Name	Hunter Schaumloffel
Project Role	Volunteer
Researcher Identifier	
Nearest person month worked	1
Contribution to Project	Data analyst for Specific Aim 2, investigating the germline genetic control of the 82 immune-oncology markers
Funding support	Volunteer status with NCI; student at Colgate University, NY

Name	Amy Zhang
Project Role	Post-baccalaureate fellow
Researcher Identifier	
Nearest person month worked	2
Contribution to Project	Preparation of datasets containing the genome-wide genotyping data linked to serum fatty acid and immune-oncology marker levels and demographic/patient data. Has started to write a manuscript
Funding support	NCI intramural program; now MD/PhD program and a volunteer with the Ambs laboratory

Name	Jason White
Project Role	PhD student
Researcher Identifier	
Nearest person month worked	4
Contribution to Project	Key person for all biospecimen-related tasks at Tuskegee University; project manager for the RNAseq and WES study with Hudson alpha; analyst of RNAseq and WES data; preparation of manuscript(s), abstracts, posters, and talks
Funding support	Tuskegee University

Name	Isra Elhussin
Project Role	PhD student
Researcher Identifier	
Nearest person month worked	2

Contribution to Project	Analysis of RNAseq data; preparation of manuscript(s), abstracts, posters, and talks
Funding support	Tuskegee University

Name	Huaitian Liu
Project Role	Staff Scientist
Researcher Identifier	
Nearest person month worked	2
Contribution to Project	Co-mentor of Isra Elhussin (PhD student from Tuskegee U) and Hunter Schaumloffel (NCI); data analysis
Funding support	NCI intramural program

Name	Balasubramanyam Karanam
Project Role	Assistant Professor
Researcher Identifier	
Nearest person month worked	2
Contribution to Project	Key personnel for the Akoya CODEX system and lead researcher of immune marker spatial expression analysis in prostate tumors; established Akoya CODEX system at Tuskegee University; biospecimen management
Funding support	Tuskegee University

Name	Michael Cook
Project Role	Principal Investigator
Researcher Identifier	
Nearest person month worked	1
Contribution to Project	Data analysis: Immune-inflammation markers; co-manager of GWAS study
Funding support	NCI intramural program; left NCI and will not be replaced

Name	Stefan Ambs
Project Role	Principal Investigator
Researcher Identifier	ORCID ID: https://orcid.org/0000-0001-7651-9309
Nearest person month worked	1
Contribution to Project	Project management including staff, service providers, and Geneva Foundation; guidance with data analysis
Funding support	NCI intramural program

Name	Clayton Yates
Project Role	Principal Investigator
Researcher Identifier	
Nearest person month worked	1
Contribution to Project	Project management including staff and service providers; guidance with project design (**Specific Aim 3**): RNAseq, DNASeq and image analysis with Akoya CODEX system
Funding support	Tuskegee University

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

PI Michael Cook left the team and will not be replaced. Remaining tasks/goals can be completed in his absence.

What other organizations were involved as partners?

We have established a collaboration with the Southern Community Cohort (SCCS) to investigate whether aspirin use among African American men is a viable strategy to prevent the development of lethal prostate cancer in these men. This collaboration also seeks to validate findings from the NCI-Maryland and NCI-Ghana study related this award in SCCS – immune and inflammation markers and their relationship to lethal prostate cancer among African American men. A grant proposal to validate some of the findings in SCCS has been submitted by Ginger Milne (Vanderbilt University) and Maeve Bailey-Whyte. We also shared part of the generated genotyping data with a consortium led by Dr. Christopher Haiman, University of Southern California. This consortium will perform the yet largest genome-wide association study to identify novel risk loci for prostate cancer among men of African ancestry by combining all existing datasets from many research institutions including the NCI. An NCI data transfer agreement has been signed. If this analysis leads to a publication, funding support by the DoD award W81XWH-18-1-0588 will be acknowledged. The research proposed by this consortium does not overlap with research aims in our award. None of these partner organizations provided financial/in-kind support.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

Not applicable

QUAD CHARTS:

Not applicable

9. APPENDICES:

PDF of 10 peer-reviewed publications (six research reports, four reviews), all published in 2021/2022, with acknowledgement of the funding support by DoD award W81XWH-18-1-0588.

Jason A. White, Ernest T. Kaninjing, Kayode A. Adeniji, Paul Jibrin, John O. Obafunwa, Chidiebere N. Ogo, Faruk Mohammed, Ademola Popoola, Omolara A. Fatiregun, Olabode P. Oluwole, Balasubramanyam Karanam, Isra Elhussin, Stefan Ambs, Wei Tang, Melissa Davis, Paz Polak, Moray J. Campbell, Kathryn R. Brignole, Solomon Rotimi, Windy Dean-Colomb, Folake T. Odedina, Damali N. Martin, and Clayton Yates. Whole-exome Sequencing of Nigerian Prostate Tumors from the Prostate Cancer Transatlantic Consortium (CaPTC) Reveals DNA Repair Genes Associated with African Ancestry. Cancer Research Communications, 2: 9, 2022.

Minas, T.Z., Candia, J., Dorsey, T.H., Baker, F., Tang, W., Kiely, M., Smith, C.J., Zhang, A.L., Jordan, S.V., Obadi, O.M., Ajao, A, Tettey, Y., Biritwum, R.B., Adjei, A.A., Mensah, J.E., Hoover, R.N., Jenkins, F.J., Kittles, R., Hs.ing, A.W., Wang, X.W., Loffredo, C.A., Yates, C., Cook, M.B., and Ambs, S. Serum proteomics links suppression of tumor immunity to ancestry and lethal prostate cancer. Nat Commun., 13: 1759, 2022. PMID: 35365620

Jenkins, F.J., Minas, T.Z., Tang, W., Dorsey, T.H., and Ambs S. Human herpesvirus 8 infection is associated with prostate cancer among IFNL4-ΔG carriers. Prostate Cancer Prostatic Dis. 2022 Apr 25. doi: 10.1038/s41391-022-00546-1. Online ahead of print. PMID: 35468990

Kiely, M., Lord, B., and Ambs, S. Immune response and inflammation in cancer health disparities. Trends in Cancer, 8: 316-327, 2022. PMID: 34965905

Kiely, M., Milne, G.L., Minas, T.Z., Dorsey, T.H., Tang, W., Smith, C.J., Baker, F., Loffredo, C.A., Yates, C., Cook, M.B., and Ambs, S. Urinary Thromboxane B2 and lethal prostate cancer in African American Men. J Natl Cancer Inst., 114: 123-129, 2022. PMID: 34264335

Kiely, M., Milne, G.L., Minas, T.Z., Dorsey, T.H., Tang, W., Smith, C.J., Baker, F., Loffredo, C.A., Yates, C., Cook, M.B., and Ambs, S. Urinary PGE-M in men with prostate cancer. Cancers, 13: 4073, 2021. PMID: 34439226

Kiely, M., and Ambs, S. Immune inflammation pathways as therapeutic targets to reduce lethal prostate cancer in African American men. Cancers, 13: 2874, 2021. PMID: 34207505

Minas, T.Z., Kiely, M., Ajao, A., and Ambs, S. 40th Anniversary Review Article: An overview of cancer health disparities: new approaches and insights and why they matter. Carcinogenesis, 42:2-13, 2021. PMID: 33185680

Tang W, Fowke JH, Hurwitz LM, Steinwandel M, Blot WJ, Ambs S. Aspirin Use and Prostate Cancer

among African-American Men in the Southern Community Cohort Study. Cancer Epidemiol Biomarkers Prev., 30: 539-544, 2021. PMID: 33293340

CANCER RESEARCH COMMUNICATIONS

AACR American Association for Cancer Research

RESEARCH ARTICLE https://doi.org/10.1158/2767-9764.CRC-22-0136 OPEN ACCESS

Whole-exome Sequencing of Nigerian Prostate Tumors from the Prostate Cancer Transatlantic Consortium (CaPTC) Reveals DNA Repair Genes Associated with African Ancestry

Jason A. White[1], Ernest T. Kaninjing[2], Kayode A. Adeniji[3], Paul Jibrin[4], John O. Obafunwa[5], Chidiebere N. Ogo[6], Faruk Mohammed[7], Ademola Popoola[3], Omolara A. Fatiregun[5], Olabode P. Oluwole[8], Balasubramanyam Karanam[1], Isra Elhussin[1], Stefan Ambs[9], Wei Tang[9], Melissa Davis[10], Paz Polak[11], Moray J. Campbell[12], Kathryn R. Brignole[13], Solomon O. Rotimi[14], Windy Dean-Colomb[1,15], Folake T. Odedina[16], Damali N. Martin[17], and Clayton Yates[1]

ABSTRACT

In this study, we used whole-exome sequencing of a cohort of 45 advanced-stage, treatment-naïve Nigerian (NG) primary prostate cancer tumors and 11 unmatched nontumor tissues to compare genomic mutations with African American (AA) and European American (EA) The Cancer Genome Atlas (TCGA) prostate cancer. NG samples were collected from six sites in central and southwest Nigeria. After whole-exome sequencing, samples were processed using GATK best practices. *BRCA1* (100%), *BARD1* (45%), *BRCA2* (27%), and *PMS2*(18%) had germline alterations in at least two NG nontumor samples. Across 111 germline variants, the AA cohort reflected a pattern *[BRCA1* (68%), *BARD1* (34%), *BRCA2* (28%), and *PMS2* (16%)] similar to NG samples. Of the most frequently mutated genes, *BRCA1* showed a statistically ($P \leq 0.05$) higher germline mutation frequency in men of African ancestry (MAA) and increasing variant frequency with increased African ancestry. Disaggregating gene-level mutation frequencies by variants revealed both ancestry-linked and NG-specific germline variant patterns. Driven by rs799917 (T>C), *BRCA1* showed an increasing mutation frequency as African ancestry increased. BRCA2_rs11571831 was present only in MAA, and BRCA2_rs766173 was elevated in NG men. A total of 133 somatic variants were present in 26 prostate cancer–associated genes within the NG tumor cohort. *BRCA2* (27%), *APC* (20%), *ATM* (20%), *BRCA1* (13%), *DNAJC6* (13%), *EGFR* (13%), *MAD1L1* (13%), *MLH1* (11%), and *PMS2* (11%) showed mutation frequencies >10%. Compared with TCGA cohorts, NG tumors showed statistically significant elevated frequencies of *BRCA2*, *APC*, and *BRCA1*. The NG cohort variant pattern shared similarities (cosign similarities ≥0.734) with Catalogue of Somatic Mutations in Cancer signatures 5 and 6, and mutated genes showed significant ($q < 0.001$) gene ontology (GO) and functional enrichment in mismatch repair and non-homologous repair deficiency pathways. Here, we showed that mutations in DNA damage response genes were higher in NG prostate cancer samples and that a portion of those mutations correlate with African ancestry. Moreover, we identified variants of unknown significance that may contribute to population-specific routes of tumorigenesis and treatment. These results present the most comprehensive characterization of the NG prostate cancer exome to date and highlight the need to increase diversity of study populations.

Significance: MAA have higher rates of prostate cancer incidence and mortality, however, are severely underrepresented in genomic studies. This is the first study utilizing whole-exome sequencing in NG men to identify West African ancestry-linked variant patterns that impact DNA damage repair pathways.

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Introduction

For men, adenocarcinoma of the prostate is the most frequently diagnosed cancer, accounting, globally, for an estimated 1,414,259 cases and 375,304 deaths in 2020. The preponderance of this mortality is for men of African ancestry (MAA), including African American (AA), Central American, Caribbean, and Sub-Saharan African men (1). The Globocan 2020-derived mortality-to-incidence ratio, "an indirect description of the general survival experience" of prostate cancer in Africa, is 0.55, relative to 0.32 and 0.17 for Asia and Europe, respectively (2, 3). Furthermore, studies of AA men showed higher incidence, worse prognoses, and higher mortality compared with European American (EA) men (4, 5). Although there is a substantial contribution of social and environmental influence on the disparity, emerging evidence from genomic profiles suggests that this disease is highly heterogenous (6), and its etiology and phenotype are influenced by enrichment of African ancestral genetic markers, with West African ancestry linked with higher Gleason grade at diagnosis (7–9).

The racial disparity in prostate cancer biology is typically characterized by increased genomic mutations, resulting in a more aggressive phenotype. For instance, West African ancestry is associated with distinctive somatic genomic mutations (10, 11). Conversely, understanding these putatively targetable genomic mutations presents opportunities for effective population-relevant and genomics-guided interventions that can improve clinical outcomes. This relies on the availability of genomic data for tumors from MAA. However, despite an upsurge in genomic data for human cancers, the data on prostate cancer from African sources are grossly underrepresented in the literature and genomics databases. For instance, AA samples account for only about 10% of The Cancer Genome Atlas (TCGA) prostate cancer sample cohorts (11, 12). Consequently, this gross underrepresentation impedes deciphering of clinically actionable genomic mutations that could be used to develop precision interventions for MAA. Hence, it is imperative to increase the representation by sequencing the tumor genome of prostate cancer in MAA.

Although Black men in the Americas generally have ancestral roots in the Atlantic coasts of Africa (13–15), the translational impact of genomics data of AA men to indigenous Africans is limited because of the varying proportions of European-related and intra-African admixtures of African American (16, 17). Such admixture and variation in germline mutations influence gene expression and phenotype (18); and are limiting factors in the understanding of the contribution of genetics to health disparities (19). Hence, studying the genomic architecture of prostate cancer in the indigenous African population is essential for advancing understanding of the contribution of African genetics to prostate cancer biology and the phenotype of this disease in the African Diaspora. To date, inadequate attention has been given to generation of genomics studies of prostate cancer among indigenous Africans. Aside from three genome-wide association studies of prostate cancer in Ghanaian (20), Ugandan (21), and South African (22) men, only Jaratlerdsiri and colleagues (23) have reported whole-genome sequence data on tumors from indigenous sub-Saharan Africans. Their analysis of prostate cancer of six South African Black men identified distinctive and elevated oncogenic driver mutations, with a high proportion of these recurrent mutations appearing early in tumorigenesis. They also showed that tumors of the African men they studied had fewer complex genomic rearrangements, loss of *PTEN*, and absent *ERG* fusions and *PIK3CA* mutations relative to AAs. Furthermore, apart from large deletions within the *BRCA2*, *DEFA1B*, and *MFF* genes, they did not report any pathogenic mutations in high-penetrance genes, such as *BRCA1*, *BRCA2*, *ATM*, and *CHK2* among the South African cohort (23, 24). Previous studies have identified, for prostate cancer of AAs, a high burden of mutations in these DNA repair genes (25, 26); suggesting that PARP inhibitors could improve clinical outcomes for men of African ancestry with prostate cancer (27). The South African study is limited by the small sample size. Furthermore, the differences observed could be due to the low contribution of South-African Khoe-San ancient ancestry genes to the AA genetic pool, which is a source of bias (16, 17). It is therefore our hypothesis that genomic profiling of prostate cancer in indigenous West African men will identify clinically actionable targets for precision intervention for MAA. The utility of clinical mutational profiling necessitates greater emphasis on identifying variants that can be used to help these underrepresented patients.

The aim of the current study was to analyze whole-exome sequencing (WES) of 45 Nigerian (NG) primary treatment-naïve formalin-fixed, paraffin-embedded (FFPE) prostate cancer and 11 NG nontumor prostate samples collected within the Prostate Cancer Transatlantic Consortium (CaPTC). Study of prostate cancer of NG men allowed us to provide genetic information of the indigenous West Africa population with the highest genetic contribution to AA men (16) and provide opportunities to investigate the shared genetic background of both groups for causal disease variants. As such, our data will be relevant for deriving actionable clinical information for prostate cancer intervention for MAA.

Materials and Methods

Sample Collection and Genomic DNA Extraction

This study utilized 45 FFPE advanced-stage, treatment-naïve primary prostate cancer and 11 NG nontumor prostate samples collected from four participating clinical sites within the CaPTC network (Fig. 1A). In accordance with the U.S. Common Rule, the archived samples used in this study were reviewed and approved by the Institutional Review Boards of their respective clinical institutions (University of Ilorin Teaching Hospital, National Hospital Abuja, Lagos State University Teaching Hospital, Federal Medical Centre, Ahmadu Bello University, and the University of Abuja) and by the Institutional Review Board at Tuskegee University (Tuskegee, AL). Because of the retrospective nature of this study and the usage of deidentified archived samples, the review boards deemed informed written consent to be unnecessary for this study. Five 10-μm-thick curls were obtained from each block with >50% tumor and ≤50% necrosis and shipped to Q2 Solutions for DNA extraction and quality analysis. Following the manufacturer's protocol, genomic DNA and total RNA were purified using Allprep DNA/RNA FFPE kits (Qiagen). DNA quality and quantity were checked with Qubit 2.0 fluorometry (Life Technologies) and with KAPA hgDNA quantification and QC kits [Kappa Biosystems (Roche)]. DNA quality and quantity thresholds were >0.2 μg and a Q129/Q41 ratio >0.00225, respectively.

Pathologic Scoring

The FFPE blocks were processed at the Pathology Biorepository Shared Service (PBSS) core at the University of Maryland, Baltimore (Baltimore, MD). For pathology review, an initial hematoxylin and eosin (H&E) slide was prepared from each block. A pathologist assessed the presence and quantity of tumor, and the presence and quantity of normal tissue. Up to two tumor and normal cores per H&E slide were circled, and the total number of cores per

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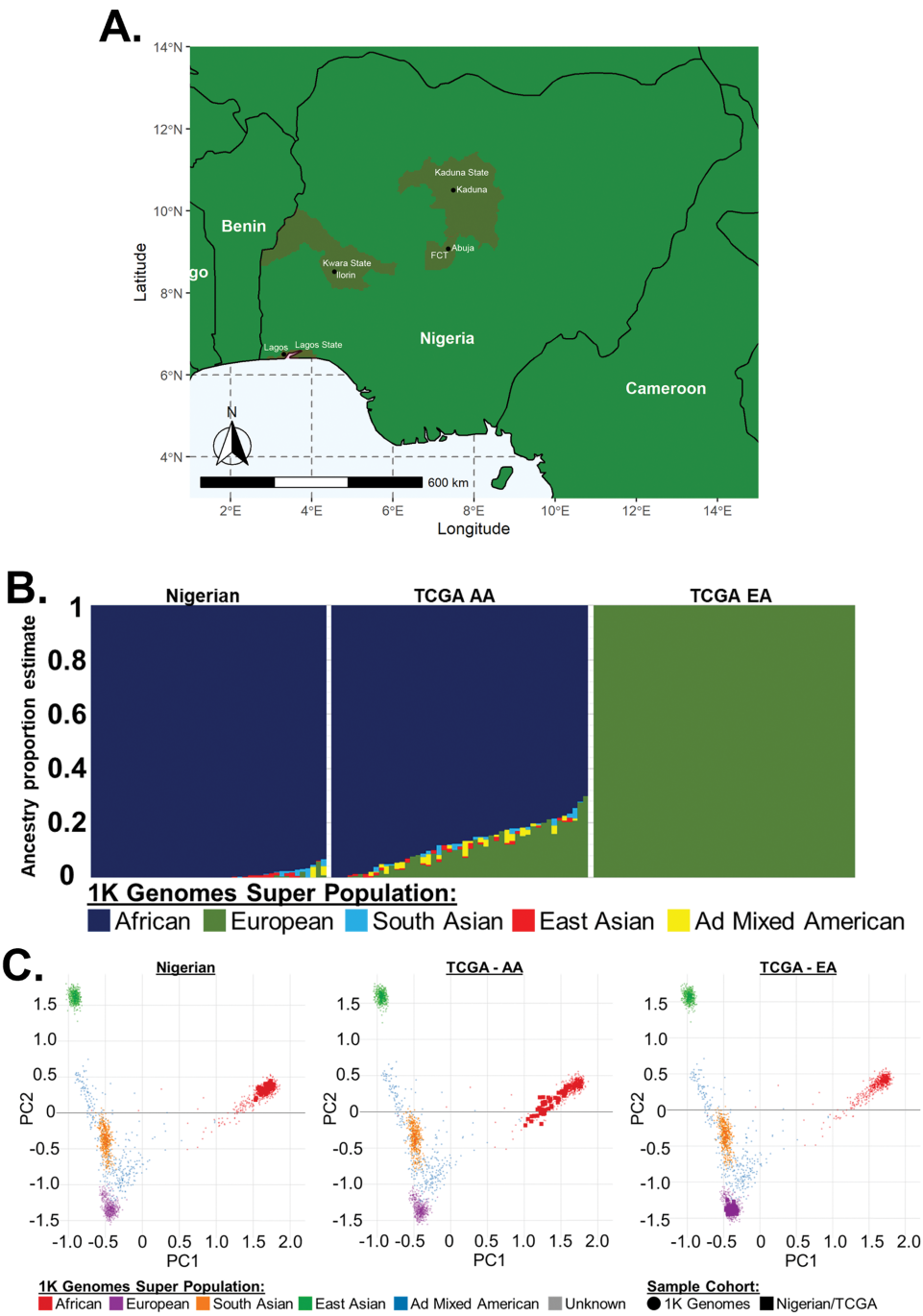


FIGURE 1 Sample collection sites and genetic admixture analysis. **A,** At clinical sites across Nigeria, 45 samples were collected. **B,** Admixture v1.3.0 was used to estimate ancestry proportions, based on reference populations from the 1000 Genomes Project phase III superpopulations. Prior to analysis, rare variants (i.e., <5% across all phase III 1000 genomes), all indels, and any SNPs that were not biallelic were removed. Samples within the CaPTC cohort had an average African proportion of 99.1%. TCGA Samples ($n = 50$) with >70% African ancestry were classified as AA; 402 TCGA samples contained >60% European admixture. Those samples were sorted by European proportion, and the top 50 samples were classified as EA and utilized in this study. The average European proportion of this group was 99.996%. **C,** Germline variants within the NG and TCGA cohorts were compared with phase III 1000 Genomes superpopulations using principal component analysis. NG samples strongly clustered with the African superpopulation. AAs samples clustered with the African superpopulation; European samples clustered with the European superpopulation.

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slide was recorded in a sample manifest. In addition, the pathologist provided the tumor core(s) Gleason score for each tumor circle. Those FFPE blocks with sufficient tumor and/or normal cores available were submitted to the PBSS research histology lab for core extraction. Using the corresponding H&E slide as a template, cores were extracted from each FFPE block by use of a manual tissue core extraction device with RNAse/DNAse-free conditions. The cores were placed in labeled RNAse/DNAse-free cryovials. A sample manifest for the extracted cores accompanied samples shipped to the NIH Laboratory of Human Carcinogenesis.

WES

As outlined in Supplementary Fig. S1, library preparation was performed using Agilent SureSelectXT Human All Exon V6 r2 Exome Kits (Agilent Technologies). Sequencing (2 × 150 bp) was performed on either an Illumina HiSeq4000 or on an Illumina NextSeq500 (Illumina), to a target of 100 (± 10) million raw data reads per each sample library. Following sequencing, raw fastq files were transferred to the NIH Biowulf supercomputing cluster and analyzed using the Center for Cancer Research Collaborative Bioinformatics Resource (CCBR) whole-exome pipeline (https://github.com/CCBR/Pipeliner). Reads were trimmed using Trimmomatic v0.33 (28) and mapped to the hs37d5 version of the human reference genome (ftp://ftp.1000genomes.ebi.ac.uk/vol1/ftp/technical/reference/phase2_reference_assembly_sequence/hs37d5.fa.gz) using BWA-MEM v07.17 (29). Binary Alignment Map (BAM) files were processed using Samtools v1.8 (http://www.htslib.org/; ref. 30), and Picard v1 (http://broadinstitute.github.io/picard/) was used to mark duplicates. GATK v3.8 (31) was used to perform indel realignment and base recalibration. Read and alignment-level quality analysis was performed using Qualimap v2.2.1 (32). Alignment quality metrics were analyzed and visualized using RStudio 1.2.5003 (http://www.rstudio.com) [R 3.6.3 (https://www.R-project.org)], the ggstatsplot v0.3.0 (https://indrajeetpatil.github.io/ggstatsplot/) package, and the compareGroups v4.2.0 (33) package.

Variant Calling and Filtration

Germline variant calling was completed using HaplotypeCaller (34), and Annovar v2019Oct24 was used for variant annotation. Variant filtration followed GATK Best practices. Cohort SNPs and indels were separated, filtered, and recombined for downstream analysis. SNP filters were Qual < 30.0, QD < 2.0, FS > 60.0, MQ < 40.0, MQRanksum < −12.5, and ReadPosRankSum < −8.0. Indel filters were Qual < 30.0, QD < 2.0, FS >200.0, and ReadPosRankSum < −20.0. Once the variants were recombined, cohort germline variants were compared with a ClinVar-derived list of known prostate cancer–associated germline variant regions (Supplementary Table S1) to target variants of known clinical importance. Germline variants with a read depth >3 and a variant allele frequency >50% were retained for downstream analysis (Supplementary Table S2). Somatic variant calling was completed using MuTect2 (35), and Annovar v2019Oct24 (36) was used for variant annotation. As described by Jones and colleagues, a single unmatched NG normal FFPE exome, sequenced using the same methods as for the tumor samples, was paired with each tumor exome to filter false-positive variant calls (37). Mutation tables were imported into RStudio for visualization and analysis using the maftools (ref. 38; v2.4.10) package. Variants were (i) screened for strand bias (Supplementary Fig. S2) using GATK FisherStrand Phred Score, (ii) separated into variants within known prostate cancer–associated genes and variants within novel prostate cancer–associated genes (Supplementary Table S3), using ClinVar and (iii) and filtered (Supplementary Fig. S3) using two filtering regimes. After filtering, retained variants within genes identified in ClinVar were considered "Known"; conversely, variants within genes not identified in ClinVar were called "Novel." Filtering steps included (i) exclusion of silent and non–protein-coding mutations, (ii) variant allele read depth ≥3, (iii) variant allele frequency >5% (10% for variants within novel prostate cancer–associated genes), (iv) dbNSFP (39) Genome Aggregation Database (gnomAD; ref. 40) exome allele frequency <0.01 (<0.001 for variants within novel prostate cancer–associated genes and variants lacking allele frequencies were retained for downstream filtration), (v) identification as Pathogenic or Uncertain in ClinVar v 20200419 (41), (vi) removal of dbSNP-annotated variants identified in NG unmatched normal samples (n = 11; Supplementary Table S4), (vii) present in genes altered in least 5% of tumors, and (viii) manual validation in the Integrative Genomics Viewer (IGV; Supplementary Fig. S4). For genes mutated across at least five prostate cancer samples, Fishers exact test was used to compare cohort mutation frequencies. The test was completed using the maftools clinicalenrichment function. P values < 0.05 were considered significant.

TCGA PRAD Data Acquisition and Analysis

Access to TCGA Prostate adenocarcinoma (PRAD) data (Accession: phs000178.v11.p8) was obtained through the database of Genotypes and Phenotypes (dbGAP). Raw sequencing files in BAM format were downloaded through the Genomic Data Commons (GDC) data transfer tool from the GDC Data Portal (https://portal.gdc.cancer.gov/). After download, the raw files were sorted using Samtools and split into their constituent forward and reverse fastq files using bedtools v2.29 (42). Once separated, the fastq files were processed through the CCBR whole-exome pipeline and analyzed using the same methods as for NG CaPTC samples.

Genetic Admixture Estimation

To ensure accurate ancestral group assignment, HaplotypeCaller (43) and Admixture v1.3.0 (44) were used to estimate ancestry proportions, based on reference populations from the 1000 Genomes Project phase III superpopulations in all TCGA and NG samples (Supplementary Fig. S5). Rare variants [i.e., <5% across all phase III (45) 1000 genomes], all indels, and any SNPs that were not biallelic were removed prior to analysis. TCGA samples (n = 57) with majority African ancestry were classified as AAs. AAs are highly admixed, which in turn increases genetic variation (17, 46). Because our NG population has greater than 90% African Ancestry, we focused on AAs within TCGA that have at least 70% African admixture (n = 50) as a comparison group, which has been used in previous reports. Thus, the AA patients with TCGA that have African admixture at or above 70%, represent 87% of the total AA samples in the prostate cancer TCGA. The EA samples that at or above >60% European admixture represent 402 TCGA samples. Those samples were sorted by European proportion, and the top 50 samples were classified as EAs and utilized in this study. The average European proportion of this group was 99.996%.

COSMIC Signature Enrichment

Using the maftools package, filtered single-nucleotide variants across each cohort were used to estimate the representation of Catalogue of Somatic Mutations in Cancer (COSMIC; cancer.sanger.ac.uk; ref. 47) mutation signatures within each tumor sample. Maftools uses cophenetic correlation and nonnegative matrix factorization to determine the optimal number of SNP signatures (across the cohort), extracts those signatures, and compares them with the known (n = 30) COSMIC signatures.

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Variant Functional Gene Ontology and Network Analyses

Filtered variants, present in at least two NG prostate cancer tumor samples, were imported into Cytoscape (48) (v. 3.7.2) to assess functional gene ontology enrichment and to visualize the GO term interaction network. Once separated, functional analysis and network construction were completed using the stringApp (ref. 49; v 1.6.0) and ClueGO (ref. 50; v. 2.5.7) plug-ins. A two-sided (enrichment/depletion) hypergeometric test with Bonferroni step-down was used to determine Reactome Pathways (v. 08.05.2020) enrichment. Analysis thresholds included enrichment significance of $P \leq 0.01$, a minimum of 5% gene inclusion and a kappa score threshold of ≥ 0.4. ClueGO uses kappa scores to determine the likelihood of GO term interactions and groupings.

Data Availability

The data generated in this study are publicly available in dbGaP (Accession phs002547.v1.p1).

Results

To determine the somatic and germline variants associated with prostate cancer in NG men, we collected samples from multiple institutions within the CaPTC. Specifically, we collected 45 intermediate (Gleason scores 4 + 3) and high-grade (Gleason scores $\geq 4 + 3$) tumors and 11 nontumor prostate samples. Samples averaged $>68\times$ coverage, 57.2 mapping quality and 263 million mapped reads per sample (Supplementary Table S5). A total of 31 (20 tumor and 11 nontumor) samples were collected from Northern Nigeria), 17 samples from Central Nigeria, and eight samples from Southwest Nigeria (Supplementary Table S6). PRAD exome data were downloaded from TCGA, using the dbGAP database as a comparison cohort (Supplementary Table S7).

Race is a poor group classifier for linking genetic variation and disease causation (51); moreover, self-reported race can obscure genetic variation due to misunderstandings about family heritage, cultural influences, and/or other societal factors (52). To ensure that our NG and TCGA cohort comparisons were not skewed by bias within self-reported race, we quantified the individual genetic admixture within each patient sample. To accomplish this, germline SNPs were compared with 1000 genomes superpopulations (African, European, South Asian, East Asian, and admixed American), and ancestry proportion estimates were calculated (Fig. 1B). NG patients showed an average genetic ancestry of 99.1% African. The genetic ancestry of TCGA AA patients was predominantly a mixture of African (50.2%–99.99%) and European admixture (1%–43%). To reduce the impact of this variance on our analysis, we selected only TCGA AA patients with $\geq$70% African ancestry ($n = 50$). TCGA EA patients showed minimal admixture, with >98.3% European ancestry. Nine patients self-identified as EAs possessed $\leq$45% European ancestry. Four of the 9 patients were majority (>50%) admixed American, 2 were majority East Asian, 2 were majority African, and 1 was predominantly (45%) European with 35% admixed American and 16% African admixture. To obtain an EA comparison group, we sorted (high to low) the cohort by European ancestry proportion and selected the top 50 TCGA EA patients. After admixture estimation and sample selection, principal component analysis plots were used to visualize the relationships between each cohort and the five 1000 Genomes superpopulations (Fig. 1C). The NG and TCGA EA cohorts clustered with their ancestral 1000 genomes superpopulations, and TCGA AA cohort clustered with the African superpopulation. Thus, data for these patients were used in subsequent analyses.

The NG cohort harbored 31 known, non-benign, germline variants. Four genes [*BRCA1* (100%), *BARD1* (45%), *BRCA2* (27%), and *PMS2* (18%)] were altered in at least two samples (Fig. 2A). These genes also showed top mutation frequencies within both TCGA cohorts (Fig. 2B and C). Across 111 germline variants, the AA TCGA cohort reflected a pattern [*BRCA1* (68%), *BARD1* (34%), *BRCA2* (28%), and *PMS2* (16%)] similar to that for NG samples. In addition, the rate of *BRCA1* mutations increased ($P \leq 0.021$) as African admixture increased (Fig. 3A; Supplementary Table S8). A total of 126 germline variants were present in the EA TCGA cohort. Disaggregating mutation frequencies down to specific variants revealed both ancestry-linked and NG-specific germline variant patterns. *BRCA1* showed an increasing mutation frequency as African admixture increased (Fig. 3A). That pattern was driven by three variants (rs799917, rs16941, and rs16942; Fig. 3B). The frequency of rs799917 was higher for men of African ancestry; rs16941 and rs16942 were lower. In esophageal squamous cell carcinoma, the BRCA1_rs799917 T>C SNP inhibits mir-638–mediated regulation of *BRCA1*, thus reducing *BRCA1* expression and increasing cancer cell proliferation (53). This variant is also linked to a higher risk of gastric, lung, and triple-negative breast cancer (54–56). BRCA1_rs16941 and BRCA1_rs16942 are variants of unknown significance (VUS). *BARD1* germline variant patterns appear to be specific to NG men. Compared with AA and EA cohorts, rs2070096 is lower and rs2070094 is higher. Of note, the BARD1_rs2070094 SNP resides within the BARD1-binding domain of *BRCA1* and may provide a protective function that enhances DNA repair by enhancing *BARD1/BRCA1* binding stability (57). BARD1_rs2070096 is a VUS. *BRCA2* germline variants displayed both ancestry-linked and NG-specific patterns. rs11571831 was present only in men of African ancestry, and rs766173 was high in NG men. Both *BRCA2* variants are classified as VUS. Although most variants in NG prostate cancer were identified as VUS, their presence and differing frequencies, compared with TCGA, provide opportunities for future investigations. Characterizing the full mutation spectrum is a first step in understanding how best to diagnose and treat this underrepresented patient population.

Somatic variant analysis of tumor-only sequencing data involves multiple nontrivial steps that are distinct from the analysis of matched tumor and normal sequencing. Therefore, we used an established pipeline that incorporated a panel of normal samples. We used an unmatched NG normal sample to filter out NG-specific germline variants (37, 58), reducing the unique NG variants by 70.8% from 2,506,254 to 730,285 variants (Supplementary Fig. S6). Within TCGA cohorts, we used each sample's patient-matched normal, which produced 11,208 unique AA variants and 15,191 unique EA variants. Because the NG cohort contained many somatic variants, we employed two filtering regimes, one for variants within known prostate cancer–associated genes (as identified in ClinVar) and one for variants within genes not associated with prostate cancer. We identified 905 variants across 25 genes known to be associated with prostate cancer, and 156 variants across 51 novel prostate cancer genes. Using the same approach, we identified 15,854 variants in TCGA AA cohort and 21,957 variants in TCGA EA cohort (Supplementary Table S9). Consistent with other sequencing studies (59), our results showed the same racial mutation patterns for *SPOP, ATM, TP53,* and *PIK3CA*. TCGA cohorts did not show recurrent mutations in genes not associated with prostate cancer. Our dual filtering approach allowed us to filter, independently, each set of variants across the NG cohort without overfiltering variants within known prostate cancer–associated genes and to identify high-confidence variants in novel prostate cancer–associated genes.

Within the NG cohort, 133 somatic variants were present in 26 prostate cancer–associated genes. Nine genes [*BRCA2* (27%), *APC* (20%), *ATM* (20%), *BRCA1*

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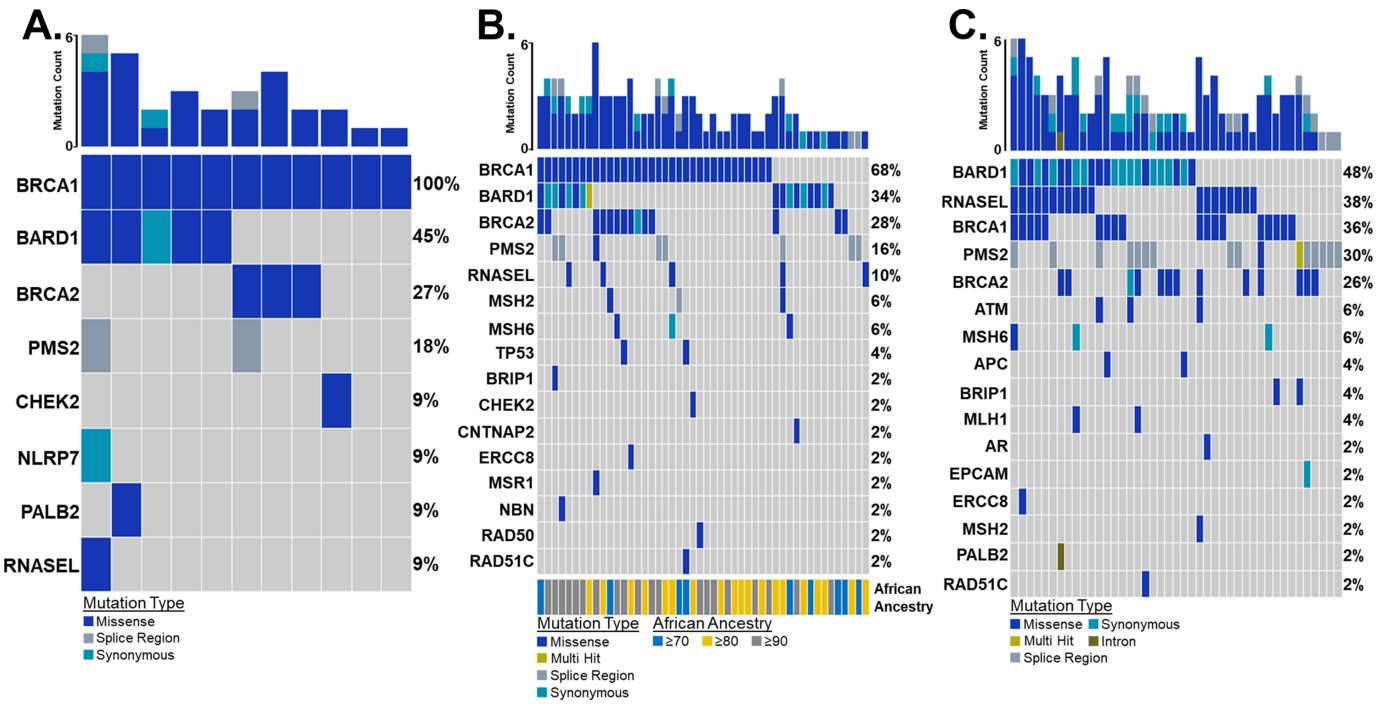


FIGURE 2 Prostate cancer germline variant oncoplot. **A,** NG germline variants detected across 11 normal samples were filtered against known ClinVar cancer variants. In at least two tumor samples, four genes known to harbor cancer-related variants were mutated. These genes included *BRCA1* (BRCA1 DNA repair associated)—100%, *BARD1*—45%, *BRCA2* (DNA repair associated)—27%, and *PMS2* (PMS1 homolog 2, mismatch repair system component)—18%. As a comparison with NG prostate cancer exome samples, TCGA prostate cancer samples ($n = 50$ AA and $n = 50$ EA) were downloaded through dbGAP and analyzed for genetic variants. **B,** In the AA cohort. eight genes showed germline mutations in at least two samples. **C,** In the EA cohort, 10 genes showed germline mutations in at least two samples.

(13%), *DNAJC6* (13%), *EGFR* (13%), *MADIL1* (13%), *MLHI* (11%), and *PMS2* (11%)] showed mutation frequencies >10% (Fig. 4A). Of NG prostate cancer, 53% showed mutations in genes (*BRCA2, ATM, BRCA1, CHEK2, TP53*, and *MSH6*) associated with genome integrity. TCGA AA and EA cohorts harbored 67 and 73 somatic variants, respectively. Across both cohorts, fifteen genes were mutated in at least two samples (*SPOP, ATM, TP53, BRAF, MED12, PIK3CA, CTNNB1, EGFR, FLCN, MYH7, PTEN,* and *TTN*). *SPOP* and *ATM* were the most frequently mutated genes in AA tumors and were mutated two times more compared with EA. Comparison of the mutation frequencies between TCGA cohorts did not show any statistically significant differences; however, AA tumors showed a significant increase in SPOP mutations compared with NG (Fig. 4B). *BRCA2, APC,* and *BRCA1* showed statistically significant increases in the NG cohort. Though not statistically significant, *ATM* had the highest mutation frequency associated with increasing African ancestry; specifically TCGA EAs had an *ATM* mutation frequency of 4%, but TCGA AA and NG cohorts had rates of 8% and 20%, respectively. Somatic mutations for NGs and AAs were distributed across the amino acid sequence of the most mutated genes. None of the variants were shared across or within cohorts. (Fig. 4C). Comparison of EA and NG cohorts showed no discernible pattern (Supplementary Fig. S7); however, the EA cohort did show a number of variants within a known SPOP hotspot.

In addition to the variants within known prostate cancer driver genes, we identified four novel mutated genes that showed mutation frequencies >10%. *CACNA2D2* had the highest mutation rate of 29% (Supplementary Fig. S8A) and showed a recurrent ($n = 13$) missense SNP of Leu54Phe (rs569543350; Supplementary Fig. S8B). *TTN* (Titin) had the second highest mutation frequency at 20%. The size of this large protein (>30,000 amino acids) renders it more susceptible to DNA repair errors, making the functional significance of these mutations unreliable, even after rigorous variant filtering (60–63). *SYNE1* was the third most frequently mutated gene (16%) in the novel prostate cancer set. This gene showed a recurrent ($n = 2$) missense SNP of Gln1491Glu. The fourth most mutated gene was *ADAMTS2*. This gene showed a recurrent ($n = 4$) in-frame insertion of Leu_Pro29dup and an overall mutation frequency of 13%. Finally, 47 other genes not known to be associated with prostate cancer were mutated in 2 or more patients; however, we did not characterize these due to their low mutation frequencies.

To validate the observed germline variant frequencies, we analyzed NG tumors using the germline variant pipeline (Supplementary Fig. S9). NG tumor samples not only possessed comparable rates of gene-level variation [*BRCA1* (100%), *BARD1* (41%), and *BRCA2* (18%)] but also showed comparable variant frequencies.

We next investigated the overall mutational patterns within each cohort to understand global somatic events. The NG cohort shared similarities (cosign similarities ≥0.734) with COSMIC signatures 5 and 6 (Fig. 4D). Five NG cohort samples had a mutational pattern similar (cosign similarity ≥0.734) to COSMIC 5. Forty cohort samples were similar (cosign similarity ≥0.796) to

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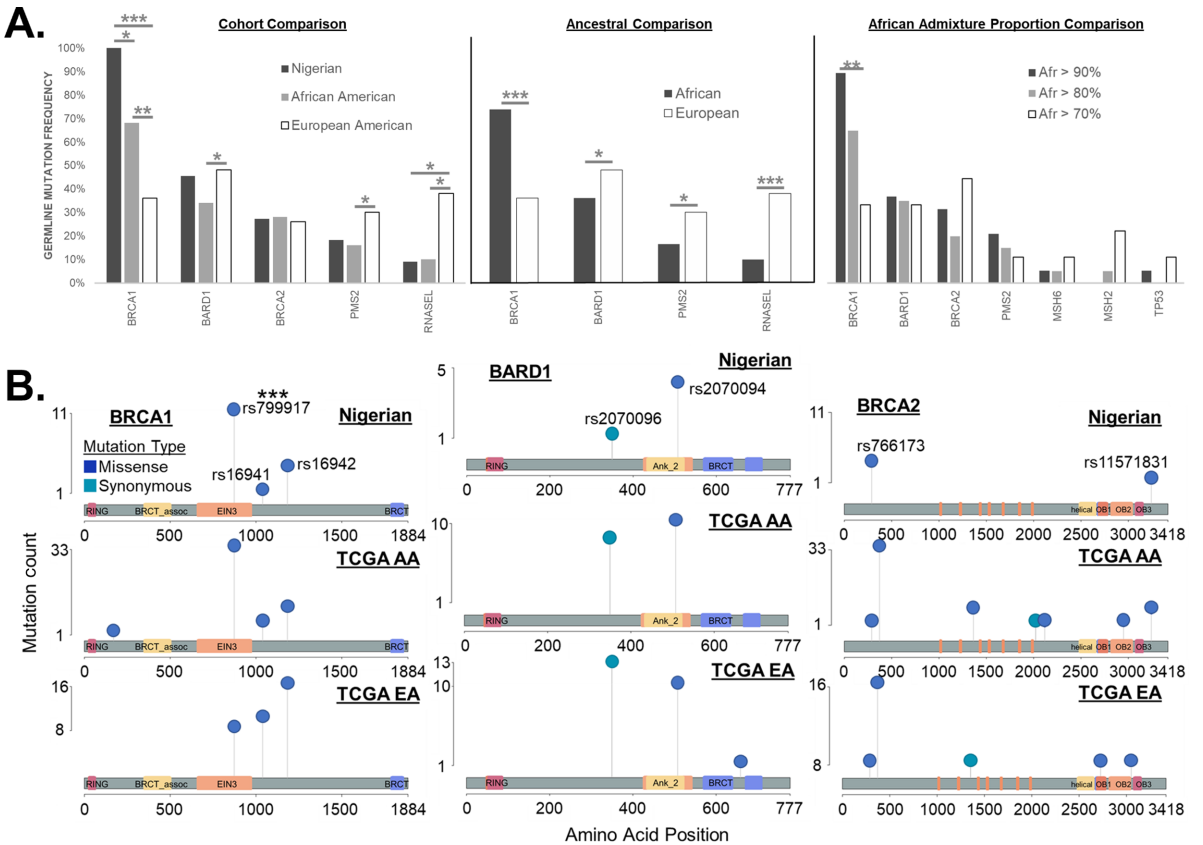


FIGURE 3 NG prostate cancer cohort germline mutations comparison with TCGA prostate cancer cohorts and lollipop plots. **A,** Prostate cancer of NG and AA men showed more *BRCA1* germline mutations ($P \leq 0.001$ and $P \leq 0.01$, respectively) compared with European men. In addition, prostate cancer of NG men showed more ($P \leq 0.036$) *BRCA1* mutations relative to AA men. In prostate cancer of EA men, *BARD1* was mutated at a higher rate ($P \leq 0.048$). *BRCA2* showed no significant difference in cohort mutation rates. AA men with greater than 90% African ancestry ($n = 19$) show a statistically significant ($P \leq 0.021$) increase in *BRCA1* germline mutations when compared with those with lower amounts of African ancestry ($n = 29$). In addition, AA men with 90% African ancestry have a statistically higher ($P = 0.012$) frequency of the *BRCA1* variant rs799917. **B,** To disaggregate mutation rates down to specific variants, lollipop plots revealed a finer variation in cohort patterns. The ancestry-linked pattern of *BRCA1* is driven by rs799917, which was more frequent ($P \leq 0.001$) for men of African ancestry. rs16941 and rs16942 were elevated in prostate cancer of European men, but that difference was not statistically significant. *BARD1* germline variants showed no significant difference in variant rates; however, the patterns appeared to be specific to NG men. Compared with AA and EA cohorts, rs2070096 was lower and rs2070094 was higher. *BRCA2* germline variants displayed no statistically different variant rates, but both ancestry-linked and NG-specific patterns were discernable. rs11571831 was present only in prostate cancer of men of African ancestry, and rs766173 was elevated in prostate cancer of NG Men. *P* values were produced via two-sided Fisher exact test groupwise comparison.

COSMIC 6 (Fig. 4E). TCGA AA mutational patterns shared similarities with COSMIC 1 and 5 (cosign similarities ≥0.645), and TCGA EA tumors shared similarities with COSMIC 1, 3, and 4 (cosign similarities ≥0.481; Supplementary Fig. S10). Within the COSMIC database, mutational signatures 1, 5, and 6 were the signatures most often observed in prostate cancer.

To determine the mechanism associated with tumorigenesis in NG prostate cancer tumors, mutated genes present in at least 2 NG patients were analyzed for functional gene ontology enrichment. NG tumors showed significant ($q < 0.001$) GO and functional enrichment in mismatch repair and nonhomologous repair deficiency pathways (Fig. 4F; Supplementary Table S10). Additional enriched pathways included PD-1 checkpoint, thyroid hormone signaling, FOXO signaling, ErB2 signaling, adherens junctions, proteoglycans, and sphingolipids.

Discussion

This study is the first to perform WES of advanced-stage, treatment-naïve primary tumors from NG men with prostate cancer. We analyzed the genomes of 45 tumors and 11 normal NG prostate samples. Because most AAs in the United States have majority West African Ancestry, we assessed genetic admixture on comparison datasets from TCGA, which contains data on AA and EA patients with prostate cancer. We identified ancestry-linked germline and somatic mutation frequencies in DNA damage repair genes (*BRCA1, BRCA2, APC,* and *ATM*), as well as three novel prostate cancer–associated genes (*CACNA2D2, SYNE1,* and *ADAMTS2*). Mutations in DNA damage repair pathways are involved in prostate cancer development and progression and are clinically targetable (64). Because men of African ancestry are severely underrepresented in genomic studies, our findings address a gap in the contribution of genetic

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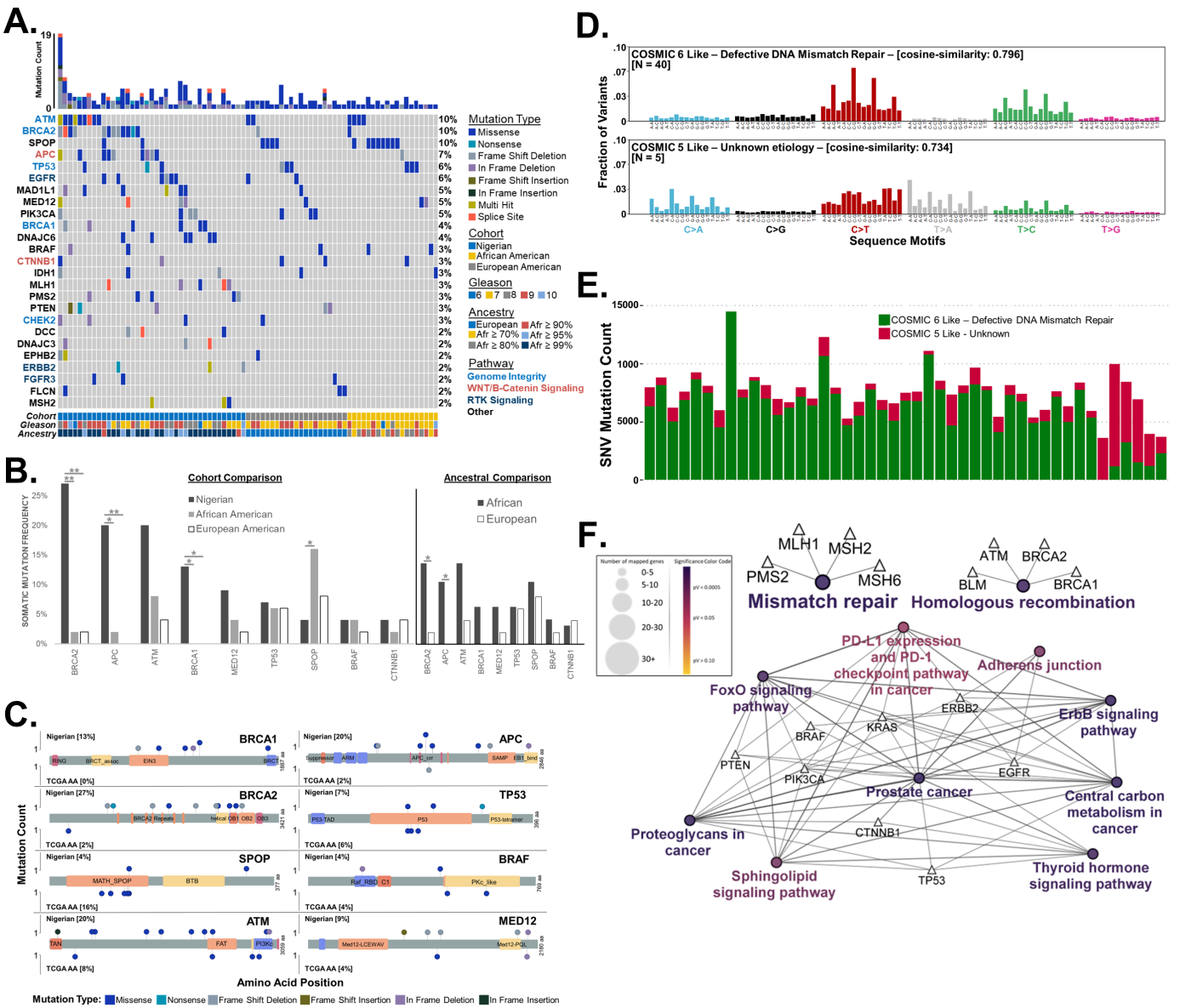


FIGURE 4 Prostate cancer somatic variants within known prostate cancer–associated genes. **A,** Variant calling within the NG cohort ($n = 45$) produced 1,168,250 variants. A total of 25 genes were known to be associated with prostate cancer harbored variants in at least two tumor samples. The most frequently mutated of these included *BRCA2* (BRCA2 DNA repair associated)—27%, *APC* (APC regulator of WNT signaling pathway)—20%, *ATM* (ATM serine/threonine kinase)—20%, *BRCA1* (BRCA1 DNA repair associated)—13%, and *DNAJC6* [DnaJ heatshock protein family (Hsp40) member C6]—13%. As a comparison with NG prostate cancer exome samples, TCGA prostate cancer samples ($n = 50$ AA and $n = 50$ EA) were downloaded through dbGAP and analyzed for genetic variants. **B,** Prostate cancer of NG men showed a significant ($P \leq 0.01$) elevation in *BRCA2* somatic mutations compared with African and EA men. A significant increase was also evident for *APC* ($P \leq 0.05$) and *BRCA1* ($P \leq 0.05$). Compared with NG men, prostate cancer of AAs were elevated ($P \leq 0.05$). NG and AA men also showed higher, but not significant, mutation frequencies of *ATM, MED12,* and *BRAF*. **C,** Somatic mutations for NGs and AAs were distributed across the amino acid sequence of the most mutated genes. None of the variants were shared across or within cohorts. **D** and **E,** SNPs in the NG prostate cancer cohort were compared with known cancer-related mutation signatures within the COSMIC. A total of 89% of NG prostate cancer mutation patterns were similar (cosign similarity ≥0.796) with COSMIC signatures 6. The remaining 11% were more like COSMIC 5. **F,** Mutated genes ($n = 83$) present in at least two NG prostate cancer tumor samples ($n = 45$) were imported into Cytoscape to assess functional gene ontology enrichment and visualize the GO term interaction network, using Kyoto Encyclopedia of Genes and Genomes pathways. Variants showed significant ($q \leq 0.000538$) GO and functional enrichment across multiple GO groups, including mismatch repair, homologous recombination, prostate cancer, and several cancer-related signaling pathways.

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variation to the incidence of prostate cancer and aggressiveness of the disease in MAA. Furthermore, these findings encourage us to identify clinically targetable sites to close the gap in health-related disparities.

We observed a high level of *BRCA1* germline mutation in prostate cancer of NG and men of African ancestry. The high rate is driven by the variant BRCA1_rs799917 T>C, which enhances disease risk in triple-negative breast (55), gastric (56), esophageal squamous cell (53), and lung (54) cancers. Results of meta-analyses, however, suggest that this variant is nonpathogenic (65–67). Because none of these studies included patients of African ancestry, the impact of this variant on that population remains poorly explored. BRCA1_rs799917 T>C alters the coding sequence of *BRCA1*, lowering *BRCA1* expression by inhibiting its interaction with miR-638 (68). In addition, because *BRCA1* is a DNA damage repair gene, it has been reported to upregulate the expression of multiple antioxidant genes and oxidoreductases, balancing cellular redox (69). Lower expression of *BRCA1*, skews this balance, which results an increase in DNA-damaging reactive oxygen species (ROS). AA men have been shown to possess lower mtDNA content, which can lead to enhanced ROS production and mitochondrial dysfunction (70). Coupled together, lower *BRCA1* expression and increased ROS production can lead to an accumulation of mutated DNA, which enhances tumorigenesis. BRCA1_rs799917 T>C has not previously been associated with prostate cancer; however, *BRCA1* germline mutations contribute to increased prostate cancer risk (71) and are associated with higher prostate cancer aggression and poorer outcomes (72). We found this trend in AA prostate cancer, which have higher frequencies of germline *BRCA1* VUS (73). This pattern is also evident in both NG and AA breast cancer tumors (74–76). Thus, our exome analysis provides evidence of distinctive germline BRCA1 mutations in prostate cancer of patients with African Ancestry.

Our analysis also showed that, in their DNA damage response (DDR) genes, NG prostate cancer tumors have higher somatic variant rates, with 53% of NG tumors having at least one somatic DDR gene mutation. NG tumors demonstrated increases in *BRCA2, APC,* and *BRCA1* mutations and SNP patterns associated with defective DNA mismatch repair. In addition, these tumors contained mutated genes that had significant gene ontology and functional enrichment across multiple GO groups, including mismatch repair and homologous recombination (HR) signaling pathways. Pathogenic mutations in DDR genes are prevalent in advanced-stage, localized prostate cancer, especially affecting genes responsible for repair by HR (77). Of note, *BRCA2* facilitates the formation of *RAD51* (RAD51 Recombinase) filaments, which are necessary for HR (78). The clinical implications of somatic *BRCA2* mutations are poorly understood; however, all *BRCA2* mutations (germline and somatic) are understood to destabilize HR, increase tumor aggression, and contribute to poor patient outcomes (79). DNA repair signalling is often impaired in cancer cells (80); however, this impairment is more substantial for MAA. Yadav and colleagues observed increased somatic mutations of *BRCA2, BRCA1,* and *ATM* in AA prostate cancer (26). AAs had a 1.24- to 2.16-fold increase in *BRCA2*, *BRCA1*, and *ATM*, as compared with EAs. The innate impairment of DNA repair in cancer cells leads to a dependence on alternative repair pathways that can be therapeutically exploited. Taking our findings, which are in line with other reports for AA men, the recent FDA approval of PARP inhibitors (specifically olaparib) and other therapies such as platinum drugs, ATR inhibitors, CHEK1/2 inhibitors, and radiotherapy may be useful for men of African Ancestry (81, 82).

Our analyses identified, in addition to somatic mutations in DDR genes, somatic mutations in novel prostate cancer–associated genes. Of NG tumors, 27% ($n = 13$) contained a recurrent *CACNA2D2* missense SNP of Leu54Phe (rs569543350). Both ClinVar and dbSNP designate this variant as having "unknown significance." *CACNA2D2* modulates the expression of functional calcium channels (83), which contribute to cancer development (84). Compared with noncancerous prostate tissue, *CACNA2D2* is expressed higher in prostate cancer tissue and can increase tumor proliferation and angiogenesis (85). *SYNE1* encodes a multi-isomeric protein that participates in connecting the nuclear envelope to the cytoskeleton. This connection is necessary for proper nuclear movement and positioning, and for cellular migration (86). Abnormal nuclear envelope structure, a feature of cancer, is thought to contribute to tumorigenesis (87). Mutations in *SYNE1* are linked to several human cancers (88). *ADAMTS2* encodes a procollagen N-proteinase that is necessary for collagen fibril assembly (89). The role of *ADAMTS2* in cancer remains poorly understood; however, the impact of collagen metabolism is well characterized (90, 91). Collagen is a structural component of the extracellular matrix, and its metabolism can affect tumor development, tumor tissue stiffness, metastasis, and treatment response. The presence of these novel prostate cancer–associated mutations is unsurprising. Non-European populations have higher rates of VUS (92–94). This is a result of the lack of diversity in research and (by extension) genomic databases (95). Non-European populations are underrepresented in research and genomic databases, which skews genomic annotations away from identifying clinically relevant variants in these populations. Further investigation into these novel mutations may expose alternative routes of disease aggression for MAA and mitigate the disparities.

These findings present the most comprehensive characterization of the NG prostate cancer exome to date and highlight the need to increase study population diversity. Although clinical genomics is a powerful tool to guide clinical interventions, the lack of non-European patients limits the capacity of these advancements to benefit men of African ancestry. Furthermore, the high level of genetic diversity within African men necessities the need for larger cohort studies to identify population-specific, recurrent mutations that contribute to prostate carcinogenesis.

Although our results are compelling, the study has some limitations. The high level of genetic diversity in Africans coupled with the lack of matched normal samples increased the number of detected somatic variants, requiring us to aggressively filter variants to limit the possibility of false positives. This conservative approach, although necessary, provides the possibility that we inadvertently excluded some true somatic variants. Moreover, the use of a nonmatched NG normal was employed to further filter out germline variants within somatic calls. These strategies reduced our ability to resolve ethnic and geographical differences within our NG cohort and required that we conduct a supervised analysis of variants within known prostate cancer driver genes. Second, we set a 70% African ancestry threshold for AA samples, (i) to make sure our AA and European TCGA comparison groups were as distinct as possible and (ii) to enrich the AA cohort for African ancestry. Setting this threshold limits the ability to determine the prevalence of the observed variant patterns in more admixed AA samples; however, a review of the available literature shows that the majority of AAs have at least that amount (96, 97). Finally, the limited representation of African patients within genomic databases and genomic research reduces our ability to determine the larger population-specific distribution of these findings. To our knowledge, this is the largest prostate cancer exome study of NG men, and, despite the limitations, provides a robust characterization of the somatic landscape within NG prostate cancer.

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Authors' Disclosures

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Authors' Contributions

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ARTICLE

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Serum proteomics links suppression of tumor immunity to ancestry and lethal prostate cancer

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There is evidence that tumor immunobiology and immunotherapy response may differ between African American and European American prostate cancer patients. Here, we determine if men of African descent harbor a unique systemic immune-oncological signature and measure 82 circulating proteins in almost 3000 Ghanaian, African American, and European American men. Protein signatures for suppression of tumor immunity and chemotaxis are elevated in men of West African ancestry. Importantly, the suppression of tumor immunity protein signature associates with metastatic and lethal prostate cancer, pointing to clinical importance. Moreover, two markers, pleiotrophin and TNFRSF9, predict poor disease survival specifically among African American men. These findings indicate that immune-oncology marker profiles differ between men of African and European descent. These differences may contribute to the disproportionate burden of lethal prostate cancer in men of African ancestry. The elevated peripheral suppression of tumor immunity may have important implication for guidance of cancer therapy which could particularly benefit African American patients.

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Men of African origin bear the highest prostate cancer burden in the U.S. and globally[1–3]. They are at an increased risk of developing fatal prostate cancer in the U.S and England[4] and present with more aggressive disease in the Caribbean and sub-Saharan Africa[2,5]. The reasons for the observed global prostate cancer health disparities are unclear but are likely related to an array of factors such as access to health care, lifestyle and environment, and ancestral and biological factors[6–8].

Previously, we and others described that tumor immunobiology differs between African-American (AA) and European-American (EA) prostate cancer patients[9–12]. A tumor-specific immune-inflammation gene expression signature was more prevalent in prostate tumors of AA than EA patients[11]. The occurrence of this signature in prostate tumors was associated with decreased recurrence-free survival[13]. Furthermore, regular use of aspirin, an anti-inflammatory drug, may reduce the risk of aggressive prostate cancer, disease recurrence and lethal disease in AA men[14,15]. Combined, these findings suggest that inflammation and host immunity may contribute to prostate cancer progression but with notable differences between AA and EA men.

Ancestral factors can influence immune-related pathways[16]. Germline genetic variant prevalence and alternative splicing in immune-inflammation-related genes can show large differences amongst population groups[17–19]. Hence, the immune-inflammation gene expression signature identified in the tumors of AA prostate cancer patients could be due to either tumor biology and the associated microenvironment, ancestral factors, or systemic differences in immune-oncology marker expression.

In this work, we test the hypothesis that a distinct systemic immune-inflammation signature exists in men of African ancestry that associates with prostate cancer using a large cohort of diverse men. Applying large-scale proteomics with Olink technology, we discover the up-regulation of circulating immune-oncological proteins that functionally relate to chemotaxis and suppression of tumor immunity and their association with West African ancestry and lethal prostate cancer. Our findings point to the clinical importance of a serum proteomic signature in prostate cancer patients that may affect men of African ancestry more so than other men.

Results

Large-scale evaluation of immune-oncological proteins in the NCI-Maryland and NCI-Ghana prostate cancer studies. To investigate if men of African descent are differentially affected by a systemic immune inflammation, we utilized two case-control studies with large representations of men of African ancestry: the NCI-Ghana and NCI-Maryland Prostate Cancer Case-Control Studies. Characteristics of the participants in the two studies have been previously described[14,20]. For our investigation, limited to men with quality-controlled (QCed) serum proteome measurements, the NCI-Ghana Prostate Cancer Case-Control study included 1143 men of whom 489 were cases and 654 were controls. Cases were older than controls (Supplementary Table 1). Controls had a slightly lower body-mass index (BMI) than cases. In addition, more controls were current smokers than cases (14% vs. 2%). High Gleason score (>7) was reported in 158 out of the 489 patients (32%). Participants in the NCI-Maryland Prostate Cancer Case-Control study included 1647 AA and EA men of whom 819 were cases and 828 were controls. Cases were slightly younger than controls. Cases and controls had similar BMI distributions. In addition, more cases were current smokers than controls (24% vs. 14%). High Gleason score (>7) was reported in 141 out of the 819 patients (17%). In the control population, Ghanaian men tended to be younger, to have a lower BMI, less likely to be diagnosed with diabetes, but with a similar prevalence of current smokers, when compared with AA and EA men.

We assayed 92 circulating immune-oncological proteins in a total of 3094 serum samples containing 1505 controls and 1432 cases along with 157 randomly selected blinded duplicates. To control for any batch effects, the serum samples were assayed in a random order along withthe 5% blind duplicates for intensity normalization (see Methods). Ninety-five percent of the samples passed stringent quality control, leaving 1482 controls (654 Ghanaian, 374 AA, and 454 EA) and 1308 cases (489 Ghanaian, 394 AA, and 425 EA) for our analysis (Supplementary Table 1). The average intra- and inter-plate coefficients of variation calculated based on duplicates were very low at 1.7% and 2.6%, respectively. In addition, the proportion of variance explained by an inter-plate batch effect was rather minimal for each of the serum proteins even before intensity normalization (Supplementary Fig. 1). Out of the 92 serum proteins, 61 had abundance levels above the lower limit of detection (LLOD) in 100% of the samples tested (Supplementary Table 2, Supplementary Fig. 2) and 78 proteins had levels above LLOD in >50% of the samples (Supplementary Fig. 2). Because 10 out of the 92 serum proteins were detected (i.e., had levels above LLOD) in <20% of the samples (Supplementary Fig. 2), only the remaining 82 proteins were included in our analysis (Supplementary Table 3). Next, we assessed how the 82 serum markers correlate with one another in Ghanaian, AA, and EA men with and without prostate cancer using Pearson's pairwise correlation analysis (Fig. 1 and Supplementary Fig. 3). The top ten observed correlations for each population group are presented in Supplementary Table 4. Most of these relationships have not previously been described. Notably, epidermal growth factor levels strongly correlated with CD40L, a marker of activated T cells, in cases [AA ($r = 0.87$), and EA ($r = 0.77$)] and controls [Ghanaian ($r = 0.71$), AA ($r = 0.83$), and EA ($r = 0.80$)]. Interleukin-8 levels correlated consistently with circulating caspase-8 in cases [Ghanaian ($r = 0.48$), AA ($r = 0.74$), and EA ($r = 0.73$)] and controls [Ghanaian ($r = 0.69$), AA ($r = 0.82$), and EA ($r = 0.80$)]. Other robust correlations include interleukin-8 with MCP3, TNFRSF4 with TNFRSF9, and CD83 with TNFRSF9.

Clinical and socio-demographic characteristics are associated with immune-oncological proteins. Cytokine levels can be influenced by environmental exposures and disease. Therefore, we investigated the association between various socio-demographic and clinical characteristics (age, BMI, education, aspirin use, smoking, diabetes, and PSA) with serum levels of immuno-oncological proteins using a multivariable linear regression model with adjustment for multicomparison analysis (Fig. 2). We restricted this analysis to the control population in the NCI-Ghana and NCI-Maryland studies to exclude the potential confounding effect of prostate cancer in the analysis. Among the exposures, aspirin use, and blood PSA levels showed only few relationships with the profile of the 82 immune-oncology markers. Other exposures and several demographics showed more robust relationships.

Aging is known to impact the immune system and is a risk factor for many diseases including cancer[21]. In our analysis, aging was most consistently associated with the level of the analytes across the three population groups, showing a significant correlation with almost half of these circulating immune-oncological proteins. For example, PGF, CXCL9, Gal9, Gal1, CX3CL1, TNFRSF12A, CCL23, MMP7, DCN, MMP12, ADGRG1, and PTN positively associated with age in all three population groups. The top-ranked biological functions that associated with these age-related proteins were cell migration and

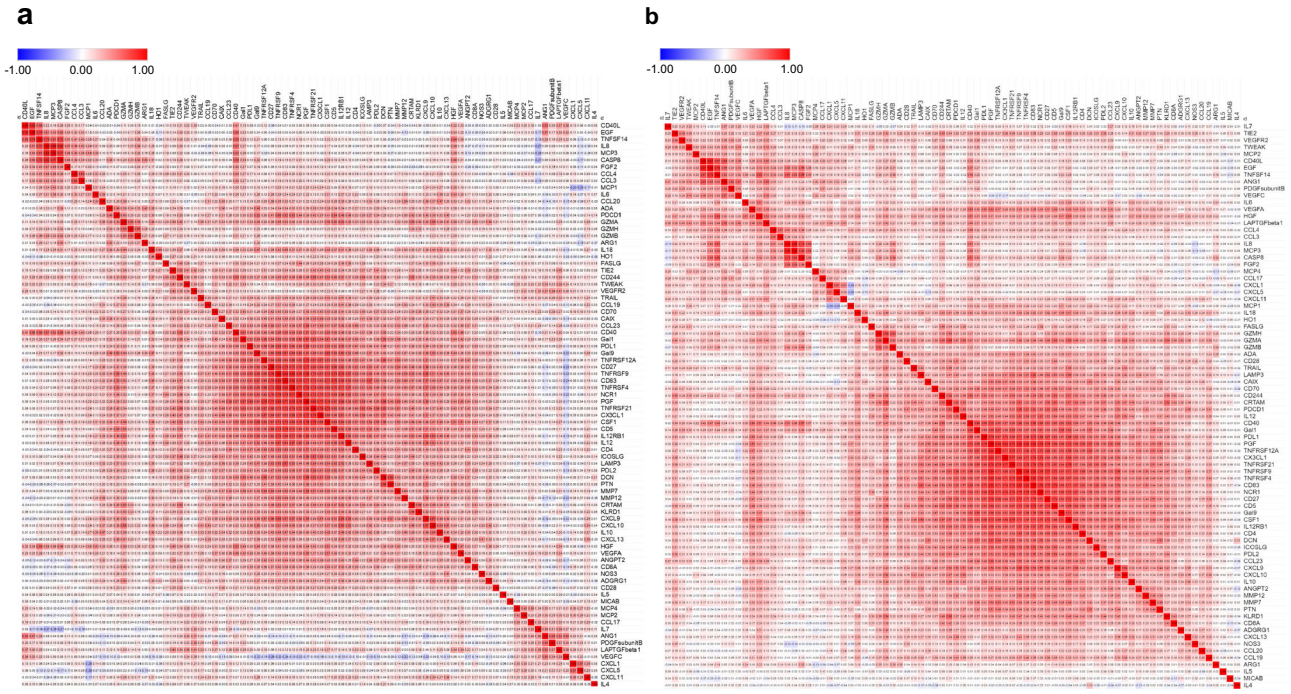


Fig. 1 Correlation matrix presenting Pearson pairwise correlations for each of the 82 serum protein pairs in African American men. Pearson pairwise correlations were estimated for each serum protein pair in African American (**a**) population controls ($n = 374$) and (**b**) prostate cancer cases ($n = 394$). Source data are provided as a Source Data file.

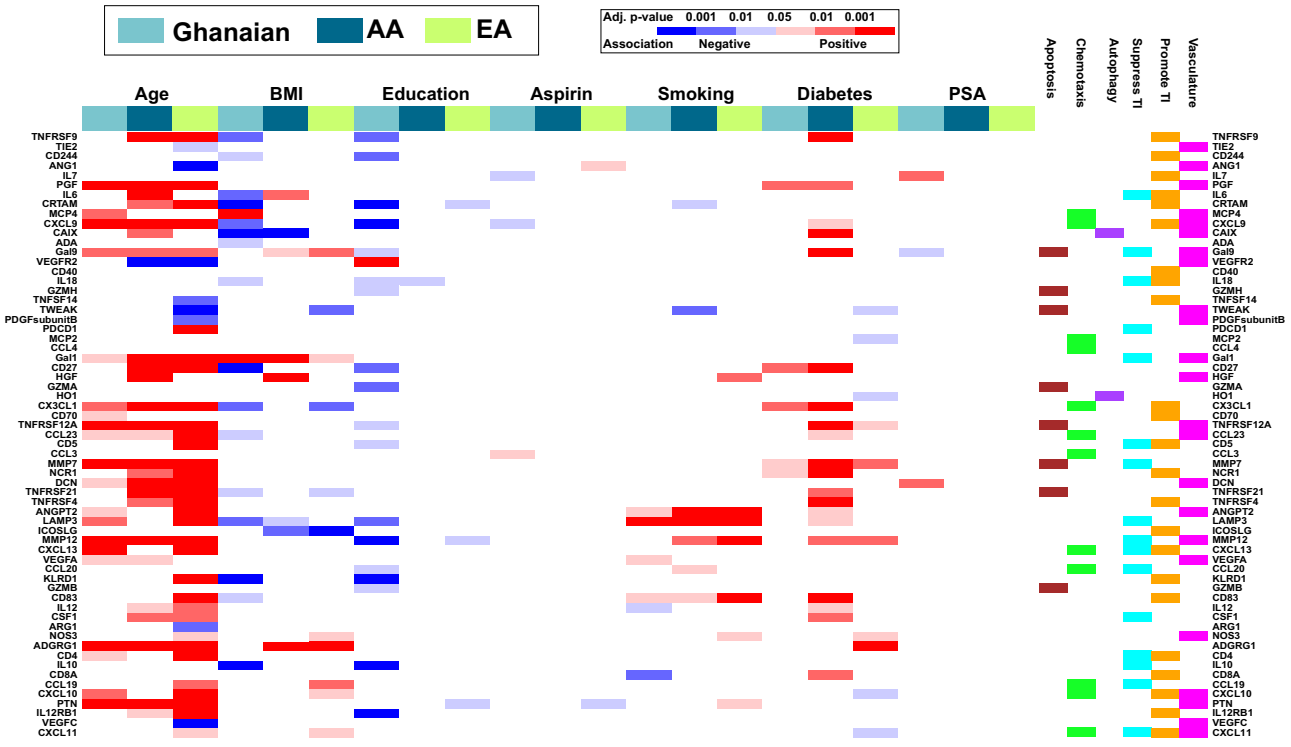


Fig. 2 Association of socio-demographic and clinical characteristics with systemic immune-oncological proteins in Ghanaian ($n = 654$), AA ($n = 374$), and EA ($n = 454$) men without prostate cancer. The association of the 82 immuno-oncological proteins (as continuous variables) with age, BMI, education, aspirin use, smoking, diabetes, and PSA was assessed in men without prostate cancer using a multivariable linear regression test. *P* values were adjusted for multiple comparison. An analyte was considered significantly associated with clinical and socio-demographic covariables if the multivariable model yielded a false discovery rate (FDR)-adjusted $P < 0.05$ on the F-statistic. Analytes that did not have a significant association with any of the clinical/sociodemographic variables in at least one of the population groups are not presented in the heatmap. Blue represents a negative association while red represents a positive association. The significance level (FDR-adjusted two-sided *P* value-based) for each association is color-coded. Source data are provided as a Source Data file. TI tumor immunity, AA African American, and EA European American.

positive regulation of cell communication (Supplementary Fig. 4). Lastly, TNFRSF9, CD27, TNFRSF21, TNFRSF4, and IL12RB1 were positively associated with age while VEGFR2, a tyrosine kinase receptor for VEGF, was negatively associated with age in EA and AA men (Fig. 2 and Supplementary Data 1).

In contrast to the positive association of many of the immune-oncological proteins with age, BMI tended to be negatively associated with these circulating immune-oncological analytes. For instance, 16 of the immune-oncological proteins negatively associated with BMI among Ghanaian men (Fig. 2). This finding may be surprising as obesity is generally thought to be associated with systemic inflammation. On the contrary, serum GAL1, a glycan binding protein that mediates the suppressive function of T_{Reg} cells[22], showed the opposite trend and was positively associated with BMI in all three population groups.

To explore how the social/behavioral environment may affect immune-oncological serum protein levels, we investigated their relationship with educational attainment. For Ghanaian men, 18 of the 82 immuno-oncological markers were negatively associated with their education level (Fig. 2). Among EA men, three of the 82 immune-oncological proteins had significant inverse relationships with the attained level of education (Fig. 2), with two of these markers showing a similar pattern among Ghanaian and EA men.

Previous studies have shown that tobacco smoking increases inflammation[23]. Herein, we assessed the association between cigarette use (never, former, vs. current smoker) on the level of immune-oncological proteins in circulation. We found that current smoking was consistently associated with significantly increased level of analytes that regulate angiogenesis (ANGPT2), antigen presentation (CD83), and autophagy (LAMP3), in all three study populations (Fig. 2).

Innate immune system-driven inflammatory processes have been implicated in the pathogenesis of diabetes[24]. In our analysis, among the proteins that showed an association with self-reported diabetes, a matrix metalloprotease enzyme, MMP7, was positively associated with diabetes in all three population groups (Fig. 2). On the other hand, CX3CL1 was positively associated with diabetes exclusively in men with African ancestry (Fig. 2 and Supplementary Data 1). CX3CL1 is known to regulate insulin secretion[25], is elevated in the serum of patients with type 2 diabetes[26], and has been implicated in diabetic nephropathy[27], validating the findings in our study.

C-reactive protein (CRP) is a commonly measured pro-inflammatory marker in the bodyand has been reported to be associated with worse prostate cancer prognosis[28,29]. Because it was not part of our marker panel, we measured blood CRP in 156 plasma samples from population controls of the NCI-Maryland study. Smoking was the only socio-demographic variable that showed association with CRP, however, the observed association was lost when adjusted for multiple testing (Supplementary Table 5). Furthermore, CRP showed positive associations with 24 of the 82 serum proteins (TNFRSF9, IL7, PGF, IL6, Gal9, GZMH, CXCL1, TNFSF14, Gal1, PDL1, HGF, HO1, CD70, TNFRSF12A, CCL3, MMP7, ANGPT2, VEGFA, CCL20, KLRD1, CSF1, CD4, MCP3, and CXCL11).

The systemic immune-oncological cytokine profile in men of African ancestry is distinct from men of European ancestry. To investigate if ancestral population group differences may influence circulating levels of the immune-oncological markers, we performed an unsupervised clustering analysis examining how the levels of the 82 immune-oncological analytes would group men without prostate cancer from Ghana and the US. Notably, these analytes tended to cluster by population group, with levels in Ghanaian men being most distant from EA men while AA samples tended to cluster in between these two groups (Fig. 3), suggesting that the ancestral background may have a significant impact on the global immune-oncological protein profile. We performed an additional statistical analysis of cluster assignments to more formally establish that the immune-oncological protein profile defined by the 82 markers is indeed different between these groups of men. We obtained the cluster assignments by cutting the hierarchical clustering dendrogram to extract K clusters (with $K = 2, 3$) and tested for differences in their distribution across the population groups (Supplementary Fig. 5). We found significant differences in cluster representation between Ghanaian, AA, and EA men with cluster enrichment by population group at $P < 1.e{-}10$, confirming that significant differences likely exist in the global immune-oncological protein profile among them.

To further evaluate the influence of ancestry, we estimated West African ancestry in AA and EA population controls of the NCI-Maryland study and its relationship with the cytokine profile. West African ancestry was determined using 100 validated ancestry informative markers[30]. The approach showed that, to some extent, the variance in the levels of several immune-oncological analytes can be strongly influenced by the degree of West African ancestry of these individuals (Fig. 4a). The variance in 39 of the analytes were significantly [false discovery rate (FDR)-adjusted $P < 0.05$] influenced by degree of West African ancestry (Supplementary Table 6, Supplementary Data 2). The levels of 37 analytes were significantly accounted for by West African ancestry even after adjusting for age, BMI, aspirin use, education, income, diabetes, and smoking status (Supplementary Table 7, Supplementary Data 3). CXCL5, CXCL1, MCP2, MCP1, CXCL11, CCL23, PTN, TWEAK, NCR1, IL18, and CCL17 were the top-ranked proteins. West African ancestry contributed to the variance with various effect sizes and explained >10% of the variance among the top 7 proteins (Supplementary Tables 6–7, Supplementary Data 2–3). For instance, 41% and 50% of the variance in the serum levels of CXCL1 and CXCL5, respectively, was accounted for by the degree of West African ancestry (Fig. 4a, Supplementary Tables 6–7, Supplementary Data 2–3).When we compared the levels of these proteins across the three population groups, we observed a significant African ancestry-related trend (Fig. 4b), with 10 of the 82 circulating immune-oncological proteins (CXCL5, CXCL1, CXCL11, MCP2, CCL17, MCP4, CD70, PDL2, MMP7, and CCL19) being significantly elevated in both Ghanaian and AA men compared to EA men (Supplementary Table 8); 13 other markers (MCP1, IL12, CCL23, CD8A, NCR1, TNFRSF4,TNFSF14, TWEAK, IL7, HGF, HO1, TNFRSF21, and ANG1) were inversely related to West African ancestry (Supplementary Table 9).

Cytokines associated with suppression of tumor immunity and chemotaxis are upregulated in men of African ancestry. Levels of many of the 82 immune-oncology markers showed a marked association with ancestry. To better define the functional implications of these population group differences, we grouped the 82 proteins into six biological processes according to Olink guidelines (Supplementary Table 10): apoptosis/cell killing, autophagy/metabolism, chemotaxis/trafficking to tumor, suppression of tumor immunity (Th2 response, tolerogenic), promotion of tumor immunity (Th1 responses), or vasculature and tissue remodeling. To gain insight on how activation of these six processes/pathways may differ by population group, we compared process/pathway sum scores between Ghanaian, AA, and EA men without prostate cancer using multicomparison-adjusted significance testing. Of these pathways, chemotaxis, promotion of

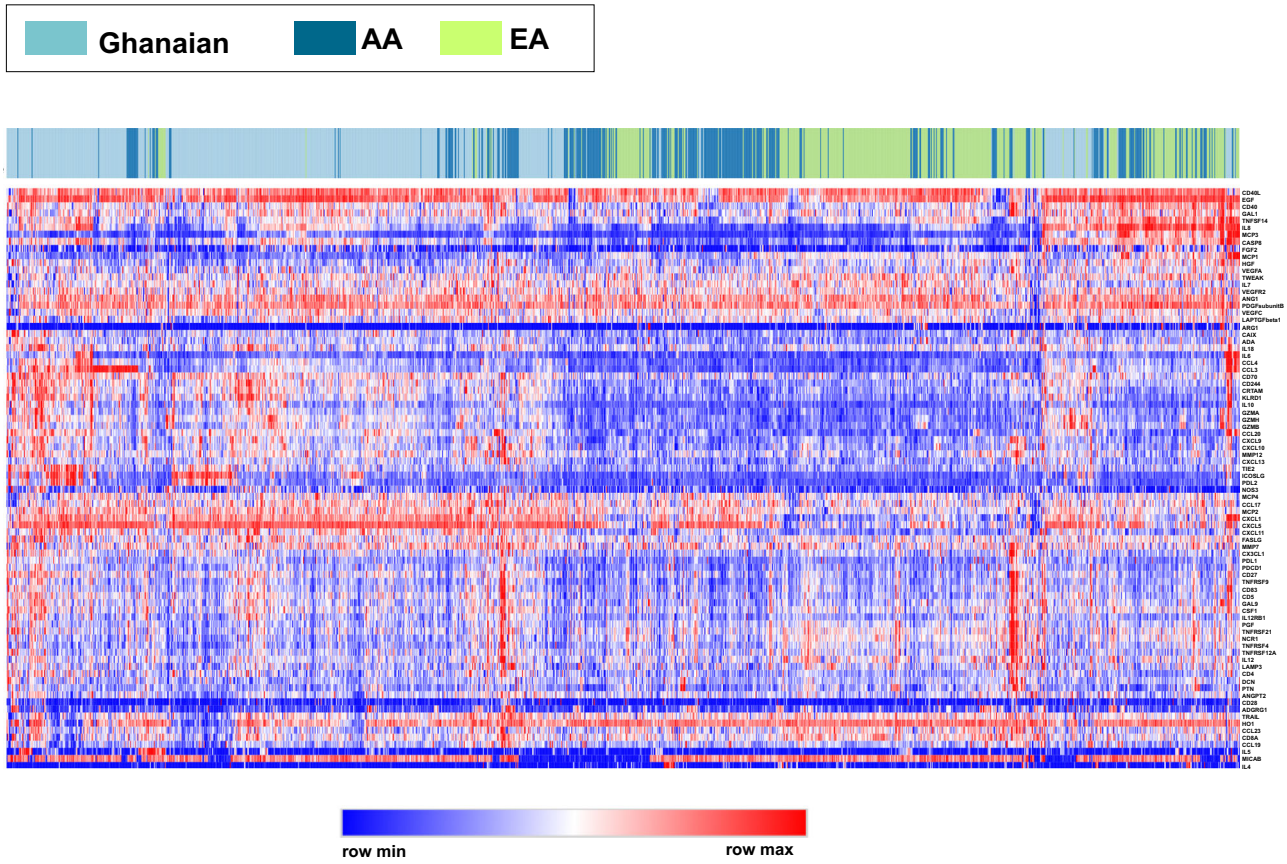


Fig. 3 Unsupervised hierarchical clustering associates circulating immune-oncological proteome profiles with population groups—Ghanaian, AA, and EA men. Heatmap showing protein profiles for men without prostate cancer. Each row represents a protein ($n = 82$), and each column corresponds to an individual [$n = 1482$ (654 Ghanaian, 374 AA, and 454 EA)]. Each individual is color-coded as Ghanaian, AA, or EA in the annotation bar on top of the heatmap. Normalized z-score of proteins abundance is depicted on a low-to-high scale (blue-white-red). Source data are provided as a Source Data file. AA African American and EA European American.

tumor immunity, and suppression of tumor immunity were different in their predicted activity between AA and EA men (Fig. 5). AA men had higher scores for chemotaxis and suppression of tumor immunity when compared to EA men, indicating higher pathway activity in AA men, but they had a lower score for promotion of tumor immunity. Ghanaian men had even higher scores for chemotaxis and suppression of tumor immunity than both AA and EA men (Fig. 5c, e), indicating a possible association with West African ancestry. The latter was corroborated with our finding that the chemotaxis and suppression of tumor immunity scores positively correlated with the proportion of West African ancestry within the NCI-Maryland cohort, even after holding the other variables constant (i.e., age, BMI, education, aspirin use, diabetes, and smoking history) in the regression analysis (for chemotaxis score: regression coefficient = 5.12 (3.75, 6.49), $P < 0.0001$; for suppression of immunity score: regression coefficient = 4.02 (2.01, 6.04), $P < 0.0001$). Even though apoptosis and vasculature-associated cytokines were not significantly different between EA and AA men, we found both processes to be elevated in the Ghanaian men.

Suppression of tumor immunity is associated with reduced survival of prostate cancer patients. Next, we examined the clinical implication of our findings and assessed the association of pathway activity with survival of prostate cancer patients or controls in the NCI-Maryland study using multivariable Cox regression modeling. As of the end of 2018, out of the 819 cases, there have been 202 deaths in our case population, of whom 103 (51%) had a cancer diagnosis as the recorded primary cause of death, and 28% of all deaths ($n = 57$) were directly attributed to prostate cancer. On the other hand, 99 of the 828 population controls had died by the end of 2018. Median survival follow-up for cases and controls were 8.6 and 6.7 years, respectively. With these data, we built a Cox regression model with the six biological processes/pathways and additional adjustments for other covariables including disease status (see Methods). Among the six pathways, only suppression of tumor immunity showed independent association with survival outcomes among the cases in a disease status-adjusted analysis (i.e., PSA, TNM Stage, Gleason score, and Gleason pattern) defined by the National Comprehensive Cancer Network (NCCN) risk score (Fig.6 with 99% CI, Supplementary Fig. 6 with 95% CI). Prostate cancer patients with an increased activity of this pathway had the highest risk of death from all causes (Fig. 6a, Supplementary Table 11). In contrast, suppression of tumor immunity was not associated with all-cause mortality of population controls (Supplementary Table 12), suggesting that the association with all-cause mortality among cases might be prostate cancer-related. Prostate cancer patients with elevated suppression of tumor immunity at diagnosis had also the highest risk of a prostate cancer-specific mortality, although statistically significant only with a 95% CI (Supplementary Fig. 6), but not with a multicomparison-adjusted 99% CI (Fig. 6b, Supplementary Table 13). Lastly, prostate cancer patients with increased suppression of tumor immunity were also significantly more likely to die from any cancer (prostate cancer or secondary cancer) following the prostate cancer diagnosis (Fig. 6c,

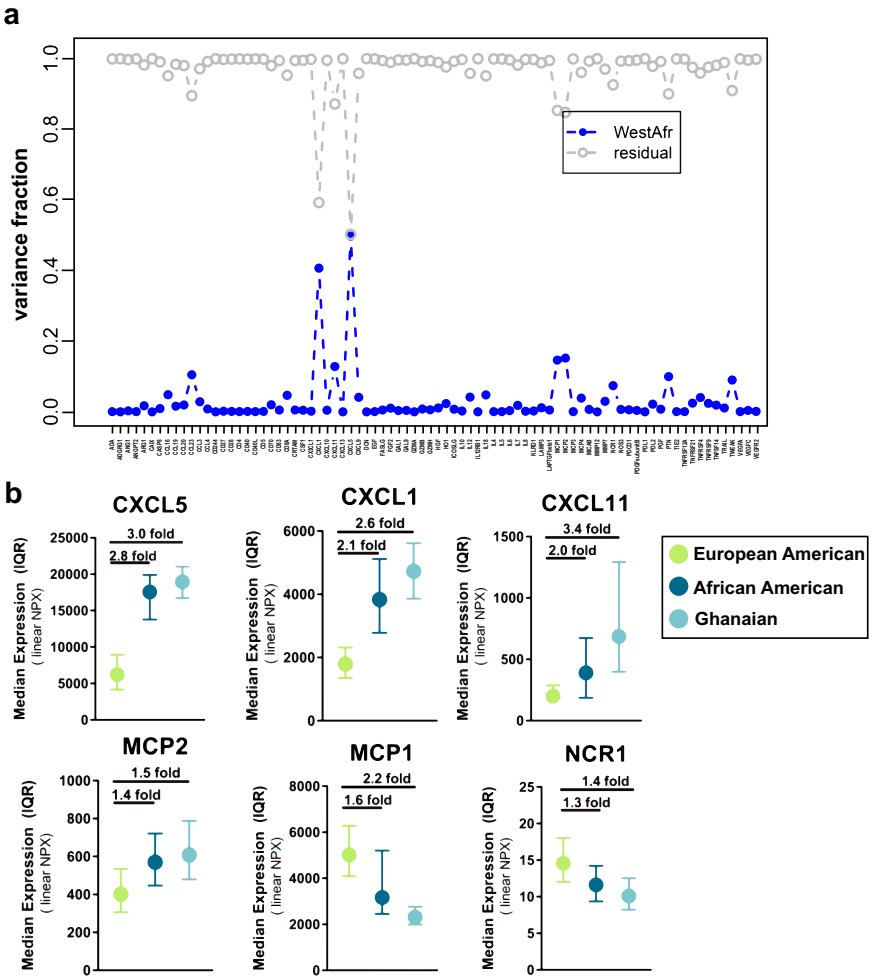


Fig. 4 Immune-oncological proteins and their relationship with West-African ancestry. a Variance analysis for the levels of each of the 82 immune-oncological cytokines assessed as a function of genetic estimation of West African admixture among men without prostate cancer within the NCI-Maryland study ($n = 795$). The blue plot represents the proportion of variance that can be explained by the degree of West-African admixture while the grey plot represents the residual variance that remains to be explained by factors other than West-African ancestry. **b** The median levels of the top six West-African ancestry-correlated immune-oncological proteins were compared between Ghanaian ($n = 654$), AA ($n = 374$), and EA ($n = 454$) men. Error bars represent inter quartile range (IQR). Linearized protein abundances (2^NPX) were used to determine median and IQR for each of the proteins. Data are presented as median ± IQR. Source data are provided as a Source Data file.

Supplementary Table 14), indicating a more general predisposition to cancer in patients with a high suppression of tumor immunity score in this hypothesis generating analysis approach.

Elevated suppression of tumor immunity is associated with metastatic prostate cancer. To further corroborate the significance of suppression of tumor immunity in the development of lethal prostate cancer, we assessed its association with prostate cancer aggressiveness per NCCN guidelines (see Methods). Information on TNM stage was only obtainable for the NCI-Maryland prostate cancer patients, hence only these cases were scored according to the NCCN guidelines. Patients with a high suppression of tumor immunity score were at substantially increased odds of being diagnosed with regional or distant metastasis (OR 3.79, 95% CI 1.59–9.04, >median vs. ≤median) (Table 1), consistent with the disease survival data. The data showed a significant trend in the association of elevated suppression of tumor immunity with disease aggressiveness (P trend = 0.004) (Table 1). Although a stratified analysis by self-reported race/ethnicity suggested that high suppression of tumor immunity was associated with metastatic prostate cancer more strongly among AA than EA men, large 95% CIs for the odds ratio precluded the observed difference across racial groups from statistical significance.

Blood levels of TNFRSF9/CD137/4-1BB and pleiotrophin predict lethal prostate cancer among AA men. To identify potential drivers of the relationship between immune-oncology markers and lethal prostate cancer, we applied a cross-validated, regularized Cox regression model using eNetXplorer (see Methods). Included in this model were the 82 immune-oncology markers and 6 patient feature covariates (age, education, BMI, smoking history, aspirin use, and diabetes). Of those patient features, education as a surrogate for socioeconomic status and health care access, BMI, smoking status, and aspirin use have previously been associated with the risk of lethal prostate cancer whereas the direction and strength association for diabetes with prostate cancer and disease outcomes is more uncertain[31,32]. Utilizing this method, we could not identify a robust predictive signature of lethal prostate cancer for EA patients. However, for AA patients, lasso regression (alpha = 1) was selected as a predictive model ($P = 0.0001$) with the best overall performance across alpha values (Supplementary Fig. 7A, Supplementary Data 4). In this model, a signature consisting of TNFRSF9 and

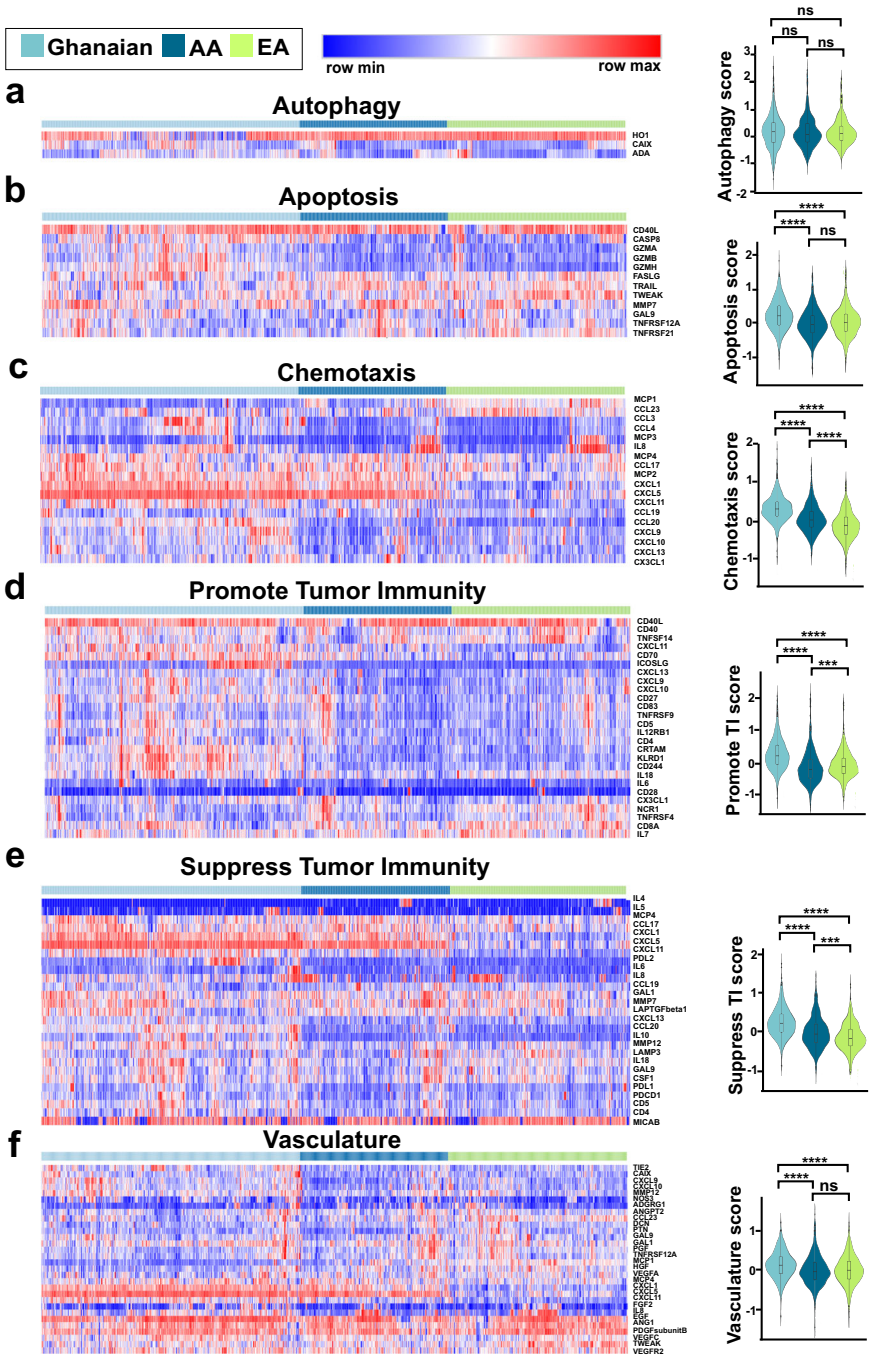


Fig. 5 Population differences in proteome-defined pathway activity scores. Shown are population differences in the abundance of proteins driving (**a**) autophagy, (**b**) apoptosis, (**c**) chemotaxis, (**d**) promotion of tumor immunity, (**e**) suppression of tumor immunity, and (**f**) vasculature. Heatmaps depict levels of process/pathway-associated proteins in relationship to population group (Ghanaian, AA, EA). Shown to the right are the mean score differences for these processes/pathways among the three population groups. Profiles for Ghanaian ($n = 654$), AA ($n = 374$), and EA ($n = 454$) men without prostate cancer are shown. The process/pathway scores are derived from the average z-scores of all the associated proteins. These scores are shown as violin plots and were compared using two-sided Wilcoxon rank sum tests. P values were adjusted for multiple comparison. FDR-adjusted P value significance was coded as <0.0001 (****), <0.001 (***), and ≥0.05 (ns). The violin plots represent median values ± IQR. Source data are provided as a Source Data file. TI tumor immunity. FDR False Discovery Rate, AA African American, and EA European American.

pleiotrophin (PTN), both positively associated with the risk of lethal disease, and regular aspirin use (negatively associated with risk) emerged as a top predictor based on two selection criteria: the feature frequency (Fig. 7a) and the weight of the features' contribution to the prediction (Fig. 7b). These three features combined predicted prostate cancer-specific mortality with an accuracy of 83.7% (SE = 3.8%). Our finding that regular aspirin use was a predictor of improved survival among AA men is consistent with previously published data from this case-control study[14] and the Southern Community Cohort Study[15]. The combination of the two proteins alone predicted prostate cancer-specific mortality with 78.2% (SE = 4.2%) accuracy. To gain additional insight of how the prediction of lethal prostate cancer by these two protein markers might be influenced by a patient's

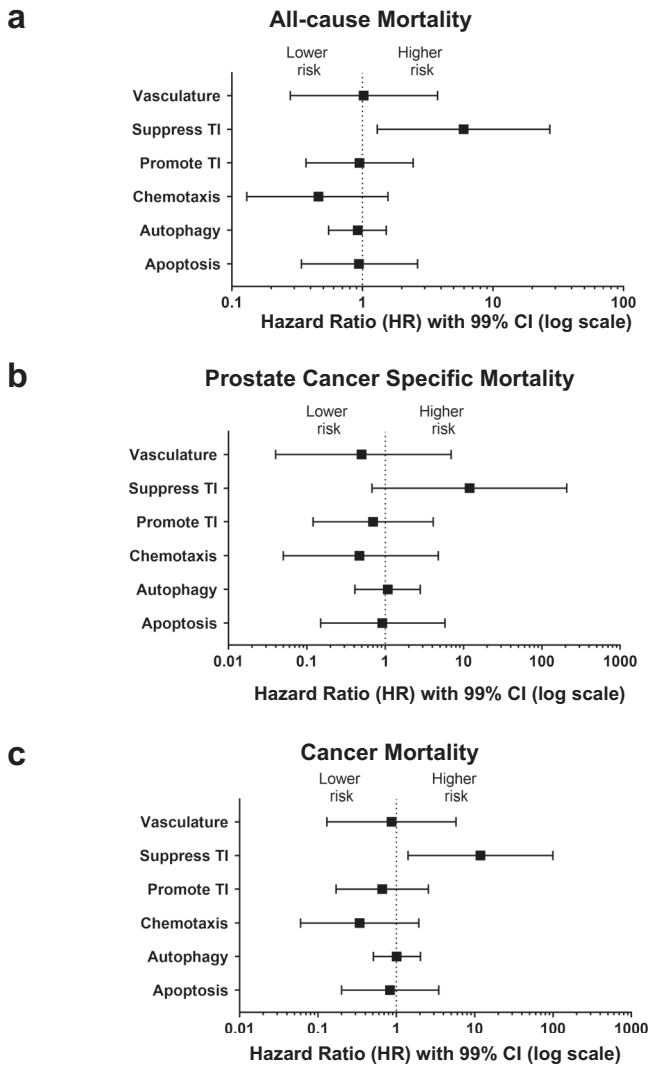


Fig. 6 Suppression of the tumor immunity pathway associates with survival of prostate cancer patients. We assessed the association of the six pathways defined by the 82 immune-oncology markers with all-cause mortality ($n = 202$), prostate cancer-specific mortality ($n = 57$), or mortality due to any cancer after a prostate cancer diagnosis ($n = 103$) out of the 819 prostate cancer patients followed. The pathway scores were evaluated as continuous predictor variables. Suppression of tumor immunity pathway was distinctively associated with all-cause mortality (**a**), prostate cancer-specific mortality (**b**), or a mortality due to any cancer after a prostate cancer diagnosis (**c**). Multivariable Cox regression analyses were used to assess if the pathways were independently associated with survival of prostate cancer patients in the NCI-Maryland study. For (**a**–**c**), the hazard ratios (HRs) were adjusted for age at study entry (years), BMI (kg/m2), self-reported race (AA/EA), education (high school or less, some college, college, professional school), income (<$10k, $10–30 K, $30–60 K, $60–90k, greater than $90k), smoking history (never, former, current), diabetes (no/yes), aspirin use (no/yes), treatment (0 = none, 1 = surgery, 2 = radiotherapy, 3 = hormone, 4 = combination), and NCCN risk score. The HRs indicate the change in risk of dying when the biological process *z*-score value increases by 1 while holding all the other biological processes' *z*-scores and covariates constant. Data are presented as hazard ratios ± 99% confidence intervals. Source data are provided as a Source Data file. TI tumor immunity and CI confidence interval.

disease status, we added the NCCN risk score to our model. Consistent with the baseline model that did not contain the NCCN risk score, lasso (alpha = 1) remained the most predictive model (Supplementary Fig. 7b, Supplementary Data 5). The NCCN risk score was the top predictor of the lethal disease. The two analytes PTN and TNFRSF9 remained the most predictive features besides the NCCN risk score, and the three features combined predicted prostate cancer-specific mortality with 90% accuracy (Supplementary Fig. 7B, Supplementary Fig. 8). In addition, AA prostate cancer patients with high levels (>median) of both TNFRSF9 and PTN in their blood at diagnosis had the worst prostate cancer-specific survival independent of disease status [(Adjusted HR = 3.09 (1.36, 7.03)] (Fig. 7c). By 10 years, 33% of cases with high levels of both TNFRSF9 and PTN died of prostate cancer compared to only 5% of cases with low levels of one or both of these proteins (Fig. 7c), highlighting the utility of these blood markers for risk stratification of AA prostate cancer patients.

Discussion

In this study, we describe differences in the expression of immune and chemotaxis-related markers in men from three population groups, with two of them—AA and Ghanaian men—having an ancestral relationship due to the trans-Atlantic slave trade. Most notably, expression of immune-oncology markers related to immune suppression were up-regulated in men of West African ancestry and were associated with prostate cancer mortality. While ancestry can explain some of the observations, other and yet unknown factors contribute to these clinically significant differences in immune function and chemotaxis.

Infections endemic to certain regions have shaped the immune response in affected populations, leaving a lasting genetic and epigenetic footprint[33]. As such, population differences in exposures to fatal pathogens have led to population heterogeneity in the immunome. It has been estimated that as many as 360 immune-related genes have been targets of positive selection and have functional variations between populations[34]. Consistent with these observations, we now report population differences in circulating immune-oncological proteins among Ghanaian, AA, and EA men. We found that the serum proteome-defined immunome of Ghanaian men resembles the immunome of AA men more so than EA men. We identified CXCL5, CXCL1, MCP2, MCP1, and CXCL11 as the top immune-oncological proteins associated with West African ancestry. Four of these chemokines (CXCL5, CXCL1, MCP1, and CXCL11) are known targets of Duffy Antigen Receptor for Chemokines (DARC) binding[35]. DARC is a non-signaling receptor that binds to both CXC and CC family of chemokines and acts as a depot for chemokines on erythrocytes and as decoy receptor on endothelial cells[36]. DARC expression modulates the susceptibility to clinical *Plasmodium vivax* malaria and loss of its expression on erythrocytes, which frequently occurs in sub-Saharan African populations due to germline genetic variants, confers resistance against malarial infection[37]. Its loss may also influence cancer susceptibility[38,39]. Consequently, these individuals lack the ability to sequester the target chemokines, leading to elevated concentration of the chemokines in circulation[40]. Accordingly, we found that CXCL5, CXCL1, and CXCL11 were 2–3-fold higher in sera of Ghanaian or AA men than EA men. Given the angiogenic properties of these chemokines[41], their role in cancer progression has been proposed[42].

As a key finding, we report that serum proteins regulating chemotaxis and suppression of tumor immunity were elevated in men of African ancestry, suggesting persistent population differences in stimulation of leukocyte recruitment and T cell mediated immune response. Such differences may predispose men of African descent to a distinct tumor microenvironment. Although the direct impact of the peripheral immunome on the prostate tumor microenvironment requires further investigation,

Table 1 A high score for suppression of tumor immunity associates with National Comprehensive Cancer Network (NCCN) Risk Score for metastatic prostate cancer.

NCCN risk score	Total OR (95% CI)[a]	AA OR (95% CI)	EA OR (95% CI)
Low	Ref.	Ref.	Ref.
Intermediate	1.04 (0.68–1.59)	0.89 (0.46–1.70)	1.18 (0.65, 2.13)
High/Very High	1.47 (0.87–2.48)	1.33 (0.59–2.98)	1.72 (0.83, 3.54)
Regional/Metastatic	**3.79 (1.59–9.04)**	**5.90 (1.43–24.34)**	3.16 (0.95, 10.50)
P value for trend	**0.004**	**0.019**	**0.040**

Bolded data indicate significant associations in the logistic regression analysis.
High suppression of tumor immunity is defined by the median score in the NCI-Maryland control population (>median vs. ≤median).
[a]Logistic regression adjusted for age at study entry, BMI (kg/m2), diabetes (no/yes), aspirin (no/yes), education (high school or less, some college, college, professional school), family history of prostate cancer (first-degree relatives, yes/no), self-reported race (not included in the stratified analysis), income (<$10k, $10–30 K, $30–60 K, $60–90k, >$90k), smoking history (never, former, current), treatment (0 = none, 1 = surgery, 2 = radiation, 3 = hormone, 4 = combination).

we and others have previously reported stark differences in the immune landscape of prostate tumors of AA men as compared to EA men[9–13]. For instance, programmed cell death ligand-1 (PD-L1), which suppresses T cell–mediated tumor immunity, was found to be overexpressed in AA prostate tumors[43]. Recent work by Awasthi et al. reported that AA prostate tumors tend to be enriched for immune pathways that are associated with poor clinical outcomes[44]. We show with our current work that elevated, peripheral suppression of tumor immunity associates with lethal prostate cancer and the underlying mechanism may possibly involve an effect on metastasis. Hence, population differences in suppression of tumor immunity may contribute to the disproportionate burden of lethal prostate cancer among men of African ancestry. On the other hand, such differences may offer a therapeutic advantage for immunotherapeutic strategies that are tailored to target immune suppressive pathways. A recent study provided a first indication that differences in the response to cancer vaccines may lead to higher survival rates among AA men[45].

Differentiating men who have lethal forms of prostate cancer from those with a more slow-growing disease remains a major challenge in clinical oncology. Risk stratification strategies are particularly needed for AA prostate cancer patients who disproportionately bear the prostate cancer burden. This study identified TNFRSF9/CD137/4-1BB and PTN as candidate predictive blood markers for prostate cancer mortality among AA patients. AA patients with high levels of both TNFRSF9 and PTN in their sera had the highest risk of dying from prostate cancer. The membrane form of TNFRSF9 possesses antitumor properties and agonistic anti-TNFRSF9 antibodies are currently in clinical trials[46,47]. On the contrary, the soluble isoform of TNFRSF9 that we measured, generated by alternative splicing[48], has been shown to antagonize antitumor immune response hence promote tumor survival most likely by acting as decoy receptor[49,50]. Regulatory T cells described as Tregs are thought to be a major source of secreted TNFRSF9[51,52]. Recently, TNFRSF9 mRNA level was shown to be a robust marker of tumor-infiltrating Tregs that suppress antitumor response[53]. Moreover, high numbers of TNFRSF9–expressing Tregs were associated with poor survival outcomes across multiple human cancers[53], consistent with our findings that serum TNFRSF9 associates with lethal prostate cancer. PTN or pleiotrophin, the second protein marker found to be associated with lethal prostate cancer in AA men, may not have the same immune function that soluble TNFRSF9 exhibits. PTN is a secreted cytokine that is developmentally regulated. Normally expressed during embryogenesis as a growth or differentiation factor, it is expressed either at very low levels or not at all in healthy adults[54,55]. PTN re-expression in adults is associated with tumor development, metastasis and angiogenesis with elevated expression reported in several cancer sites[56–58]. This increased expression of PTN has been associated with poor prognosis in colorectal cancer[56], hepatocellular carcinoma (HCC)[58] and gliomas[57]. Several hypotheses have been pursued to find out how PTN exerts pro-metastatic effects. For example, it is proposed that PTN promotes cancer progression through increased vascular endothelial growth factor deposition at the vasculature leading to vascular disruption[57]. PTN may upregulate lipid synthesis, contributing to hepatic stenosis and progression of HCC[58]. Serum PTN is a candidate biomarker for occurrence of breast[59] and small cell lung cancers[60] and has been shown to associate with metastatic prostate cancer[61], consistent with the findings in this study. In prostate cancer, PTN regulates mesenchymal and epithelial proliferation, with PTN itself being regulated by the androgen receptor during prostate development[62]. To the best of our knowledge, a potential relationship between PTN and TNFRSF9 has not been described. However, PTN has been implicated in the induction of TNF-α expression in peripheral blood mononuclear cells demonstrating a link between PTN and the TNF superfamily in the circulation[63].

The immune and inflammatory environment in the circulation has been implicated as a potential influencer of metastasis. Evidence is emerging to indicate that inflammation-activated platelets are pro-metastatic, instigating the formation of a pre-metastatic niche. *Lucotti et al.* recently demonstrated reduced metastasis in lung cancer models through inhibition of intravascular COX-1-derived thromboxane A2 (TXA2) from platelets via aspirin treatment[64]. Consistent with the animal model data, we recently reported an association between elevated urinary TXB2 (the stable metabolite of TXA2) and metastatic prostate cancer in AA men with prostate cancer[65], suggesting a distinct inflammatory environment and platelet activity in these men. Platelets can disrupt immunosurveillance of the metastatic cascade through cloaking of natural killer (NK) cells, preventing the NK cells from patrolling and inducing tumor-cell cytolysis[66]. Pre-clinical studies have also implicated other immune cells including macrophages[67], Tregs[68], and neutrophils[69,70] as promoters of the metastatic process through protection of tumor cells in the circulation and promotion of tumor cell seeding. Thus, the immune-oncological profile in men of African ancestry may develop in an environment of systemic chronic inflammation and promote metastasis. Future research should test this hypothesis.

Our study has strength and limitations. The major strength is the large sample size, the measurement of 82 immune-oncology markers with a robust technology, and the inclusion of men from Ghana and the U.S. Moreover, we applied multiple testing adjustments in reporting the significance of our observations. However, we collected blood samples in Ghana and the U.S. Although blood sample collection in Ghana followed a protocol

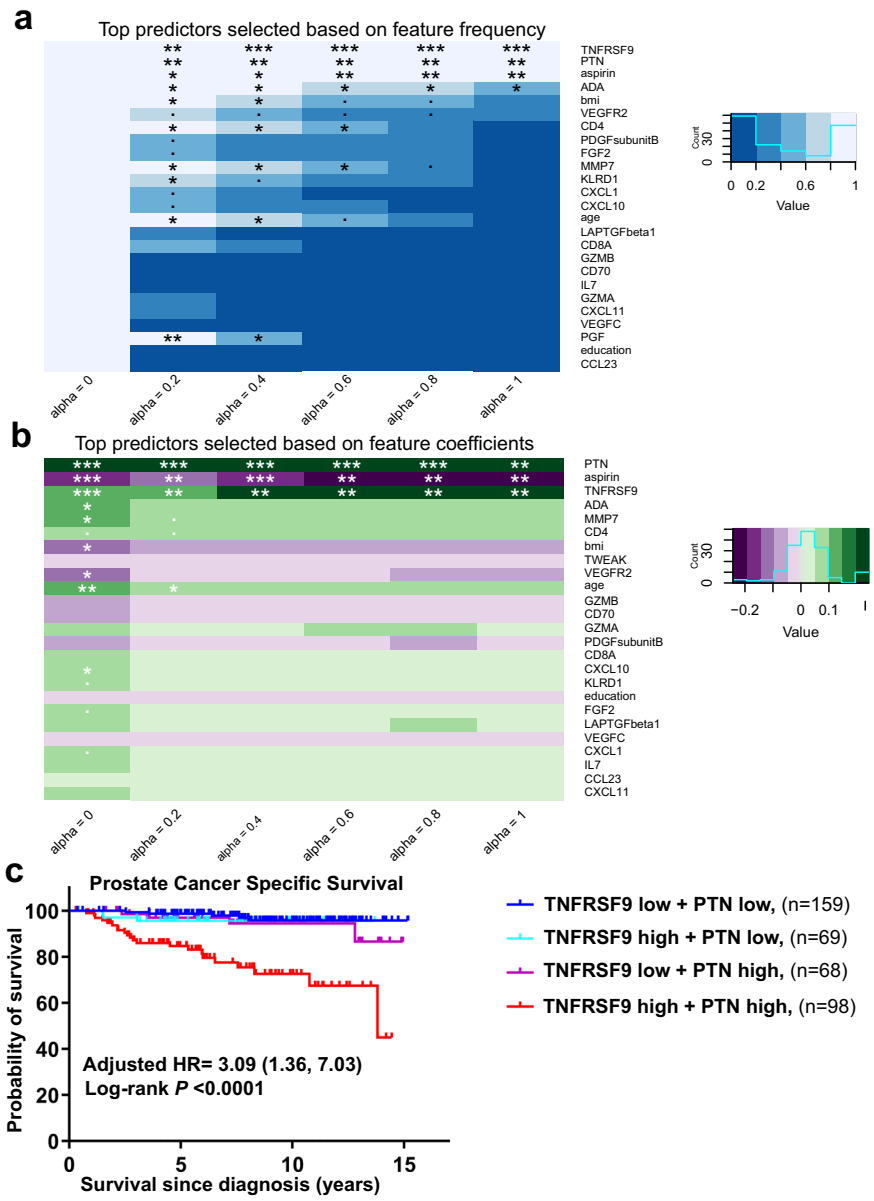


Fig. 7 A signature of two serum markers is predictive of lethal prostate cancer in AA patients. Cross-validated, regularized Cox regression models with different elastic net mixture parameters from ridge (alpha = 0) to lasso (alpha = 1) were implemented to identify a predictive proteomic signature. One-sided *P* values were obtained empirically by comparing feature frequencies and coefficients against those obtained from random permutations of the response[74]. **a** Heatmaps of feature frequencies across alpha. Features were ranked by *P* value for alpha = 1. **b** Heatmaps of feature coefficients across alpha. Features were ranked by *P* value for alpha = 1. **c** Kaplan–Meier plot comparing prostate cancer-specific mortality of AA cases with high levels (>median) of both TNFRSF9/CD137/4-1BB and pleiotrophin (PTN) vs. low levels of either or both proteins. Two-sided log-rank test was used to determine if there were statistically significant survival differences. Adjusted hazard ratio (HR) compares the risk of prostate cancer mortality for those with high levels of both TNFRSF9 and PTN vs. the remaining AA cases. HR estimates were adjusted for potential confounding factors: age, BMI, education, income, smoking history, diabetes status, aspirin use, treatment, and NCCN risk score. In (**a** and **b**), *P* value significance was coded as <0.001 (***), <0.01 (**), <0.05 (*),and <0.1 (.). The exact *P* values are found in the Source Data file. Source data are provided as a Source Data file. HR hazard ratio.

that applied standards of practice in the U.S., we cannot exclude that serum preparation and shipping influenced the performance of the immune-oncology marker measurements, yet we are not aware of such an influence. In our survival analysis, we included 57 events for prostate cancer-specific deaths, 103 events for any cancer deaths, and 202 events for all-cause mortality among men with prostate cancer. In these analyses, we found that an elevated suppression of tumor immunity score to beconsistently associated with decreased survival. Yet, additional studies will be needed to further confirm the association of the suppression of tumor immunity signature with lethal prostate cancer.

In conclusion, it is a key finding of our study that suppression of tumor immunity is increased in Ghanaian and AA men, when compared to EA men, and associates with lethal prostate cancer. As such, these findings provide an insight into potential causes of the prostate cancer health disparity. The current study has a large representation of men of African descent who were profiled for their immune-oncological proteome. With the advent of an increasing number of immunotherapies in the drug development pipeline, studies like ours may inform clinical research on population differences in the immune landscape that need to be considered when designing therapies that exploit the immune response.

Methods

NCI-Maryland prostate cancer case-control study. This study and the eligibility criteria have been previously described[14,71]. Race/ethnicity was assigned based on self-identification as either black or AA or as white or EA. The study was initiated to test the primary hypothesis that environmental exposures and ancestry-related factors contribute to the excessive prostate cancer burden among AA men. The study was approved by the NCI (protocol # 05-C-N021) and the University of Maryland (protocol #0298229) Institutional Review Boards and all participants signed an informed consent. Cases were recruited at the Baltimore Veterans Affairs Medical Center and the University of Maryland Medical Center. A total of 976 cases (489 AA and 487 EA men) were recruited into this study between 2005 and 2015. Controls were identified through the Maryland Department of Motor Vehicle Administration database and were frequency-matched to cases on age and race. A total of 1034 population controls were recruited (486 AA and 548 EA men). At the time of enrollment, both cases and controls were administered a survey by a trained interviewer and a blood sample was collected. Serum samples were available for 846 cases (407 AA and 439 EA) and 846 controls (382 AA and 464 EA), therefore only these individuals were used for the study herein. Most of the 846 cases (85%) were recruited within a year of the disease diagnosis with a median of 5.1 months between disease diagnosis and blood collection.

NCI-Ghana prostate cancer case-control study. This case-control study has been previously described[20]. The study was designed to study lifestyle, environmental, and genetic risk factors for prostate cancer in African men. The study was approved by institutional review boards at the University of Ghana (protocol #001/01-02) and at the National Cancer Institute (protocol #02CN240). Prior to study enrollment, all participants signed an informed consent. Prostate cancer cases were recruited at Korle Bu Teaching Hospital in Accra, Ghana between 2008 and 2012. The cases were diagnosed using Digital Rectal Exam (DRE) and PSA tests, followed by biopsy confirmation. Immediately after diagnosis and before treatment, cases were consented and asked to submit blood specimen and questionnaire data. Controls were identified through probability sampling using the 2000 Ghana Population and Housing Census data to recruit ~1000 men aged 50–74 years in the Greater Accra region between 2004 and 2006. These men were confirmed to not have prostate cancer by PSA testing and DRE. Serum samples were available for 586 prostate cancer cases and 659 population controls; hence, only these individuals were used for the study herein.

Serum sample processing. The participants in the two studies provided blood samples at time of recruitment. For the NCI-Maryland study, most blood samples were processed the same day, but always within 48 h, after storage in a refrigerator. For the NCI-Ghana study, blood samples were processed within 6 h. In this study, population controls provided fasting blood. Serum was prepared using standard procedures and aliquots were stored at −80 °C. Serum samples were shipped from Ghana to the NCI in dry ice boxes.

Serum protein measurement. Serum levels of 92 immuno-oncology panel proteins were measured simultaneously using a proprietary multiplex Proximal Extension Assay by Olink Proteomics (Boston). Olink utilizes a relative quantification unit, Normalized Protein eXpression (NPX), which is in a Log2-format. Serum samples from NCI-MD study (846 cases and 846 controls) and NCI-Ghana study (586 cases and 659 controls) were completely randomized and were assayed in that order. In addition to the built-in internal controls, 5% blinded duplicates were randomly selected and were randomized along with the original set of samples. Protein levels were intensity normalized to adjust for batch effect. Because all our samples were randomized across plates, a global adjustment was used to center the values for each assay around its median and across all plates. Ninety-five percent of the samples passed a stringent quality control (1647 from the NCI-MD study: 819 cases and 828 controls; 1143 from NCI-Ghana study: 489 cases and 654 controls)—with coefficients of variation among duplicates at <10% for every marker. These are men reported in Supplementary Table 1. Out of the 92 proteins assayed, IL33, IL35, IL21, IL2, IFNβ, IL13, IL1α, CXCL12, IFNγ, and TNF were detected in <20% of the samples, hence the remaining 82 proteins were used for subsequent analysis (Supplementary Table 3).

Functional annotation and biological processes scores. Proteins were grouped into six biological processes based on their respective biological roles following the Olink guideline: apoptosis/cell killing, autophagy/metabolism, chemotaxis/trafficking to tumor, suppression of tumor immunity (Th2 response, tolerogenic), promotion of tumor immunity (Th1 responses), or vasculature and tissue remodeling. Apoptosis, autophagy, chemotaxis, suppression of tumor immunity, promotion of tumor immunity, or vasculature scores were calculated for each study participant as the mean z-score value for the proteins belonging to the respective biological process. For survival analysis, the biological process/pathway scores were evaluated as continuous variables. To evaluate the association of suppression of tumor immunity with aggressive prostate cancer, we grouped suppression of tumor immunity scores into low (≤median) and high (>median) with cutoffs determined using the distribution of the score among population controls of the NCI-Maryland study.

Prostate specific antigen (PSA) measurement. For the cases in the NCI-Maryland cohort, PSA levels were obtained from medical record. For the controls of the NCI-Maryland study, total PSA was measured from stored serum aliquots using the human total PSA ELISA Kit (Abcam, ab188388). About 7% ($n = 56$) of the controls in the NCI-Maryland cohort had PSA >2.5 ng/ml, while only 3% ($n = 27$) had blood PSA over 4 ng/ml. For the controls in the NCI-Ghana study, close to 20% ($n = 132$) had a PSA >2.5 ng/ml, while about 11% ($n = 73$) had PSA over 4 ng/ml.

C-reactive protein (CRP) measurement. Plasma CRP was assayed using an ELISA assay (cat# ab99995, Abcam, United States) according to the manufacturer's instructions. Two microliters of plasma samples were added to 398 µL of 1x Diluent D, followed by a second 1:200 dilution steps for each sample. One-hundred microliters of CRP standard (0–600 pg/mL) and the diluted samples were loaded as duplicates into pre-coated 96-well plates. Samples were incubated overnight at 4 °C with gentle shaking, followed by incubations with the anti-human CRP antibody and the horseradish peroxidase-streptavidin solution. CRP was quantified measuring absorbance at 450 nm with a microplate reader.

West African ancestry estimation for participants in the NCI-Maryland case-control study. Genomic DNA was isolated from buffy coats (DNeasy Blood & Tissue Kit - Qiagen) or mouthwash samples (standard phenol-chloroform technique). Isolated DNA was genotyped for 100 ancestry informative markers using the Sequenom MassARRAY iPLEX platform, as previously described[30]. Single nucleotide polymorphism (SNP) genotype calls were generated using Sequenom TYPER software for 1505 of the 1647 (91%) individuals from the NCI-Maryland prostate cancer study with QCed serum proteomics data (i.e., 710 cases and 795 controls). A genotype concordance rate of >99% was observed for all markers. Admixture estimates for each study participant were calculated using a model-based clustering method as implemented in the program STRUCTURE v2.3. We applied STRUCTURE v2.3 with an admixture model estimating K (number of sub populations) from 2 to 5 with 100 iterations and parental population genotypes from West Africans, Europeans, and Native Americans, yielding three admixture estimations (West African, European, Native American). For a subset (83%) of the NCI-Maryland study participants with QCed serum proteomics data (i.e., for 1367 men: 658 cases and 709 controls), additional West African ancestry estimates were provided by the Cancer Genomics Research Laboratory/NCI-Leidos from a genome-wide association study using the Infinium HumanOmni5-Quad BeadChip array. Here, we employed the SNP weights approach for ancestry estimation which is a python-based software for ancestry inference using genome-wide SNP weights precomputed from external reference panels[72]. We interrogated a total of 55,446 genotype SNPs after applying linkage disequilibrium-based pruning and minor allele frequency filtering and those served as input SNPs. SNP weights will check for reference allele and strand between the pre-computed SNP weights and input genotypes. It then uses the intersection of the SNP weights and input genotypes to perform ancestry inference. The West African ancestry estimates using the two approaches were similar ($r = 0.98$) and can be found in Supplementary Data 6.

Association of clinical/socio-demographic characteristics with immune-oncological proteins. The association of age, BMI, education, aspirin use, smoking, diabetes, and PSA levels with the relative abundance of individual analytes (as continuous value) was assessed by means of multivariable linear regression models implemented by the function lm in the base R package stats (version 3.6.1). These variables were chosen because they have either been linked to prostate cancer risk and survival or may influence the status of inflammation and host immunity. For each analyte, we fitted the formula "analyte ~ age + bmi + education + aspirin + smoking + diabetes + PSA", which yielded the model's F-statistic and associated F-statistic p value, as well as the intercept and regression coefficients with their associated standard errors (SE) and P values. F-statistic P values were adjusted by FDR across all models; moreover, within each model, regression coefficient P values were also FDR-adjusted. Full regression results for each cohort are provided as Supplementary Data 1. In Fig. 2, an analyte (as response variable) was considered significantly associated with clinical and socio-demographic covariables if the multivariable model yielded an FDR-adjusted P value < 0.05 on the F-statistic. If this condition was satisfied, the association between the target analyte and each individual covariable was characterized by the corresponding FDR-adjusted P value and coefficient. In order to assess the statistical significance of cohort differences in the association between analytes and clinical/socio-demographic characteristics, we fitted each analyte to a model with interaction terms according to the formula "analyte ~ age*Gh + bmi*Gh + education*Gh + aspirin*Gh + smoking*Gh + diabetes*Gh + PSA*Gh + age*Afr + bmi*Afr + education*Afr + aspirin*Afr + smoking*Afr + diabetes*Afr + PSA*Afr", where the intercept and non-interacting terms were implicitly also included in the model. Here, two dummy variables were introduced: Gh (defined as 1 for Ghanaian subjects, 0 otherwise) and Afr (defined as 1 for Ghanaian and African American subjects, 0 for European Americans). As described above, models were selected based on significance of the FDR-adjusted p value < 0.05 on the F-statistic; if this condition was satisfied, the association between the target analyte and each individual covariable was characterized by the corresponding FDR-adjusted P value and coefficient.

Analysis of variance as a function of genetic estimation of West African admixture. Variance analysis for the levels of each of the 82 immune-oncological cytokines were simultaneously assessed as a function of genetic estimation of West African admixture among men without prostate cancer from the NCI-Maryland study. The analysis was implemented by the function aov in the base R package stats (version 3.6.1).

Analysis of (dis)similarity across cohorts. Using the distance metric $d = 1\text{-}r$, where r is Pearson's correlation between pairs of subjects, unsupervised hierarchical clustering was performed using average linkage and visualized as a heatmap with cohort annotations (Fig. 3), generated using Broad Institute's web-based matrix visualization and analysis platform Morpheus (https://software.broadinstitute.org/morpheus). To avoid spurious effects from outliers in heatmap plots, each protein's range of abundance values were set to saturate at the 1st and 99th percentiles. To account for widely different abundance ranges for different proteins in the assay, each protein's measured abundances across all subjects were z-score transformed. The hierarchical clustering dendrogram was cut to extract K clusters (with K = 2, 3). The association between cluster labels andpopulation groups was tested via Fisher's or chi-squared tests performed on the resulting contingency tables (Supplementary Fig. 5).

Gene ontology (GO) enrichment analysis. GO terms with an enrichment in proteins of interest were identified using Over-Representation Analysis (ORA) as part of the web tool WebGestalt (WEB-based Gene SeT AnaLysis Toolkit). Enriched gene sets were further processed using affinity propagation (R package apcluster) to cluster gene sets according to functional similarity.

Survival analysis. Information on patient survival was only obtainable for the NCI-Maryland prostate cancer patients. Survival data was obtained from the National Death Index database for both cases and controls in the NCI-Maryland study. We calculated survival for cases from date of diagnosis to either date of death or to the censor date of December 31, 2018. We built a multivariable Cox regression model with all biological processes scores and adjustment for other covariables to estimate adjusted HRs and 95% or 99% CIs for all-cause mortality, cancer-related mortality, and prostate cancer-specific mortality. We adjusted for the following potential confounding factors: age at study entry (years), BMI (kg/m^2), self-reported race (AA/EA), education (high school or less, some college, college, professional school), income (>$10k, $10–30 K, $30–60 K, $60–90k, > $90k), smoking history (never, former, current), diabetes (no/yes), aspirin use (no/yes), treatment (0 = none, 1 = surgery, 2 = radiotherapy, 3 = hormone, 4 = combination), and disease status defined by the NCCN risk score. Missing values for education ($n = 1$), smoking history ($n = 5$), and income ($n = 63$) were imputed using the R package missForest, which implements nonparametric missing value imputation based on random forests. In the overall survival analysis of population controls, we calculated survival from the date of interview to either date of death or to the censor date of December 31st, 2018. We applied the Cox regression model to estimate adjusted HR and 95% CI and adjusted for all the confounding factors listed above except for treatment. Missing values for education ($n = 1$), smoking history ($n = 7$), and income ($n = 67$) were imputed using the R package missForest. The reported HRs indicate the change in risk of dying when the biological process z-score value increases by 1 while holding all the other biological processes' z-scores and covariates constant.

Classification of cases using National Comprehensive Cancer Network (NCCN) risk score. Cases were assigned to risk groups based on the patients' TNM stage, Gleason score, Gleason pattern, and PSA level at diagnosis according to the 2019 NCCN guideline for prostate cancer[73]. Information on TNM stage was only obtainable for the NCI-Maryland prostate cancer patients, hence only these cases were scored. Cases were categorized as localized, regional, and metastatic prostate cancer based on their clinical parameters at the time of diagnosis. Localized prostate cancer cases were further classified into low, intermediate, high, and very high risk based on the likelihood of their disease to progress to lethal prostate cancer per the 2019 NCCN guideline[73]. Prostate cancer cases with lymph node involvement but no distant metastasis at diagnosis were classified as regional prostate cancer while those with distant metastasis at the time of diagnosis were classified as metastatic prostate cancer. For our analysis, we condensed these risk groups into four categories (low, intermediate, high/very high, and regional/metastatic).

Developing a predictive proteomic signature of lethal prostate cancer. The analysis was restricted to the cases from NCI-Maryland study for whom we had survival data. We stratified by self-reported race/ethnicity into AA cases (360 censored, 34 prostate cancer deaths) and EA cases (402 censored, 23 prostate cancer deaths). To identify a multi-analyte proteomic signature that is predictive of lethal prostate cancer, 88 features were evaluated [82 immune-oncological proteins along with six demographic/clinical variables (education, age, BMI (kg/m^2), smoking history, diabetes, and aspirin use)]. Missing values for education ($n = 1$) and smoking history ($n = 5$) were imputed using R package missForest. R package eNetXplorer (version 1.1.2)[74] was implemented to build cross-validated, regularized Cox regression models with different elastic net mixture parameters from ridge (alpha = 0) to lasso (alpha = 1). Alpha was selected based on overall performance assessed as a function of the fivefold cross-validated quality function (concordance) and the empirical P value generated from comparing the model against a statistical ensemble of null models created by random permutations of the response (i.e., survival time/status randomized across subjects in the cohort). These results comprise 10,000 Cox regression elastic net realizations arising from 200 randomly generated folds, each of them compared against 50 null model permutations. Features' performance as predictors was evaluated using two different, but complementary selection criteria: feature coefficients and feature frequencies. The feature frequency measure captures the significance of how often a feature is chosen in an in-bag model. When it is chosen, the feature coefficient measure captures the significance of the feature's weight in the in-bag model. See the publication by *Candia et al.* for more details on this method[74]. Using only the significant protein features from both selection criteria, a multivariate Cox regression model was run. Risk stratification was used to generate Kaplan–Meier plots and log-rank tests of significance.

Statistical analysis. Data analyses were performed using Stata/SE 16.0 and R statistical packages. An association was considered statistically significant with $P < 0.05$ or FDR-adjusted $P < 0.05$ in instances where correction for multiple testing was required.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

Clinical, demographic and molecular data used for this study (i.e., self-reported race, degree of West African ancestry, age, BMI, education, income, aspirin use, diabetes use, smoking status, NCCN risk score, PSA, treatment type, proteomics data, and survival data) are deposited at the Open Science Framework at https://osf.io/327ha (https://doi.org/10.17605/OSF.IO/327HA)[75] and as a public GitHub repository at https://github.com/juliancandia/ProstateCancerProteomics (https://doi.org/10.5281/zenodo.5815262)[76]. The individual West African ancestry estimates for participants in the NCI-Maryland study obtained with either 100 ancestry informative markers or 55446 GWAS-based SNPs can be found in Supplementary Data 6. Individual raw genotype data cannot be shared through open access due to NIH rules that do not allow it because the participants did not consent to sharing this type of data. Source data are provided with this paper. The remaining data are available within the paper, Supplementary Information, and Supplementary Data. Source data are provided with this paper.

Code availability

The scripts used in our bioinformatics pipeline to perform data analysis and visualization are available as a public GitHub repository at https://github.com/juliancandia/ProstateCancerProteomics (https://doi.org/10.5281/zenodo.5815262)[76].

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Author contributions

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ARTICLE OPEN

Basic Research

Human herpesvirus 8 infection is associated with prostate cancer among *IFNL4-ΔG* carriers

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BACKGROUND: The dinucleotide germline variant, *rs368234815-ΔG*, in the *IFNL4* gene (*IFNL4-ΔG)* has been associated with prostate cancer among men at increased risk of sexually transmitted infections and reported to impair viral clearance. Human herpesvirus 8 (HHV-8) seropositivity has been associated with prostate cancer in Tobago.

METHODS: We examined whether the association of HHV-8 with prostate cancer is *IFNL4-ΔG*-dependent among 728 *IFNL4-ΔG*-genotyped cases and 813 genotyped population-based controls from the NCI-Maryland Prostate Cancer Case-Control study. Associations between HHV-8 and prostate cancer were assessed in multivariable unconditional logistic regression models. We calculated adjusted odds ratios (OR) and stratified the analysis into men harboring the *IFNL4-ΔG*-variant and non-carriers (ΔG/ΔG or ΔG/TT vs. TT/TT).

RESULTS: HHV-8 seropositivity was higher in cases than controls (11% vs. 6%) and this association was restricted to carriers of the ΔG allele (OR 2.19: 95% CI:1.38–3.48) in both African American (OR 1.96; 95% CI:1.08–3.56) and European American men (OR 2.59; 95% CI:1.20–5.56).

CONCLUSIONS: HHV-8 seropositivity is associated with increased odds of prostate cancer in men harboring the *IFNL4* rs368234815-ΔG variant. This study describes HHV-8 infection as a candidate prostate cancer risk factor in men with the *IFNL4-ΔG* genotype and supports the hypothesis that *IFNL4-ΔG* is a susceptibility factor that contributes to prostate cancer.

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INTRODUCTION

Prostate cancer is the most common cancer among men in the United States and is second to lung cancer as a cause of cancer death [1]. The occurrence of prostate cancer is not uniform among the different US population groups. African American (AA) men experience a higher incidence and mortality than European American (EA) and Asian men [2, 3]. Despite several decades of research, the reason behind the excessive prostate cancer burden among men of African descent remains incompletely understood but we have learned that West African genetic ancestry is one underlying risk factors [4–6]. Other causes for the disease include age and family history of prostate cancer [7–10]. Modifiable risk factors such as diet and lifestyle may account for the many prostate cancers globally [11], with supporting evidence from migration studies that the environment modulates prostate cancer risk [12, 13].

Chronic inflammation is a proposed risk factor for prostate cancer and associates with aggressive disease [14, 15]. Inflammation may also contribute to prostate cancer disparities [16, 17]. While environmental exposures, such as infections, can promote inflammation, ancestral factors may additionally influence inflammatory processes and the response to infections [18, 19]. Sexually transmitted infections (STIs) including common viral infections are candidates for establishing chronic inflammation in the prostate by either repeated exposures to the infectious agent or establishment of a persistent infection [20].

Human herpesviruses are prime contenders for STIs causing chronic inflammation. Herpesviruses establish life-long latent infections and periodically reactivate resulting in viral replication and, depending on the virus, clinical disease. Several studies have failed to find associations between prostate cancer risk and infections with several herpesviruses, including herpes simplex virus, Epstein-Barr virus, and cytomegalovirus [21–24]. We, however, found that men from Tobago with prostate cancer were significantly more likely to be human herpesvirus 8 (HHV-8) seropositive than age matched controls (OR 2.24; 95% CI 1.29–3.90) [25]. Other studies including prospective US cohorts have failed to find any association between HHV-8 seropositivity and prostate cancer risk [26, 27]. HHV-8 is sexually transmitted and the causative agent of Kaposi's sarcoma and pleural effusion lymphomas [28]. Notably, HHV-8 can establish latent infections in the prostate glandular epithelium and the infection was found associated with an increased macrophage infiltrate [29, 30]. Thus, HHV-8 may serve as one of the infectious agents that others have hypothesized as responsible for establishing chronic infection, leading to increased inflammation in the prostate and an elevated risk of prostate cancer.

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Interferon lambda 4 (*IFNL4*) is a type III interferon discovered in 2013 which can serve as a negative regulator of IFN responses [31]. Recently, a germline dinucleotide polymorphism, rs368234815 (ΔG and TT alleles) in the interferon lambda 4 gene has been reported to modify the association of sexual history with prostate cancer [32, 33]. Upon induction, both ΔG and TT variants produce *IFNL4* mRNA. However, IFN-λ4 protein can only be produced in the presence of the ΔG allele because the TT allele encodes a premature stop codon for translation into protein [31]. The *IFNL4-ΔG* variant, which results in the expression of IFN-λ4 protein, is strongly linked to an inability to clear certain chronic viral infections such as hepatitis C virus [31, 34, 35] and cytomegalovirus [36, 37]. The *IFNL4-ΔG* variant is also more prominent among African (78%) than European (30%) or Asian men (0–10%) [31]. Similarly, we find that the *IFNL4-ΔG* variant has a 61.7% allele frequency among AA men and 33.7% allele frequency among EA men in the NCI-Maryland Prostate Cancer Case-Control study. Thus, the presence of this polymorphism may predispose to prostate cancer in the context of a pro-inflammatory infection but may affect AA men more so than EA men because of its high germline prevalence among AA men.

To examine whether an interaction between HHV8 and *IFNL4-ΔG* may increase the risk of prostate cancer, we investigated the combined effect of an HHV-8 infection history and *IFNL4-ΔG* on prostate cancer in the NCI-Maryland Prostate Cancer Case-Control Study.

MATERIALS AND METHODS

NCI-Maryland prostate cancer case-control study

This case-control study has been previously described [33, 38]. A key purpose of this study was to investigate the role of environmental and genetic factors in prostate cancer development among AA men. Prior to interview, all participants signed informed consent for participation. All study forms and procedures were approved by the NCI (protocol # 05-C-N021) and the University of Maryland (protocol #0298229) Institutional Review Boards. Cases were recruited at the Baltimore Veterans Affairs Medical Center and the University of Maryland Medical Center. The use of data and samples from this study was approved as an exempt study by the University of Pittsburgh Institutional Review Board (PRO18060266). Eligibility criteria included the following: diagnosis with prostate cancer within two years prior to enrollment, residence in Maryland or adjacent counties in Pennsylvania, Delaware, Virginia, or District of Columbia, 40–90 years old at the time of enrollment, born in the United States, either AA or EA by self-report, can be interviewed in English, had a working home phone number, physically and mentally fit to be interviewed, not severely ill, and not residing in an institution such as prison, nursing home, or shelter. A total of 976 cases (489 AA and 487 EA men) were recruited into the study between 2005 and 2015. Controls were identified through the Maryland Department of Motor Vehicle Administration database and were frequency-matched to cases on age and race. The controls also had the same eligibility criteria as cases with the exception that they could not have a personal history of cancer (other than non-melanoma skin cancer), radiation therapy, or chemotherapy. A total of 1034 population controls were recruited (486 AA and 548 EA men). At the time of enrollment, both cases and controls were administered a survey by a trained interviewer and a blood sample or mouthwash rinse/buccal cells was collected. The survey asked about their demographics, tobacco use, nutrition, medical history, family history of cancer, prostatitis, or benign prostatic hypertrophy, occupational history, socioeconomic status, anthropometry, and sexual history. For the current study, a total of 1729 participants (851 cases, 878 controls) were included in the analysis, based on availability of sera and survey data, and the exclusion of HHV8 test results because of quality control failure (1.2%). Because *IFNL4-ΔG* genotyping data was not obtained for 188 participants who did not provide germline DNA, only 1541 participants (728 cases and 813 controls) were included in the combined assessment of HHV-8 serology and the *IFNL4-ΔG* genotype in prostate cancer development. The 188 participants without genotyping data had similar characteristics as the genotyped participants. In the study, we report prostate-specific antigen (PSA) levels for the control population. PSA measurements in the stored sera samples were performed years after recruitment of these controls when recruitment into the study was completed. We did not use the PSA measurements for selection of controls.

HHV-8 serology

HHV-8 serology was obtained for 1751 participants, but 22 results were excluded from further analysis because of quality control failure (1.2%, with high background making seropositivity determination impossible). HHV-8 serology was determined using a well-defined immunofluorescence assay (IFA) [39–41]. The inter-assay reliability with the HHV-8 IFA is 93.4%. All serum samples were tested at least twice in a blinded fashion and assessed microscopically by the same reader. The cutoff for a positive serologic result was 1:50.

Genotyping of IFNL4 rs368234815

This assay has been previously described [33]. Genomic DNA was isolated from buffy coats, when available, using the DNeasy Blood &Tissue Kit (Qiagen, Germantown, MD). For a subset of men (100 cases and 112 controls), genomic DNA was obtained from a mouthwash sample using the Gentra Puregene Buccal Cell Kit (Qiagen). Genotyping of rs368234815 was done with a custom-designed assay purchased from ThermoFisher (Waltham, MA) and was performed using ABI 7900 (Applied Biosystems, (Waltham, MA) according to the standard protocol. Genotype concordance among duplicates was 97%. There was no deviation from the Hardy–Weinberg equilibrium among controls. Genotyping success rate was 98.6%. The genotyping failure rate was similarly low for DNA extracted from blood and mouthwash samples (1.4%).

Prostate cancer classification

To define high grade disease, Gleason grading system was used to stratify prostate cancer into "low-grade" (Gleason score ≤ 7) or "high-grade" (Gleason score > 7). Tumor stage at diagnosis was obtained from medical records and stage 1 or 2 disease was defined as localized and stage 3 or 4 as advanced prostate cancer. Cases with a combination of low-grade tumors and localized disease were defined as "nonaggressive" prostate cancer, while cases with either high-grade tumors or advanced disease were defined as having "aggressive" prostate cancer.

Statistics

All statistical analyses were performed using the Stata/SE 16.0 statistical software package (Stata Corp, College Station, TX). All statistical tests were two-sided and $P < 0.05$ was considered statistically significant. We evaluated whether HHV-8 is associated with prostate cancer and then stratified by race/ethnicity (AA vs. EA) and *IFNL4* rs368234815 (ΔG/ΔG or ΔG/TT vs. TT/TT), or by aggressive vs. nonaggressive disease. To assess the association of HHV-8 with prostate cancer, unconditional logistic regression was used to compute odds ratios (OR) and 95% confidence intervals (CI). We adjusted for the following potential confounding factors: age at enrollment, body mass index at enrollment (BMI, kg m^{-2}), education (high school or less, some college, college, professional school), family history of prostate cancer (first degree relatives, yes/no), smoking history (never, former, current), condom use (usually use, yes/no), aspirin use (regular user, yes/no), and *IFNL4* rs368234815 (ΔG/ΔG or ΔG/TT vs. TT/TT) or race/ethnicity as applicable.

RESULTS

Clinical and demographic characteristics of participants

Characteristics of the study participants are shown in Table 1. Cases and controls had similar age distributions (Wilcoxon rank-sum test, $P = 0.15$). Cases had slightly lower BMI than controls (Wilcoxon rank-sum test, $P = 0.028$). More cases, compared to controls, had a first-degree family history of prostate cancer (10% vs. 7%, $P = 0.019$, Chi-square test), whereas fewer cases than controls had a college or graduate-level degree (34% vs. 51%, $P < 0.001$, Chi-square test). Additionally, more cases were current smokers than controls (24% vs. 14%, $P < 0.001$, Chi-square test). Among the 851 cases, 121 men (14%) had advanced disease (stage 3 or 4 prostate cancer). High Gleason score (>7) was reported in 145 patients (17%). Both AA and EA men had similar Gleason score distribution, but AA men tended to have higher blood PSA levels at diagnosis. A somewhat greater proportion of

Table 1. Characteristics of study participants.

	Controls			Cases[a]		
	All ($n = 878$)	**AA[b] ($n = 406$)**	**EA[c] ($n = 472$)**	**All ($n = 851$)**	**AA ($n = 412$)**	**EA ($n = 439$)**
Demographic factors						
Age[d]						
Median (IQR[e]) in years	64 (12)	64 (10)	66 (13)	64 (11)	63 (10)	65 (11)
BMI						
Mean (SD[f]) in kg/m2	28 (6.5)	29.6 (5.4)	27.9 (4.9)	27.4 (5.6)	27.9 (5.2)	28.0 (4.3)
Education, N (%)						
High school or less	209 (24)	119 (29)	90 (19)	313 (37)	196 (48)	117 (27)
Some college	220 (25)	119 (29)	101 (21)	250 (29)	136 (33)	114 (26)
College	226 (26)	87 (21)	139 (29)	159 (19)	53 (13)	106 (24)
Graduate	222 (25)	80 (20)	142 (30)	128 (15)	26 (6)	102 (23)
Did not provide	1 (<1)	1 (<1)	–	1 (<1)	1 (<1)	–
Annual household income, N (%)						
Less than $10,000	25 (3)	19 (5)	6 (1)	79 (9)	63 (15)	16 (4)
$10,000–$29,999	115 (13)	72 (18)	43 (9)	226 (27)	143 (35)	83 (19)
$30,000–$59,999	200 (23)	89 (22)	111 (24)	188 (22)	95 (23)	93 (21)
$60,000–$90,000	205 (23)	96 (24)	109 (23)	130 (15)	41 (10)	89 (20)
>$90,000	266 (30)	92 (23)	174 (37)	161 (19)	31 (8)	130 (30)
Don't know/Refused	67 (8)	38 (9)	29 (6)	67 (8)	39 (9)	28 (6)
Health factors						
Family history of prostate cancer[g], *N (%)*						
No	817 (93)	381 (94)	436 (92)	765 (90)	376 (91)	389 (89)
Yes	61 (7)	25 (6)	36 (8)	86 (10)	36 (9)	50 (11)
Smoking status[h], *N* (%)						
Never	349 (40)	158 (39)	191 (40)	291 (34)	119 (29)	172 (39)
Former	398 (45)	167 (41)	231 (49)	355 (42)	155 (38)	200 (46)
Current	122 (14)	78 (19)	44 (9)	200 (24)	135 (33)	65 (15)
Did not provide	9 (1)	3 (1)	6 (1)	5 (<1)	3 (1)	2 (<1)
Diabetes, *N* (%)						
No	677 (77)	286 (70)	391 (83)	671 (79)	297 (72)	374 (85)
Yes	201 (23)	120 (30)	81 (17)	180 (21)	115 (28)	65 (15)
Regular aspirin use, *N* (%)						
No	377 (43)	197 (49)	180 (38)	433 (51)	238 (58)	195 (44)
Yes	501 (57)	209 (51)	292 (62)	418 (49)	174 (42)	244 (56)
Gleason grade group, *N* (%)						
Gleason grade group 1 (6 or less)	NA	NA	NA	363 (43)	167 (41)	196 (45)
Gleason grade group 2 (3 + 4)	NA	NA	NA	233 (27)	118 (29)	115 (26)
Gleason grade group 3 (4 + 3)	NA	NA	NA	108 (13)	57 (14)	51 (12)
Gleason grade group 4 (8)	NA	NA	NA	83 (10)	41 (10)	42 (9)
Gleason grade group 5 (9 or 10)	NA	NA	NA	62 (7)	28 (7)	34 (8)
Not available	NA	NA	NA	2 (<1)	1 (<1)	1 (<1)
Stage[i], *N* (%)						
I	NA	NA	NA	163 (19)	63 (15)	100 (23)
II	NA	NA	NA	567 (67)	297 (72)	270 (62)
III	NA	NA	NA	69 (8)	24 (6)	45 (10)
IV	NA	NA	NA	52 (6)	28 (7)	24 (5)
PSA[j]						
Median (IQR) in ng/ml	0.4 (0.6)	0.4 (0.6)	0.4 (0.6)	6.3 (5.6)	6.8 (7.6)	6.0 (4.7)
IFNL4 rs368234815 genotype, *N* (%)						
IFNL4 TT/TT	255 (31)	59 (15)	196 (46)	225 (31)	59 (16)	166 (45)
IFNL4 ΔG/TT	361 (44)	185 (48)	176 (41)	317 (44)	157 (44)	160 (43)

Table 1. continued

	Controls			Cases[a]		
	All ($n = 878$)	AA[b] ($n = 406$)	EA[c] ($n = 472$)	All ($n = 851$)	AA ($n = 412$)	EA ($n = 439$)
IFNL4 ΔG/ΔG	197 (24)	139 (36)	58 (13)	186 (26)	142 (40)	44 (12)
HHV-8 seroprevalence, *N* (%)						
Positive	57 (6)	30 (7)	27 (6)	92 (11)	52 (13)	40 (9)
Negative	821 (94)	376 (93)	445 (94)	759 (89)	360 (87)	399 (91)

[a]Cases recruited within 2 years after disease diagnosis with a median interval between diagnosis and enrollment of 5.1 months.
[b]AA: African-American.
[c]EA: European-American.
[d]Age at study interview.
[e]IQR: Interquartile range.
[f]SD: Standard deviation.
[g]First-degree relative with prostate cancer.
[h]Smoking status describes cigarette smoking.
[i]Pathologically confirmed using American Joint Committee on Cancer (AJCC) 7th Edition.
[j]PSA: Prostate specific antigen.

EA men (15%), compared with AA men (13%), presented with an advanced stage disease in this case-control study.

HHV-8 and prostate cancer

The HHV-8 seroprevalence among study participants was 9.4%, with an increased seropositivity among AA men (11.1%) compared to EA men (7.9%; unadjusted OR 1.40; 95% CI: 1.001–1.97, AA vs. EA men). To determine if HHV-8 seroprevalence is associated with prostate cancer among study participants, univariate and multivariable analyses were performed with and without stratification by self-reported race/ethnicity (Table 2). As shown in Table 1, HHV-8 seroprevalence was higher among men with prostate cancer (11%) when compared to the control group (6%; unadjusted OR 1.75; 95% CI: 1.23–2.46) and remained significantly elevated among cases in the confounder-adjusted logistic regression analysis that included *IFNL4* rs368234815 (ΔG/ΔG or ΔG/TT vs. TT/TT) as covariate (adjusted OR 1.76; 95% CI: 1.20–2.59; Table 2). After stratification by race/ethnicity, the association of HHV-8 seropositivity with prostate cancer remained significant only among AA men (adjusted OR 1.91; 95% CI: 1.11–3.27). However, among EA men, a similar trend was observed (adjusted OR 1.57: 95% CI: 0.90–2.75).

Influence of *IFNL4* rs368234815 genotype on the association between HHV-8 and prostate cancer

IFNL4 rs368234815 genotype frequencies are shown in Table 1. There is a significant overrepresentation of homozygote *IFNL4-ΔG* carriers among AA men, yielding a *ΔG* allele frequency of 61.7% in AA vs. 33.7% in EA men, consistent with the literature [31]. To determine if the *IFNL4* rs368234815 genotype modifies the association between HHV-8 and prostate cancer, we stratified the participants into those carrying a ΔG allele (ΔG/TT or ΔG/ΔG) vs. those without the ΔG allele (TT/TT). The association of HHV-8 seropositivity with prostate cancer was restricted to carriers of the ΔG allele (adjusted OR 2.19: 95% CI: 1.38–3.48) in the multivariable analysis that did not stratify by race/ethnicity (Table 3). Carriers of the TT genotype were not at increased odds of prostate cancer due to HHV-8 seropositivity (adjusted OR 1.03: 95% CI: 0.51–2.11). Further stratification by race/ethnicity showed that HHV-8 was significantly associated with prostate cancer only among carriers of the ΔG allele in both EA (OR 2.59; 95% CI: 1.20–5.56) and AA men (OR 1.96; 95% CI:1.08–3.56). Although the OR point estimate for the association of HHV-8 with prostate cancer in men with *IFNL4-ΔG* was higher in EA than AA men (2.59 vs. 1.96), the 95% CIs largely overlapped, suggesting comparable risks. However, among AA men, HHV-8 showed a trend towards an association among *IFNL4* TT carriers, albeit not statistically significant. The association of the *IFNL4-ΔG* genotype with prostate cancer risk among men who were HHV-8 seropositive suggests the existence of a gene–environment interaction.

HHV-8 and risk of aggressive prostate cancer

To determine if the association between HHV-8 and prostate cancer risk differed between aggressive vs. non-aggressive prostate cancer, we looked at nonaggressive and aggressive prostate cancer as separate outcomes. The association of HHV-8 with aggressive prostate cancer was slightly stronger (OR 1.77; 95% CI: 1.06–2.96) than the association of HHV-8 with non-aggressive prostate cancer (OR 1.51; 95% CI: 1.02–2.22), yet the confidence intervals largely overlapped (Supplementary Table 1). In the analysis stratified by race/ethnicity, we found a significant association between HHV-8 and the risk for aggressive prostate cancer among AA men (OR 2.69; 95% CI: 1.27–5.68), but not among EA men (OR 1.38; 95% CI: 0.59–3.23), in the multivariable analysis (Table 4). The association of HHV-8 with non-aggressive prostate cancer was weaker and did not reach statistical significance in either group of men. In contrast, HHV-8 was associated with both aggressive (OR 2.62; 95% CI: 1.36–5.06) and non-aggressive prostate cancer (OR 2.06; 95% CI: 1.25–3.38) among men carrying at least one ΔG allele (Table 5), again indicating that HHV-8 is a prostate cancer risk factor if men harbor the *IFNL4-ΔG* germline variant—independent of race/ethnicity.

DISCUSSION

Here, we report that HHV-8 seropositivity is associated with prostate cancer in men harboring the *IFNL4* rs368234815-ΔG germline variant, suggesting a gene–environment interaction that increases prostate cancer risk. Although our findings do not show how HHV-8 may affect prostate cancer biology, the presence of *IFNL4-ΔG* may interfere with the clearance of an HHV-8 infection, thereby conferring the risk of developing prostate cancer.

Chronic viral infections are thought to commonly establish an oncogenic proinflammatory environment. Chronic low-grade inflammation has been proposed as a driving force behind the initiation and development of prostate cancer [42]. While an infectious agent as the cause of prostate cancer has not been conclusively established, Nelson and colleagues hypothesized that host immune inflammatory responses to infections may serve as the causative process for prostate cancer [20]. Hayes et al. [43] reported an elevated risk of prostate cancer among men with STIs, yet other studies could not make this association [44].

HHV-8 establishes latent infections in the host which can lead to chronic inflammation due to the expression of a subset of proteins termed the latency proteins [28]. Thus, we hypothesized that an HHV-8 infection may increase prostate cancer risk in susceptible individuals, potentially leading to population differences in prostate cancer risk associated with HHV-8. Detection of HHV-8-specific antibodies does not indicate when the individual was infected with HHV-8 but does demonstrate that they are latently infected since all human herpesviruses establish latent infections in the host. Our previous studies investigating HHV-8 and prostate cancer in the Caribbean Island of Tobago supported this hypothesis. In Tobago, HHV-8 seroprevalence was significantly higher among Tobago men (31.5%) compared to EA men in the US (13%) or U.S. blood donors overall (4.3%) [25, 45]. Because HHV-8 establishes a chronic, latent infection in the glandular epithelium of the prostate, the virus' presence results in an increased macrophage infiltrate [29, 30].

In the present study, we have extended our earlier studies on HHV-8 and prostate cancer from Tobago to the U.S. HHV-8 (as determined by seropositivity) was found to be significantly associated with prostate cancer in the NCI-Maryland Prostate Cancer Case Control Study. This association was most obvious among AA men. Nevertheless, the increased odds of prostate cancer were chiefly associated with the occurrence of the *IFNL4-ΔG* germline variant, and this relationship was principally independent of race/ethnicity. Noteworthy, this is the first study to demonstrate an association between HHV-8 and prostate cancer risk among AA men. In smaller studies investigating HHV-8 and prostate cancer risk in the US, we previously failed to find an association, but these studies were primarily focused on EA men [26, 27] and did not consider *IFNL4-ΔG* as an important modulator of the association between HHV-8 and prostate cancer risk. Moreover, those studies were limited in sample size and lacked sufficient power given the relatively low seroprevalence of HHV-8 in the U.S (4.3–13%) [25, 41], when compared to Tobago (31.5%) [25]. Additionally, the prevalence of *IFNL4-ΔG* is lower in EA men than men of African ancestry [31, 33].

The present study also extends the observations from an earlier study that found the *IFNL4-ΔG* genotype to be associated with prostate cancer among men of an increased risk of sexually transmitted infections [33]. Based on the findings, the authors proposed that an infectious viral agent(s), capable of establishing a chronic infection in the background of the *IFNL4-ΔG* genotype, resulted in the increased prostate cancer risk. IFN-λ4 is involved in the innate immune response and its expression is associated with an inability to clear certain viral infections such as hepatitis C virus [31, 34, 35] and cytomegalovirus [36, 37]. Thus, it is plausible that *IFNL4-ΔG* may increase prostate cancer risk by interfering with the clearance of a viral infection like HHV-8.

Our study has limitations. They include the case-control design, not allowing us to evaluate whether HHV-8 seropositivity precedes prostate cancer development. In addition, we could not evaluate the relationship of HHV-8 seropositivity with prostate cancer for all men in the NCI-Maryland study because we could not collect sera samples and germline DNA from a subset of the men, leading to the inclusion of 1541 men in the analysis out of the 2010 recruited men (76.7%). Future studies are needed that use a prospective design to establish HHV-8 as a prostate cancer risk factor among men with the *IFNL4-ΔG* genotype.

In summary, the present study provides first evidence that HHV-8 seropositivity is associated with increased odds of prostate cancer in men harboring the *IFNL4* rs368234815-ΔG germline variant, consistent with previous data from this group. The observations suggest that HHV-8 represents an infectious viral agent that establishes a chronic infection in the prostate in an *IFNL4-ΔG* genotype-dependent manner, and thereby increases prostate cancer risk. The expression of IFN-λ4 protein may prevent the host from clearing the chronic infection, as has been suggested [34], leading to prostate cancer.

Table 2. Association between HHV8 and prostate cancer stratified by self-reported race/ethnicity.

	Total				African-American				European-American				
	Control *N* (%)	Case *N* (%)	Univariable OR (95% CI)	Multivariable[a] OR (95% CI)	*P*-value	Control *N* (%)	Case *N* (%)	Univariable OR (95% CI)	Multivariable[b] OR (95% CI)	*P*-value	Control *N* (%)	Case *N* (%)	Univariable OR (95% CI)	Multivariable[b] OR (95% CI)	*P*-value
HHV8															
No	821 (94)	759 (89)	Ref.	Ref.		376 (93)	360 (97)	Ref.	Ref.		445 (94)	399 (91)	Ref.	Ref.	
Yes	57 (6)	92 (11)	1.75 (1.23, 2.46)	**1.76 (1.20, 2.59)**	**0.004**	30 (7)	52 (13)	1.81 (1.13, 2.90)	**1.91 (1.11, 3.27)**	**0.019**	27 (6)	40 (9)	1.65 (1.00, 2.74)	1.57 (0.90, 2.75)	0.113

Bolded data indicate significant associations in the multivariable logistic regression analysis.
OR denotes Odds Ratio; 95% CI denotes 95% confidence interval.
[a]Unconditional logistic regression adjusted for body mass index at study enrollment (BMI, kg/m^2), age at study entry, education (high school or less, some college, college, professional school), family history of prostate cancer (first degree relatives, yes/no), smoking history (never, former, current), condom use (usually use, yes/no), aspirin use (regular user, yes/no), IFNL4 rs368234815 genotype (ΔG/ΔG or ΔG/TT vs. TT/TT), and race.
[b]Unconditional logistic regression adjusted for body mass index at study enrollment (BMI, kg/m^2), age at study entry, education (high school or less, some college, college, professional school), family history of prostate cancer (first degree relatives, yes/no), smoking history (never, former, current), condom use (usually use, yes/no), aspirin use (regular user, yes/no), and IFNL4 rs368234815 genotype (ΔG/ΔG or ΔG/TT vs. TT/TT).

Table 3. Association between HHV-8 and prostate cancer after stratification by *IFNL4* rs368234815 genotype.

Total										
	IFNL4 ΔG/TT or ΔG/ΔG					**IFNL4 TT/TT**				
	Control *N* (%)	**Case *N* (%)**	**Univariable OR (95% CI)**	**Multivariable[a] OR (95% CI)**	***P*-value**	**Control *N* (%)**	**Case *N* (%)**	**Univariable OR (95% CI)**	**Multivariable[a] OR (95% CI)**	***P*-value**
HHV-8										
No	527 (94)	441 (88)	Ref.	Ref.		237 (93)	205 (91)	Ref.	Ref.	
Yes	31 (6)	62 (12)	2.39 (1.53, 3.75)	**2.19 (1.38, 3.48)**	**0.001**	18 (7)	20 (9)	1.28 (0.66, 2.49)	1.03 (0.51, 2.11)	0.922
European-American										
	IFNL4 ΔG/TT or ΔG/ΔG					**IFNL4 TT/TT**				
	Control *N* (%)	**Case *N* (%)**	**Univariable OR (95% CI)**	**Multivariable[b] OR (95% CI)**	***P*-value**	**Control *N* (%)**	**Case *N* (%)**	**Univariable OR (95% CI)**	**Multivariable[b] OR (95% CI)**	***P*-value**
HHV-8										
No	223 (95)	180 (88)	Ref.	Ref.		182 (93)	157 (95)	Ref.	Ref.	
Yes	11(5)	24 (12)	2.70 (1.29, 5.67)	**2.59 (1.20, 5.56)**	**0.015**	14 (7)	9 (5)	0.75 (0.31, 1.77)	0.76 (0.30, 1.88)	0.547
African-American										
	IFNL4 ΔG/TT or ΔG/ΔG					**IFNL4 TT/TT**				
	Control *N* (%)	**Case *N* (%)**	**Univariable OR (95% CI)**	**Multivariable[b] OR (95% CI)**	***P*-value**	**Control *N* (%)**	**Case *N* (%)**	**Univariable OR (95% CI)**	**Multivariable[b] OR (95% CI)**	***P*-value**
HHV-8										
No	304 (94)	261 (87)	Ref.	Ref.		55 (93)	48 (81)	Ref.	Ref.	
Yes	20 (6)	38 (13)	2.21 (1.26, 3.90)	**1.96 (1.08, 3.56)**	**0.027**	4 (7)	11 (19)	3.15 (0.94, 10.55)	1.45 (0.38, 5.52)	0.579

Bolded data indicate significant associations in the multivariable logistic regression analysis.
OR denotes odds ratio; 95% CI denotes 95% confidence interval.
[a]Unconditional logistic regression adjusted for body mass index at study enrollment (BMI, kg/m^2), age at study entry, education (high school or less, some college, college, professional school), family history of prostate cancer (first degree relatives, yes/no), smoking history (never, former, current), condom use (usually use, yes/no), aspirin use (regular user, yes/no), and race.
[b]Unconditional logistic regression adjusted for body mass index at study enrollment (BMI, kg/m^2), age at study entry, education (high school or less, some college, college, professional school), family history of prostate cancer (first degree relatives, yes/no), smoking history (never, former, current), condom use (usually use, yes/no), and aspirin use (regular user, yes/no).

Table 4. Association of HHV-8 with aggressive vs. non-aggressive prostate cancer stratified by self-reported race/ethnicity.

	African-American					European-American				
	Control *N* (%)	**Aggressive Case *N* (%)**	**Non-aggressive Case *N* (%)**	**Aggressive Case Multivariable[a] OR (95% CI)**	**Non-Aggressive Case Multivariable[a] OR (95% CI)**	**Control *N* (%)**	**Aggressive Case *N* (%)**	**Non-aggressive Case *N* (%)**	**Aggressive Case Multivariable[a] OR (95% CI)**	**Non-Aggressive Case Multivariable[a] OR (95% CI)**
HHV-8										
No	376 (93)	83 (84)	277 (89)	Ref.	Ref.	445 (94)	106 (92)	293 (90)	Ref.	Ref.
Yes	30 (7)	16 (16)	36 (12)	**2.69 (1.27, 5.68)**	1.59 (0.89, 2.84)	27 (6)	9 (8)	31 (10)	1.38 (0.59, 3.23)	1.75 (0.95, 3.21)

Bolded data indicate significant associations in the multivariable logistic regression analysis.
OR denotes Odds Ratio; 95% CI denotes 95% confidence interval.
[a]Unconditional logistic regression adjusted for body mass index at study enrollment (BMI, kg/m^2), age at study entry, education (high school or less, some college, college, professional school), family history of prostate cancer (first degree relatives, yes/no), smoking history (never, former, current), condom use (usually use, yes/no), aspirin use (regular user, yes/no), and IFNL4 rs368234815 genotype (ΔG/ΔG or ΔG/TT vs. TT/TT).

Table 5. Association of HHV-8 with aggressive vs. non-aggressive prostate cancer stratified by *IFNL4* rs368234815 genotype.

	IFNL4 ΔG/TT or ΔG/ΔG					IFNL4 TT/TT				
	Control *N* (%)	**Aggressive Case *N* (%)**	**Non-aggressive Case *N* (%)**	**Aggressive Case Multivariable[a] OR (95% CI)**	**Non-Aggressive CaseMultivariable[a] OR (95% CI)**	**Control *N* (%)**	**Aggressive Case *N* (%)**	**Non-aggressive Case *N* (%)**	**Aggressive Case Multivariable[a] OR (95% CI)**	**Non-Aggressive Case Multivariable[a] OR (95% CI)**
HHV-8										
No	527 (94)	109 (87)	332 (88)	Ref.	Ref.	237 (93)	56 (90)	149 (91)	Ref.	Ref.
Yes	31 (6)	17 (13)	45 (12)	**2.62 (1.36, 5.06)**	**2.06 (1.25, 3.38)**	18 (7)	6 (10)	14 (9)	1.26 (0.46, 3.46)	1.00 (0.45, 2.22)

Bolded data indicate significant associations in the multivariable logistic regression analysis.
OR denotes Odds Ratio; 95% CI denotes 95% confidence interval.
[a]Unconditional logistic regression adjusted for body mass index at study enrollment (BMI, kg/m^2), age at study entry, education (high school or less, some college, college, professional school), family history of prostate cancer (first degree relatives, yes/no), smoking history (never, former, current), condom use (usually use, yes/no), aspirin use (regular user, yes/no), and race.

DATA AVAILABILITY

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

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AUTHOR CONTRIBUTIONS

FJ and SA were involved with project conception and design. FJ, TM, WT and TD were involved with development of methodologies. FJ, TM, WT and SA were involved with analyses and interpretation of data. FJ, TM and SA were involved with writing and review of the manuscript. FJ and SA were involved with study supervision.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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Trends in Cancer

Special issue: Cancer health disparities

Review

Immune response and inflammation in cancer health disparities

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Cancer death rates vary among population groups. Underserved populations continue to experience an excessive burden of lethal cancers that is largely explained by health-care disparities. However, the prominent role of advanced-stage disease as a driver of cancer survival disparities may indicate that some cancers are more aggressive in certain population groups than others. The tumor mutational burden can show large differences among patients with similar-stage disease but differences in race/ethnicity or residence. These dissimilarities may result from environmental or chronic inflammatory exposures, altering tumor biology and the immune response. We discuss the evidence that inflammation and immune response dissimilarities among population groups contribute to cancer disparities and how they can be targeted to reduce these disparities.

Highlights

Immunological differences in tumor biology, immune response, and systemic inflammation between population groups are contributing to a population-specific disease etiology in many major cancers. These differences may contribute to the cancer disparities experienced by people of African descent.

Treatments targeting systemic inflammation and immune response, such as aspirin use for cancer prevention and the use of cancer vaccines such as sipuleucel-T, may show increased efficacy in African Americans.

The immune-modulatory and metastasis-promoting function of platelets can be targeted using nonsteroidal anti-inflammatory drugs (NSAIDs).

Increased clinical trial diversity is required to comprehensively evaluate current and emerging therapies with respect to immune function and a chronic inflammatory environment. This may lead to better characterization of therapy response and adverse effects across population groups.

Introduction

Despite a global decline in cancer death rates, patients of African descent bear a disproportionate burden of cancer lethality for some of the most common cancer types (Box 1) [1]. In the USA, incidence and mortality rates for prostate cancer, lung cancer, and colorectal cancer (CRC) are higher for African Americans (AAs) than for European Americans (EAs) (http://seer.cancer.gov/statfacts/html/prost.html) [2]. Although age-adjusted incidence rates for breast cancer are slightly higher for EA women (140.4/100 000) than for AA women (124.9), age-adjusted mortality rates are increased in AA women (26.8/100 000) compared with EA women (19.3) (https://seer.cancer.gov/statfacts/html/breast.html). Deciphering the sources of these disparities is challenging and multifaceted. Studies have shown that differences in access to health care and delays in treatment contribute to the overburden of prostate and breast cancer deaths in populations of African descent [3–5]. Even after adjusting for disparities in access to health care, however, these disparities remain [6], suggesting that additional causes contribute to the poorer survival outcomes in these historically understudied populations.

The prominent role of advanced-stage disease in explaining some of the survival health disparities in the USA [7] raises the question of whether some patient groups may develop more aggressive disease than others because of environmental exposures, persistent chronic inflammation, comorbidities, or ancestral factors. Intrinsic differences in tumor biology and inflammatory pathway responses are well established for certain cancer types, resulting in a population-specific disease etiology [8–11]. Immune response can differ across population groups due to ancestral factors and environmental exposures [12]. Distinct germline genetic variations and alternative splicing in immune-inflammation-related genes have been detected between different population groups [13]. Study participants of African and European ancestry showed a differential immune response to infections potentially due to adaptations after exposure to infectious pathogens endemic to different regions of the world [14]. Circulating levels of some cytokines have been shown to differ between population groups because of ancestry and environmental influences [15]. These differential immune responses may have

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Box 1. The burden of global cancer disparities for people of African descent

All have not benefitted equally from the global decline in cancer death rates. Disparities in cancer incidence and mortality rates are evident for many major cancer sites. Socioeconomic inequalities, significant differences in risk factor exposures, and late-stage diagnosis are all contributing factors. Cancer patients in sub-Saharan Africa and in the central and southern parts of Asia and the Americas bear a disproportionate burden of cancer lethality for some of the most common cancer types. This is also the case for certain patient groups in the USA [1]. Cancer patients from Africa or of African descent continue to experience a higher burden of cancer incidence and mortality for many of the world's most common cancers. For example, men in sub-Saharan Africa and the Caribbean, and men of African descent in the USA and England, continue to have higher rates of fatal prostate cancer than other men [83]. In the USA, AAs also have the highest death rates from lung cancer [69]. In breast cancer, the disparity between AA and EA women in the USA persists, equating to a 40–50% increase in breast cancer death among AA women compared with EA women [2]. Breast cancer is also a leading cause of cancer death in sub-Saharan Africa. Expanded access to care, more diverse participation in clinical trials, and the enactment of policies to dismantle systemic racism are all needed to fully tackle the burden of cancer disparities on people of African descent.

consequences and contribute to cancer disparities because of their impacts on cancer progression and therapy response.

In this review, we present recent advances that describe immunological differences in cancer biology between population groups but focus on the comparison between AA and EA patients, as almost all existing data have been generated from research on these two patient groups. We discuss breast cancer, lung cancer, CRC, and prostate cancer as they are the top-four most-commonly diagnosed cancers worldwide and leading causes of cancer death [1]. We highlight the evidence that inflammation and immune response dissimilarities contribute to cancer disparities and discuss how these distinctions can be targeted through prevention and therapy to reduce these disparities. Furthermore, we evaluate the evidence that some of these differences may specifically influence the metastatic process.

Breast cancer

Inflammatory signaling may excessively affect AA breast cancer patients: an opportunity for targeted prevention and therapeutic intervention

Systemic inflammation increases cancer risk. Although anti-inflammatory drugs, like aspirin, have a rather modest protective effect in preventing breast cancer in an unselected population, they may reduce the risk of estrogen receptor (ER)-negative breast cancer in AA women [16], consistent with a distinct role for inflammation in the development of aggressive disease among these women (Figure 1). There is a growing appreciation for the role of inflammatory signaling molecules

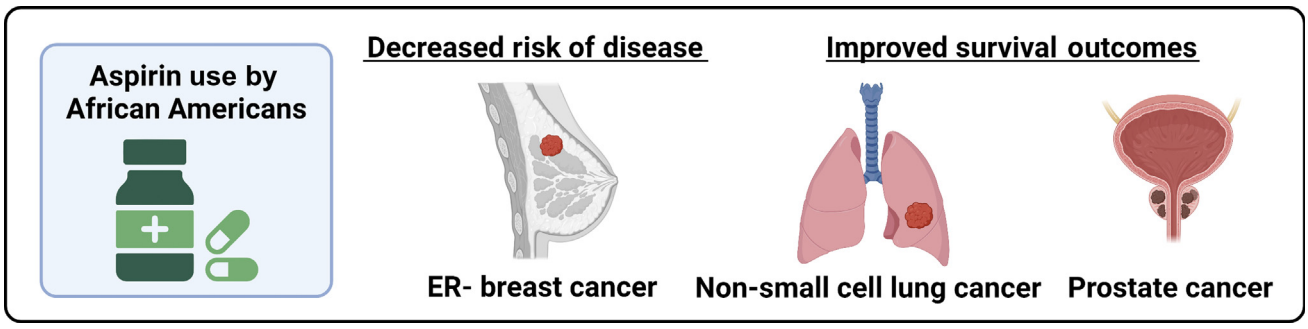


Figure 1. Aspirin may have a more robust benefit as a cancer prevention tool for African Americans (AAs) in certain cancer types. In breast cancer, AA women who are aspirin users show a decreased risk of estrogen receptor (ER)$^-$ breast cancer [16]. For lung and prostate cancer, aspirin is associated with a reduced risk of mortality in AAs [10,62]. These findings require validation but show promise for aspirin, and perhaps other drugs targeting inflammation, as a precision-medicine prevention approach to reduce the existing survival disparities for certain cancer types.

in regulating the immune response to an evolving breast tumor. One important cancer risk factor, obesity, impacts AA and Latina breast cancer patients more than EA patients, and leads to an inflammatory tumor microenvironment in breast tumors and the expansion of protumorigenic crown-like structures (CLSs) that comprise activated macrophages and dead adipocytes [17]. CLSs are more commonly found in breast tumors of AA patients and their presence tends to be associated with decreased breast cancer survival [18,19]. Recent studies have also identified significant differences in T cell signatures across population groups, including a unique T cell exhaustion signature that may have implications for decreased survival in AA women (Box 2).

Proinflammatory molecules contribute to mechanisms associated with breast cancer risk and progression and have been shown to differ by population group [20,21]. A previously described five-gene classifier in the tumor stroma of AA women, comprising *PSPHL*, *CXCL10*, *CXCL11*, *ISG20*, and *GMDS* [22], has the hallmark of an interferon-gamma (IFNγ) signaling signature, suggesting that stromal IFN signaling is increased in these patients. IFNγ enhances tumor immunogenicity by serving as the master regulator of the T helper type 1 (Th1) response and enhances patients' response to immune therapy [23]. Tumors with an IFN-stimulated gene signature are susceptible to the inhibition of adenosine deaminase acting on RNA (ADAR1) [24]. These inhibitors are currently being developed as cancer therapy agents. Thus, AA women with breast cancer and the IFN signature may show more favorable responses to both immune therapies and inhibitors of ADAR1 (Figure 2). Last, the finding that inflammation-inducible nitric oxide synthase (NOS2) is linked to poor survival in ER-negative breast cancer [25,26] provided key support for a clinical trial (Phase Ib/II, NCT02834403; https://clinicaltrials.gov/ct2/show/NCT02834403) that targets refractory triple-negative breast cancer (TNBC) with a NOS inhibitor together with taxane chemotherapy. AA breast cancer patients may also respond favorably to this therapy and should be recruited into those trials.

Immunotherapy of breast cancer may reduce the survival health disparity

Unlike other solid organ tumors, breast cancer has long been considered more immunologically 'cold', particularly the ER-positive disease, although recent advances have shown that other

Box 2. T cells and breast cancer disparities

Historically, investigations of breast cancer were the first that described differences in the immune content of tumors between patient groups comparing women of African and European descent, noticing increased numbers of tumor-infiltrating macrophages in AA and African women [84,85]. Others reported increased densities of $CD8^+$ T cells in breast tumors of young AA women and AA women with aggressive forms of breast cancer [86]. This supports the hypothesis that a more active and prolonged inflammatory immune response exists in individuals of African descent and promotes more aggressive breast cancer.

Various studies have provided solid evidence for marked differences in the tumor immune environment between AA and EA women with breast cancer [32,86,87], including an overall higher immune content score in AA patients as demonstrated by increased T cell tumor infiltrates [32,87]. Additional studies found indirect evidence for differences in the abundance of Tregs and Th2 cells in AA patients compared with EA patients [87,88]. Another study further postulated that expression of atypical chemokine receptor 1 (*DARC*/*ACKR1*), which is controlled by a West African ancestral genotype, rs2814778, shapes the immune environment of breast tumors [87]. Most interesting, however, is the observation of a signature of T cell exhaustion that occurs commonly in AA breast cancer patients. The signature distinguished these patients from EA patients [32]. In this report, the authors found a higher immune cell content in AA breast tumors, but also observed that exhausted $CD8^+$ T cells accumulate in the same tumors. Using a derived T cell exhaustion signature, they confirmed an association of this signature with poor survival. Thus, although the immune cell content might be higher in AA breast tumors, the antitumor immune response in AA patients might be inhibited because of T cell exhaustion.

Although no studies have investigated differences in response to immunotherapy based on differential $CD8^+$ T cell exhaustion signatures between AA and EA women, one study has shown that exhausted CD8 T cells predict the response to anti-PD-1 therapy in women with ER-positive breast cancer [89]. These provocative findings should be taken a step further and investigated in the context of differential T cell exhaustion signatures by population group and ER status.

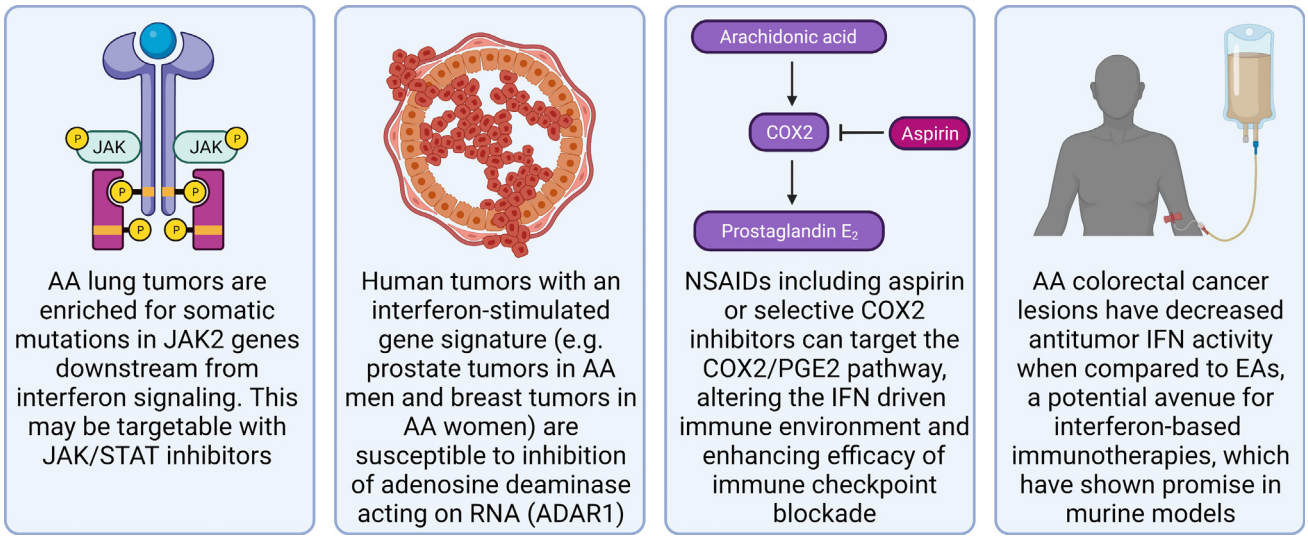


Figure 2. Targeting enhanced interferon (IFN) signaling in tumors and systemic inflammation to improve survival among all cancer patients, but with a potential impact in reducing survival health disparities [10,24,52,65]. Abbreviations: AA, African American; ADAR1, adenosine deaminase acting on RNA; COX2, cyclooxygenase 2; EA, European American; NSAIDs, nonsteroidal anti-inflammatory drugs; PGE2, prostaglandin E_2.

breast cancer subtypes, like TNBC, have higher densities of tumor-infiltrating lymphocytes [27], making it a potential target for immunotherapy. Multiple studies have shown that the immune content in breast tumors associates with survival in ER-negative breast cancer, pointing to the critical importance of the immune environment in therapy response [28,29]. Cytotoxic chemotherapy has been the main systemic treatment for women with TNBC, including the use of anthracyclines and taxanes. Nonetheless, immunotherapies have recently shown promising results in improving progression-free survival for those with TNBC [30]. Immunotherapies currently in use to treat select groups of patients with TNBC include programmed cell death protein 1 (PD-1) (pembrolizumab) and programmed death-ligand 1 (PD-L1) (atezolizumab) inhibitors, which enhance the immune system's ability to shrink the tumor using T cell-related mechanisms (discussed extensively in [31]). These therapies could significantly improve survival outcomes for women of African descent, and likely other patient groups, as accumulation of exhausted effector $CD8^+$ T cells in aggressive ER-negative breast tumors has now been revealed for AA patients [32]. These tumors may respond well to PD-1/PD-L1 inhibitors. Given the increased prevalence of ER-negative breast cancer among AA women, enhanced recruitment of these patients into clinical trials evaluating immune therapies is warranted. They may lead to higher response rates among these patients than in other patients, improving their survival and thereby reducing the existing survival health disparity in breast cancer.

Prostate cancer

Inflammation and immune response are upregulated in prostate tumors of AA men

Low-grade inflammation and associated immune cells can provoke prostate cancer progression through a milieu of signaling pathways involving both innate and adaptive immune systems [33]. Unlike TNBC, prostate tumors are seen as immunologically cold tumors that do not respond well to immunotherapy. However, high levels of certain immune cell types in these tumors, including $CD4^+$ T cells, tumor-associated macrophages, myeloid-derived suppressor cells, and natural killer (NK) cells, were found to be associated with worse prognosis [34–37]. An elevated immune content score in prostate tumors is associated with inferior survival outcomes, whereas upregulated PD-L2 expression predicted worse distant-metastasis-free survival and was associated

with inferior radiotherapy outcomes, suggesting that it may be a therapeutic target of interest either alone or in combination with radiotherapy [34]. Elevated release of the cytokines interleukin (IL)-6 and IL-8 is also associated with adverse prostate cancer outcomes, as shown in additional studies [38,39].

AA tumors display a distinct immunological profile compared with EA tumors, with overall higher immune cell content, enrichment of immune oncological pathways and epithelial-to-mesenchymal transition, and a lower DNA damage repair capacity [40–42]. *In vitro* studies show that stromal cells from AA men with prostate cancer have enhanced expression of inflammatory mediators including TrKB, BDNF, VEGF, and IL-6 and higher immune content in the tumor microenvironment [43]. These rather robust population differences in the immune environment may provide a new understanding of tumor biology that can be used to guide decisions on therapy. This gained knowledge may spur novel therapeutic approaches that can further improve prostate cancer outcomes in these men [44]. For example, men of African descent with metastatic castration-resistant prostate cancer (mCRPC) showed higher survival rates when treated with the cancer vaccine sipuleucel-T [45]. Baseline and post-treatment samples from men who received sipuleucel-T showed increased systemic inflammation in AA men compared with EA men [46]. However, the data did not reveal why AA men would have this advantageous response to the vaccine, so further studies are required.

An immune-inflammation gene expression signature, featuring upregulation of IFN pathway genes, occurs more often in prostate tumors of AA than EA men. This signature is a marker of decreased disease-free survival in prostate cancer and has previously been linked to acquired resistance to radiation and chemotherapy in breast cancer [47]. The precise origin of the immune-inflammation signature remains unknown, but its presence is associated with an IFNλ4 genotype (rs368234815-ΔG) that is most common in individuals of West African ancestry and influences host viral response [48]. Upregulation of the immune-inflammation signature in AA tumors indicates a mechanism by which either inflammatory ancestral factors or an as-yet-unknown infectious agent may contribute adversely to prostate cancer outcomes. Future studies should investigate why AA men develop this signature in their tumors as a potential opportunity for cancer prevention.

The influence of systemic inflammation on prostate cancer metastasis

mCRPC has a median survival of just 3 years and should therefore remain a key research focus (Box 3) [49]. When investigating inflammation as a driver of prostate cancer, much of the focus has been on the tumor and tumor microenvironment. However, recent studies highlight the importance of systemic inflammation and the immune environment in the circulation as influencers of the metastatic process, with particular relevance to men of African descent. Preclinical studies have implicated immune cells including macrophages [50], T regulatory cells (Tregs) [51], platelets [52], and neutrophils [53] as promoters of the metastatic process through the protection of tumor cells in circulation as well as the promotion of tumor-cell seeding and colonization.

Levels of circulating immune cells may predict treatment response for mCRPC. It has been shown that abiraterone/enzalutamide-sensitive patients display different baseline levels of immune content in peripheral blood compared with treatment-resistant patients, including higher levels of plasma-derived proinflammatory mediators during treatment [54]. Patients with enzalutamide-resistant CRPC have higher expression of PD-L1/2 in peripheral blood dendritic cells, suggesting suppression of immune response in enzalutamide-resistant CRPC through induction of PD-L1/2 in circulating immune cells [55]. Two distinct CRPC bone metastasis

Box 3. Prostate cancer metastasis

The growth of prostate tissue is regulated by androgen hormones through activation of the androgen receptor. Androgen deprivation therapy is the standard treatment for advanced prostate cancer. Nonetheless, androgen mutations or alternative androgen production and oncogenic signaling can contribute to resistance to hormonal therapy [54]. If prostate tumors are not sensitive to hormone treatment or develop resistance to androgen therapy, castration-resistant disease is diagnosed. Metastasis is a process where single tumor cell clones detach and disseminate from the primary tumor, travel through the blood and/or lymph vasculature, and grow into a secondary tumor at a distant site. mCRPC is a lethal disease with a poor relative 5-year survival of 30% (http://seer.cancer.gov/statfacts/html/prost.html). The most common metastatic site for prostate cancer is bone, but it can also be found in the lymph nodes, liver, lung, and brain. Current treatments for mCRPC are chemotherapy, irradiation, immunotherapy, and hormonal therapy with limited success. The use of PARP inhibitors, PD-1 inhibitors, and combination strategies with current treatments show promise in clinical trials but improved management and selection of suitable patients for these treatments is required to improve outcomes [44].

The odds are stacked against circulating tumor cells developing into a metastasis, with many being lost in the bloodstream or failing to initiate growth. However, when conditions are right, with a combination of growth factors and immune response from tumor and stromal cells, tumor clones can escape from the primary tumor, evade the systemic immune system, survive the bloodstream, and set up micrometastasis in a secondary location. There are many factors that promote prostate cancer metastasis, including the release of cytokines and chemokines by cells in the tumor, the immune system, and the tumor microenvironment. This promotes prostate cancer metastasis through facilitation of the epithelial-to-mesenchymal transition [50], contribution to resistance [54], angiogenesis, and the creation of favorable conditions to guide the homing of tumor cells to secondary growth sites, such as the creation of a premetastatic niche, the promotion of extravasation, and endothelial attachment (reviewed in [90]). Platelet-derived factors in the systemic circulation may also promote metastasis in prostate cancer [62].

AA men with prostate cancer may have distinct immune and inflammatory responses to both established and proposed therapeutic strategies [44]. A strong focus on recruiting this population group for clinical studies targeting mCRPC could lead to additional discoveries of how immune response differences can be targeted with either already-available or novel treatments for this lethal condition.

molecular subtypes differentiated by their androgen receptor activity and cellular immune response have been described [56].

The metastatic process relating to inflammation and the immune environment outside the tumor may affect the survival and seeding of circulating cancer cells at distant sites, with possible implications for disparate outcomes across population groups. Aspirin, the anti-inflammatory drug, has shown benefit in preventing aggressive prostate cancer, perhaps most robustly in men of African descent, suggesting that systemic inflammation might drive adverse outcomes in this patient population (Figure 1) [57,58]. The preventive benefits of aspirin have been attributed to inhibition of the inflammatory cyclooxygenase (COX) signaling pathway. Elevation of prostaglandin E_2 (PGE_2) and PGE_2 metabolite (PGE-M), both products of the COX pathway, have been associated with many cancer types [59,60], but an association with prostate cancer is not supported [61]. Instead, elevated urinary thromboxane B2 (TXB2), the stable metabolite of the eicosanoid thromboxane A2 (TXA2), was associated with metastatic prostate cancer [62]. Elevated urinary TXB2 was also associated with increased mortality but exclusively in AA men. Aspirin inhibits TXA2/TXB2 synthesis and inhibits the prometastatic effects of platelet-derived COX1/TXA2 in animal models of lung metastasis [63].

Platelets are now regarded as active drivers of metastatic progression. Thrombocytosis is associated with higher risk of CRC death, most strongly in AAs [64]. Precision targeting of inflammatory pathways, such as COX2/PGE_2 axis inhibition, can modulate the tumor microenvironment and enhance the response to immunotherapy [65]. AAs already have a demonstrably better response to some immunotherapies, so this combination treatment may address survival disparities [45]. Inhibition of platelets and platelet-derived factors including COX-1/TXA2, but also transforming growth factor (TGF)-β, IL-6, and VEGF, is a promising approach to target disparities in cancer outcomes for AAs [64]. Aspirin has the potential to

prevent lethal prostate cancer through the inhibition of prometastatic, platelet-derived TXA2/B2 synthesis in the circulation [64].

Systemic inflammation as a driver of prostate cancer progression is likely to be linked to altered systemic immunity. Although unpublished, it was found that levels of immune-oncological proteins in the blood are distinctly associated with the population groups in which they are measured, suggesting a potential role in cancer disparities. Here, the immunome of Ghanaian men resembles the immunome of AA men more than EA men, presumably due to the shared ancestral relationship. Proteins involved in chemotaxis and the suppression of tumor immunity were significantly elevated in both Ghanaian and AA men compared with EA men. Suppression of a tumor-immunity signature comprising several systemic immune-oncological markers, of which many are proinflammatory, is associated with metastatic prostate cancer, and this finding was most robust among the AA cases.

The exact cause of elevated inflammation in AA men is unknown. Genetic ancestry in immune response to pathogens [14], proinflammatory diet [66], and comorbidities, such as diabetes [67], that are more prevalent in AA men have all been implicated. These differences in immune-inflammation pathways may be contributing to the disproportionate burden of prostate cancer lethality in AA men, but may also offer opportunities for targeted therapies (discussed extensively in [44]), including targeting the patients with anti-inflammatory drugs, cancer immunotherapies beyond sipuleucel-T, or ADAR1 inhibitors.

Lung cancer

Systemic inflammatory markers are associated with lung cancer

Like other cancers, the reduction or elimination of disparities in lung cancer incidence and mortality is impeded because our current knowledge of lung cancer biology is derived primarily from populations of European descent. Smoking, asbestos, and infectious diseases are known to cause inflammation in the lung with established links to carcinogenesis. With different smoking patterns in AAs compared with EAs, the etiology of lung cancer appears to differ between groups. However, despite this strong rationale, compared with other cancer sites fewer studies have assessed the role of systemic inflammation and the immune response in the disparate outcomes experienced by AAs with lung cancer. For the studies that have been completed, a picture is cautiously emerging of a distinct immune-inflammation landscape that could possibly be utilized to address these disparities.

Using a nested case-control study design, a positive association has been found between the circulating plasma inflammation markers IFNλ, IL-12/IL-23p40, IL-6, IL-8, and C-reactive protein (CRP) and lung cancer diagnosis. However, data from this study do not support the utility of these associations in risk stratification [68]. Continuing this line of inquiry, elevated inflammatory mediators were also associated with having lung cancer. Levels of the mediators were distinct by population group and may offer clinical utility as biomarkers of disease [69]. The study described inflammatory proteins that were commonly associated with lung cancer in EAs and AAs (CRP, IL-6, IFNγ, and IL-8), but also inflammatory proteins that were distinct to population groups, with SAA, sTNFRII, and CXCL9 associated with lung cancer in EAs only and IL-1β, IL-10, IL-15, IP-10, MIP-1α, MCP-4, and TNF-β associated with lung cancer in AAs only. The results were not driven by genetic ancestry or menthol cigarette use, and ultimately the origin of this inflammation–lung cancer association remains to be determined. However, IL-6, IL-15, and MCP-4 were identified as potential biomarkers of early-stage lung cancer. The observation may have important clinical utility and should be validated prospectively because biomarkers to differentiate between benign and malignant nodules are lacking in lung cancer. Suitable

biomarkers are needed with urgency, particularly for AAs who are at highest risk of lung cancer. Targeting of IL-6 is one option that may be worth exploring further (Figure 3).

Despite recent updates to the US Preventive Services Task Force screening guidelines, expanding eligibility to include more AAs, current models suggest that, although more lung cancer deaths in AAs will be averted, the disparity between EAs and AAs undergoing screening may not be targeted comprehensively [70]. The identification and incorporation of measurements of appropriate biomarkers that detect early-stage disease in AAs, in combination with low-dose computed tomography, which has a high rate of false positivity [71], could help to minimize health disparities and improve patient outcomes in this population.

Aspirin use has been shown to reduce the risk of non-small cell lung cancer in men only and was also associated with improved survival, but only among AA men and women [72]. Similar to previously outlined findings in prostate [62] and breast [16] cancers, this points to racial differences in systemic inflammatory processes contributing to cancer progression, which are being curtailed by aspirin use (Figure 1). It is unclear whether global genetic ancestry is an underlying factor in inflammation and the population-specific aspirin effect or whether other factors explain the prevalence of inflammation in AAs. Future studies that include participants of African ancestry should at a minimum explore how genetic factors, such as somatic mutations and functional polymorphisms, may modulate the response to aspirin.

For biological targets within the tumor, the global somatic mutation landscape is comparable in AAs and EAs, but AA lung adenocarcinomas are enriched for somatic mutations in the *PTPRT* and *JAK2* genes compared with EA tumors [10]. This is of interest because both genes are downstream signal transducers of the oncogene STAT3, through cytokine and IFN signaling, and are potentially targetable with JAK inhibitors. Further work to determine immune response and inflammation by population group may offer opportunities to reduce disparities in lung cancer through targeting these pathways.

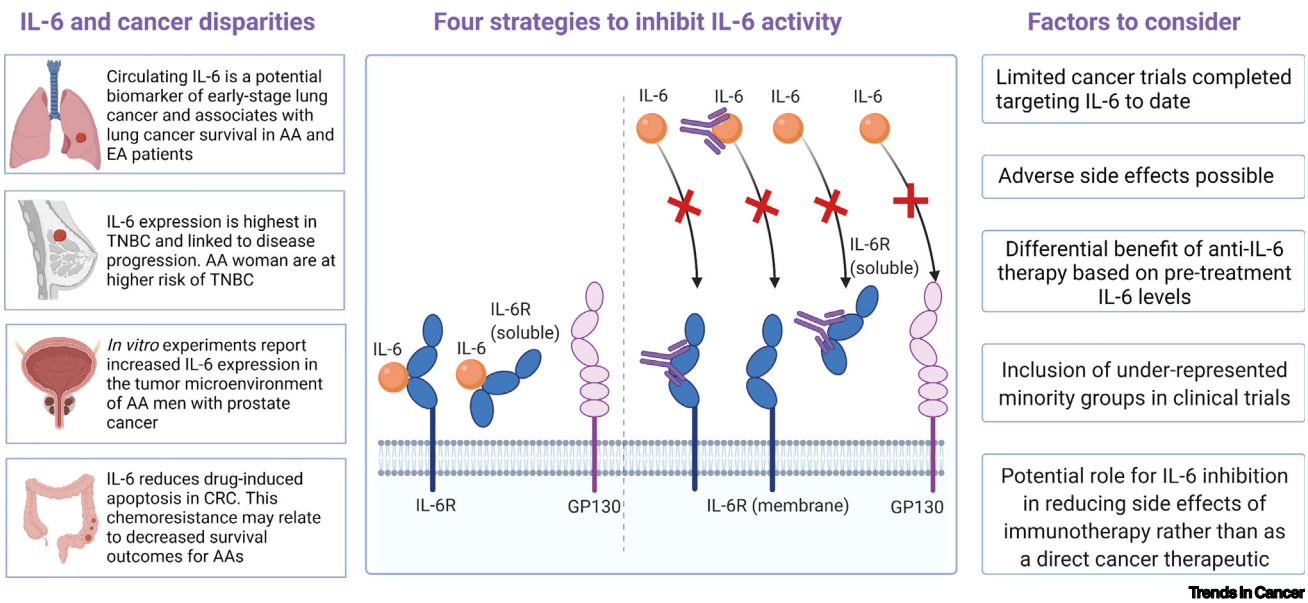


Figure 3. Anti-interleukin-6 (IL-6) therapy as an example of the potential reduction of cancer outcome disparities through an immune-therapy approach [31,43,69,82]. Abbreviations: AA, African American; CRC, colorectal cancer; EA, European American; TNBC, triple-negative breast cancer.

Colorectal cancer

Distinct immunological landscape may contribute to poorer survival outcomes experienced by AAs compared with EAs

Significant disparities have been observed in CRC, following a comparable trend of increased incidence and mortality in men and women of African ancestry. Reasons for this disparity have not been studied to the extent of other cancers, but a more advanced stage at diagnosis, differences in treatment, lower screening rates, and differences in tumor biology are all believed to be contributors to the poorer outcomes for AAs [73,74]. Additionally, among biological risk factors for CRC, obesity has been shown to contribute significantly [75], with AAs engaging in more high-risk CRC behaviors including being more likely to smoke and having reduced physical activity and an unbalanced diet [76]. It is well known that obesity leads to inflammation, which can initiate and exacerbate cancer development. Obesity-related inflammation may also contribute to racial disparities in CRC incidence and mortality through changes in tumor immune response.

Recent studies comparing AA and EA CRC patients have revealed distinct immunological landscapes that may contribute to the poorer survival outcomes experienced by AAs. A more immunosuppressive environment has been described in preinvasive colorectal lesions from AAs compared with EAs. Differences related to IFN signaling were also described by these authors, where AA patients had lower densities of cells labeled with IFNγ [52]. Another recent study showed tumor-related differences in the IFN regulatory factor (IRF) family of transcription factors, key players in the IFN signaling pathway, where IRF3 and IRF7 were upregulated in adenocarcinomas compared with normal tissues and were significantly associated with poor prognosis [77]. These and other studies suggest potential prognostic value in IFN-related biomarkers in not only colon cancer, but breast, prostate, and lung cancers, with particular importance in the tumor biology of AA patients. Additionally, as significant differences in IFN-related gene expression have been observed between population groups, the development of therapeutics targeting these proteins and their signaling pathways may help to ameliorate the increased mortality burden for populations of African ancestry, as they more often develop aggressive cancers.

Other investigations into population differences in the immune microenvironment in CRC have described an immunologically cold tumor microenvironment in AA patients, including reduced densities of cells labeled as $CD4^+$ T cells, NK cells, Th17 cells, and mast cells, indicative of reduced effector cell activity in AA lesions [52]. Similarly, reduced numbers of macrophages and $CD8^+$ T cells but increased numbers of B cells have been reported in AA tumors compared with EA tumors [78]. Interestingly, this T cell suppression tumor environment can also be observed in AA women with ER-negative breast tumors in the form of a distinct T cell exhaustion signature, which is associated with poorer outcomes in this population. Further studies into this signature in CRC patients is warranted. and if found may partially explain differences in mortality between AAs and EAs. With an increased understanding of immune profile differences between AA and EA CRC tumors, we can leverage this information for the development of targeted therapeutics to reduce mortality in overburdened population groups.

Concluding remarks

Outcome disparities in certain major cancers that impact underserved populations have been recognized and studied for many years. While thought to enhance the risk of lethal disease, the immunological and inflammatory differences in cancer biology between population groups described in this review may also offer valuable opportunities for precision therapeutic strategies to address these disparate outcomes. In the tumor, the increased presence of an immune-inflammation signature could be targeted by therapeutics in current development (e.g., ADAR1

Outstanding questions

How can we apply a precision-medicine approach to reduce cancer outcome disparities that target the tumor immune environment?

What is the contribution of the immune-inflammation environment in tumors of AA patients to cancer outcome disparities? If we target this environment, can we reduce the excessive cancer mortality in this patient group?

What are the causes of the distinct immune-inflammation signatures that associate with cancer biology in certain populations but not others?

Do tumors traditionally considered 'cold' deserve another shot at immunotherapy? Prostate cancer, CRC, and breast cancer (ER^- subtype) have shown elevated immune responses. Could immunotherapy alone or in combination with other treatments be advantageous to patients with elevated immune activity, such as AA men with prostate cancer?

How can $CD8^+$ T cell exhaustion signatures, recently characterized in AA breast and colorectal tumors, be leveraged to improve cancer outcomes in populations of African ancestry?

Why do AA men with prostate cancer respond more robustly to the cancer vaccine sipuleucel-T?

Why do AA men with prostate cancer respond more robustly to aspirin as a strategy to prevent mortality? Is inhibition of platelets by aspirin responsible for this observation. Do AA men with higher platelet counts respond the best to aspirin treatment?

How does the social environment contribute to the increased burden of cancer-promoting systemic inflammation and immune deficiency in underserved populations?

How can we ensure equitable access to clinical trials evaluating immune therapies? Should trials be mandated to recruit from population groups representative of the population being served by the clinical trial site?

inhibitors, NOS inhibitors together with taxane chemotherapy). However, it is important to recognize the prohibitive expense of novel treatments such as immunotherapy, which are out of reach for many patients from underserved groups who often do not have access to adequate health care and health insurance.

Furthermore, beyond the immediate tumor, differences in levels of immune cell types, inflammatory proteins, and cytokines in the tumor microenvironment and circulation show potential as biomarkers to guide interventions and disease targets. Inhibition of elevated platelets and platelet-derived factors, including COX-1/TXA2, using nonsteroidal anti-inflammatory drugs (NSAIDs) highlights a potential strategy against metastasis through the targeting of systemic inflammation (see Outstanding questions).

Emerging single-cell sequencing technologies will further allow us to identify characteristics of immune subpopulations in cancerous tissues with a greater resolution, moving forward our efforts in precision medicine by better tailoring cancer treatments to the tumor microenvironment, with an aim of improving outcomes in patient groups that have long suffered from excessive cancer mortalities.

Last, the participation of AAs in clinical trials has historically been low, with AA women having an 11-fold-lower participation rate than EAs [79]. Closing this participation gap may lead to advances in cancer immune therapeutics, benefiting AA and other patients but with an impact on reducing cancer health disparities. While the reasons for this gap in trial participation are multifactorial, including historical mistrust of the medical profession due to major ethical breaches in the past, a higher prevalence of comorbidities, and a lack of access to academic medical centers involved in trials, the issue needs to be immediately addressed through tailored recruitment efforts to ensure the generalizability of trial observations across population groups and equitable access to cutting-edge treatments [80,81].

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Declaration of interests

No interests are declared.

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Article

Urinary Thromboxane B2 and Lethal Prostate Cancer in African American Men

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Abstract

Background: Thromboxane A2 (TXA2) is a platelet- and cyclooxygenase-derived eicosanoid that has been linked to metastasis. We investigated the role of TXA2 in the development of lethal prostate cancer in African American (AA) and European American (EA) men. **Methods:** We measured urinary 11-dehydrothromboxane B2 (TXB2), a stable metabolite of TXA2, with mass spectrometry. Samples were obtained from 977 cases and 1022 controls at time of recruitment. We applied multivariable logistic and Cox regression modeling to examine associations of TXB2 with prostate cancer and patient survival. The median survival follow-up was 8.4 years, with 246 deaths among cases. Aspirin use was assessed with a questionnaire. Race was self-reported. **Results:** Urinary TXB2 was inversely associated with aspirin use. High (>median) TXB2 was associated with prostate cancer in AA (adjusted odds ratio [OR] = 1.50, 95% confidence interval [CI] = 1.13 to 2.00) but not EA men (OR = 1.07, 95% CI = 0.82 to 1.40), suggesting upregulated TXA2 synthesis in AA men with prostate cancer. High TXB2 was positively associated with metastatic prostate cancer (OR = 2.60, 95% CI = 1.08 to 6.28) compared with low (≤median) TXB2. Furthermore, high TXB2 was also associated with all-cause (adjusted hazard ratio = 1.59, 95% CI = 1.06 to 2.40) and prostate cancer-specific mortality (hazard ratio = 4.74, 95% CI = 1.62 to 13.88) in AA men only. **Conclusions:** We report a distinct association of TXB2 with prostate cancer outcomes in AA men. In this high-risk group of men, upregulation of TXA2 synthesis may promote metastasis and lethal disease. Our observation identifies a potential benefit of aspirin in preventing lethal prostate cancer through inhibition of TXA2 synthesis.

Systemic low-grade inflammation and an inflammatory tumor microenvironment are candidate risk factors for prostate cancer that promote aggressive disease (1-3). Prostate tumors in African American (AA) men harbor a distinct immune and inflammation signature consistent with a unique immunobiology and the activation of inflammation pathways (4-6). These observations, coupled with our previous findings that regular use of aspirin is associated with decreased odds of lethal prostate cancer in AA men (7,8), suggest that low-grade chronic inflammation might be a driver of adverse outcomes in AA prostate cancer patients.

The preventative benefits of aspirin have been attributed to inhibition of the arachidonic acid signaling pathway (9,10). Arachidonic acid is broken down to eicosanoids by the cyclooxygenase (COX) 1 and 2 enzymes. These enzymes and subsequent production of these eicosanoids are important for normal physiological processes, including the modulation of immune responses and regulation of blood clotting. However, as a promoter of inflammatory responses, eicosanoid synthesis is commonly upregulated in cancer and may contribute to cancer progression (11).

Thromboxane A2 (TXA2), an eicosanoid produced primarily via COX1 in activated platelets, orchestrates platelet aggregation. TXA2, activated and elevated at times of inflammation, contributes to carcinogenesis through roles in vasoconstriction, endothelial adhesion (12), cell motility (13), and cell

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proliferation (14). Importantly, platelet-derived TXA2 is pro-metastatic (15). Aspirin may reduce metastatic cancer and inhibit the pro-metastatic effects of platelet-derived COX1 or TXA2, as shown in an animal model of lung metastasis (15-17).

There have been no studies investigating the relationship of TXA2 or TXB2 formation with adverse prostate cancer outcomes. Hence, we assessed the role of TXA2 levels in the development of lethal prostate cancer in a diverse study population .

Methods

Study Population

The National Cancer Institute (NCI)-Maryland prostate cancer case-control study has been described (7,18). The study was initiated to test the primary hypothesis that environmental exposures and ancestry-related factors contribute to the excessive prostate cancer burden among AA men. Before the interview, all individuals signed informed consent for participation. All study forms and procedures were approved by the NCI (protocol # 05-C-N021) and the University of Maryland (protocol #0298229) institutional review boards. Research followed the ethical guidelines set by the Declaration of Helsinki. Cases were recruited at the Baltimore Veterans Affairs Medical Center and the University of Maryland Medical Center through arrangements with physicians. Controls were identified through the Maryland Department of Motor Vehicle Administration database and were frequency matched to cases on age and race. This article follows the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines for the reporting of observational studies. See the Supplementary Methods (available online) for exclusion and inclusion criteria, questionnaire, and biospecimen information.

Laboratory Assay for Urinary TXB2 Measurement

Because TXA2 is unstable, its primary, more stable metabolite, urinary 11-dehydrothromboxane B2 (TXB2), was measured by the Eicosanoid Core Laboratory at Vanderbilt University Medical Center (Nashville, TN). See the Supplementary Methods (available online) for more details about this assay and its quality control performance. It has previously been shown that urinary TXB2 correlates well with platelet-derived TXA2 synthesis (19).

Assessment of Aspirin Use

Our survey evaluated aspirin use with the following question: “Have you taken aspirin or aspirin-containing compounds (such as Bufferin, Anacin, Ascriptin, Excederin) regularly—at least 1 pill per week for 2 months during the past 5 years?” Responses were no, yes, or do not know.

Statistical Analysis

Data analysis was performed using the Stata/SE 16.0 statistical software package (StataCorp). All statistical tests were 2-sided. An association was considered statistically significant with P less than .05. For analysis, we assessed TXB2 as either a continuous measure or assigned TXB2 values to quartiles (Q1-Q4, Q1 being the lowest, Q4 being the highest) and median ($\leq$median or $>$median) with cutoff points determined using the distribution of TXB2 values among all controls. TXB2 data analyzed as a continuous measure were log2 transformed. The nonparametric Kruskal-Wallis or the Mann-Whitney test was used to determine differences in TXB2 levels across groups. Furthermore, cases were assigned to risk groups according to National Comprehensive Cancer Network (NCCN) Risk Score classification, which stratifies patients into pretreatment recurrence risk groups according to the clinical tumor stage, biopsy Gleason score, and serum prostate-specific antigen level (20). We condensed these risk groups into 4 categories (low, intermediate, high or very high, and regional or metastatic).

Unconditional logistic regression models were used to calculate adjusted odds ratios (OR) and 95% confidence intervals (CI) to assess the association of TXB2 with use of aspirin, case status, or the NCCN risk score. We adjusted for the following potential confounding factors: age at study entry, body mass index, diabetes, aspirin use, education, family history of prostate cancer, self-reported race, smoking history, treatment, disease stage, and Gleason score (see the Supplementary Methods, available online, for more information). To test for a statistical interaction between aspirin use and TXB2 levels, we applied the multivariable logistic regression model with and without the interaction term and examined statistical significance with the likelihood ratio test. A P less than .05 was considered as statistical evidence for effect modification.

We applied the Cox regression model to estimate adjusted hazard ratios (HR) and 95% CI for all-cause mortality and prostate cancer-specific mortality in cases. Median survival follow-up was 8.4 years. In the analysis of all-cause mortality, median follow-up time to death from any cause was 4.52 years for AA men and 5.99 years for EA men. In the analysis of prostate cancer-specific survival, median follow-up time to death from prostate cancer was 2.75 years for AA men and 7.7 years for EA men. We adjusted for potential confounding factors (defined in the Supplementary Methods, available online). We calculated survival for cases and controls from date of diagnosis to either date of death or to the censor date of December 31, 2018. We confirmed nonviolation of the proportionality assumption based on the goodness-of-fit test using Schoenfeld residuals. In a sensitivity analysis, the Fine-Gray competing risk model was used to estimate whether the association of TXB2 with prostate cancer survival was influenced by other causes of death. For survival analysis with the Kaplan-Meier method, the log-rank test was used to examine differences in all-cause and prostate cancer–specific mortality according to TXB2 levels.

Results

Clinical and Demographic Characteristics of Participants in the NCI-Maryland (NCI-MD) Prostate Cancer Case-Control Study

Demographic characteristics of the enrolled participants are shown in Supplementary Table 1 (available online) with the disease characteristics of the cases. The study enrolled 977 cases (490 AA and 487 EA) and 1022 population controls (479 AA and 543 EA) from the greater Baltimore area in Maryland. Race or ethnicity was self-reported as part of the eligibility screener and with the survey. The distributions of age and body mass index were very similar in cases and controls. Controls had higher levels of education, with 24.9% reporting a graduate school qualification compared with 14.6% of cases. Cases had a higher proportion of current smokers at 24.8% compared with 14.5% of controls .

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Urinary TXB2 Levels Among Cases and Controls and Their Association With Aspirin Use

Urinary TXB2 was statistically significantly upregulated in AA men with prostate cancer (Figure 1, A). Among controls, EA men tended to have higher levels than AA men ($P = .03$). With TXB2 data distributed into quartiles according to control values and stratified by race, a higher proportion of AA cases presented with the most elevated (Q4) TXB2 levels compared with controls. There was a statistically significant difference in Q4 TXB2 levels between cases and controls for AA men ($P < .001$) but not for EA men ($P = .35$) (Figure 1, B).

Next, we investigated whether intake of the most common antiinflammatory drug, aspirin, would have the anticipated inhibitory effect on urinary TXB2. Data on aspirin use was available for 99.1% of our cases (968 of 977), with 49.6% reporting regular aspirin use, which is generally consistent with aspirin use in this age group (21). However, broken down by self-reported race, 43.1% of AA men reported to have taken aspirin compared with 56.9% of EA men. Aspirin use was associated with decreased TXB2 in both cases and controls (Table 1). For cases who used aspirin, the adjusted odds of having high urinary TXB2 levels (Q4) were statistically significantly reduced compared with nonusers (OR = 0.27, 95% CI = 0.18 to 0.41) (Table 1), and this observation remained when data were stratified by race (AA men: OR = 0.20, 95% CI = 0.11 to 0.36; EA men: OR = 0.39, 95% CI = 0.21 to 0.70), suggesting strong inhibition of TXB2 formation by aspirin in men with prostate cancer. There was also a strong inhibition of TXB2 by aspirin use in controls (OR = 0.21, 95% CI = 0.14 to 0.32) (Table 1). This was consistent for both AA and EA controls. These findings agree with previous work showing inhibition of TXA2 by aspirin through inhibition of the COX1 and TXA2 pathway (15). Notably, however, the aspirin inhibitory effect tended to be more robust among both AA cases and controls than EA cases and controls.

TXB2 and Aggressive Prostate Cancer

We used unconditional logistic regression to determine the odds of having prostate cancer when TXB2 is elevated. The adjusted OR for prostate cancer was 1.24 (95% CI = 1.02 to 1.50) for men with high (>median) TXB2 compared with low TXB2 (≤median) (Table 2), pointing to a moderate but statistically significant association with the disease. To further understand the importance of this finding for AA men, we stratified the analysis by race. In this analysis, a statistically significant association between high TXB2 and having prostate cancer was observed

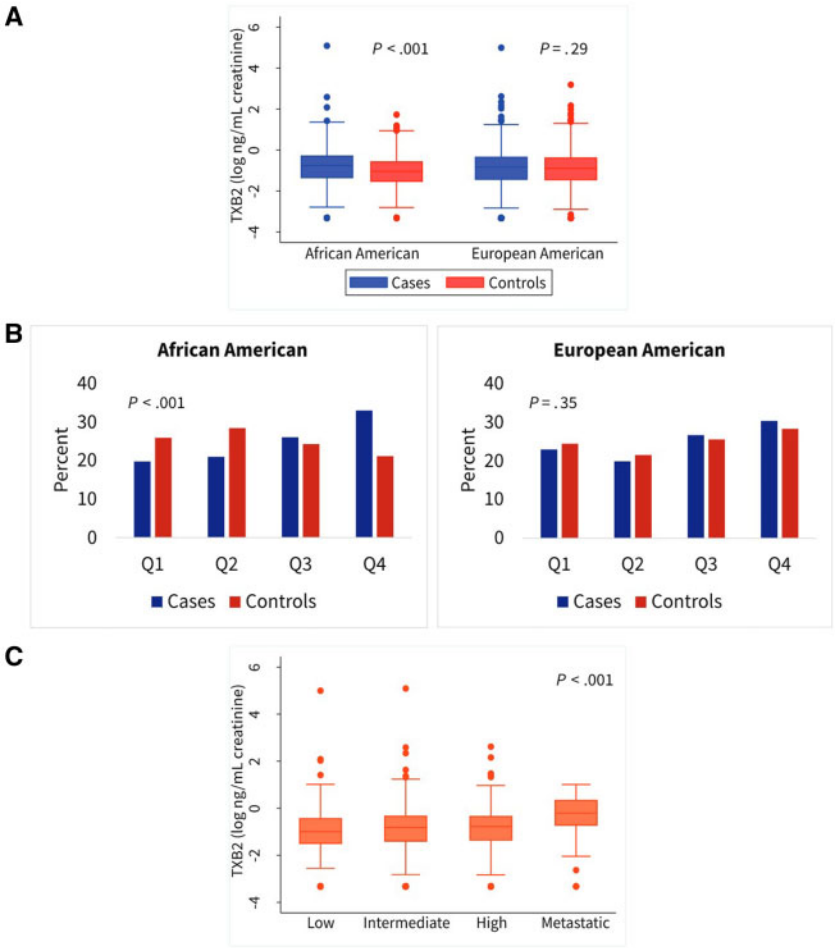


Figure 1. Urinary 11-dehydrothromboxane B2 (TXB2) levels in African American and European American men and their association with prostate cancer and National Comprehensive Cancer Network (NCCN) risk scores. **A)** Urinary TXB2 levels in cases and controls shown as a continuous measure, stratified by race. **B)** Bar charts show distribution of cases and controls stratified into African American and European American men across TXB2 quartiles (Q). Q cutoff points originate from the combined control groups and are 0.35, 0.505, and 0.738 ng TXB2/mg creatinine. **C)** Shown are urinary TXB2 levels across 4 NCCN risk score categories. Two-sided Mann-Whitney (A), χ^2 (B), and Kruskal-Wallis (C) tests were applied for statistical significance testing. The **error bars** in (A) and (C) represent the 95% confidence interval.

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Table 1. Inverse association of regular aspirin use with urinary TXB2 levels in cases and controls

	Aspirin use								
	All			African American			European American		
TXB2	No, No. (%)	Yes, No. (%)	OR (95% CI)	No, No. (%)	Yes, No. (%)	OR (95% CI)	No, No. (%)	Yes, No. (%)	OR (95% CI)
Cases									
Q1[a]	72 (15.1)	132 (27.9)	Ref[b]	35 (12.9)	60 (29.6)	Ref[b]	37 (18.0)	72 (26.7)	Ref[b]
Q2	85 (17.9)	107 (22.6)	0.72 (0.46 to 1.14)[b]	52 (19.2)	46 (22.7)	0.47 (0.24 to 0.83)[b]	33 (16.1)	61 (22.6)	1.16 (0.58 to 2.30)[b]
Q3	128 (26.9)	123 (26.0)	0.47 (0.31 to 0.73)[b]	74 (27.3)	51 (25.1)	0.31 (0.17 to 0.56)[b]	54 (26.3)	72 (26.7)	0.70 (0.37 to 1.31)[b]
Q4	191 (40.1)	111 (23.5)	0.27 (0.18 to 0.41)[b]	110 (40.6)	46 (22.7)	0.20 (0.11 to 0.36)[b]	81 (39.5)	65 (24.1)	0.39 (0.21 to 0.70)[b]
P_{trend}			<.001			<.001			<.001
Controls									
Q1	71 (16.4)	180 (31.8)	Ref[c]	38 (16.6)	84 (35.4)	Ref[c]	33 (16.3)	96 (29.1)	Ref[c]
Q2	86 (19.9)	163 (21.8)	0.69 (0.46 to 1.04)[c]	57 (24.9)	75 (31.7)	0.48 (0.27 to .84)[c]	29 (14.3)	88 (26.7)	0.91 (0.49 to 1.68)[c]
Q3	129 (29.9)	121 (21.3)	0.31 (0.21 to 0.46)[c]	66 (28.8)	47 (19.8)	0.27 (0.15 to 0.48)[c]	63 (31.0)	74 (22.4)	0.32 (0.18 to 0.56)[c]
Q4	146 (33.8)	103 (18.2)	0.21 (0.14 to 0.32)[c]	68 (29.7)	31 (13.1)	0.14 (0.08 to 0.27)[c]	78 (38.4)	72 (21.8)	0.27 (0.15 to 0.47)[c]
P_{trend}			<.001			<.001			<.001

[a]Quartile (Q) cutoff points are 0.35, 0.505, and 0.738 ng TXB2/mg creatinine. CI = confidence interval; OR = odds ratio; Ref = reference.
[b]Logistic regression analysis adjusted for age at study entry, body mass index (kg/m^2), diabetes (yes or no), education (high school or less, some college, college, professional school), family history of prostate cancer (first-degree relatives, yes or no), self-reported race (not included in stratified analysis), disease stage (1 = stage I, 2 = stage IIA and IIB, 3 = stage III, 4 = stage IV), Gleason score (0 = Gleason ≤7 and 1 = Gleason >7), smoking history (never, former, current), and treatment (0 = none, 1 = surgery, 2 = radiation, 3 = hormone, 4 = combination).
[c]Logistic regression analysis adjusted for age at study entry, BMI (kg/m^2), diabetes (yes or no), education (high school or less, some college, college, professional school), family history of prostate cancer (first-degree relatives, yes or no), self-reported race (not included in stratified analysis), and smoking history (never, former, current).

only among AA men (OR = 1.50, 95% CI = 1.13 to 2.00) but not EA men (OR = 1.07, 95% CI = 0.82 to 1.40). We previously reported that aspirin use associates with a reduced risk of developing advanced prostate cancer among AA in our study cohort (7). Given this observation, we conducted a mediation analysis to estimate direct effects of aspirin use on prostate cancer risk and indirect effects via TXB2. From this analysis, we estimate that the indirect effect of TXB2 as a mediator of prostate cancer risk accounts for almost one-half of the protective effect of aspirin use on prostate cancer risk in AA men (Supplementary Table 2, available online).

We further investigated the association between TXB2, aggressive disease, and metastasis, and assigned men with prostate cancer into NCCN risk groups as described under the Methods and shown in Supplementary Table 3 (available online). In the unadjusted analysis, the highest median level of TXB2 associated with prostate cancer patients who had developed regional and distant metastatic disease (Figure 1, C). This association remained statistically significant using an adjusted logistic regression model. High levels of TXB2 associated with prostate cancer only among cases who were in the risk group for regional and distant metastatic disease (Table 3) (OR = 2.60, 95% CI = 1.08 to 6.28, >median vs ≤median). We did not find an association of TXB2 with localized disease, consistent with the role of TXA2 or TXB2 signaling in metastasis (15). These observations suggest a distinct relationship between high TXB2 levels and lethal prostate cancer.

Association of High TXB2 With Survival and the Effect of Aspirin Use

To build on our findings that high TXB2 is associated with prostate cancer among AA men, we next examined if there is an association between high levels of TXB2 and survival outcomes in our population. As of the end of 2018, there have been 246 deaths in our case population, of whom 47.6% had a cancer

Table 2. Association of urinary TXB2 levels with prostate cancer

	Odds of case status		
TXB2	Control, No. (%)	Case, No. (%)	OR (95% CI)[a]
All cases			
≤Median[b]	500 (50.0)	397 (41.8)	Ref
>Median	499 (50.0)	554 (58.2)	1.24 (1.02 to 1.50)
African American			
≤Median	254 (54.5)	193 (40.6)	Ref
>Median	212 (45.5)	282 (59.4)	1.50 (1.13 to 2.00)
European American			
≤Median	246 (46.2)	204 (42.9)	Ref
>Median	287 (53.8)	272 (57.1)	1.07 (0.82 to 1.40)

[a]Unconditional logistic regression adjusted for age at study entry, body mass index (kg/m^2), diabetes (no or yes), aspirin (no or yes), education (high school or less, some college, college, professional school), family history of prostate cancer (first-degree relatives, yes or no), self-reported race (not included in stratified analysis), and smoking history (never, former, current). CI = confidence interval; OR = odds ratio; Ref = reference.
[b]Median cutoff point is 0.505 ng TXB2/mg creatinine.

diagnosis as the recorded primary cause of death, and 26.8% of all deaths (n = 66) were directly attributed to prostate cancer. In agreement with the literature, AA men in the NCI-MD study were more likely to die after a prostate cancer diagnosis than EA men (all-cause mortality, adjusted HR = 1.59, 95% CI = 1.20 to 2.10) (Supplementary Table 4, available online). Moreover, a higher proportion of them died because of prostate cancer (prostate cancer-specific mortality, adjusted HR = 1.71, 95% CI = 0.97 to 3.01) (Supplementary Table 5, available online), corroborating that AA patients are at an increased risk of lethal prostate cancer (22).

To examine the role of TXA2 in this survival disparity, we investigated the relationship of urinary TXB2 with vital status and survival among the men in our study. An initial analysis showed that a greater proportion of the all-cause mortality among cases

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Table 3. Association of high urinary TXB2 with NCCN Risk Score for metastatic prostate cancer

NCCN risk score	OR (95% CI)[a]	P[b]
Low	Ref	
Intermediate	1.49 (0.98 to 2.26)	.06
High or very high	1.34 (0.80 to 2.26)	.27
Regional or metastatic	2.60 (1.08 to 6.28)	.03

[a]Unconditional logistic regression adjusted for age at study entry, body mass index (kg/m^2), diabetes (no or yes), aspirin (no or yes), education (high school or less, some college, college, professional school), family history of prostate cancer (first-degree relatives, yes or no), self-reported race, smoking history (never, former, current), and treatment (0 = none, 1 = surgery, 2 = radiation, 3 = hormone, 4 = combination). CI = confidence interval; NCCN = National Comprehensive Cancer Network; OR = odds ratio; Ref = reference.
[b]P value was calculated using 2-sided Wald statistical test.

associated with high TXB2 (>median) (Supplementary Table 6, available online). This relationship was most pronounced in AA men, where 70.6% of the case deaths associated with high TXB2 compared with 57.3% of EA cases, reaching statistical significance only among the AA cases. Additionally, we did not find a relationship between TXB2 levels and vital status in the controls (Supplementary Table 6, available online). We then investigated whether high TXB2 was associated with survival outcomes in our case population. In the Kaplan-Meier (Supplementary Figure 1, available online) and multivariable-adjusted Cox regression survival analyses (Table 4), there was a marginal but positive association between high TXB2 levels and an increased all-cause mortality after a prostate cancer diagnosis (HR = 1.33, 95% CI = 0.99 to 1.79). However, when the data were stratified by self-reported race, high TXB2 was associated with an elevated all-cause mortality in AA men (HR = 1.59, 95% CI = 1.06 to 2.40) but not in EA men (HR = 1.11, 95% CI = 0.70 to 1.76). Associations were observed with both dichotomized and continuous TXB2 level data in the survival analysis, although the continuous data additionally supported an association among EA men (Table 4). Lastly, this relationship of urinary TXB2 with all-cause mortality was uniquely observed among men with prostate cancer but not in our control population (Supplementary Table 7, available online).

Although all-cause mortality is an important outcome measure for prostate cancer patients with advanced disease, prostate cancer-specific survival is the most rigorous outcome determinant for prostate cancer patients. We found that high TXB2 was associated with lethal prostate cancer in AA men (HR = 4.74, 95% CI = 1.62 to 13.88 with data dichotomized at the median) but not in EA men (HR = 1.12, 95% CI = 0.34 to 3.66) (Table 5; Supplementary Figures 2 and 3, available online). Similar relationships were observed when we used continuous TXB2 data in the survival analysis. Additional Fine-Gray competing risk analysis showed that the association of TXB2 with lethal prostate cancer was largely independent of competing causes of death (subdistribution HR = 3.83, 95% CI = 1.29 to 11.35) (Table 5).

As a final point, we explored the possibility of an interaction between aspirin use and TXB2 on survival. Although a statistically significant interaction was not found in the analysis of all-cause and prostate cancer-specific survival, stratification of cases by aspirin use status (yes or no) revealed disparate outcomes. The association between high TXB2 and all-cause mortality remained statistically significant only in AA men who did not use aspirin (Supplementary Table 8, available online) but not in aspirin users. Our observations suggest that aspirin may reduce all-cause mortality by decreasing TXA2 levels in AA patients with an otherwise upregulated pro-metastatic COX1 and TXA2 pathway .

Discussion

Determining which prostate cancers will develop into lethal disease is an unmet clinical need. The choice is currently between active surveillance or immediate intervention with surgery or radiation, which can lead to complications. Although AA men are at increased risk of aggressive disease, consensus has not been reached on the appropriateness of active surveillance (23,24). Identifying novel markers of aggressive disease and targets for therapy would therefore be important for men of African descent who experience a disproportionately high burden of prostate cancer lethality (22,25).

Here, we report a distinct association of upregulated TXB2 with lethal prostate cancer in AA men. Furthermore, because TXA2 and TXB2 formation are inhibited through aspirin use, as shown by our and other data, inhibitors of the COX1 and TXA2 signaling pathway, like aspirin, might be used to reduce the excess prostate cancer mortality among AA patients. Our findings further corroborate the hypothesis that a systemic low-grade inflammation is prevalent in AA men with prostate cancer, and these elevated inflammatory processes are contributing to adverse outcomes for this population.

A reduction of metastasis due to aspirin use has been established in both murine models and clinical studies, but until

Table 4. Association of urinary TXB2 levels with all-cause mortality among prostate cancer patients

	All cases			African American			European American		
TXB2	Alive, No. (%)	Dead, No. (%)	HR (95% CI)[a]	Alive, No. (%)	Dead, No. (%)	HR (95% CI)[a]	Alive, No. (%)	Dead, No. (%)	HR (95% CI)[a]
≤Median[b]	313 (43.6)	82 (33.2)	Ref	153 (44.3)	39 (28.7)	Ref	160 (42.9)	43 (39.6)	Ref
>Median	396 (56.4)	158 (66.8)	1.33 (0.99 to 1.79)	186 (55.7)	95 (71.3)	1.59 (1.06 to 2.40)	210 (57.1)	63 (60.4)	1.11 (0.70 to 1.76)
Continuous[c]	—	—	1.24 (1.09 to 1.41)	—	—	1.31 (1.10 to 1.56)	—	—	1.21 (1.01 to 1.45)

[a]Unconditional Cox regression adjusted for age at study entry, body mass index (kg/m^2), diabetes (no or yes), aspirin (no or yes), education (high school or less, some college, college, professional school), family history of prostate cancer (first-degree relatives, yes or no), self-reported race (not included in stratified analysis), smoking history (never, former, current), treatment (0 = none, 1 = surg, 2 = radiation, 3 = hormone, 4 = combination), disease stage (1= stage I, 2 = stage IIA and IIB, 3 = stage III, 4 = stage IV), and Gleason score (0 = Gleason ≤7 and 1 = Gleason >7). CI = confidence interval; OR= odds ratio; Ref = reference.
[b]Median cutoff point is 0.505 ng TXB2/mg creatinine.
[c]TXB2 as a continuous, log2-transformed variable.

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Table 5. Association of urinary TXB2 levels with prostate cancer–specific mortality

	All cases				African American				European American			
TXB2	Death from PC, No. (%)	Death from other cause, No. (%)	HR[a] (95% CI)	SHR[a] (95% CI)	Death from PC, No. (%)	Death from other cause, No. (%)	HR[a] (95% CI)	SHR[a] (95% CI)	Death from PC, No. (%)	Death from other cause, No. (%)	HR[a] (95% CI)	SHR[a] (95% CI)
≤Median[b]	20 (30.8)	62 (35.4)	Ref	Ref	7 (17.5)	32 (34.1)	Ref	Ref	13 (52.0)	30 (37.1)	Ref	Ref
>Median	45 (69.2)	113 (64.6)	1.69 (0.88 to 3.24)	1.58 (0.82 to 3.05)	33 (82.5)	62 (65.9)	4.74 (1.62 to 13.88)	3.83 (1.29 to 11.35)	12 (48.0)	51 (62.9)	1.12 (0.34 to 3.66)	0.84 (0.26 to 2.74)
Continuous[c]	—	—	1.33 (1.02 to 1.75)	—	—	—	1.59 (1.07 to 2.36)	—	—	—	1.35 (0.90 to 2.01)	—

[a]Cox regression model and Fine and Gray competing risks regression models adjusted for age at study entry, BMI (kg/m^2), diabetes (no or yes), aspirin (no or yes), education (high school or less, some college, college, professional school), family history of prostate cancer (first-degree relatives, yes or no), self-reported race (not included in stratified analysis), smoking history (never, former, current), treatment (0 = none, 1 = surgery, 2 = radiation, 3 = hormone, 4 = combination), disease stage (1 = stage I, 2 = stage IIA and IIB, 3 = stage III, 4 = stage IV), and Gleason score (0 = Gleason ≤7 and 1 = Gleason >7). CI = confidence interval; HR = hazard ratio; OR= odds ratio; Ref = reference; SHR = subdistribution hazard ratio.

[b]Median cutoff point is 0.505 ng TXB2/mg creatinine.

[c]TXB2 as a continuous, log2-transformed variable.

recently the molecular mechanism remained elusive (16,17,26). Lucotti et al. (15) used a murine model of lung cancer to demonstrate suppression of an early metastatic niche in the vasculature via inhibition of the COX1 and TXA2 pathway. Aspirin inhibited this metastatic process. Although very intriguing, similar experimental data for prostate cancer and bone metastasis have not been reported. Nevertheless, our study further supports the observations from the lung cancer model, providing epidemiological evidence that high TXA2 levels may increase the odds of developing metastatic prostate cancer in AA men.

Aspirin use has been associated with a decreased prostate cancer–specific mortality (8,27). The mechanism for this association has yet to be established; however, our findings suggest involvement of arachidonic acid signaling, specifically inhibition of TXA2, in AA men. We did not find an association between TXB2 levels and prostate cancer–specific mortality among EA men in our study. The reasons for this are unclear and will have to be investigated in future studies. Consistent with other studies, AA men in the NCI-MD study were less likely to report taking aspirin regularly compared with EA men (27). This is important in the context of our findings, where high TXB2 associated with increased risk of mortality for AA men could possibly be mitigated by aspirin use. Through platelet aggregation, TXA2 is a mediator of cardiovascular disease (CVD). Irreversible inhibition of platelet COX1-derived TXA2 with low-dose aspirin affords protection against vascular thrombotic events (28). This established link between TXA2 and CVD is relevant to this study, because use of low-dose aspirin may afford protection from both CVD events and lethal prostate cancer in AA men.

Our study has limitations. Firstly, the case-control study design is retrospective, so it remains unknown if elevated TXA2 is a risk factor for prostate cancer development. Secondly, the question remains as to why AA men with prostate cancer have higher TXB2 levels than EA men. Current lines of enquiry include whether increased arachidonic acid signaling may contribute to higher incidence of CVD comorbidities in AA men (29). Higher dietary ingestion of arachidonic acid or linoleic acid may also increase levels of TXB2. Thirdly, aspirin use data were self-reported. However, our findings that aspirin use may reduce TXA2 or TXB2 levels is very much in agreement with the published literature providing strong support of the correctness of the self-reported aspirin use data. Lack of dose information for aspirin use prevents us from making any conclusions as to what dose is required to inhibit TXA2 formation. However, it is known that low-dose aspirin inhibits COX1, and the antimetastatic effects of aspirin are attributed to both low and high doses (15,16). Lastly, in our analysis of the relationship of TXB2 with metastatic disease, we could not further stratify by race because too few men presented with metastatic prostate cancer in our study population (AA = 30, EA = 22) at the time of enrollment.

In conclusion, this study identifies a novel association between high urinary TXB2 and aggressive prostate cancer as well as adverse survival outcomes for AA men. These observations need further validation, but they are consistent with our previous findings of a prevalent immune-inflammation signature and an inverse association of aspirin use with lethal prostate cancer in these patients (6–8). Our study highlights the potential benefit of aspirin for prevention of lethal prostate cancer in this high-risk group of men through inhibition of TXA2 synthesis.

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Notes

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Author contributions: MK: Conceptualization; Investigation: Data curation; Methodology; Formal Analysis; Writing—original draft; Writing—review and editing, GM: Methodology, TZM: Conceptualization; Writing—review and editing, TD: Data curation; Project administration, WT: Supervision; Writing—review and editing, CJS: Writing—review and editing, FB: Project administration, CL: Methodology; Writing—review and editing, CY: Writing—review and editing, MBC: Methodology; Writing—review and editing, SA: Conceptualization; Methodology; Funding acquisition; Resources; Writing—review and editing; Supervision.

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Data Availability

The data underlying this article will be shared on reasonable request to the corresponding author.

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Review

Immune Inflammation Pathways as Therapeutic Targets to Reduce Lethal Prostate Cancer in African American Men

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Simple Summary: Men of African descent are twice as likely to die of prostate cancer than other men. While equal access to care is the key target to improve cancer survival, it is now known that there are differences in disease biology and risk factor exposure across population groups. These differences could be causatively linked to the existing prostate cancer health disparities. In this review, we will discuss the candidate role of inflammation and the immune response as contributing factors to the excessive burden of lethal prostate cancer among men of African ancestry. Furthermore, we will introduce the concept that these immunogenic vulnerabilities could be exploited to address the adverse outcomes experienced by these men. Lastly, we will summarize how these immunogenic and inflammatory differences could be targeted using current treatments to improve survival for men of African descent.

Abstract: Despite substantial improvements in cancer survival, not all population groups have benefitted equally from this progress. For prostate cancer, men of African descent in the United States and England continue to have about double the rate of fatal disease compared to other men. Studies suggest that when there is equal access to care, survival disparities are greatly diminished. However, notable differences exist in prostate tumor biology across population groups. Ancestral factors and disparate exposures can lead to altered tumor biology, resulting in a distinct disease etiology by population group. While equal care remains the key target to improve survival, additional efforts should be made to gain comprehensive knowledge of the tumor biology in prostate cancer patients of African descent. Such an approach may identify novel intervention strategies in the era of precision medicine. A growing body of evidence shows that inflammation and the immune response may play a distinct role in prostate cancer disparities. Low-grade chronic inflammation and an inflammatory tumor microenvironment are more prevalent in African American patients and have been associated with adverse outcomes. Thus, differences in activation of immune–inflammatory pathways between African American and European American men with prostate cancer may exist. These differences may influence the response to immune therapy which is consistent with recent observations. This review will discuss mechanisms by which inflammation may contribute to the disparate outcomes experienced by African American men with prostate cancer and how these immunogenic and inflammatory vulnerabilities could be exploited to improve their survival.

Keywords: prostate cancer; African American; inflammation; health disparity

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Although cancer death rates have declined in the United States and other countries [1], disparities in cancer risk and outcomes persist, disproportionately affecting the systematically underserved and race/ethnic minoritized populations [2,3]. Prostate cancer is a key example of this with men of African descent in the United States and England continuing to have 2–3 times higher rates of fatal disease than other men [2]. Studies suggest that when there is equal access to care, survival disparities in prostate cancer are greatly diminished [4,5]. However, these investigations do not explain the notable differences in prostate cancer incidence, nor did they consider the now well-known differences in

prostate cancer incidence, nor did they consider the now well-known differences in tumor biology across population groups. As shown recently, ancestral factors and disparate exposures may lead to distinct tumor biology in prostate cancer patients, resulting in a population-specific disease etiology [6–9]. While equal care remains the key target to improve survival, additional efforts should be made to gain comprehensive knowledge of the tumor biology in prostate cancer patients of African descent. Such an approach may identify novel intervention strategies for high risk groups in the era of precision medicine.

A growing body of evidence supports the hypothesis that inflammation plays a fundamental role in prostate cancer disparities. Key differences in activation of immune–inflammatory pathways between African American and European American men with prostate cancer are emerging and these biological processes may influence how African American men respond to therapy, as suggested by recent findings from clinical trials with the cancer vaccine, Sipuleucel T [10]. It is the aim of this review to discuss the candidate role of inflammation and the immune response as contributing factors to the excessive burden of lethal prostate cancer among men of African ancestry (Figure 1). Furthermore, we will introduce the concept that these immunogenic vulnerabilities could be exploited to address the adverse outcomes experienced by the high-risk African American population.

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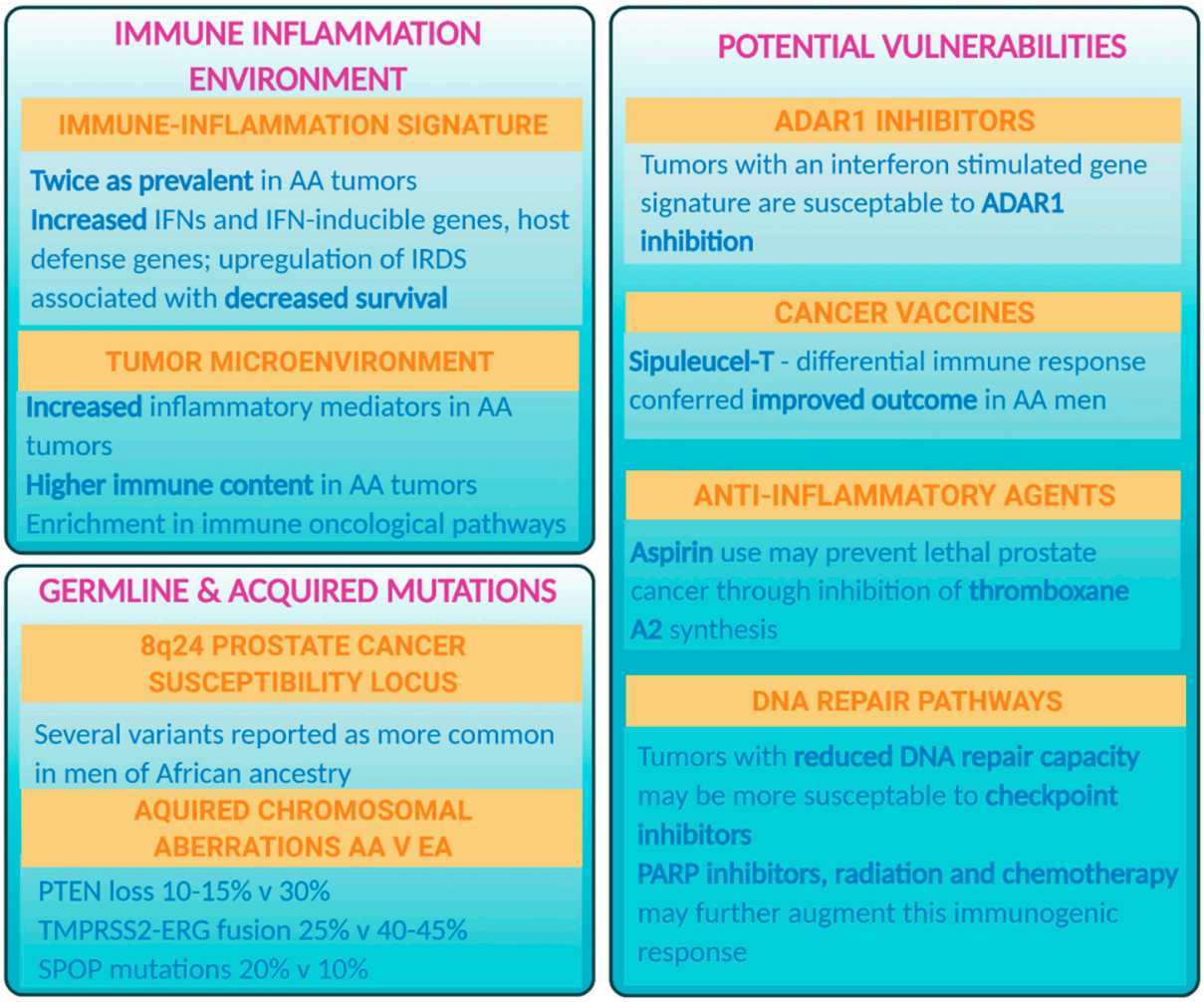


Figure 1. Key differences in the tumor immune–inflammation environment and the mutational spectrum between African American and European American men with prostate cancer. These differences open up potential vulnerabilities which preliminary studies have indicated could be exploited with treatment options, with some of them having demonstrated favorable responses in African American men.

1. The Mutational and Immune–Oncologic Landscape of Prostate Tumors Differs between Populations

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Prostate cancer displays large geographical differences in occurrence, with low incidence rates in East Asia and high rates in Western countries. Recognized risk factors for the disease include age, family history of the disease, race/ethnicity, and germline genetics [11–14]. It has been assumed that modifiable risk factors such as diet and lifestyle account for the majority of prostate cancers globally [15]. There is strong evidence from migration studies that the environment modulates prostate cancer risk [16,17]. Yet, there are few environmental factors that have consistently been linked to prostate cancer [11,18]. Notably, while prostate cancer is the leading cause of cancer death among men in many countries globally, sub-Saharan Africa and the Caribbean have more than double the age-standardized rates of mortality compared to other regions of the world, including North America and Europe [3,19]. This observation led to the hypothesis that ancestral factors may predispose men of sub-Saharan African ancestry to prostate cancer and a more aggressive disease [3,19]. Recent observations revealing the association of genetic ancestral factors with prostate cancer risk support this hypothesis [20–24]. Moreover, men of African ancestry are at an increased risk of developing fatal prostate cancer in the United States and England [2] and present with more aggressive disease in the Caribbean and sub-Saharan Africa [3,25]. The causes of the observed global prostate cancer health disparities are still being investigated but certainly include delayed diagnosis and lack of access to health care, ancestral, lifestyle, and environmental risk factors, and likely tumor biological differences [21,26,27].

Prostate cancer is a heterogeneous disease, in which inherited factors may account for about 40 to 50% of the cases [28]. Several familial susceptibility genes have been described, including *RNASEL*, *BRCA1*, *BRCA2*, and *HOXB13* [28–32]. *RNASEL*, or ribonuclease L, encodes a component of the interferon-regulated 2–5A system that functions in the antiviral roles of interferons [33], suggesting the importance of immune function in prostate cancer susceptibility. Most of the inherited risk for prostate cancer arises from common genetic variants [14]. More than 200 disease susceptibility loci are now known [24], but not all of them confer risk in men of African ancestry [34]. Numerous studies have examined the possibility of low penetrance genes contributing to the excessive burden of prostate cancer in African American men. To date, the best characterized risk locus for prostate cancer is located at 8q24. Multiple common variants within this locus increase the risk of prostate cancer in many populations [13,35–38]. As shown by several studies, this locus confers an even higher risk for prostate cancer in men of West African ancestry, when compared with men of European and East Asian ancestry, partly explained by variants that were only found in men of African ancestry [13,20,34,35,39,40]. Thus, the 8q24 region accounts for some of the excessive disease risk among men of African ancestry.

Prostate cancer can be classified into genomic subtypes, such as those with ETS-fusion gene arrangements and other subtypes that are negative for ETS-fusion gene arrangements, and either overexpress the *SPINK1* oncogene or carry *SPOP*, *FOXA1*, or *IDH1* mutations, or represent a triple-negative subtype (negative for *ERG*- and other *ETS*-fusions and *SPINK1*-negative) [6,41]. Early-stage prostate cancer contains few recurrent mutations in cancer-related genes (e.g., *ETS* gene fusions) [42,43]. Instead, prostate tumors are characterized by allelic gains of the *MYC* gene and deletions of the *NKX3–1*, *PTEN*, *Rb*, and *TP53* tumor suppressors [44]. Yet, there is strong evidence of prominent population differences in the acquisition of genetic alterations for prostate cancer. Reports showed that prostate tumors from patients of either European, African, or Asian descent exhibit notable differences in acquired chromosomal aberrations (e.g., *ERG* fusion and *PTEN* loss) and subtype distribution [6–9], indicating disparities in disease etiology and mutational events among these population groups. Comparing African American with European American patients [6,45–47], significant differences were observed in the frequency of *TMPRSS2–ERG* fusions (about 25% African American vs. 40–45% European American), *SPOP* mutations (about 20% African American vs. 10% European American), and *PTEN* deletions (about

10–15% African American vs. 30% European American). Chinese prostate cancer patients acquire mutations in *FOXA1* at a high frequency (about 40%), as shown by a recent report [9]. This gene is infrequently mutated in European-ancestry populations (<10%).

Chronic inflammation has been described as a prostate cancer risk factor that is associated with aggressive disease [48,49]. We found that aspirin use significantly reduces the risk of advanced prostate cancer in African American men [50]. Yet, no study has assessed whether these men commonly develop a systemic inflammatory process that increases the risk of prostate cancer progression and mortality. While environmental exposures, such as infections, promote systemic inflammation, ancestral factors may also influence inflammatory processes and the response to infections [51,52].

2. Inflammation as a Possible Driver of Aggressive Prostate Cancer in African American Men

The immune–inflammation signature that was initially described by Wallace et al. to be prevalent in prostate tumors of African American patients is central to the hypothesis that inflammation is a candidate driver of prostate cancer disparities [53]. Subsequently observed by others [54] and validated in TCGA [55], this signature includes upregulation of genes in the interferon (IFN) signaling pathway and contains elements of a viral mimicry signature. Further investigations of this signature in prostate tumors from African American men describe a signature which corresponds to a previously described "interferon-related DNA damage resistance signature", also termed IRDS [56,57]. Detection of IRDS is a marker of decreased disease-free survival in prostate cancer and has been linked to acquired resistance to radiation and chemotherapy in breast cancer. Thus, upregulation of this signature in African American tumors indicates a mechanism by which either inflammatory ancestral factors or a yet unknown infectious agent may contribute adversely to prostate cancer outcomes. Even though the presence of IRDS in a tumor may indicate an adverse outcome, this signature may also constitute a vulnerability. Tumors with an interferon-stimulated gene signature were reported to be highly susceptible to inhibition of adenosine deaminase acting on RNA (ADAR1) [58,59].

Despite the fact that we know it occurs about twice as often in African American prostate tumors when compared to European American tumors [56], the precise origin of this immune inflammation signature remains unknown. However, presence of the signature is associated with an interferon-λ4 ΔG genotype [56]. This genotype is responsible for production of the interferon lambda 4 protein (IFNL4) and is most common in people of West African ancestry and influences host viral response [56,60]. In this context, the signature may have origins in either infection history [61], pro-inflammatory diets [62], changes to the epigenome [63], or reactivation of endogenous retroviral sequences which have been reported in African American prostate cancer patients [64].

Multiple studies reported upregulation of inflammatory mediators in the tumor microenvironment (TME) of African American prostate cancer patients, many of which have implications for disease prognosis [65–69]. Gillard et al. investigated the role of the stroma in prostate cancer disparities by isolating prostate fibroblasts from the TME of African American and European American men and culturing prostate cancer cell lines in conditioned fibroblast media [65]. They found enhanced expression of proinflammatory mediators including TrKB, BDNF, VEGF, and IL6 by tumor cells when the conditioned media was obtained from fibroblasts of African American origin as compared to European origin. This implicates the stromal environment in African American men as a potential driver of prostate cancer progression through elevation of inflammatory mediators. Weiner et al. report higher immune content in the TME of prostate tumors from African American men compared to European American men with the proportion of plasma cells contributing the greatest difference in quantity across three independent cohorts [68]. These high intra tumoral counts of plasma cells were further associated with increased metastasis-free survival in both a Johns Hopkins Medical Institute and the TCGA cohort, implicating plasma cells as candidate regulators of the immune responsiveness in African American men with prostate cancer. High plasma cell levels correlated with increased IgG expression

and IFN signaling, and B cell and natural killer (NK) cell activity in tumors of these patients, showing a possible link between high plasma cells and increased immune activity. High IgG expression and NK cell activity also showed clinical significance as they were associated with increased metastasis-free survival. Our group previously detected a B cell signature in prostate tumors from current smokers, but smoking is thought to increase the risk of metastasis [70]. Collectively, these findings suggest a regulatory network between intratumor immune cells, inflammatory cytokines, and cells in the TME. Such a network, if clearly defined, may have potential as a biomarker of responsiveness to immunotherapy and targets to improve outcomes among African American patients.

In support of these findings, Awasthi and colleagues reported distinct changes in immune pathways including overall higher immune cell content, enrichment of immune oncological pathways, and lower DNA damage repair in prostate tumors of African American men compared to European American men [66]. After exploring discovery and validation cohorts of immune-related genes, the authors focused on 38 genes that were differentially expressed between the two population groups. Of these genes, 26 with the most robust gene expression differences were identified as being consistently associated with major immune biological pathways, including IFN signaling and cytokine signaling based on discovery and validation with two separate pathway analysis tools. As a standout, the proinflammatory gene *IFITM3* (IFN inducible transmembrane protein 3) was the only gene overexpressed in African American prostate tumors that predicted increased risk of biochemical recurrence only for African American men with prostate cancer, but not European American men.

The cause of this elevated immune–inflammation response is still under investigation. Numerous studies have shown that population differences in genetic ancestry can contribute to population differences in cancer susceptibility through processes that may involve inflammation. Genetic ancestry and natural selection are known to contribute to population differences in immune response to pathogens [52,71]. Furthermore, relationships of ancestry with expression levels of inflammatory cytokines are well documented in human populations [72,73]. As a modifiable risk factor, a pro-inflammatory diet that associates with high-grade prostate cancer is more commonly consumed by African American than European American men [62] and may lead to systemic inflammation. Other inducers of systemic inflammation may include stress exposures. Stress signaling transduces its biological effects through hypersecretion of the corticotrophin-releasing hormone and activation of the peripheral autonomic and sympathetic nervous system, which has direct effects on tumor biology and immune response, promoting inflammation, angiogenesis, mesenchymal differentiation, and metastasis [74]. As a final example, co-morbidities including chronic infections and diabetes can be excessively high in African American men [75–77]. They are frequently associated with increased inflammatory processes which could contribute to cancer development. This suggests that there could be a role for both biological and environmental factors in the elevated immune–inflammation pathways that are reported in the prostate tumors from men of African descent, as previously discussed [78].

3. African American Men May Have a Differential Response to Certain Therapies for Metastatic Prostate Cancer

The peer-reviewed literature now provides some evidence that men of African descent may respond differently across the gamut of both standardized and emerging options of care for prostate cancer, including radiation, hormone therapy, chemotherapy, and immunotherapy. Differences in immune response may play a key role in many of these observations. Metastatic castration-resistant prostate cancer (mCRPC) is a main cause of lethal prostate cancer and therefore remains a key focus for research. Despite patients with mCRPC having multiple treatment options targeting a variety of mechanisms (Figure 2), median overall survival is still only around 3 years [79]. This further highlights the need for inclusion of diverse biospecimens in scientific studies and historically understudied populations in clinical trials to determine who is benefitting optimally from these currently approved treatments.

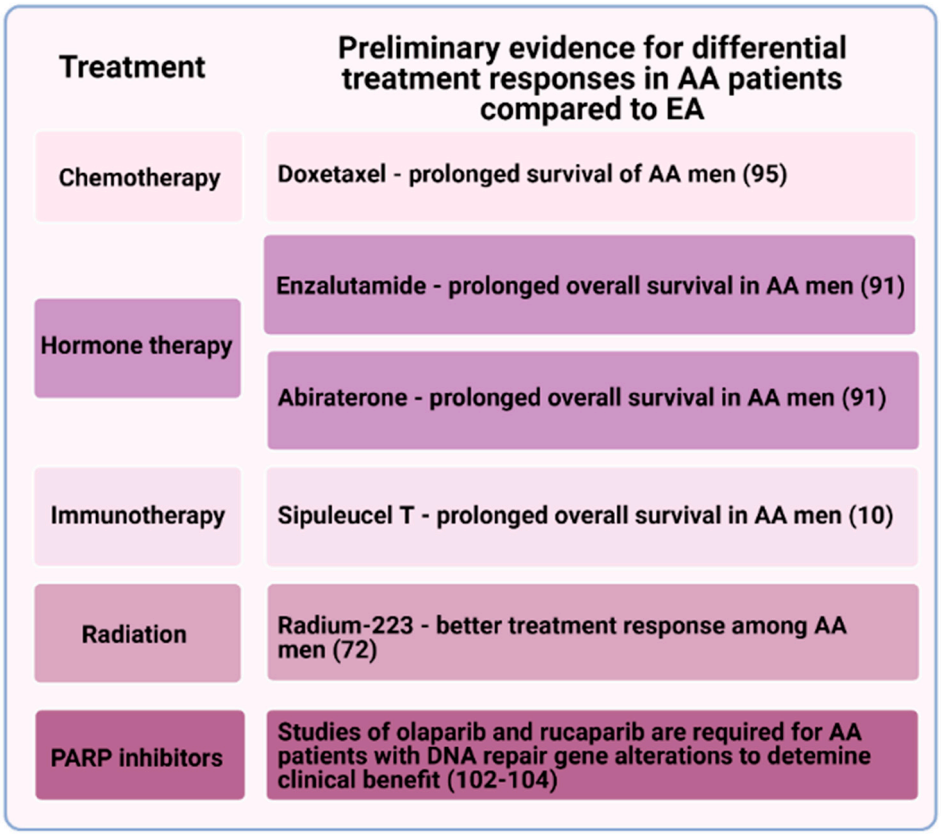


Figure 2. Preliminary evidence for certain treatment response differences between African American (AA) and European American (EA) patients with metastatic castration-resistant prostate cancer (mCRPC). Current approved treatment modalities for mCRPC, how clinical responses may differ between the two patient groups, and where additional studies are warranted. For PARP inhibitor use, the pathologic role of germline variants of unknown significance in DNA repair genes that commonly occur in AA men needs to be investigated.

4. Radiation

Radium-223 is an approved therapeutic option for mCRPC patients with symptomatic bone metastases. Zhao et al. examined the response to radium-223 treatment in men from a Veteran Affairs cohort with mCRPC [80]. With equal access to care across the cohort, this group found that African American men may have a better response to this treatment compared to European American men, resulting in a 25%–35% decreased risk of mortality in this equal access to care study. African American men in this study were more likely to have received docetaxel beforehand and the improved response to therapy was despite the African American cases being more likely to not start radium treatment until further along in the disease course. Patients harboring DNA damage repair mutations have prolonged overall survival after radium-223 treatment compared to patients who do not have these alterations [81–83]. This is also the subject of another clinical trial currently in the recruitment stage (NCT04489719). With Awasthi et al. reporting decreased DNA damage repair capacity in prostate tumors of African American men, it can be speculated that inactivating mutations that decrease the DNA damage repair capacity in tumors from African American men may contribute to the positive outcomes post treatment with radium-223 [66].

A recent, small, phase II trial (NCT02463799) found combining radium-223 treatment with Sipuleucel-T increased progression-free survival and overall survival in men with mCRPC [84]. Now that studies have shown better responses from African American men treated with radium-223 and Sipuleucel-T separately [10,80], a planned larger trial may

may inform on whether African American men may benefit synergistically from this combination approach.

5. Immunotherapy

Immunotherapy has not been as successful in treating prostate cancer as with other hematologic or solid cancers and clinical trials show a modest [85] to no effect [79,86,87] on survival. This has been attributed to prostate cancer not being as immunogenic as other cancers. However, recent studies indicate a potential role for immunotherapy in certain patient groups with prostate cancer. Precision medicine strategies targeting immunotherapy to those men with the best response is the preferred goal. Evidence is currently being built to support the hypothesis that African American men may have a differential and perhaps superior response to certain treatments due to changes in immune cell response and a differing tumor biology.

Tumors from men of African descent may have a heightened response to immunotherapies, and specifically to cancer vaccines, as assumed from the presence of an interferon signature in their tumors and increased immune content in the TME [56,66]. Studies have shown that young people who self-report as African American mounted an increased immune response to vaccination [88,89]. Sartor et al. recently reported that African American men with mCRPC who were treated with the cancer vaccine, Sipuleucel-T, in the PROCEED trial/registry, had significantly better survival than the European American patients [10]. Median overall survival was 35.3 months for African American men compared to 25.8 months for European American men, in a PSA-matched set. This difference became even greater when measured in patients with a baseline PSA below the median, with median overall survival of 54.3 months in African American men versus 33.4 months in European American men. Increased activation of dendritic cells is a proposed mechanism of action of the vaccine and in agreement with this, activated dendritic cells in localized tumors have subsequently been associated with improved distant metastasis free survival [90]. Mechanistically, evidence points towards a complex interplay of immune cells with tumor biology which may predict prognosis and response to therapy. However, the lack of tumor specimens from African American men means that more work must be done to capitalize on the differences in the immune landscape which may improve response to treatment in this population.

Generally, poor immunogenicity has resulted in little success for PD-L1 blockade in treatment of prostate cancer [87,91]. This has been attributed in part to relatively low PD-L1 expression from tumor cells [92,93]. However, this is not consistent across the literature, with studies also reporting increased PD-L1 expression and association with biochemical recurrence [94] and shorter metastasis free survival [95]. Petitprez et al. provide preliminary evidence that a composite assessment of both PD-L1 and CD8 expression in localized prostate cancer may be a good strategy for predicting outcomes in mCRPC [95]. A group in Norway reported high PD-L1 expression in post-prostatectomy, hormone-naïve tumor epithelial cells with a non-significant trend towards an inverse association between PD-L1 expression and biochemical failure-free survival [96]. However, clinical trials investigating the effect of PD-L1 inhibition reported no significant clinical benefit. Yet, they have typically not included men of African descent [87].

Recent work has focused on PD-L1 expression on tumor-infiltrating immune cells. Bishop et al. reported enzalutamide-resistant prostate cancer patients showing increased PD-L1 expression on dendritic cells and high PD-L1 T cells when compared to enzalutamide-sensitive or treatment-naïve patients [97]. African American ethnicity and an aggressive cancer phenotype have been associated with prediction of tumor PD-L1 positivity in hormone-naïve tumors [98], suggesting a potential benefit for immunotherapy in African Americans at high risk of aggressive disease, but this has not been replicated yet [66]. When tumors are enzalutamide-sensitive, McNamara et al. preliminarily reported increased overall survival for African American, chemotherapy-naïve men with mCRPC treated with abiraterone or enzalutamide compared to European American men [99]. Overall survival

was 918 days for African Americans compared to 781 days for European Americans. This study in a Veteran Affairs population was retrospective in design, again pointing to the value of equal access to care across populations. Thus, additional work is warranted, including measurement of PD-L1 in tumor samples from African American men post various treatment regimens to account for increased immunogenic response to therapy.

6. Other Treatment Opportunities

Historically, participation of African American men in clinical trials has been low. Reasons for this are multifactorial but include historical mistrust of the medical profession as a result of systemic racism and major ethical breaches in the past [100]. A higher prevalence of comorbidities and a lack of access to academic medical centers involved in trials may also prevent access to trials [100–102]. This prevents generalizability across population groups when reporting clinical trial data. A recent example highlights the need to include diverse population groups and possibly stratify clinical trial participants by race to get a fuller picture of treatment response. Halabi et al. completed a meta-analysis of survival outcomes for African American versus European American men in phase III clinical trials treating mCRPC with docetaxel [103]. With just 6% of African American participants, they reported that while overall median survival was similar, a pooled hazard ratio of 0.81 (95% CI, 0.72 to 0.91) post adjustment for baseline prognostic factors estimated that African American men may have a significantly decreased risk of death compared to European American men. This was despite African American men having baseline characteristics known to be prognostic of overall survival including statistically significantly worse performance status, higher testosterone levels, higher PSA levels, and lower hemoglobin levels.

It is assumed by many that the prostate cancer biology in men of African ancestry is intrinsically more aggressive—at least for a subset of patients—leading to a survival health disparity in the population [104]. Yet, this does not mean that African American men would not respond as well as European American men to most standard therapies. In fact, it appears that the treatment responses of African American and European American men are mostly similar. Yet, tumors in African American men could still respond better to certain therapies compared to the average response among European American men. Some of the treatments or combination treatments discussed in this review were the subject of small clinical trials and so these therapeutic options might not be widely offered yet in the clinic. Therefore, the findings require further evaluation in larger studies but do suggest that there is a potential role for these treatments in reducing the survival disparities observed in prostate cancer. African American men are less likely to be recruited into clinical trials and may not have the opportunities to avail of these new therapeutic options.

7. Germline and Somatic Mutations in DNA Repair Pathways

A proposed feature of prostate tumors in African American men that may play a prominent role in differential response to treatment is a deficiency in DNA damage repair capacity. Both germline and somatic alterations to DNA damage repair pathways have now been found in prostate tumors across multiple studies [66,105–107]. Tumors from African American men were reported to have a significantly lower level of DNA repair capacity when compared to those from European American men. Notably, these tumors seemed to have an increased radiosensitivity [66].

Germline mutations in DNA repair genes have a higher occurrence in metastatic prostate cancer when compared to localized prostate cancer [105,106]. BRCA1/2 pathogenic variants have been associated with more aggressive prostate cancer and adverse survival outcomes [108,109]. DNA repair gene mutations may contribute to aggressive disease in African American men. Acquired somatic mutations may differ among patient groups, with Yadav et al. reporting that prostate tumors from African American men were twice as likely to have at least one mutation in nucleotide excision repair pathway genes compared to European American (89% vs. > 40%) [107]. Petrovics and colleagues reported that germline

variants in DNA repair genes of unknown significance had an increased frequency in African American men (4.6%) compared to European American men (1.6%) [110]. As the significance of these is undetermined, there is an opportunity to investigate whether they play a pathogenic role in prostate cancer. The same authors also reported that just 0.7% of men with localized prostate cancer carried pathogenic variants of BRCA1/2 mutations, but this increased over 4-fold to 3.1% in patients with metastatic and advanced disease, indicating that the presence of known BRCA1/2 pathogenic variants is linked to disease status [110]. Because the FDA-approved PARP inhibitors, olaparib and rucaparib, have shown success in prolonging overall survival in mCRPC patients with mutations in these DNA damage response genes [111–113], they should be made available to all African American men with prostate cancer who carry these mutations.

Altered DNA damage repair pathways may sensitize tumors to immunotherapeutic approaches. Several clinical trials across many cancer sites including metastatic prostate cancer are currently underway, targeting DNA damage repair-deficient tumors with checkpoint inhibitors (extensively reviewed by Bever et al.) [114]. Mechanistically, in prostate cancer, the stimulator of the IFN genes (STING) pathway has been linked to the recruitment and activation of interferon-related genes in vitro, increasing sensitivity to the immune checkpoint inhibitor PD-L1 in DNA repair-deficient tumors [115–118]. As a low DNA repair capacity may increase tumor genomic instability and tumor mutational burden, this again might constitute a vulnerability to immunotherapeutic strategies [119]. It has been suggested that enhancement of this genomic instability through use of radiation, chemotherapy, or PARP inhibitors could augment the immunotherapeutic response [114,120,121].

8. Anti-Inflammatory Drug Aspirin for Prevention of Adverse Outcomes in African American Men with Prostate Cancer

While the research community plays catch-up with ensuring proper representation of population groups in clinical trials and precision medicine studies, strategies for the prevention of lethal prostate cancer and reduction in adverse outcomes are of paramount importance to men of African descent who continue to experience a disproportionally high mortality from prostate cancer. An extensive body of evidence including both preclinical and clinical studies led the United States Preventative Services Task Force to recommend the anti-inflammatory drug aspirin for prevention of colorectal cancer for an albeit narrow category of adults [122–124]. In keeping with the hypothesis that inflammation is one of the drivers of the prostate cancer disparities, our group explored the link between regular use of aspirin and prostate cancer in African American men. We found that regular aspirin use significantly reduces the risk of both advanced prostate cancer and disease recurrence in these men [50]. The finding is consistent with a similar observation in a previous study [125]. Inhibition of the pro-inflammatory cyclooxygenase/thromboxane A2 pathway has been identified as a potential mechanism of action for aspirin in the prevention of metastatic cancer [126]. Using a retrospective cohort, we found a distinct association between high urinary 11-dehydrothromboxane B2 (the stable metabolite of thromboxane A2) and aggressive prostate cancer as well as adverse survival outcomes for African American men. Importantly, our ongoing research showed high 11-dehydrothromboxane B2 was inversely correlated with aspirin use, indicating a potential benefit of aspirin in preventing lethal prostate cancer through inhibition of TXA2 synthesis.

Lastly, data prospectively obtained in the Southern Community Cohort Study suggested that aspirin use is tentatively associated with a reduced prostate cancer mortality in African American men [127]. Hurwitz et al. also observed this inverse relationship between aspirin use and prostate cancer mortality in both African American and European American men using the ARIC cohort [128], again pointing to the potential benefit of aspirin use for men at high risk of fatal prostate cancer.

9. Conclusions

Elevated inflammatory processes in African American men with prostate cancer are a candidate biological driver of disparate disease risks. There is a need for more clinical trials

specifically focused on the treatment response of African American men with metastatic prostate cancer [129]. Improved inclusion of minority populations in trials is essential to further enhance our knowledge of how inflammation and the immune response and alterations to molecular pathways may govern the response to emerging therapies across all patient groups. With evidence now building that suggests increased clinical benefit with certain therapies among African American men when compared to European American men, targeting inflammatory processes and the immune system could be an important strategy to reduce lethal disease in high-risk populations such as men of African ancestry.

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Review

Immune Inflammation Pathways as Therapeutic Targets to Reduce Lethal Prostate Cancer in African American Men

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Simple Summary: Men of African descent are twice as likely to die of prostate cancer than other men. While equal access to care is the key target to improve cancer survival, it is now known that there are differences in disease biology and risk factor exposure across population groups. These differences could be causatively linked to the existing prostate cancer health disparities. In this review, we will discuss the candidate role of inflammation and the immune response as contributing factors to the excessive burden of lethal prostate cancer among men of African ancestry. Furthermore, we will introduce the concept that these immunogenic vulnerabilities could be exploited to address the adverse outcomes experienced by these men. Lastly, we will summarize how these immunogenic and inflammatory differences could be targeted using current treatments to improve survival for men of African descent.

Abstract: Despite substantial improvements in cancer survival, not all population groups have benefitted equally from this progress. For prostate cancer, men of African descent in the United States and England continue to have about double the rate of fatal disease compared to other men. Studies suggest that when there is equal access to care, survival disparities are greatly diminished. However, notable differences exist in prostate tumor biology across population groups. Ancestral factors and disparate exposures can lead to altered tumor biology, resulting in a distinct disease etiology by population group. While equal care remains the key target to improve survival, additional efforts should be made to gain comprehensive knowledge of the tumor biology in prostate cancer patients of African descent. Such an approach may identify novel intervention strategies in the era of precision medicine. A growing body of evidence shows that inflammation and the immune response may play a distinct role in prostate cancer disparities. Low-grade chronic inflammation and an inflammatory tumor microenvironment are more prevalent in African American patients and have been associated with adverse outcomes. Thus, differences in activation of immune–inflammatory pathways between African American and European American men with prostate cancer may exist. These differences may influence the response to immune therapy which is consistent with recent observations. This review will discuss mechanisms by which inflammation may contribute to the disparate outcomes experienced by African American men with prostate cancer and how these immunogenic and inflammatory vulnerabilities could be exploited to improve their survival.

Keywords: prostate cancer; African American; inflammation; health disparity

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Although cancer death rates have declined in the United States and other countries [1], disparities in cancer risk and outcomes persist, disproportionately affecting the systematically underserved and race/ethnic minoritized populations [2,3]. Prostate cancer is a key example of this with men of African descent in the United States and England continuing to have 2–3 times higher rates of fatal disease than other men [2]. Studies suggest that when there is equal access to care, survival disparities in prostate cancer are greatly diminished [4,5]. However, these investigations do not explain the notable differences in prostate cancer incidence, nor did they consider the now well-known differences in

prostate cancer incidence, nor did they consider the now well-known differences in tumor biology across population groups. As shown recently, ancestral factors and disparate exposures may lead to distinct tumor biology in prostate cancer patients, resulting in a population-specific disease etiology [6–9]. While equal care remains the key target to improve survival, additional efforts should be made to gain comprehensive knowledge of the tumor biology in prostate cancer patients of African descent. Such an approach may identify novel intervention strategies for high risk groups in the era of precision medicine.

A growing body of evidence supports the hypothesis that inflammation plays a fundamental role in prostate cancer disparities. Key differences in activation of immune–inflammatory pathways between African American and European American men with prostate cancer are emerging and these biological processes may influence how African American men respond to therapy, as suggested by recent findings from clinical trials with the cancer vaccine, Sipuleucel T [10]. It is the aim of this review to discuss the candidate role of inflammation and the immune response as contributing factors to the excessive burden of lethal prostate cancer among men of African ancestry (Figure 1). Furthermore, we will introduce the concept that these immunogenic vulnerabilities could be exploited to address the adverse outcomes experienced by the high-risk African American population.

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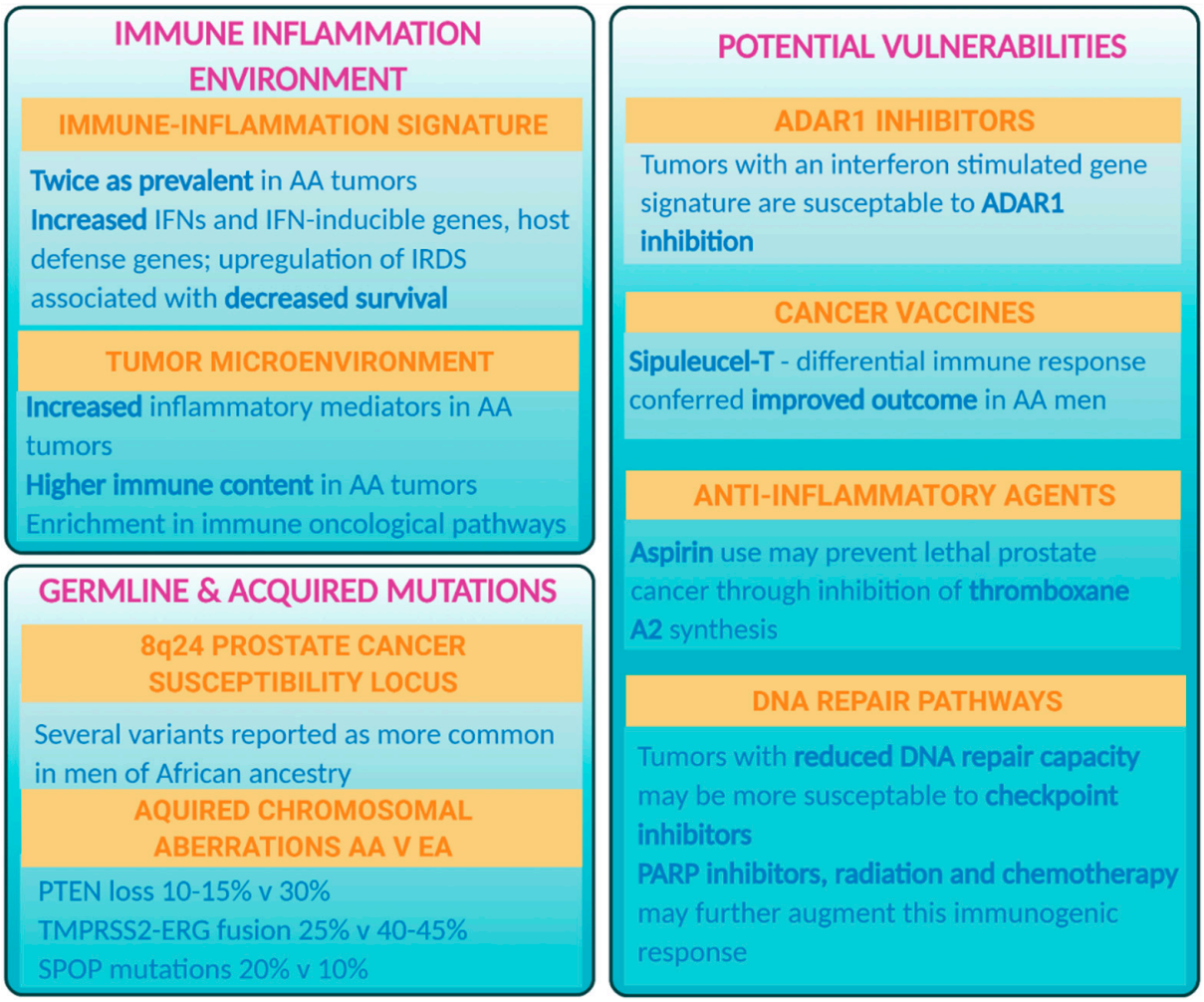


Figure 1. Key differences in the tumor immune–inflammation environment and the mutational spectrum between African American and European American men with prostate cancer. These differences open up potential vulnerabilities which preliminary studies have indicated could be exploited with treatment options, with some of them having demonstrated favorable responses in African American men.

1. The Mutational and Immune–Oncologic Landscape of Prostate Tumors Differs between Populations

Prostate cancer displays large geographical differences in occurrence, with low incidence rates in East Asia and high rates in Western countries. Recognized risk factors for

1. The Mutational and Immune–Oncologic Landscape of Prostate Tumors Differs between Populations

Prostate cancer displays large geographical differences in occurrence, with low incidence rates in East Asia and high rates in Western countries. Recognized risk factors for the disease include age, family history of the disease, race/ethnicity, and germline genetics [11–14]. It has been assumed that modifiable risk factors such as diet and lifestyle account for the majority of prostate cancers globally [15]. There is strong evidence from migration studies that the environment modulates prostate cancer risk [16,17]. Yet, there are few environmental factors that have consistently been linked to prostate cancer [11,18]. Notably, while prostate cancer is the leading cause of cancer death among men in many countries globally, sub-Saharan Africa and the Caribbean have more than double the age-standardized rates of mortality compared to other regions of the world, including North America and Europe [3,19]. This observation led to the hypothesis that ancestral factors may predispose men of sub-Saharan African ancestry to prostate cancer and a more aggressive disease [3,19]. Recent observations revealing the association of genetic ancestral factors with prostate cancer risk support this hypothesis [20–24]. Moreover, men of African ancestry are at an increased risk of developing fatal prostate cancer in the United States and England [2] and present with more aggressive disease in the Caribbean and sub-Saharan Africa [3,25]. The causes of the observed global prostate cancer health disparities are still being investigated but certainly include delayed diagnosis and lack of access to health care, ancestral, lifestyle, and environmental risk factors, and likely tumor biological differences [21,26,27].

Prostate cancer is a heterogeneous disease, in which inherited factors may account for about 40 to 50% of the cases [28]. Several familial susceptibility genes have been described, including *RNASEL*, *BRCA1*, *BRCA2*, and *HOXB13* [28–32]. *RNASEL*, or ribonuclease L, encodes a component of the interferon-regulated 2–5A system that functions in the antiviral roles of interferons [33], suggesting the importance of immune function in prostate cancer susceptibility. Most of the inherited risk for prostate cancer arises from common genetic variants [14]. More than 200 disease susceptibility loci are now known [24], but not all of them confer risk in men of African ancestry [34]. Numerous studies have examined the possibility of low penetrance genes contributing to the excessive burden of prostate cancer in African American men. To date, the best characterized risk locus for prostate cancer is located at 8q24. Multiple common variants within this locus increase the risk of prostate cancer in many populations [13,35–38]. As shown by several studies, this locus confers an even higher risk for prostate cancer in men of West African ancestry, when compared with men of European and East Asian ancestry, partly explained by variants that were only found in men of African ancestry [13,20,34,35,39,40]. Thus, the 8q24 region accounts for some of the excessive disease risk among men of African ancestry.

Prostate cancer can be classified into genomic subtypes, such as those with ETS-fusion gene arrangements and other subtypes that are negative for ETS-fusion gene arrangements, and either overexpress the *SPINK1* oncogene or carry *SPOP*, *FOXA1*, or *IDH1* mutations, or represent a triple-negative subtype (negative for *ERG*- and other *ETS*-fusions and *SPINK1*-negative) [6,41]. Early-stage prostate cancer contains few recurrent mutations in cancer-related genes (e.g., *ETS* gene fusions) [42,43]. Instead, prostate tumors are characterized by allelic gains of the *MYC* gene and deletions of the *NKX3–1*, *PTEN*, *Rb*, and *TP53* tumor suppressors [44]. Yet, there is strong evidence of prominent population differences in the acquisition of genetic alterations for prostate cancer. Reports showed that prostate tumors from patients of either European, African, or Asian descent exhibit notable differences in acquired chromosomal aberrations (e.g., *ERG* fusion and *PTEN* loss) and subtype distribution [6–9], indicating disparities in disease etiology and mutational events among these population groups. Comparing African American with European American patients [6,45–47], significant differences were observed in the frequency of *TMPRSS2–ERG* fusions (about 25% African American vs. 40–45% European American), *SPOP* mutations (about 20% African American vs. 10% European American), and *PTEN* deletions (about

10–15% African American vs. 30% European American). Chinese prostate cancer patients acquire mutations in *FOXA1* at a high frequency (about 40%), as shown by a recent report [9]. This gene is infrequently mutated in European-ancestry populations (<10%).

Chronic inflammation has been described as a prostate cancer risk factor that is associated with aggressive disease [48,49]. We found that aspirin use significantly reduces the risk of advanced prostate cancer in African American men [50]. Yet, no study has assessed whether these men commonly develop a systemic inflammatory process that increases the risk of prostate cancer progression and mortality. While environmental exposures, such as infections, promote systemic inflammation, ancestral factors may also influence inflammatory processes and the response to infections [51,52].

2. Inflammation as a Possible Driver of Aggressive Prostate Cancer in African American Men

The immune–inflammation signature that was initially described by Wallace et al. to be prevalent in prostate tumors of African American patients is central to the hypothesis that inflammation is a candidate driver of prostate cancer disparities [53]. Subsequently observed by others [54] and validated in TCGA [55], this signature includes upregulation of genes in the interferon (IFN) signaling pathway and contains elements of a viral mimicry signature. Further investigations of this signature in prostate tumors from African American men describe a signature which corresponds to a previously described "interferon-related DNA damage resistance signature", also termed IRDS [56,57]. Detection of IRDS is a marker of decreased disease-free survival in prostate cancer and has been linked to acquired resistance to radiation and chemotherapy in breast cancer. Thus, upregulation of this signature in African American tumors indicates a mechanism by which either inflammatory ancestral factors or a yet unknown infectious agent may contribute adversely to prostate cancer outcomes. Even though the presence of IRDS in a tumor may indicate an adverse outcome, this signature may also constitute a vulnerability. Tumors with an interferon-stimulated gene signature were reported to be highly susceptible to inhibition of adenosine deaminase acting on RNA (ADAR1) [58,59].

Despite the fact that we know it occurs about twice as often in African American prostate tumors when compared to European American tumors [56], the precise origin of this immune inflammation signature remains unknown. However, presence of the signature is associated with an interferon-λ4 ΔG genotype [56]. This genotype is responsible for production of the interferon lambda 4 protein (IFNL4) and is most common in people of West African ancestry and influences host viral response [56,60]. In this context, the signature may have origins in either infection history [61], pro-inflammatory diets [62], changes to the epigenome [63], or reactivation of endogenous retroviral sequences which have been reported in African American prostate cancer patients [64].

Multiple studies reported upregulation of inflammatory mediators in the tumor microenvironment (TME) of African American prostate cancer patients, many of which have implications for disease prognosis [65–69]. Gillard et al. investigated the role of the stroma in prostate cancer disparities by isolating prostate fibroblasts from the TME of African American and European American men and culturing prostate cancer cell lines in conditioned fibroblast media [65]. They found enhanced expression of proinflammatory mediators including TrKB, BDNF, VEGF, and IL6 by tumor cells when the conditioned media was obtained from fibroblasts of African American origin as compared to European origin. This implicates the stromal environment in African American men as a potential driver of prostate cancer progression through elevation of inflammatory mediators. Weiner et al. report higher immune content in the TME of prostate tumors from African American men compared to European American men with the proportion of plasma cells contributing the greatest difference in quantity across three independent cohorts [68]. These high intra tumoral counts of plasma cells were further associated with increased metastasis-free survival in both a Johns Hopkins Medical Institute and the TCGA cohort, implicating plasma cells as candidate regulators of the immune responsiveness in African American men with prostate cancer. High plasma cell levels correlated with increased IgG expression

and IFN signaling, and B cell and natural killer (NK) cell activity in tumors of these patients, showing a possible link between high plasma cells and increased immune activity. High IgG expression and NK cell activity also showed clinical significance as they were associated with increased metastasis-free survival. Our group previously detected a B cell signature in prostate tumors from current smokers, but smoking is thought to increase the risk of metastasis [70]. Collectively, these findings suggest a regulatory network between intratumor immune cells, inflammatory cytokines, and cells in the TME. Such a network, if clearly defined, may have potential as a biomarker of responsiveness to immunotherapy and targets to improve outcomes among African American patients.

In support of these findings, Awasthi and colleagues reported distinct changes in immune pathways including overall higher immune cell content, enrichment of immune oncological pathways, and lower DNA damage repair in prostate tumors of African American men compared to European American men [66]. After exploring discovery and validation cohorts of immune-related genes, the authors focused on 38 genes that were differentially expressed between the two population groups. Of these genes, 26 with the most robust gene expression differences were identified as being consistently associated with major immune biological pathways, including IFN signaling and cytokine signaling based on discovery and validation with two separate pathway analysis tools. As a standout, the proinflammatory gene *IFITM3* (IFN inducible transmembrane protein 3) was the only gene overexpressed in African American prostate tumors that predicted increased risk of biochemical recurrence only for African American men with prostate cancer, but not European American men.

The cause of this elevated immune–inflammation response is still under investigation. Numerous studies have shown that population differences in genetic ancestry can contribute to population differences in cancer susceptibility through processes that may involve inflammation. Genetic ancestry and natural selection are known to contribute to population differences in immune response to pathogens [52,71]. Furthermore, relationships of ancestry with expression levels of inflammatory cytokines are well documented in human populations [72,73]. As a modifiable risk factor, a pro-inflammatory diet that associates with high-grade prostate cancer is more commonly consumed by African American than European American men [62] and may lead to systemic inflammation. Other inducers of systemic inflammation may include stress exposures. Stress signaling transduces its biological effects through hypersecretion of the corticotrophin-releasing hormone and activation of the peripheral autonomic and sympathetic nervous system, which has direct effects on tumor biology and immune response, promoting inflammation, angiogenesis, mesenchymal differentiation, and metastasis [74]. As a final example, co-morbidities including chronic infections and diabetes can be excessively high in African American men [75–77]. They are frequently associated with increased inflammatory processes which could contribute to cancer development. This suggests that there could be a role for both biological and environmental factors in the elevated immune–inflammation pathways that are reported in the prostate tumors from men of African descent, as previously discussed [78].

3. African American Men May Have a Differential Response to Certain Therapies for Metastatic Prostate Cancer

The peer-reviewed literature now provides some evidence that men of African descent may respond differently across the gamut of both standardized and emerging options of care for prostate cancer, including radiation, hormone therapy, chemotherapy, and immunotherapy. Differences in immune response may play a key role in many of these observations. Metastatic castration-resistant prostate cancer (mCRPC) is a main cause of lethal prostate cancer and therefore remains a key focus for research. Despite patients with mCRPC having multiple treatment options targeting a variety of mechanisms (Figure 2), median overall survival is still only around 3 years [79]. This further highlights the need for inclusion of diverse biospecimens in scientific studies and historically understudied populations in clinical trials to determine who is benefitting optimally from these currently approved treatments.

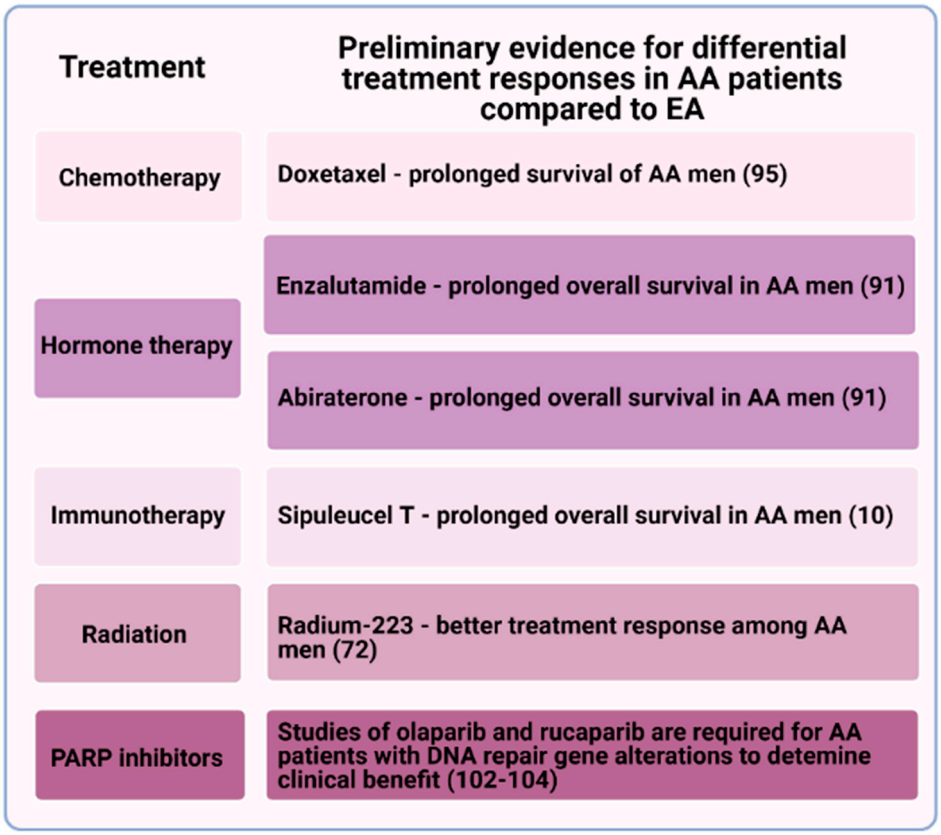


Figure 2. Preliminary evidence for certain treatment response differences between African American (AA) and European American (EA) patients with metastatic castration-resistant prostate cancer (mCRPC). Current approved treatment modalities for mCRPC, how clinical responses may differ between the two patient groups, and where additional studies are warranted. For PARP inhibitor use, the pathologic role of germline variants of unknown significance in DNA repair genes that commonly occur in AA men needs to be investigated.

4. Radiation

Radium-223 is an approved therapeutic option for mCRPC patients with symptomatic bone metastases. Zhao et al. examined the response to radium-223 treatment in men from a Veteran Affairs cohort with mCRPC [80]. With equal access to care across the cohort, this group found that African American men may have a better response to this treatment compared to European American men, resulting in a 25%–28% decreased risk of mortality in this equal access to care study. African American men in this study were more likely to have received docetaxel beforehand and the improved response to therapy was despite the African American cases being more likely to not start radium treatment until further along in the disease course. Patients harboring DNA damage repair mutations have prolonged overall survival after radium-223 treatment compared to patients who do not have these alterations [81–83]. This is also the subject of another clinical trial currently in the recruitment stage (NCT04489719). With Awasthi et al. reporting decreased DNA damage repair capacity in prostate tumors of African American men, it can be speculated that inactivating mutations that decrease the DNA damage repair capacity in tumors from African American men may contribute to the positive outcomes post treatment with radium-223 [66].

A recent, small, phase II trial (NCT02463799) found combining radium-223 treatment with Sipuleucel-T increased progression-free survival and overall survival in men with mCRPC [84]. Now that studies have shown better responses from African American men treated with radium-223 and Sipuleucel-T separately [10,80], a planned larger trial may

may inform on whether African American men may benefit synergistically from this combination approach.

5. Immunotherapy

Immunotherapy has not been as successful in treating prostate cancer as with other hematologic or solid cancers and clinical trials show a modest [85] to no effect [79,86,87] on survival. This has been attributed to prostate cancer not being as immunogenic as other cancers. However, recent studies indicate a potential role for immunotherapy in certain patient groups with prostate cancer. Precision medicine strategies targeting immunotherapy to those men with the best response is the preferred goal. Evidence is currently being built to support the hypothesis that African American men may have a differential and perhaps superior response to certain treatments due to changes in immune cell response and a differing tumor biology.

Tumors from men of African descent may have a heightened response to immunotherapies, and specifically to cancer vaccines, as assumed from the presence of an interferon signature in their tumors and increased immune content in the TME [56,66]. Studies have shown that young people who self-report as African American mounted an increased immune response to vaccination [88,89]. Sartor et al. recently reported that African American men with mCRPC who were treated with the cancer vaccine, Sipuleucel-T, in the PROCEED trial/registry, had significantly better survival than the European American patients [10]. Median overall survival was 35.3 months for African American men compared to 25.8 months for European American men, in a PSA-matched set. This difference became even greater when measured in patients with a baseline PSA below the median, with median overall survival of 54.3 months in African American men versus 33.4 months in European American men. Increased activation of dendritic cells is a proposed mechanism of action of the vaccine and in agreement with this, activated dendritic cells in localized tumors have subsequently been associated with improved distant metastasis free survival [90]. Mechanistically, evidence points towards a complex interplay of immune cells with tumor biology which may predict prognosis and response to therapy. However, the lack of tumor specimens from African American men means that more work must be done to capitalize on the differences in the immune landscape which may improve response to treatment in this population.

Generally, poor immunogenicity has resulted in little success for PD-L1 blockade in treatment of prostate cancer [87,91]. This has been attributed in part to relatively low PD-L1 expression from tumor cells [92,93]. However, this is not consistent across the literature, with studies also reporting increased PD-L1 expression and association with biochemical recurrence [94] and shorter metastasis free survival [95]. Petitprez et al. provide preliminary evidence that a composite assessment of both PD-L1 and CD8 expression in localized prostate cancer may be a good strategy for predicting outcomes in mCRPC [95]. A group in Norway reported high PD-L1 expression in post-prostatectomy, hormone-naïve tumor epithelial cells with a non-significant trend towards an inverse association between PD-L1 expression and biochemical failure-free survival [96]. However, clinical trials investigating the effect of PD-L1 inhibition reported no significant clinical benefit. Yet, they have typically not included men of African descent [87].

Recent work has focused on PD-L1 expression on tumor-infiltrating immune cells. Bishop et al. reported enzalutamide-resistant prostate cancer patients showing increased PD-L1 expression on dendritic cells and high PD-L1 T cells when compared to enzalutamide-sensitive or treatment-naïve patients [97]. African American ethnicity and an aggressive cancer phenotype have been associated with prediction of tumor PD-L1 positivity in hormone-naïve tumors [98], suggesting a potential benefit for immunotherapy in African Americans at high risk of aggressive disease, but this has not been replicated yet [66]. When tumors are enzalutamide-sensitive, McNamara et al. preliminarily reported increased overall survival for African American, chemotherapy-naïve men with mCRPC treated with abiraterone or enzalutamide compared to European American men [99]. Overall survival

was 918 days for African Americans compared to 781 days for European Americans. This study in a Veteran Affairs population was retrospective in design, again pointing to the value of equal access to care across populations. Thus, additional work is warranted, including measurement of PD-L1 in tumor samples from African American men post various treatment regimens to account for increased immunogenic response to therapy.

6. Other Treatment Opportunities

Historically, participation of African American men in clinical trials has been low. Reasons for this are multifactorial but include historical mistrust of the medical profession as a result of systemic racism and major ethical breaches in the past [100]. A higher prevalence of comorbidities and a lack of access to academic medical centers involved in trials may also prevent access to trials [100–102]. This prevents generalizability across population groups when reporting clinical trial data. A recent example highlights the need to include diverse population groups and possibly stratify clinical trial participants by race to get a fuller picture of treatment response. Halabi et al. completed a meta-analysis of survival outcomes for African American versus European American men in phase III clinical trials treating mCRPC with docetaxel [103]. With just 6% of African American participants, they reported that while overall median survival was similar, a pooled hazard ratio of 0.81 (95% CI, 0.72 to 0.91) post adjustment for baseline prognostic factors estimated that African American men may have a significantly decreased risk of death compared to European American men. This was despite African American men having baseline characteristics known to be prognostic of overall survival including statistically significantly worse performance status, higher testosterone levels, higher PSA levels, and lower hemoglobin levels.

It is assumed by many that the prostate cancer biology in men of African ancestry is intrinsically more aggressive—at least for a subset of patients—leading to a survival health disparity in the population [104]. Yet, this does not mean that African American men would not respond as well as European American men to most standard therapies. In fact, it appears that the treatment responses of African American and European American men are mostly similar. Yet, tumors in African American men could still respond better to certain therapies compared to the average response among European American men. Some of the treatments or combination treatments discussed in this review were the subject of small clinical trials and so these therapeutic options might not be widely offered yet in the clinic. Therefore, the findings require further evaluation in larger studies but do suggest that there is a potential role for these treatments in reducing the survival disparities observed in prostate cancer. African American men are less likely to be recruited into clinical trials and may not have the opportunities to avail of these new therapeutic options.

7. Germline and Somatic Mutations in DNA Repair Pathways

A proposed feature of prostate tumors in African American men that may play a prominent role in differential response to treatment is a deficiency in DNA damage repair capacity. Both germline and somatic alterations to DNA damage repair pathways have now been found in prostate tumors across multiple studies [66,105–107]. Tumors from African American men were reported to have a significantly lower level of DNA repair capacity when compared to those from European American men. Notably, these tumors seemed to have an increased radiosensitivity [66].

Germline mutations in DNA repair genes have a higher occurrence in metastatic prostate cancer when compared to localized prostate cancer [105,106]. BRCA1/2 pathogenic variants have been associated with more aggressive prostate cancer and adverse survival outcomes [108,109]. DNA repair gene mutations may contribute to aggressive disease in African American men. Acquired somatic mutations may differ among patient groups, with Yadav et al. reporting that prostate tumors from African American men were twice as likely to have at least one mutation in nucleotide excision repair pathway genes compared to European American (89% vs. > 40%) [107]. Petrovics and colleagues reported that germline

variants in DNA repair genes of unknown significance had an increased frequency in African American men (4.6%) compared to European American men (1.6%) [110]. As the significance of these is undetermined, there is an opportunity to investigate whether they play a pathogenic role in prostate cancer. The same authors also reported that just 0.7% of men with localized prostate cancer carried pathogenic variants of BRCA1/2 mutations, but this increased over 4-fold to 3.1% in patients with metastatic and advanced disease, indicating that the presence of known BRCA1/2 pathogenic variants is linked to disease status [110]. Because the FDA-approved PARP inhibitors, olaparib and rucaparib, have shown success in prolonging overall survival in mCRPC patients with mutations in these DNA damage response genes [111–113], they should be made available to all African American men with prostate cancer who carry these mutations.

Altered DNA damage repair pathways may sensitize tumors to immunotherapeutic approaches. Several clinical trials across many cancer sites including metastatic prostate cancer are currently underway, targeting DNA damage repair-deficient tumors with checkpoint inhibitors (extensively reviewed by Bever et al.) [114]. Mechanistically, in prostate cancer, the stimulator of the IFN genes (STING) pathway has been linked to the recruitment and activation of interferon-related genes in vitro, increasing sensitivity to the immune checkpoint inhibitor PD-L1 in DNA repair-deficient tumors [115–118]. As a low DNA repair capacity may increase tumor genomic instability and tumor mutational burden, this again might constitute a vulnerability to immunotherapeutic strategies [119]. It has been suggested that enhancement of this genomic instability through use of radiation, chemotherapy, or PARP inhibitors could augment the immunotherapeutic response [114,120,121].

8. Anti-Inflammatory Drug Aspirin for Prevention of Adverse Outcomes in African American Men with Prostate Cancer

While the research community plays catch-up with ensuring proper representation of population groups in clinical trials and precision medicine studies, strategies for the prevention of lethal prostate cancer and reduction in adverse outcomes are of paramount importance to men of African descent who continue to experience a disproportionally high mortality from prostate cancer. An extensive body of evidence including both preclinical and clinical studies led the United States Preventative Services Task Force to recommend the anti-inflammatory drug aspirin for prevention of colorectal cancer for an albeit narrow category of adults [122–124]. In keeping with the hypothesis that inflammation is one of the drivers of the prostate cancer disparities, our group explored the link between regular use of aspirin and prostate cancer in African American men. We found that regular aspirin use significantly reduces the risk of both advanced prostate cancer and disease recurrence in these men [50]. The finding is consistent with a similar observation in a previous study [125]. Inhibition of the pro-inflammatory cyclooxygenase/thromboxane A2 pathway has been identified as a potential mechanism of action for aspirin in the prevention of metastatic cancer [126]. Using a retrospective cohort, we found a distinct association between high urinary 11-dehydrothromboxane B2 (the stable metabolite of thromboxane A2) and aggressive prostate cancer as well as adverse survival outcomes for African American men. Importantly, our ongoing research showed high 11-dehydrothromboxane B2 was inversely correlated with aspirin use, indicating a potential benefit of aspirin in preventing lethal prostate cancer through inhibition of TXA2 synthesis.

Lastly, data prospectively obtained in the Southern Community Cohort Study suggested that aspirin use is tentatively associated with a reduced prostate cancer mortality in African American men [127]. Hurwitz et al. also observed this inverse relationship between aspirin use and prostate cancer mortality in both African American and European American men using the ARIC cohort [128], again pointing to the potential benefit of aspirin use for men at high risk of fatal prostate cancer.

9. Conclusions

Elevated inflammatory processes in African American men with prostate cancer are a candidate biological driver of disparate disease risks. There is a need for more clinical trials

specifically focused on the treatment response of African American men with metastatic prostate cancer [129]. Improved inclusion of minority populations in trials is essential to further enhance our knowledge of how inflammation and the immune response and alterations to molecular pathways may govern the response to emerging therapies across all patient groups. With evidence now building that suggests increased clinical benefit with certain therapies among African American men when compared to European American men, targeting inflammatory processes and the immune system could be an important strategy to reduce lethal disease in high-risk populations such as men of African ancestry.

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40TH ANNIVERSARY REVIEW ARTICLE

An overview of cancer health disparities: new approaches and insights and why they matter

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Abstract

Cancer health disparities remain stubbornly entrenched in the US health care system. The Affordable Care Act was legislation to target these disparities in health outcomes. Expanded access to health care, reduction in tobacco use, uptake of other preventive measures and cancer screening, and improved cancer therapies greatly reduced cancer mortality among women and men and underserved communities in this country. Yet, disparities in cancer outcomes remain. Underserved populations continue to experience an excessive cancer burden. This burden is largely explained by health care disparities, lifestyle factors, cultural barriers, and disparate exposures to carcinogens and pathogens, as exemplified by the COVID-19 epidemic. However, research also shows that comorbidities, social stress, ancestral and immunobiological factors, and the microbiome, may contribute to health disparities in cancer risk and survival. Recent studies revealed that comorbid conditions can induce an adverse tumor biology, leading to a more aggressive disease and decreased patient survival. In this review, we will discuss unanswered questions and new opportunities in cancer health disparity research related to comorbid chronic diseases, stress signaling, the immune response, and the microbiome, and what contribution these factors may have as causes of cancer health disparities.

Introduction

Cancer death rates in the United States (U.S.) reached their high point in the 1990s (1). They have been declining from that time on because of reduced tobacco use among adults, more widespread cancer screening and early detection, and improved cancer therapies (1). Declines in deaths from lung cancer, melanoma, and other leading cancers, like breast, colorectal, and prostate cancer, account for much of the advances in reducing the U.S. cancer mortality. These improvements are more pronounced among younger than older Americans (2). Nevertheless, cancer health disparities persevere. In this review, we will first summarize our understanding of cancer health disparities in the U.S. and abroad and then evaluate the contribution that comorbid chronic diseases, chronic stress exposure, population differences in immune response, and a dysbiosis may have as causes of these disparities (Figure 1). The advent of COVID-19 infections reinforced the notion that diseases other than cancer influence cancer survival and may contribute to an excessive mortality in underserved communities.

Cancer health disparities in the United States and globally

Cancer disparities continue to persist across geographic areas, socioeconomic strata, and different racial and ethnic groups. Rural communities experience higher death rates from lung, cervical, and colorectal cancers than urban communities because of poverty, health risk behavior, and lower vaccination and screening rates (3), consistent with the widening disparity in life expectancy between rural and urban areas (4).

Low educational attainment is an indicator of socioeconomic deprivation and strongly correlates with elevated all-cause death rates in the general population. 40–50% of all pre-mature

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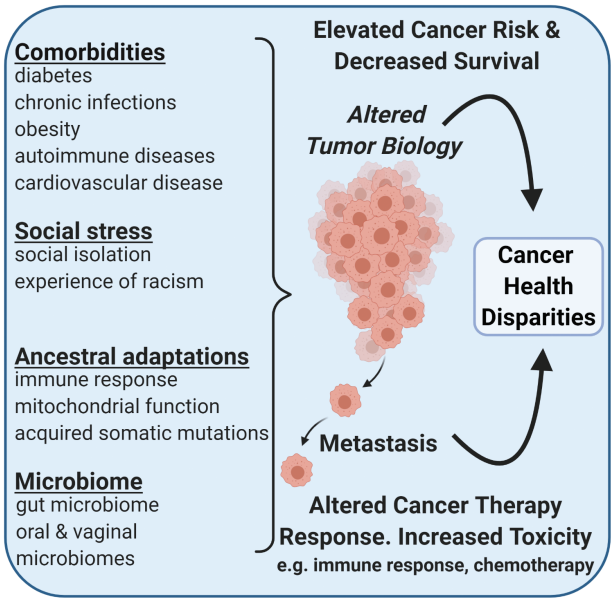


Figure 1. Comorbid chronic diseases, stress exposure, population differences in immune response, and dysbiosis are factors that contribute to cancer health disparities.

deaths might not occur if all segments of the U.S. population would experience the death rates of college graduates (5). Socioeconomic status is a key determinant of cancer mortality as well. About a quarter of all cancer deaths may not occur if all Americans were college-educated (6). Cancer survival increases with higher socioeconomic status for all U.S. racial and ethnic groups (7). Yet, socioeconomic patterns in cancer mortality have changed markedly over time (8). Into the 1980s, socioeconomic status positively correlated with U.S. cancer mortality rates, showing a higher risk of cancer deaths among the affluent. This correlation has now turned into the opposite direction, with affluent Americans being less likely of dying from cancer because of advances in disease prevention, early cancer detection, and cancer therapy that benefit patients with private health insurance more so than others. Presently, socioeconomic inequalities contribute most strongly to the excess mortality from lung, colorectal, cervical, stomach, and liver cancer among Americans who live in deprived areas (8). While the prostate cancer mortality did not vary much by socioeconomic status in the past, an inverse socioeconomic gradient appears now to exist (8,9). Neighborhood socioeconomic deprivation can further be linked to shortened telomere length, an indicator of pre-mature aging, and lethal cancer (10–12).

Global disparities in cancer incidence and mortality rates are evident for most cancer sites and indicate socioeconomic inequalities and significant differences in risk factor exposure (13). Rates of cancers including breast, colorectal, and prostate vary greatly between high-income and low-income countries, geographic areas, and race/ethnic groups. Differences in health care and modifiable risk factor exposure are major drivers of these global disparities, as shown by migration studies for breast and other cancers (14–16). Lung cancer is the leading cause of cancer death worldwide but is prominently under-represented in sub-Saharan Africa because of a low smoking prevalence. Prostate cancer is the most common cancer among men worldwide but shows large geographical differences in occurrence, with low incidence rates in East Asia and high rates in Western countries. With the westernization of lifestyles in East Asia, the incidence difference has narrowed (17). Notably, prostate cancer is the leading cause of cancer death among men in sub-Saharan Africa and the Caribbean (18), which led to the hypothesis that genetic ancestral factors may predispose men of sub-Saharan African ancestry to prostate cancer and a more aggressive disease. Recent findings are consistent with this hypothesis (19–22). Cervical cancer is a major cause of cancer deaths among women in sub-Saharan Africa and South-East Asia because of human papillomavirus infections and delayed disease detection. Stomach and esophageal cancer are two other cancers with high incidence and mortality rates in Eastern Asia. *Helicobacter pylori* and salted foods are major risk factors for stomach cancer. This cancer is particularly common on the Korean peninsula due to a combination of regional dietary risk factors and chronic *H. pylori* infections whereas Malawi in Eastern African is especially impacted by esophageal cancers, having the highest global disease rates due to factors that have yet to be identified. Lastly, the burden of liver cancer is greatest in Northern and Western Africa and South East Asia and is a primary cause of cancer death in Mongolia. Chronic hepatitis B & C virus infections and exposure to aflatoxin are key causes of the disease in these areas while heavy alcohol use and non-alcoholic fatty liver disease are drivers of the increasing liver cancer incidence in many high-income countries.

Cancer health disparities between population groups in the United States

Large differences in cancer incidence and mortality do also exist between U.S. population groups (1,2). These disparities are largely explained by differences in access to health care, diet, lifestyle, cultural barriers, and disparate exposures to pathogens and carcinogens (23,24). Disparities in liver cancer occur across U.S. states and race/ethnic groups (25,26). This cancer affects American Indians/Alaska Natives, American Asians, and Hispanic Americans more so than African Americans and European Americans. American Indians/Alaska Natives have the lowest 5-year cancer survival across all cancer types and experience elevated rates for many malignancies and major risk factors, like comorbid conditions, when compared to European Americans (1,27,28). In contrast, Hispanics/Latinos and Asian Americans tend to have lower cancer incidence rates than other U.S. population groups. Asian Americans, by themselves a rather heterogenous population group, have the lowest cancer-specific mortality by reasons that are yet unclear but may relate to better treatment responses (29). Among Hispanics/Latinos, infection-related cancers are over-represented and women and men are more likely to be diagnosed with late stage cancer when compared to U.S. European Americans (25). While prostate cancer is generally less common among Hispanic/Latino men, it is the leading cause of cancer death among men in Puerto Rico, indicating heterogeneity in cancer risk within the Hispanic population. African Americans disproportionately bear the cancer burden and have the highest death rates from malignancies of the breast, gastrointestinal tract, lung, and prostate, and develop multiple myeloma more commonly than other population groups (23,30). Reasons of why these specific cancer disparities exist have been extensively reviewed (31–37). Therefore, they will not be the focus of this review. Nonetheless, cancer risk profiles among African Americans are not uniform and vary whether they are Sub-Saharan African-, Caribbean-, or U.S.-born (38,39). African Americans have an excess risk of developing early-onset cancer, which is reminiscent of disease presentation in Africa (40); however, African populations and African Americans in the U.S. are generally younger than the U.S.

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European American population which may bias cancer-onset comparisons (41). In recent years, cancer incidence and death rates declined faster among African Americans than European Americans, a very positive development that is mainly due to reductions in lung, colorectal, and prostate incidence and mortality (2,30). Barriers still exist and current lung cancer screening guidelines may often exclude African American smokers at increased risk of lung cancer (42). Moreover, men of African ancestry continue to have 2–3-times higher absolute rates of fatal prostate cancer in both the U.S. and England (43).

The differences in cancer survival between U.S. race/ethnic groups and their underlying causes have been investigated. This research showed that disparities in stage at diagnosis may have the largest contribution to these survival disparities, followed by socioeconomic factors and marital status as other key contributing factors (44,45). The importance of marital status suggests that social isolation and stress may contribute to these racial/ethnic disparities. Still, private insurance provides the single most protective effect against being diagnosed with advanced stage disease, emphasizing the importance of access to health care in reducing the cancer survival health disparity among U.S. population groups (46).

Influence of sex and gender on cancer risk and outcomes

Sex and gender are modifiers of health and contribute to disparities in disease development and outcome (47). Men are at an increased risk of dying from cancer (1,2). Many non-reproductive cancers show a 2:1 male predominance worldwide. Sex hormone signaling and Y chromosome-encoded oncogenes are drivers of sex- and gender-related cancer disparities. Sex differences in cancer genetics have been recognized (48). The androgen receptor has key roles in the progression of liver diseases like fatty liver, cirrhosis, and liver cancer, consistent with a 2:1 to 7:1 male predominance in the liver cancer incidence globally (49). The response to cancer therapy may differ between women and men. For example, the therapy benefit from immune checkpoint inhibitors is sex-dependent and these therapies provide more benefit to men (50). Although sex is a well-established modifier of cancer risk, the biology of sex-related cancer disparities remains incompletely understood. Nonetheless, it has been recommended that clinicians should consider sex and gender in their approach to diagnosis, prevention, and treatment of diseases (47). To end with, there are also cancer health disparities related to sexual behavior. For example, anal cancer incidence rates are increasing in both men and women across the globe and will require population-based preventive measures including advocacy for safe sexual behaviors and human papillomavirus vaccination (51).

Impact of health care access and the Affordable Care Act on cancer health disparities

Access to health care and health insurance coverage are key determinants of receipt of cancer care and cancer survival (52). A survival disparity for African American men with prostate cancer exists in the U.S. population, but is not observed in clinical trials or for men served by the Veteran Affairs equal-access health care system (53), highlighting the importance of equal access to health care in reducing cancer health disparities. Furthermore, insurance status provides the single most protective effect against the diagnosis of metastatic cancer (46). In 2010, the Patient Protection and Affordable Care Act, also termed "the Affordable Care Act", was signed into law. Its primary goal was to improve health insurance coverage (54). The preliminary impact of this legislation has now been assessed. Disparities in the percentage of uninsured patients have been diminished in Medicaid expansion states under the Affordable Care Act (55–57). Americans living in areas of greater deprivation and rurality still have lower rates of recommended cancer screening than others (58). With the Affordable Care Act, however, colorectal cancer screening uptake seems to have increased, albeit modestly (59), yet race/ethnic disparities persist (60). On the other hand, Medicaid expansion shows consistent relationships with lower odds of having either advanced stage or metastatic cancer at diagnosis among low-income Americans (55,56,61). It also increased care affordability among cancer survivors in Medicare expansion states, but not in nonexpansion states, and increased utilization of cancer surgery by low-income Americans (57,62). Still, race/ethnic disparities remain (62), and Medicaid expansion may not have lowered the disparity in breast cancer mortality between African American and European American women (63). With the continuation of an impact by the Affordable Care Act on both secondary prevention of cancer and cancer care, future analyses of Surveillance, Epidemiology, and End Results program data should provide more clarity to what extent the Affordable Care Act has reduced cancer survival health disparities in low-income communities and across race/ethnic groups.

Chronic diseases modify cancer risk and survival and contribute to health disparities

Comorbidities in cancer patients are chronic diseases that commonly co-occur with cancer because of shared risk factors (64). Common comorbid diseases include obesity, diabetes, and metabolic syndrome, cardiovascular, liver, and autoimmune diseases, chronic infections, but also dysbiosis and neurological and stress-related disorders. They influence cancer diagnosis, tumor biology and metastasis, and the utilization of cancer therapy. Comorbidities do not affect all segments of the US populations equally. American Indians and African Americans have significantly higher rates of comorbidities, when compared to other U.S. population groups (27). Four of these comorbidities, obesity, diabetes, chronic kidney disease, and hypertension, contribute disproportionally to the mortality disparity between African Americans and European Americans. Although not a chronic condition, COVID-19 infections have recently been associated with an excessive mortality among African Americans (65) and cancer patients (66).

Diabetes, hyperinsulinemia, and obesity are closely related comorbid conditions. They are all cancer risk factors (67,68). Because these conditions are more prevalent in underserved and minority populations, one would predict that they contribute to a disproportionate cancer burden in these communities. However, the evidence that link comorbidities to cancer health disparities remains rather sparse, partly because these investigations were either not done or focused on only a few comorbid conditions. Diabetes approximately doubles the risk for liver and pancreas cancer and is additionally associated with the risk of breast, colorectal, endometrial, esophageal, and gallbladder cancer (67,69). Diabetes-related advanced glycation end products have been linked to a cancer health disparity (70). Diabetes is thought to promote cancer development and progression through insulin and insulin-like growth factor signaling, oxidative stress, and excessive inflammation (71). This comorbidity is excessively high among African Americans and in the Hispanic/Latino community (27,72). Insulin resistance and the metabolic syndrome have been found to contribute to disparities in breast cancer

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outcomes between African American and European American women (73,74). Diabetes also increases the risk of pancreatic cancer in African American and Hispanic/Latino (75), however, the data do not indicate that the conferred risk is higher in these two population groups than in European-Americans.

Comorbidities are associated with an elevated cancer mortality. They impede the participation of cancer patients in clinical trials and adversely affect trial participation (76). Accordingly, clinical trial participation of U.S. minorities remains low (77,78), which may partly relate to barriers in enrollment due to comorbidities. The presence of a comorbidity will influence treatment selection and the use of surgery and chemotherapy (79,80). Cancer patients with a comorbidity are generally less likely to receive curative treatment than those without the comorbidity (81). These deaths are preventable with lifestyle changes and other intervention strategies that target these chronic diseases. Moreover, the negative impact of comorbidities on cancer outcomes tends to increase with the number and severity of the comorbidities. Their impact is generally larger for cancers that have otherwise better survival. Thus, future cancer health disparity research should develop an increased focus on comorbidities and how they contribute to existing U.S. cancer outcome disparities.

Mechanisms linking stress exposure to cancer metastasis and survival and disparate outcomes

The concept of a public health exposome was developed for targeted community health intervention and includes exposure to stressors, their signaling, and the causes of the stress exposure (82). Posttraumatic stress because of a cancer diagnosis may disproportionally affect minority populations (83). Social adversity in early life can lead to decreased glucocorticoid and increased pro-inflammatory signaling in humans (84). Intrauterine stress exposures associate with a shortened telomere length in young adulthood (85), which may predispose these individuals to premature aging and cancer. Perceived experiences of racism show relationships with breast cancer and cancer-promoting health behaviors, such as increased tobacco and alcohol consumption (86,87). In breast tumors, social isolation may lead to reprogramming of tumor biology (88,89). Thus, stress exposures may alter cancer susceptibility and disproportionally affect socially deprived and minority populations (Figure 2).

Behavioral comorbidities (e.g. depression, fatigue, anxiety, cognitive impairment) are prevalent in cancer patients and a target for therapy (90). Cancer patients have higher rates of depression than most Americans (91). Major depression affects about 5–8% of the U.S. population but approximately 15% of cancer patients. Race- and gender-based discrimination and social isolation of the elderly are common events and create chronic stress exposures in affected individuals. Chronic stress and depressive disorders are associated with an increased cancer mortality (92–94). They are cancer risk factors and have been linked to elevated concentrations of circulating pro-inflammatory cytokines and chemokines (90–92).

Stress exposures and depression transduce their biological effects through the hypothalamic-pituitary-adrenal axis. This signaling pathway is characterized by hypersecretion of the

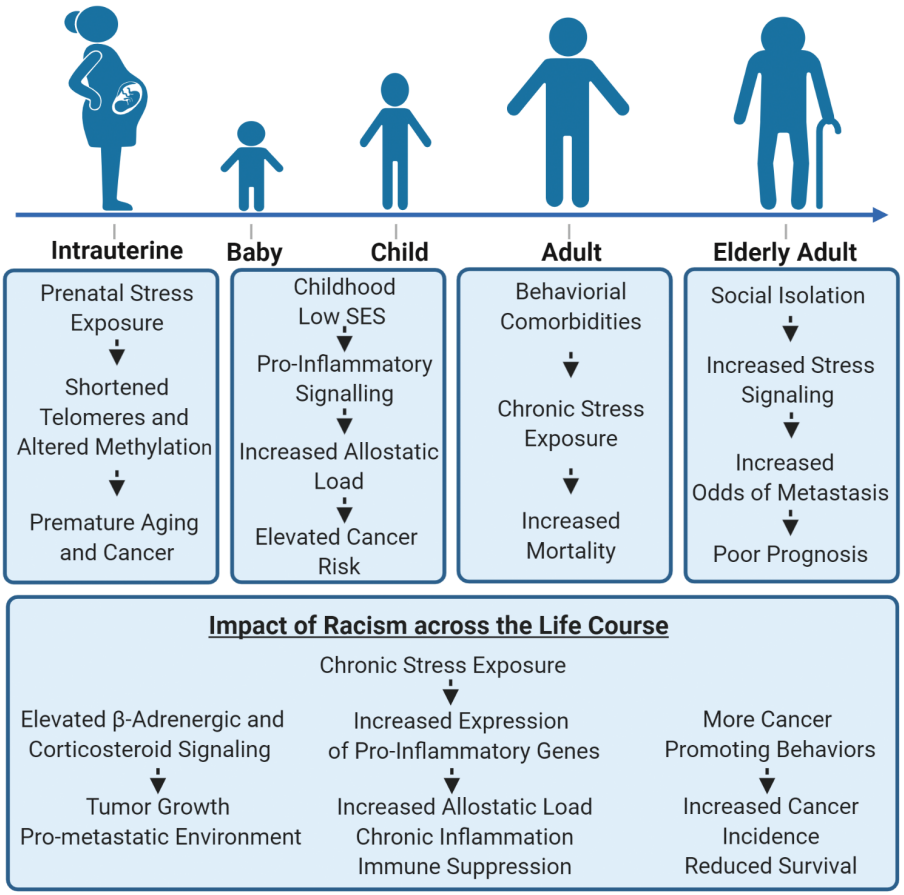


Figure 2. Stress exposure over the life course and its potential impact on socially deprived and minority populations.

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corticotrophin-releasing hormone and activation of the peripheral autonomic and sympathetic nervous system, which has direct effects on tumor biology and immune response, promoting inflammation, angiogenesis, mesenchymal differentiation, and metastasis (95). Chronic stress influences tumor biology through two major pathways involving catecholamines (adrenaline, noradrenaline) and glucocorticoids (96). Socially isolated ovarian cancer patients were found to have elevated tumor noradrenaline levels (97). In mouse models of ovarian and breast cancer, chronic stress promotes invasive tumor growth and metastasis in a β-adrenergic signaling-dependent manner (98–100). Here, catecholamines activate β-adrenergic signaling in cancer cells and tumor-associated macrophages (95,99), leading to a pro-metastatic tumor microenvironment. Consistent with these observations, a pro-metastatic niche has been described for breast tumors from socially isolated women (101) and a decrease in chronic depression may slow metastasis in breast cancer patients (102). In other studies, social stress was found to up-regulate inflammatory gene expression in monocytes through β-adrenergic signaling (103). Likewise, African Americans with exposure to racial discrimination showed up-regulation of these genes (104).

Social isolation may contribute to racial and ethnic differences in cancer survival. Ellis *et al*. reported that marital status is a contributing factor to these survival disparities (45). Being married provides a survival benefit while being unmarried, a surrogate for social isolation, is a risk factor. There are other studies that link stress exposure and β-adrenergic signaling to cancer survival. β-adrenergic receptor expression may predict a poor prognosis for breast cancer patients (105). β-blocker use after a disease diagnosis reduces disease recurrence and improves survival of breast cancer patients (106), while regular users of the β-blocker, propranolol, are less likely to develop advanced breast cancer and have a reduced breast cancer-specific mortality (107). Beta-blocker use has been associated with improved recurrence-free survival in triple-negative breast cancer as well (108). Together, these data indicate that stress may alter breast cancer biology through activation of the pro-metastatic catecholamine pathway, leading to an aggressive disease in a subpopulation of patients who would benefit from stress management. Lastly, a high prevalence of major depression has been reported for African American men with prostate cancer (109). This condition and other social stress exposures may predispose these men to aggressive disease as it has recently been shown that stress-related signaling pathways are up-regulated in prostate tumors that progressed into lethal disease (110). In summary, it is well documented that stress exposures, which impact underserved and minority communities more so than affluent communities, can adversely affect tumor biology, cancer survival, and quality of life of cancer patients (Figure 2). Yet, a knowledge gap persists. Still few studies have examined the impact of various stress exposures in minority and socially deprived communities using large and well-designed studies. These studies should be conducted as the detrimental impact of chronic stress and depression in cancer patients is preventable using community engagement, psychosocial support, and therapies like β-adrenergic blocking agents.

Ancestry and population differences in immune response as underlying factors of cancer health disparities

Differences in pan-cancer mitochondrial function were found to distinguish African American from European American cancer patients, suggesting an ancestral link (111). Recent observations have shown that population differences in genetic ancestry can contribute to population differences in cancer susceptibility (19,20,112–114). Genetic ancestry and natural selection are underlying causes of population differences in immune response to pathogens (115,116). Those differences may relate to cancer (37,117). Relationships of ancestry with expression levels of inflammatory cytokines are evident in human populations (118,119). These differences may contribute to lung cancer disparities (120,121). Two studies investigated gene expression variations between subjects of European and West African ancestry using lymphoblastoid cell lines (122,123) and observed that these variations can cluster in cancer-related pathways and influence pathway signaling. Thus, genetic differences among population groups may lead to population-specific susceptibilities for common diseases, like certain cancers, because of their effect on the transcriptome (114,124).

One mechanism by which ancestry-related factors affect cancer outcomes is by inducing an adverse tumor biology (125). Research has now documented that tumors from patients of either African, Asian, or European descent show notable differences in acquired somatic mutations (126). Two large studies investigated the relationship of African and European ancestry with mutational signatures and gene expression across 33 cancer sites in the Cancer Genome Atlas (TCGA) database and reported associations of African ancestry with somatic mutations that tended to be cancer type-specific (127,128). At a pan-cancer level, the mutational burden of tumors and associated signatures were not significantly different between patients from these two ancestries, nor were there significant differences in chromosome arm-level copy number alterations. *TP53* mutations were enriched in African American patients in a subset of cancers, most notable in breast cancer, whereas genomic alterations in genes of the phosphatidylinositol 3-kinase pathway were less frequent in this patient group. After adjusting for tumor subtype differences between African American and European American patients, few significant associations between ancestry and either tumor somatic mutations or chromosomal aberrations remained (128). Notably, mutations in the gene, *FBXW7*, showed a pan-cancer association with African ancestry. *FBXW7* is a tumor suppressor gene that is involved in the proteasome-mediated degradation of many oncoproteins such as cyclin E, c-Myc, Mcl-1, mTOR, Jun, Notch, and AURKA (129). Mutations in other genes, such as *VHL*, *PBRM1*, *HRAS*, and *NFE2L2*, showed only cancer-specific associations with ancestry.

Other investigators focused on specific cancer types, such as breast, colorectal, lung, and prostate cancer. The breast cancer studies reported an overall increased mutation frequency, and specifically for *TP53*, and fewer *PIK3CA* mutations in African American and Nigerian women, together with an over-representation of triple negative breast tumors among these women (130,131). The latter is consistent with many previous reports (40,132). Breast tumors from Nigerian women were also characterized by the occurrence of *GATA3* mutations and a homologous recombination deficiency signature. A smaller study of triple-negative breast tumors that applied whole genome sequencing identified the over-representation of *CTNNA1* deletions in African American patients (133). Among patients with colorectal cancer, African Americans seem to acquire *KRAS*, *EPHA6*, and *FLCN* mutations more frequently than other patients whereas *APC* loss-of-function and oncogenic *BRAF* mutations may manifest less frequently in their tumors (33,134–136). Lung cancer is the most fatal cancer and is highly heterogenous as a disease and presents with geographic differences

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in acquired mutations and the therapeutic response of lung cancer patients (31). Mutations in the gene encoding the epidermal growth factor (*EGFR*) are generally more prevalent in non-small cell lung tumors from smokers and nonsmokers of East Asian ancestry (137,138) whereas mutations in *KEAP1* and *CDC27* are over-represented in lung adenocarcinomas from patients of European ancestry when compared to East Asian patients, independent of smoking history (138). Furthermore, lung adenocarcinomas from European ancestry patients featured a comparatively high genomic instability score, perhaps explaining some of the reported ethnicity-related differences in survival outcome among non-small cell lung cancer patients (139). Research into racial/ethnic differences in lung cancer mutational profiles has been extended to African Americans. While one study did not find significant differences between African American and European American lung cancer patients (140), another study discovered the distinct occurrence of *PTPRT* and *JAK2* mutations in lung adenocarcinomas among African Americans and their association with increased STAT3 signaling (141).

A role of tumor biology and the immune response in cancer health disparity: the example of prostate cancer

The most prominent population differences in tumor biology have been reported for prostate cancer. This disease can be classified into subtypes, such as those with ETS-fusion gene arrangements and other subtypes that are negative for ETS-fusion gene arrangements and either overexpress the SPINK1 oncogene or carry a *SPOP* mutation (142,143). Localized prostate cancer contains few recurrent mutations in oncogenes or tumor suppressor genes (144,145). Instead, prostate tumors are characterized by gene fusions (e.g. ETS gene fusions), allelic gains of the *MYC* gene, and deletions of the *PTEN*, *TP53*, and *NKX3-1* tumor suppressors, with additional common changes in DNA methylation that increase aggressiveness (146,147). Multiple reports have now shown that prostate tumors from patients of either European, African, or Asian descent exhibit notable differences in acquired chromosomal aberrations (e.g. *ERG* fusion events and *PTEN* loss) and subtype distribution (143,148–150), indicating disparities in disease etiology and mutational events among these population groups. Chinese prostate cancer patients were found to acquire mutations in *FOXA1* at a high frequency (41%) (150). By contrast, this gene is mutated at <10% in European-ancestry populations. Comparing African American with European American patients in TCGA, significant differences were observed in the frequency of *TMPRSS2-ERG* fusions (29.3% African American versus 39.6% European American), *SPOP* mutations (20.3% African American versus 10% European American), and *PTEN* deletions (11.5% African American versus 30.2% European American), consistent with other studies in the United States and Africa (143,151–153). The application of whole genome sequencing to the disease in African men, currently performed on only few tumors (154), should provide further insight into the etiology of prostate cancer in Africa. Currently, we do not know how the disease in Sub-Saharan Africa relates to the disease in men of African ancestry in the United States, the Caribbean, or in European and South American countries. However, whole genome sequencing already revealed an elevated tumor mutational burden in prostate cancer patients from South Africa and the frequent loss of the *LSAMP* locus in African American patients (154,155).

As a key discovery of the study of prostate tumors in African American men, *Wallace et al.* was the first to describe a prevalent immune-inflammation signature in prostate tumors of African American patients (156), followed by others (157). This finding has been validated in TCGA (127). The signature contains elements of a viral mimicry signature and could be functionally related to the previously describe interferon-related DNA damage resistance signature, also termed IRDS (158,159). Thus, tumors with this signature may not respond as well to radiation and chemotherapy as tumors without the signature, as was shown for breast cancer (159). Yet, these tumors may have an improved response to immunotherapies, and specifically to cancer vaccines, and perhaps ADAR1 inhibitors (160). In agreement with our hypothesis, Sartor *et al.* recently reported that African American men with metastatic castration-resistant prostate cancer who were treated with the cancer vaccine, Sipuleucel-T, in the PROCEED trial had significantly better survival than the European American patients (161). Our group explored the link between regular use of aspirin and prostate cancer in African American men and found that regular aspirin use significantly reduces the risk of both advanced prostate cancer and disease recurrence in these men (162). The finding is consistent with a similar observation in a previous study (163) and the hypothesis that inflammation is a driver of tumor biology in African American men. There is only a weak association of the immune-inflammation signature with previously described germline genetic risk loci for prostate cancer (127); however, we described a significant relationship with the presence of the interferon-λ4 ΔG genotype that is common in West African ancestry populations and influences the host viral response (124,158). The precise origin of the signature remains poorly understood and may include an infection history in the context of the interferon-λ4 ΔG genotype (164), dietary factors (165), or changes to the epigenome, manifesting in the re-activation of endogenous retroviral sequences (166,167). We described up-regulation of *HERV-K* retroviral sequences in African American prostate cancer patients (166). In addition, a pro-inflammatory diet that associates with high-grade prostate cancer is more commonly consumed by African American than European American men (165). Others described the up-regulation of the transcription factor, Kaiso, in prostate tumors of African-American men (168). Kaiso regulates pathways related to epithelial-to-mesenchymal transition, apoptosis, and inflammation and may have a significant role in the cancer biology of prostate and breast cancer patients of African descent.

The presence of a distinct immune-inflammation signature has been reported for breast tumors in African American patients as well. Such a signature describes a subset of triple-negative breast tumors (169). Recruitment of tumor-associated macrophages is elevated in breast tumors of African and African-American women, as described by us and others (170–173). Moreover, Martin *et al.* observed an increased microvessel density in these tumors (170). An elevated tumor vascularization in African-American breast cancer patients was confirmed by Lindner *et al.* (174). Tumor angiogenesis correlates with breast cancer metastasis and poor survival (175). In Nigerian breast cancer patients, a prominent interferon signature was detected in luminal-type tumors whereas macrophage infiltration was more commonly observed in the basal subtype tumors (131). Hence, current data suggest that inflammation-induced breast cancer progression could be more prevalent in patients of African descent and may relate to increased inflammatory cytokine levels in these women (119,125).

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Microbiome and cancer health disparities: impact of geography, ethnicity, and genetics on the human microbiome composition

The gut microbiome affects human health (176,177). A dysbiosis can increase cancer risk and modify the cancer therapy response (178–181). Diet and genetics shape the gut microbiome (182–184) and may contribute to cancer health disparities through their effects on the gut microbiome (Figure 3). Likewise, comorbidities may confer their cancer risk through effects on the gut microbiome (185,186). Hence, there is evidence that a dysbiosis can be a cause of cancer (179). An altered microbiome and the accumulation of microbiome-derived metabolites have been reported for various human cancers (187–189). Alterations to the human microbiome can induce an aggressive tumor biology (190), linking the microbiome to cancer survival outcomes.

Geographic location and ethnicity strongly associate with the diversity of the gut microbiome (191,192) although geography (e.g. rural versus urban) usually confers a larger effect than ethnicity (193,194). Dissimilarities in the gut microbiota among ethnic groups with a shared environment have been reported, as shown for Amsterdam, a city in the Netherlands (192). Here, the gut microbiome diversity was significantly associated with ethnicity. Other factors, besides ethnicity, influenced the microbiome diversity. Nevertheless, ethnicity was the strongest determinant of gut microbiome diversity in models that included other non-dietary and dietary factors. Similarly, a U.S. study reported that ethnicity captures the gut microbiome with a stronger effect size than body mass, age, and sex, albeit the effect of all these factors was not as impactful as geographic location (194). Microbial community richness was greatest in Hispanics and decreased further from European Americans to Asian-Pacific Islanders to African Americans. However, the authors pointed out that there is more similarity than dissimilarity in the gut microbiome between the four studied U.S. population groups, thus the differences were comparably small. In addition, ethnicity may influence only a subset of the gut microbiome while other microbiome components remained unrelated to the ancestral background. Lastly, immigrants into the United States acquire a "westernized" gut microbiome (195), which is reminiscent of findings from migration studies that immigrants tend to acquire cancer rates of their new home country within two generations (15,196).

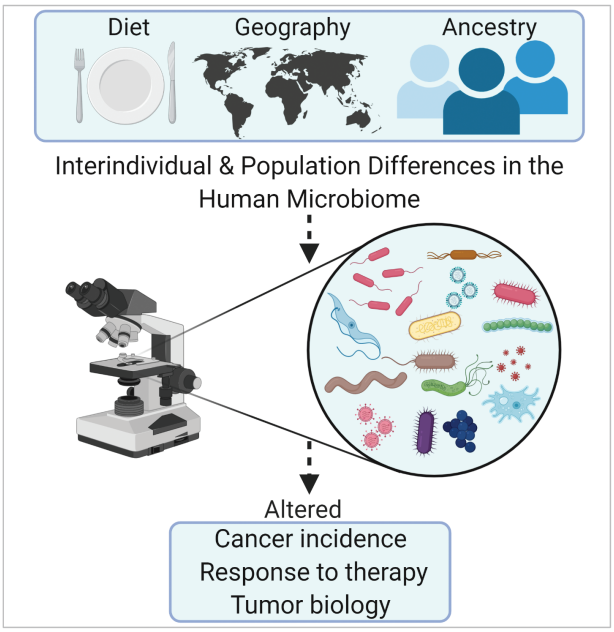


Figure 3. Diet, geographic location, and ethnicity strongly associate with the diversity of the gut microbiome and may increase the risk of dysbiosis, a cancer risk factor.

Cancer health disparity research has just begun to investigate the contribution of the microbiome to disparities in cancer risk and survival. Observations are sparse and validation of findings is non-existent. Differences in both the oral and vaginal microbiome have been reported comparing subjects of African and European descent (197,198). These studies did not include cancer patients. An exploratory investigation reported a rich bacterial content in high-risk prostate tumors from 6 men of South African ancestry when compared to 16 Australian men (199). In a study of breast cancer, differences in the breast tumor microbiome were observed comparing African American with European American women. Only 12 of the 64 tumors in the study came from African American women. Previously, the microbiome of breast tumors has been described from TCGA data but a separate analysis of African American tumors was not performed (200). Lastly, a large study of the non-cancerous colonic mucosa from 197 African Americans and 132 European Americans with or without colorectal cancer described a robust association of sulfidogenic bacteria with being African American, regardless of disease status (201). Abundance of these bacteria has previously been linked to diet (202) and the up-regulation of these bacteria in the African American study participants might have been related to their high intake of dietary fat and protein, as the authors concluded.

As shown by these few studies, cancer disparity-related differences in the gut, oral, and vaginal microbiome may exist. Future investigations are needed to assess the microbiome as an underlying factor or potential driver of cancer health disparities.

Conclusions and outlook

Minority, immigrant, and other underserved populations continue to experience an excessive cancer burden not only due to barriers in access to health care, but also because of disparate exposure to carcinogens, pathogens, co-morbidities, environmentally induced stress, and ancestry-related risk factors (Figure 1). These factors, singularly or in combination, are the likely causes of cancer health disparities in the U.S. and globally. There is convincing evidence from migration and epidemiological studies that the environment defines cancer risk but there is also indication that population differences in genetic ancestry can lead to population differences in cancer susceptibility.

Genetic ancestry and natural selection are underlying causes of population differences in immune response. Those differences may relate to cancer risk and therapy response. Current data suggest that inflammation-induced cancer progression could be more prevalent in patients of African descent, manifesting in a distinct tumor immune environment. Inflammation-induced cancer progression can be targeted by therapy. Tumors with an immune-inflammation signature may respond favorably to immune therapy.

Comorbidities influence cancer diagnosis, tumor biology and metastasis, and the utilization of cancer therapy. Many comorbidities are cancer risk factors. They do not affect all segments of the US populations equally. Because these conditions are more prevalent in underserved and minority populations, one would predict that they contribute to a disproportionate cancer burden in these communities. Yet, the evidence that link

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comorbidities to cancer health disparities remains sparse. Thus, future cancer health disparity research should develop an increased focus on cancer comorbidities.

Chronic stress and depressive disorders are associated with an increased cancer mortality and directly influence tumor biology (Figure 2). Chronic stress after a cancer diagnosis may disproportionally affect minority populations. Likewise, social isolation and perceived experiences of racism show relationships with cancer-promoting health behaviors and cancer development. Thus, stress exposures may alter cancer susceptibility and disproportionally affect socially deprived and minority populations. Still, few studies have examined the impact of these exposures in minority and socially deprived communities using large and well-designed studies. These studies should be conducted as the detrimental impact of chronic stress and depression in cancer patients is preventable using community engagement, psychosocial support, and therapeutic approaches. RESPOND is such study that focuses on prostate cancer among African American men and investigates the impact of social stress (https://respondstudy.org/).

Geographic location and ethnicity strongly associate with the diversity of the gut microbiome (Figure 3). Recent advances have shown that the microbiome is causatively linked to cancer. A dysbiosis can increase cancer risk and modify cancer therapy response. Diet and genetics shape the gut microbiome and may contribute to cancer health disparities through their effects on the gut microbiome. Cancer disparity-related differences in the gut, oral, and vaginal microbiome may exist. Future investigations are needed to assess the microbiome as an underlying factor or potential driver of cancer health disparities.

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Aspirin Use and Prostate Cancer among African-American Men in the Southern Community Cohort Study

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ABSTRACT

Background: The association of aspirin use with prostate cancer has been investigated, but few studies included African-American men. Here, we analyzed the relationship of aspirin intake with prostate cancer risk and mortality among African-American men in the Southern Community Cohort Study (SCCS).

Methods: SCCS recruited 22,426 African-American men between 2002 and 2009. Aspirin use was assessed at enrollment. Our exposures of interest were any aspirin use (regular strength, low-dose or baby aspirin, or half tablets of aspirin) and regular strength aspirin. Each exposure variable was compared with non-users. Associations between aspirin use and prostate cancer risk and mortality were examined with Cox proportional hazards models.

Results: At enrollment, 5,486 men (25.1%) reported taking any aspirin and 2,634 men (12.1%) reported regular strength aspirin use. During follow-up (median, 13 years), 1,058 men developed prostate cancer, including 103 prostate cancer–specific deaths. Aspirin use was not associated with prostate cancer development [adjusted HR, 1.07; 95% confidence interval (CI), 0.92–1.25 for any aspirin use and HR, 0.97; 95% CI, 0.78–1.19 for regular strength aspirin], but was suggestively associated with reduced prostate cancer mortality (HR, 0.66; 95% CI, 0.39–1.14 for any aspirin use and HR, 0.41; 95% CI, 0.17–1.00 for regular strength aspirin).

Conclusions: Aspirin use at enrollment was tentatively associated with reduced prostate cancer mortality, but not risk, among African-American men in SCCS.

Impact: Prospective SCCS data suggest that aspirin use may help prevent lethal prostate cancer among this high-risk group of men.

Introduction

Men of African ancestry have an excess risk of developing and dying from prostate cancer (1–6). We have had limited success in reducing this health disparity. Our group and others have described previously an immune inflammation signature that is prevalent in prostate tumors of African-American men, but absent in most European-American men (7–12). This gene signature associated with an increased risk of recurrent disease (11), suggesting that potential inhibitors of this inflammation-related signature, such as an anti-inflammatory drug like aspirin, may prevent prostate cancer progression in African-American men. In agreement with the hypothesis, we reported that aspirin use at time of disease diagnosis was associated with fewer cases having advanced-stage prostate cancer and a lower risk of disease recurrence among African-American men in the NCI-Maryland Prostate Cancer Case–Control Study (13). There have been numerous studies investigating the association of regular aspirin intake with prostate cancer risk (14–17) and disease mortality and survival (18–21) among European-American men, with several reporting an association with reduced mortality, but few studies have included African-American men. Here, we pursued the hypothesis that use of aspirin prior to a disease diagnosis reduces prostate cancer risk and mortality among African-American men in the Southern Community Cohort Study (SCCS), a large cohort study that prospectively recruited low-income and predominately African-American participants to investigate the causes of cancer health disparities (22).

Materials and Methods

SCCS

SCCS focused on the recruitment of a low-income, predominantly African-American population from a 12-state area of the Southeast (22). Accordingly, 59% of the recruited African-American men came from households with less than $15,000 of annual household income and 21% from households with annual incomes between $15,000 and $25,000 at time of recruitment (23). Recruitment began in March 2002 and was completed in September 2009. Informed consent was obtained from all study participants, and the study was approved by the institutional review boards of the involved institutions. Participants were asked to complete an in-person interview at enrollment. About 85,000 men and women ages 40–79 years were recruited into this study. To obtain follow-up data on cancer development, procedures for data linkage, processing, and quality control were established with the 12-state cancer registries covering the SCCS catchment area (Alabama, Arkansas, Florida, Georgia, Kentucky, Louisiana, Mississippi, North Carolina, South Carolina, Tennessee, Virginia, and West Virginia). These registries provide the primary source of identifying incident cancer diagnoses and disease characteristics. Information on disease staging followed the 7th edition of the American Joint Committee on Cancer (AJCC) tumor–node–metastasis (TNM) system for clinical stage, abbreviated as I–IV. We

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Note: Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (http://cebp.aacrjournals.org/).

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defined aggressive prostate cancer as stage IV (T4) or N1 or M1 by AJCC, or Gleason score ≥ 8 (high grade), as described previously (24). Cohort member deaths were identified through annual linkages with both the Social Security Administration and the National Death Index (NDI). For this study, SCCS state cancer registries reporting was completed through December 31, 2016 and NDI reporting through December 31, 2018. We examined the SCCS dataset for all self-reported African-American men ($n = 22{,}426$). Of these men, 1,058 developed prostate cancer. During the study follow-up (median, 13 years; maximum, 16.8 years), a total of 6,627 deaths occurred, including 103 prostate cancer–related deaths as defined by International Classification of Diseases 10th criteria using NDI data. All participants provided written informed consent for the studies that were conducted. The research followed the ethical guidelines set by the Declaration of Helsinki.

Assessment of aspirin use

We pursued two exposures of interests, any aspirin use and regular strength aspirin use. The SCCS baseline survey evaluated aspirin use with the following questions: (i) in the past year, have you taken the following medication regularly? By regularly, we mean at least two times per week for 1 month or more: regular aspirin (such as Anacin, Bayer, Bufferin, Excedrin, etc.) with no or yes; low-dose aspirin, baby aspirin, or half tablets of aspirin with no or yes. (ii) How many years have you taken this type of medication regularly? Number of years was calculated. (iii) When you took this regularly, what is the average number of pills you took per week? Number of pills was calculated. A total of 97.4% of the participants (21,851/22,426) answered the questions on aspirin use at enrollment. We created aspirin use categories on the basis of question (i). The use of regular aspirin was defined as "regular strength aspirin use" in the study, whereas "any aspirin use" was defined as taking either regular aspirin, low-dose or baby aspirin, or half tablets of aspirin. Use was then categorized as either "no" or "yes." For all analyses, nonusers of aspirin were men who did not report any aspirin use. The aspirin category was further categorized as either "taken more than seven pills per week" or "taken seven pills per week or less" based on question (iii). Duration of aspirin use was based on question (ii). Duration was then categorized as either "less than or equal to 3 years," or "more than 3 years," as done previously (13).

Statistical analysis

Cox proportional hazards regression models with age as the time scale were used to calculate HRs for disease risk and disease-specific mortality. Our exposures of interest were any aspirin use and regular strength aspirin use. Each exposure variable was separately compared with nonusers. Models to assess disease risk and mortality contained the same covariates [age, year of enrollment (categorical), education (without high school degree, high school degree, college degree, and graduate degree), household income (<$15,000, $15,000–25,000, >$25,000–50,000, >$50,000–100,000, and >$100,000), family history of prostate cancer including father and brother (yes/no), smoking status (current, former, and never), diabetes (yes/no), body mass index (BMI; as six categories <18.5, 18.5–25, 25–30, 30–35, 35–40, and >40 kg/m^2), benign prostate hyperplasia (BPH) (yes/no), prostate cancer screening by PSA test (yes/no) and digital rectal exam (DRE; yes/no), acetaminophen (yes/no), and other NSAID use (yes/no)]. In the analysis of disease risk, men contributed follow-up time from enrollment until incident cancer, death, or last follow-up. We performed additional secondary analyses after grouping patients by disease stage (TNM I/II vs. III/IV), by Gleason score [≤7 (low/medium grade) vs. ≥8 (high grade)], and by disease aggressiveness (T4 or N1 or M1 or Gleason score ≥ 8). In these stratified analyses, the other cases were removed (e.g., when data for early-stage disease were analyzed, cases with late-stage disease were removed). Models were adjusted for potential confounders, as aforementioned. Individuals who did not answer the aspirin survey questions or with missing values for the two exposure variables were excluded from the analysis. Missing data for covariates were imputed as mean values. Because PSA levels were measured in a subset of men at baseline, we compared these PSA levels between aspirin users and nonusers to evaluate an aspirin effect.

In the mortality analysis, we compared the risk of fatal prostate cancer by aspirin use among men without prostate cancer at enrollment. Men contributed follow-up time from enrollment until death or last follow-up at December 31, 2018. Deaths from causes other than prostate cancer were censored. Subdistribution HRs (SHR) were calculated using Fine and Grey regression to examine the impact of aspirin use on the cumulative probability of prostate cancer–related death in the presence of competing events.

Tests for trend related to frequency and duration of aspirin use were performed by calculating P values in regression models, with aspirin use coded as an ordinal variable. Data analysis was performed using the R statistical software, version 3.6.0. All statistical tests were two-sided. An association was considered statistically significant with $P < 0.05$.

Data availability

The data underlying this article were obtained through a data access agreement with the SCCS (https://www.southerncommunitystudy.org/research-opportunities.html). These and derived data generated in this research will be shared in agreement with data access rules set by the SCCS and can be obtained from the corresponding author upon request.

Results

Demographics and clinicopathologic features

The study recruited 22,426 African-American men, of whom 1,058 developed prostate cancer on follow-up. At enrollment, 5,486 men (25.1%) reported taking any aspirin and 2,634 men (12.1%) reported taking regular strength aspirin. Characteristics of the African-American men by aspirin use are shown in **Table 1**. The median age at enrollment was 49 (interquartile range, 11) years, with a median follow-up time of 13 years. Baseline characteristics of men who used or did not use aspirin differed significantly with respect to age, education, and smoking status (**Table 1**). Aspirin users were more likely to have an elevated BMI (28.5 vs. 26.1 for any aspirin and 28.1 vs. 26.1 for regular strength aspirin), a history of diabetes (33.8% vs. 12.9% for any aspirin and 27.5% vs. 12.9% for regular strength aspirin), BPH (9.4% vs. 3.8% for any aspirin and 7.2% vs. 3.8% for regular strength aspirin), or family history of prostate cancer (6% vs. 4.3% for any aspirin and 5.9% vs. 4.3% for regular strength aspirin). Among men who were diagnosed with prostate cancer, 120 (11.3%) men with disease stage information presented with an advanced-stage disease (TNM III/IV) and 169 (16%) with an aggressive disease (T4 or N1 or M1 or Gleason score ≥ 8).

Aspirin use and prostate cancer risk

In the multivariable-adjusted Cox regression analysis, aspirin use at enrollment was not associated with prostate cancer risk [HR, 1.07; 95% confidence interval (CI), 0.92–1.25 for any aspirin use and HR, 0.97; 95% CI, 0.78–1.19 for regular strength aspirin use; **Table 2**]. Associations remained null when examined by frequency of use (HR, 1.03; 95% CI, 0.77–1.39 for less than daily use and HR, 1.07; 95% CI, 0.91–

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Table 1. Baseline characteristics of African-American men in the SCCS by aspirin use.

	No aspirin		Any aspirin		Regular strength aspirin	
	(*n*)	(%)	(*n*)	(%)	(*n*)	(%)
Total	16,365	74.9[a]	5,486	25.1	2,634	12.1
Age median (IQR)	48 (10)		53 (13)		52 (12)	
BMI median (IQR)	26.1 (7)		28.5 (7.8)		28.1 (7.8)	
Education						
Less than high school	5,526	33.8[b]	1,823	33.2	913	34.7
High school	6,903	42.2	2,028	37.0	991	37.6
College	2,751	16.8	992	18.1	471	17.9
More than college	1,085	6.6	548	10.0	227	8.6
Household income						
<15,000	10,138	61.9	2,900	52.9	1,477	56.1
15,000–25,000	3,453	21.1	1,176	21.4	539	20.5
25,000–50,000	1,840	11.2	855	15.6	386	14.7
50,000–100,000	620	3.8	381	6.9	156	5.9
>100,000	153	0.9	113	2.1	38	1.4
Family history of prostate cancer						
No	15,606	95.4	5,123	93.4	2,460	93.4
Yes	703	4.3	331	6.0	156	5.9
Tobacco use						
Current	10,017	61.2	2,457	44.8	1,343	51.0
Former	2,814	17.2	1,640	29.9	701	26.6
Never	3,475	21.2	1,335	24.3	559	21.2
Diabetes						
No	14,242	87.0	3,629	66.2	1,906	72.4
Yes	2,112	12.9	1,853	33.8	725	27.5
BPH						
No	15,689	95.9	4,936	90.0	2,432	92.3
Yes	627	3.8	516	9.4	189	7.2
PSA screening						
No	9,076	55.5	2,067	37.7	1,180	44.8
Yes	6,402	39.1	3,109	56.7	1,299	49.3
DRE screening						
No	7,611	46.5	1,760	32.1	978	37.1
Yes	8,666	53.0	3,681	67.1	1,636	62.1

Abbreviation: IQR, interquartile range.
[a]Percentage of all men in study ($n = 21,851$).
[b]Percentage of men within aspirin group [no aspirin ($n = 16,365$), any aspirin ($n = 5,486$), and regular strength aspirin ($n = 2,634$)] for all listed variables. Missing data not included in percentages.

1.26 for daily use; $P_{trend} = 0.41$) and duration of use (HR, 1.07; 95% CI, 0.89–1.28 for ≤3 years of use and HR, 1.05; 95% CI, 0.84–1.30 for >3 years of use; $P_{trend} = 0.45$). No significant association was observed between any aspirin use and risk of advanced-stage, high-grade, or aggressive disease. Only use of regular strength aspirin showed a suggestive association with a reduced risk of advanced-stage disease (HR, 0.70; 95% CI, 0.34–1.41), but this association was not statistically significant.

Aspirin use and disease-specific mortality

In this analysis, we assessed the risk of fatal prostate cancer related to baseline aspirin use among men without prostate cancer. During follow-up, 103 men developed fatal prostate cancer. In the multivariable-adjusted Cox regression analysis, aspirin use at enrollment tentatively associated with a reduced prostate cancer mortality (HR, 0.66; 95% CI, 0.39–1.14 for any aspirin use and HR, 0.41; 95% CI, 0.17–1 for regular strength aspirin use; **Table 3**). There was not much of an influence of competing risks of death on the risk of fatal prostate cancer, as shown by the SHRs in the Fine–Gray competing risk regression model (**Table 3**).

Discussion

Men of African ancestry are a high-risk population for prostate cancer and have an excess risk of developing lethal disease (1, 2, 25, 26). Using the NCI-Maryland Prostate Cancer Case–Control Study, we reported previously that intake of aspirin at diagnosis was inversely associated with advanced-stage prostate cancer and disease recurrence among these men (13). Here, we extended this study and examined the relationship between self-reported aspirin use at enrollment in SCCS and prostate cancer risk and mortality among African-American men. We did not observe an association between aspirin use and prostate cancer risk, but aspirin use tended to be associated with a lower prostate cancer mortality. Our observations are plausible as recent mechanistic observations and epidemiologic data showed that aspirin could have promising effects on reducing metastasis and cancer mortality (27, 28).

This is the first study that specifically investigated the relationship between aspirin use and prostate cancer mortality among African-American men using a prospective design. SCCS recruited both African-American and European-American men from a predominantly

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Table 2. Associations between aspirin use at enrollment and prostate cancer risk among African-American men in SCCS.

	Events	PY	HR (95% CI)[a]	*P*	HR (95% CI)[b]	*P*
Overall						
No use	690	162,857	Reference		Reference	
Any use	332	50,057	1.11 (0.97–1.27)	0.14	1.07 (0.92–1.25)	0.40
Regular strength	136	25,091	1.01 (0.84–1.21)	0.95	0.97 (0.78–1.19)	0.75
Less than daily	54	10,934	1.00 (0.75–1.32)	0.97	1.03 (0.77–1.39)	0.84
Daily and more	269	37,850	1.13 (0.98–1.31)	0.09	1.07 (0.91–1.26)	0.42
					P_{trend}	0.41
≤3 years	189	30,283	1.09 (0.92–1.28)	0.31	1.07 (0.89–1.28)	0.48
>3 years	135	18,417	1.12 (0.93–1.36)	0.24	1.05 (0.84–1.30)	0.68
					P_{trend}	0.45
TNM stage I/II						
No use	420	162,857	Reference		Reference	
Any use	214	50,057	1.12 (0.94–1.32)	0.21	1.03 (0.85–1.26)	0.74
Regular strength	90	25,091	1.06 (0.84–1.34)	0.61	0.98 (0.75–1.28)	0.88
TNM stage III/IV						
No use	90	162,857	Reference		Reference	
Any use	30	50,057	0.83 (0.54–1.28)	0.40	1.00 (0.63–1.60)	0.98
Regular strength	9	25,091	0.54 (0.27–1.07)	0.08	0.70 (0.34–1.41)	0.31
Gleason ≤ 7						
No use	404	162,857	Reference		Reference	
Any use	181	50,057	1.08 (0.90–1.29)	0.41	0.95 (0.77–1.17)	0.64
Regular strength	76	25,091	0.98 (0.77–1.26)	0.88	0.90 (0.68–1.20)	0.48
Gleason ≥ 8						
No use	86	162,857				
Any use	44	50,057	1.15 (0.79–1.68)	0.46	1.36 (0.89–2.08)	0.15
Regular strength	16	25,091	0.93 (0.54–1.59)	0.78	0.95 (0.51–1.75)	0.87
Aggressive[c]						
No use	116	162,857	Reference		Reference	
Any use	53	50,057	1.04 (0.74–1.46)	0.80	1.17 (0.80–1.71)	0.42
Regular strength	19	25,091	0.82 (0.51–1.34)	0.44	0.84 (0.49–1.46)	0.54

Abbreviation: PY, person-years.
[a]HR was adjusted for age.
[b]HR was adjusted for age, enrollment year, education, income, family history of prostate cancer, smoking status, diabetes, BMI, BPH, PSA and DRE screening, acetaminophen, and NSAIDs other than aspirin.
[c]T4 or N1 or M1 or Gleason score ≥ 8.

low-income background. Because only 22 of the European-American men progressed into fatal prostate cancer (vs. 103 African-American men), we did not examine the relationship of aspirin use with prostate cancer mortality among these men. There have been previous reports showing that regular aspirin intake may reduce the risk of prostate cancer (14–17), although a robust protective relationship may only exist with the aggressive disease (29–31) and disease mortality and survival (18–20). Nonetheless, data for men of African ancestry remain sparse. Hurwitz and colleagues investigated the relationship of aspirin use with prostate cancer in the Atherosclerosis Risk in Communities Study, a prospective study that included 5,060 European-American men and 1,534 African-American men (19). In this cohort, aspirin use was inversely associated with prostate cancer mortality, but did not associate with disease incidence, which is consistent with our findings in SCCS. An additional race-stratified analysis, although limited by the relative low number of African-American men who participated in this study, showed a suggestive protective effect of aspirin against prostate cancer mortality among the African-American men (adjusted HR, 0.41; 95% CI, 0.14–1.20). This observation is again consistent with our findings in this study.

Table 3. Association between aspirin use at enrollment and prostate cancer mortality among African-American men in SCCS.

	Events	PY	HR (95% CI)[a]	*P*	HR (95% CI)[b]	*P*	SHR (95% CI)	*P*
No aspirin use	74	2,368,439	Reference		Reference		Reference	
Any aspirin use[c]	25	744,964	0.65 (0.41–1.03)	0.07	0.66 (0.39–1.14)	0.14	0.69 (0.39–1.25)	0.22
Regular strength	6	369,317	0.36 (0.16–0.84)	0.02	0.41 (0.17–1.00)	0.05	0.43 (0.16–1.13)	0.09

Abbreviation: PY, person-years.
[a]HR was adjusted for age.
[b]HR was adjusted for age, enrollment year, education, income, family history of prostate cancer, smoking status, diabetes, BMI, BPH, PSA and DRE screening, acetaminophen, and NSAIDs other than aspirin.
[c]Any aspirin use category also includes regular strength events.

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Aspirin may protect against lethal prostate cancer among African-American men by suppressing an immune inflammation signature in their cancerous prostate (7, 11, 12), as we hypothesize. Aspirin may also exert a more general protection by suppressing metastasis (32). Treating patients with prostate cancer with celecoxib, a selective cyclooxygenase-2 (COX2) inhibitor, did not confer a survival benefit in the STAMPEDE trial (33). Aspirin is thought to have cancer preventive activity by inhibiting the same pathway and prostaglandin synthesis. Yet, in contrast to COX2-specific inhibitors, aspirin irreversibly inhibits both COX1 and 2 activity by acetylation. Acetylation of COX1, which is the main enzyme activity in platelets, blocks the production of thromboxane A2. It has now been shown in an animal model of lung metastasis that aspirin inhibits the metastatic spread by blocking the formation of a metastatic intravascular niche that depends on platelet-derived thromboxane A2 (27), although comparable data for prostate cancer and bone metastasis are still missing. Still, such a mechanism would explain why aspirin may inhibit lethal prostate cancer more so than the localized disease, as observed in our study and the study by Hurwitz and colleagues (19). In addition to its ability to inhibit prostaglandin synthesis, aspirin can turn on the production of anti-inflammatory lipid mediators, lipoxins, which makes aspirin further distinct in function from other NSAIDs (34, 35). Finally, aspirin has additional anti-inflammatory actions that cannot be attributed to its ability to inhibit prostaglandin biosynthesis, such as blocking leukocyte trafficking to inflamed tissues. As such, aspirin may have unique cancer preventive activities and may distinctly inhibit metastasis and lethal cancer.

Aspirin is commonly used in the U.S. population, which is primarily for prevention of cardiovascular disease. Its usage increases among the elderly population (36). A recent survey reported an estimated use of about 50% among U.S. adults ages 45–75 years (37). The observation indicates that aspirin use can be underreported when enrollment data are used. Aspirin effects in cancer prevention have been linked to dosage and duration, although a dosage or duration effect for the relationship of aspirin use with the risk of lethal prostate cancer remains to be determined (18–20). We stratified aspirin into any aspirin use and regular strength aspirin use, but could not stratify further because of inadequate statistical power. Regular strength aspirin is usually a 325 mg dose of aspirin (38), whereas our any aspirin use group comprised additional users of low-dose aspirin (about 50%). Accordingly, we found that the inverse relationship of aspirin use with prostate cancer mortality is somewhat stronger among regular strength aspirin users than any aspirin users, consistent with a dose effect, however, the CIs largely overlapped and there was no significant difference between the two groups.

There are several limitations to our study. First, we did not assess the exact daily dose of aspirin beyond the number of tablets per day. Also, we did not collect information on the reasons why participants were taking aspirin, however, most aspirin use is for prevention of cardiovascular disease among the elderly in the United States and follows recommendations by primary care physicians (37). Second, we did not receive information on disease characteristics for all men with prostate cancer. Accordingly, we had missing data on disease stage and Gleason score for 25%–30% of the patients, limiting our ability to analyze the relationship between aspirin use and advanced disease in this study. Third, studies have reported that aspirin leads to lower blood PSA, leading to a potential underestimate of disease occurrence among aspirin users in the prostate cancer risk analysis (39). We observed a rather modest effect of aspirin use on PSA levels in SCCS, with a median PSA reduction of 9.1% among all men who were regular strength aspirin users and only a 2.5% reduction among men who were aspirin users at baseline and later developed prostate cancer (Supplementary Fig. S1). Fourth, to abrogate a possible confounding effect of access to healthcare on the relationship between aspirin use and prostate cancer mortality, we adjusted a series of factors, such as age, socioeconomic status (education and income), smoking status, medical history of diabetes, BMI, BPH, acetaminophen, and other NSAID use, and PSA and DRE screening, in the multivariable Cox regression model. In our mortality analysis, we cannot adjust for received prostate cancer therapy. However, other studies have reported rather modest differences in obtained primary prostate cancer treatment comparing aspirin users with nonusers (40), and our adjustments for socioeconomic status and PSA screening should capture differences in primary care and treatment, if they exist. Nevertheless, we cannot exclude residual confounding as an underlying factor for our observations. We could not adjust for cardiovascular risk factors or some medications as these data were not collected in SCCS. Finally, the mortality analysis was limited by a small number of events, which may have prevented us to observe a more definite relationship between aspirin use and a reduced prostate cancer mortality. Additional studies are needed to replicate and strengthen our findings.

Conclusions

Self-reported aspirin use associated with a decreased prostate cancer–specific mortality among African-American men in SCCS. Yet, uncertainty remains about the strength of this association. In contrast, our data did not indicate an association between aspirin use and the risk of developing the disease. Our observations suggest that aspirin for prevention should be further evaluated as an opportunity to decrease lethal prostate cancer in these men.

Authors' Disclosures

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Authors' Contributions

W. Tang: Data curation, software, formal analysis, validation, investigation, visualization, methodology, writing-original draft, project administration, writing-review and editing. **J.H. Fowke:** Conceptualization, resources, supervision. **L.M. Hurwitz:** Supervision, investigation, methodology, writing-review and editing. **M. Steinwandel:** Resources, data curation. **W.J. Blot:** Conceptualization, resources, data curation, supervision. **S. Ambs:** Conceptualization, supervision, funding acquisition, investigation, project administration, writing-review and editing.

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