AWARD NUMBER:	W81XWH-18-1-0588
TITLE:	A Precision Medicine Study of How Inflammation May Underlie the Excessive Burden of Prostate Cancer in Men of African Ancestry
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CONTRACTING ORGANIZATION:	The Geneva Foundation, Tacoma, WA
REPORT DATE:	October 2021
TYPE OF REPORT:	Annual Report
PREPARED FOR:	U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012
DISTRIBUTION STATEMENT:	Approved for Public Release; Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
October 2021	Annual Report	30Sep2020-29Sep2021

4. TITLE AND SUBTITLE

A Precision Medicine Study of How Inflammation May Underlie the Excessive Burden of Prostate Cancer in Men of African Ancestry

5a. CONTRACT NUMBER W81XWH-18-1-0588

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
The Geneva Foundation
917 Pacific Ave. # 600
Tacoma, WA 98402

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

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
Oncology, Cancer, Prostate Cancer

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	95	19b. TELEPHONE NUMBER *(include area code)*

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

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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 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. We also proposed to assess the genetic and ancestral basis of prostate cancer-associated inflammation using a genome-wide association approach. 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/Summary

We continued our research addressing all aims, tasks, and subtasks of the award. We are still experiencing the negative impact of the COVID-19 pandemic, yet less so than in 2020. NIH laboratory research continues to be restricted to follow a safe working environment. However, our laboratory is in the fortunate position that we can focus on data analysis when laboratory research cannot be conducted. For **Specific Aim 1**, all laboratory-based tasks have previously been completed. All current tasks relate to data analysis, data interpretation, presenting the data, and publishing the findings in the peer-reviewed scientific literature. We published two research papers (*JNCI* PMID: 34264335*, Cancers* PMID: 34439226) reporting the association of arachidonic acid metabolites, thromboxane B2 and PGE-M, with prostate cancer and disease survival. We published a related paper, in collaboration with investigators from the Southern Community Cohort Study (SCCS), showing that aspirin use at time of recruitment associates with a reduced prostate cancer mortality among the African-American men in SCCS (*CEBP* PMID: 33293340). This is of interest because aspirin inhibits the oncogenic signaling of

arachidonic acid metabolites. Furthermore, we have submitted a revised manuscript to *Nature Communications* reporting a serum proteomics signature that links suppression of tumor immunity to ancestry and lethal prostate cancer. This manuscript contains the key findings from our immune-oncology marker studies. Work has begun investigating the association of 24 circulating fatty acids with prostate cancer in Ghanaian, African-American and European-American men. We expect to publish the finalized findings in 2022. Preliminary findings are discussed in this report. For **Specific Aim 2**, we received all GWAS genotyping data, thus completed the laboratory-based tasks. We now received the delivery of a finalized data file with additional imputed data for missing genotypes from the bioinformatics group with the Cancer Genomics Research Laboratory (October 15, 2021). With these data in hand, we will proceed with the quantitative trait locus analysis to describe the genetic and ancestral influence on immune-oncology marker expression and blood levels of circulating fatty acids and how these germline genetic-related relationships may influence prostate cancer risks. We aim on finishing these analyses and to publish them in 2022. 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 have now been resolved. We could proceed with the sequencing tasks using the company, HudsonAlpha, as the service provider. However, we are still facing problems with the quality of the extracted nucleic acids. While HudsonAlpha delivered good quality RNA-seq data for 167 tumors, we are still struggling in obtaining good quality whole exome sequencing data for a similar number of tumors. Yet, we still anticipate finishing the analysis of the RNA-seq and whole exome sequencing data, and to submit manuscripts, in 2022.

Completed tasks and progress in the reporting period.

During the past 36 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, laboratory work and data acquisition has been completed for those aims. Thus, major milestones have been achieved.

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 has been completed. 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 (**Table 1**) and NCI-Ghana studies (**Table 2**). In total, about 3190 measurements (including blinded duplicates) were performed for each assay type (**Table 3**). For the 92 immune-inflammation markers, measurements of duplicates showed very small sample-to-sample variation (**Table 3**), 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.

Table 1. Characterstics of prostate cancer cases and population controls of NCI-MD Study used for the DoD research project

Demographics	Cases[a] All (n=846)	Cases[a] AA[b] (n=407)	Cases[a] EA[c] (n=439)	Population Controls All (n=846)	Population Controls AA (n=382)	Population Controls EA (n=464)
Age[d]						
Median (IQR[e]) in years	64 (11)	63 (11)	65 (11)	65 (12)	64 (10)	66.5 (13
BMI						
Mean(SD[f]) in kg/m^2	28.0 (4.7)	28.0 (5.2)	28.0 (4.3)	28.7 (5.2)	29.7 (5.5)	27.8 (4.5
Education, *N(%)*						
High school or less	304 (35)	191(47)	113 (26)	196 (23)	111(29)	85 (18)
Some college	249(29)	135 (33)	114 (26)	206 (24)	109 (29)	97 (21)
College	162 (19)	53 (13)	109 (25)	221 (26)	84 (22)	137 (30)
Graduate	130 (15)	27 (7)	103 (23)	222 (26)	77 (20)	145 (31)
Did not provide	1(<1)	1(<1)	-	1(<1)	1(<1)	
Baseline Health Factors						
Family history of prostate cancer[g], *N (%)*						
No	759 (90)	371 (91)	388 (88)	788 (93)	360 (94)	428 (92)
Yes	87 (10)	36 (9)	51 (12)	58 (7)	22 (6)	36 (8)
Smoking status[h], *N (%)*						
Current	199 (24)	133 (33)	66 (15)	113 (13)	70 (18)	43 (9)
Former	350 (41)	155 (38)	195 (44)	378 (45)	157 (41)	221 (48)
Never	292 (35)	116 (29)	176 (40)	346 (41)	152 (40)	194 (42)
Did not provide	5 (<1)	3 (<1)	2 (<1)	9 (1)	3 (<1)	6 (1)
Stage[i], *N(%)*						
T1	164 (19)	64 (16)	100 (23)			
T2	560 (66)	289 (71)	271 (62)			
T3	68 (8)	24 (6)	44 (10)			
T4	54 (6)	30 (7)	24 (5)			
Gleason score, *N (%)*						
≤7	702 (83)	338(83)	364 (83)			
>7	144 (17)	69 (17)	75 (17)			
Disease aggressiveness, *N (%)*						
Nonaggressive disease[j]	634 (75)	308 (76)	326 (74)			
Aggressive disease[k]	212 (25)	99 (24)	113 (26)			
PSA						
Median (IQR) in ng/ml	6.3 (5.8)	6.9 (7.6)	6 (4.8)	0.4 (0.6)	0.4 (0.6)	0.4 (0.6)

[a] Cases recruited within 2 years after disease diagnosis with an average interval between diagnosis and enrollment of 6.7 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] Cases with pathologically confirmed T1 or T2 and Gleason score ≤7
[k] Cases with pathologically confirmed T3 or T4 or Gleason score >7
[l] PSA: Prostate specific antigen

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 (**Table 3**), 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 (**Table 3**). 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.

Table 2. Characterstics of prostate cancer cases and population controls of NCI-Ghana Study used for DoD research project

Demographics	Cases (n=659)	Controls (n=659)
Age		
Median (IQR[a]) in years	70 (11)	59 (11)
BMI		
Mean(SD[b]) in kg/m^2	25.4 (4.6)	24.3 (4.4)
Education, *N(%)*		
Primary	92 (14)	146 (22)
Middle (junior secondary)	197 (30)	293 (44)
Secondary (senior secondary)	127 (19)	126 (19)
Higher	239 (36)	87 (13)
Did not provide	4 (<1)	7 (1)
Baseline Health Factors		
Smoking status[c], *N (%)*		
Current	16 (2)	95 (14)
Former	206 (31)	188 (29)
Never	427 (65)	348 (53)
Did not provide	10 (2)	28 (4)
Gleason score, *N (%)*		
≤7	415 (63)	
>7	205 (31)	
Did not provide	39 (6)	
PSA[d]		
Median (IQR) in ng/ml	44.2 (96)	0.98 (1.46)

[a]IQR: Interquartile range
[b]SD: Standard deviation
[c]Smoking status describes cigarette smoking
[d]PSA: Prostate specific antigen

Table 3: Completed Assays for Plasma Markers in the DoD Research Project

	Number of Analytes	# Analytes detected in all samples	# Analytes detected in 50% samples	QC criteria	Passed QC	Average CV (duplicates and across plates)	# cases	# controls	# total (with blinded duplicates)
Olink Immune-inflammation markers	92	61	78	Internal controls on each plate	95%	1.7% intra 2.6% inter	1520	1518	3195
OmegaQuant Omega-3 fatty acids	24	24	24	CLIA-certified assay at lab	100%	8.7%	1520	1518	3192
Leidos LPS assay	1		LPS detectable in 14% of samples	Random duplicates; added positive control samples	99.4%	Average 2.8%; 28% with high LPS	1520	1518	3190

With these data for the blood and urine markers in hand, Tsion Minas and Maeve Bailey-Whyte, two postdoctoral fellows, began to analyze them starting in the second half of 2019. The main analysis of the 82 immune-oncology markers has been completed and a manuscript is in revision with *Nature Communications*. More details will follow. Additional work with these data may generate another manuscript, yet with lower impact. The analysis of the urinary arachidonic acid metabolites has also been completed and two research papers were published. More details will follow. For the analysis of the 24 fatty acids, Tsion Minas has been joined by Brittany Lord, an NCI Cancer Prevention Fellow who arrived in the Ambs laboratory at the beginning of 2021. This project is still ongoing but should be completed in early 2022. More details will follow.

Immune-oncology marker studies

To address **Specific Aim 1**, **Major Task 2 &3**, Tsion concentrated her efforts on analyzing the immune-oncology markers and their association with African ancestry and prostate cancer. Her analyses included 82 of the 92 markers, excluding those that were detected in less than 20% of the study population.

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 (**Figure 1**). 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.

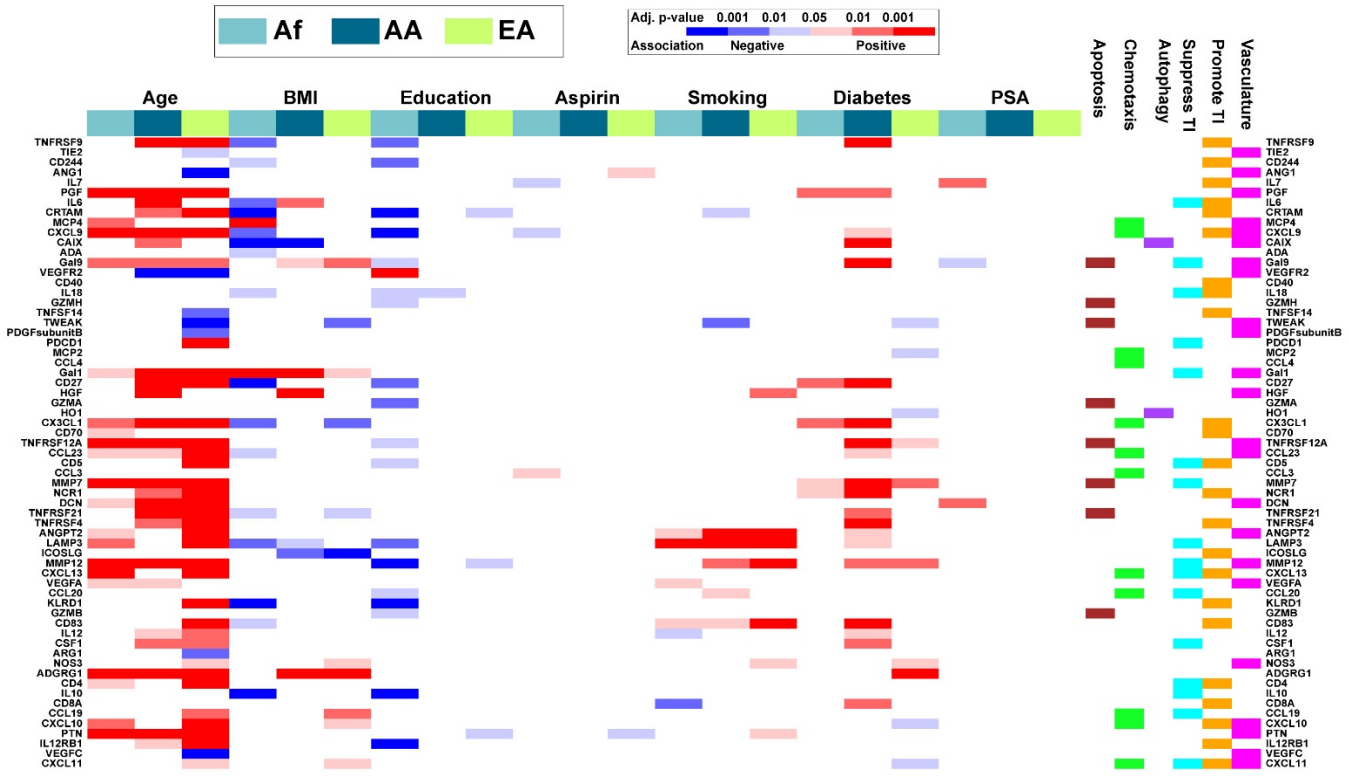


Fig. 1. Association of socio-demographic and clinical characteristics with systemic immune-oncological proteins in Ghanaian (Af), AA, and EA men without prostate cancer. The association of the 82 immuno-oncological proteins (as continuous variables) with age, BMI, education, aspirin use, smoking, diabetes, and PSA was assessed in men without prostate cancer using a multivariable linear regression model. An analyte was considered significantly associated with clinical and socio-demographic covariables if the multivariable model yielded an 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 negative association while red represents positive association. The significance level (FDR-adjusted *P* value-based) for each association is color-coded. TI = tumor immunity.

Aging is known to impact the immune system and is a risk factor for many diseases including cancer. 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 (**Figure 1**). For example, PGF, CXCL9, Gal9, Gal1, CX3CL1, TNFRSF12A, CCL23, MMP7, DCN, MMP12, ADGRG1, and PTN positively associated with age in all three population groups. 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. This finding may be surprising as obesity is generally thought to be associated with systemic inflammation. LAMP3 was inversely associated with BMI exclusively in men of African ancestry, suggesting that ancestral factors may influence the relationship between BMI and expression of this marker. LAMP3 is a member of lysosomal associated membrane glycoprotein family that have a myriad of roles including lysosomal exocytosis and cholesterol homeostasis. On the contrary, serum GAL1, a glycan binding protein that mediates the suppressive function of T_{Reg} cells, 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. Among European-American men, three of the 82 immune-oncological proteins had significant inverse relationships with the attained level of education, with two of these markers showing a similar pattern among Ghanaian and European-American men.

Previous studies have shown that tobacco smoking increases inflammation. 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 (**Figure 1**).

Innate immune system-driven inflammatory processes have been implicated in the pathogenesis of diabetes. 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 (**Figure 1**). Others, including PGF and CX3CL1 were positively associated with diabetes exclusively in men with African ancestry. CX3CL1 is known to regulate insulin secretion, is elevated in the serum of patients with type 2 diabetes, and has been implicated in diabetic nephropathy, validating the findings in our study.

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 European-American men while samples from African-American men tended to cluster in between these two groups

(**Figure 2**), suggesting that the ancestral background may have a significant impact on the global immune-oncological protein profile.

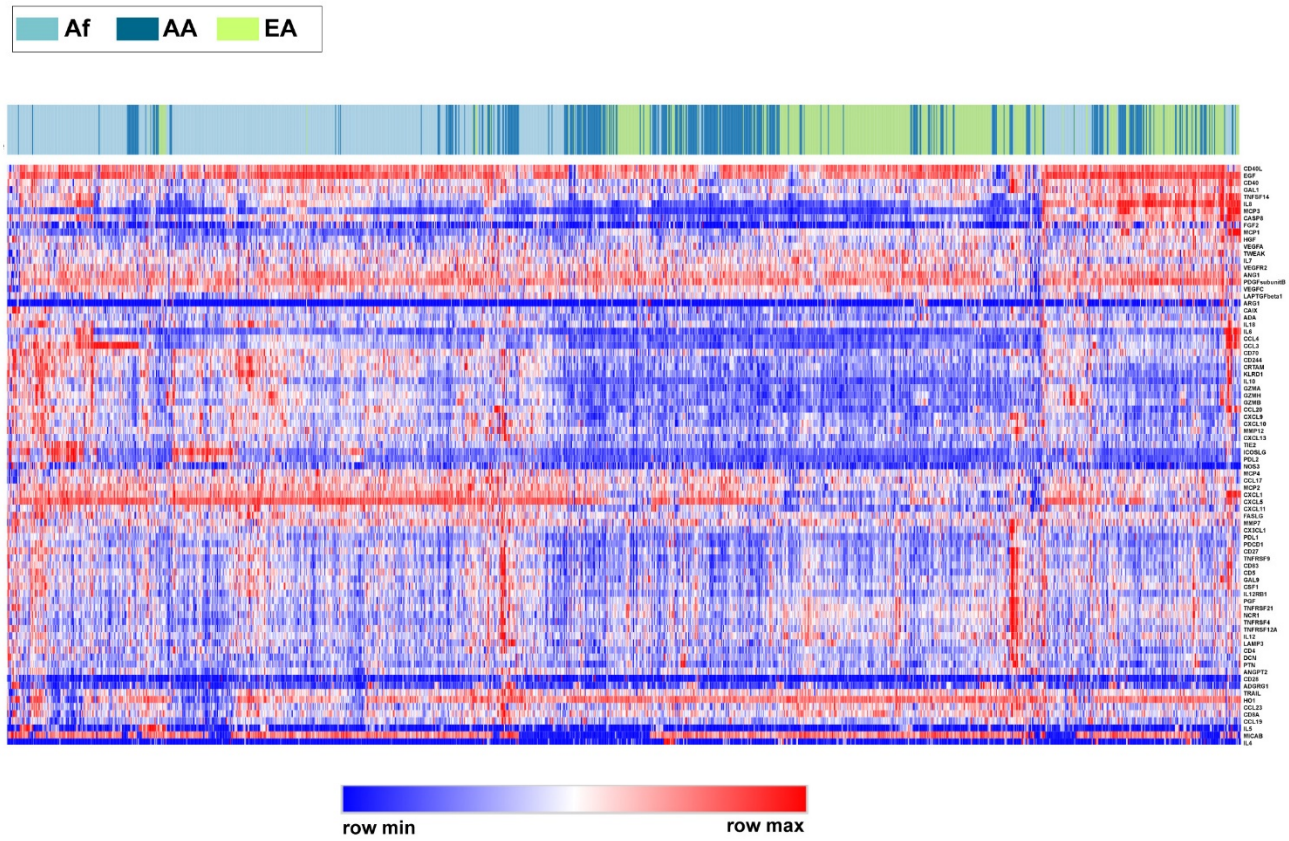


Fig. 2. Unsupervised hierarchical clustering associates circulating immune-oncological proteome profiles with population groups - Ghanaian (Af), AA, and EA men. Heatmap showing protein profiles for men without prostate cancer. Each row represents a protein (n=82), and each column corresponds to an individual [n=1482 (654 Af, 374 AA, and 454 EA)]. Each individual is color-coded as Af, AA, or EA in the annotation bar on top of the heatmap. Normalized z-score of proteins abundance are depicted on a low-to-high scale (blue-white-red).

To further evaluate the influence of ancestry, we estimated West African ancestry in African-American and European-American 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. 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 (**Figure 3A**). The variance in 39 of the analytes were significantly (FDR-adjusted P <0.05) influenced by degree of West African ancestry. 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. 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. For instance, 41% and 50% of the variance in the serum levels of CXCL1 and CXCL5, respectively, was accounted for by degree of West African ancestry (**Figure**

3A). When we compared levels of these proteins across the 3 population groups, we observed a significant West African ancestry-related trend (**Figure 3B**), 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 African-American men compared to European-American men; thirteen other markers (MCP1, IL12, CCL23, CD8A, NCR1, TNFRSF4, TNFSF14, TWEAK, IL7, HGF, HO1, TNFRSF21, and ANG1) were inversely related to West African ancestry.

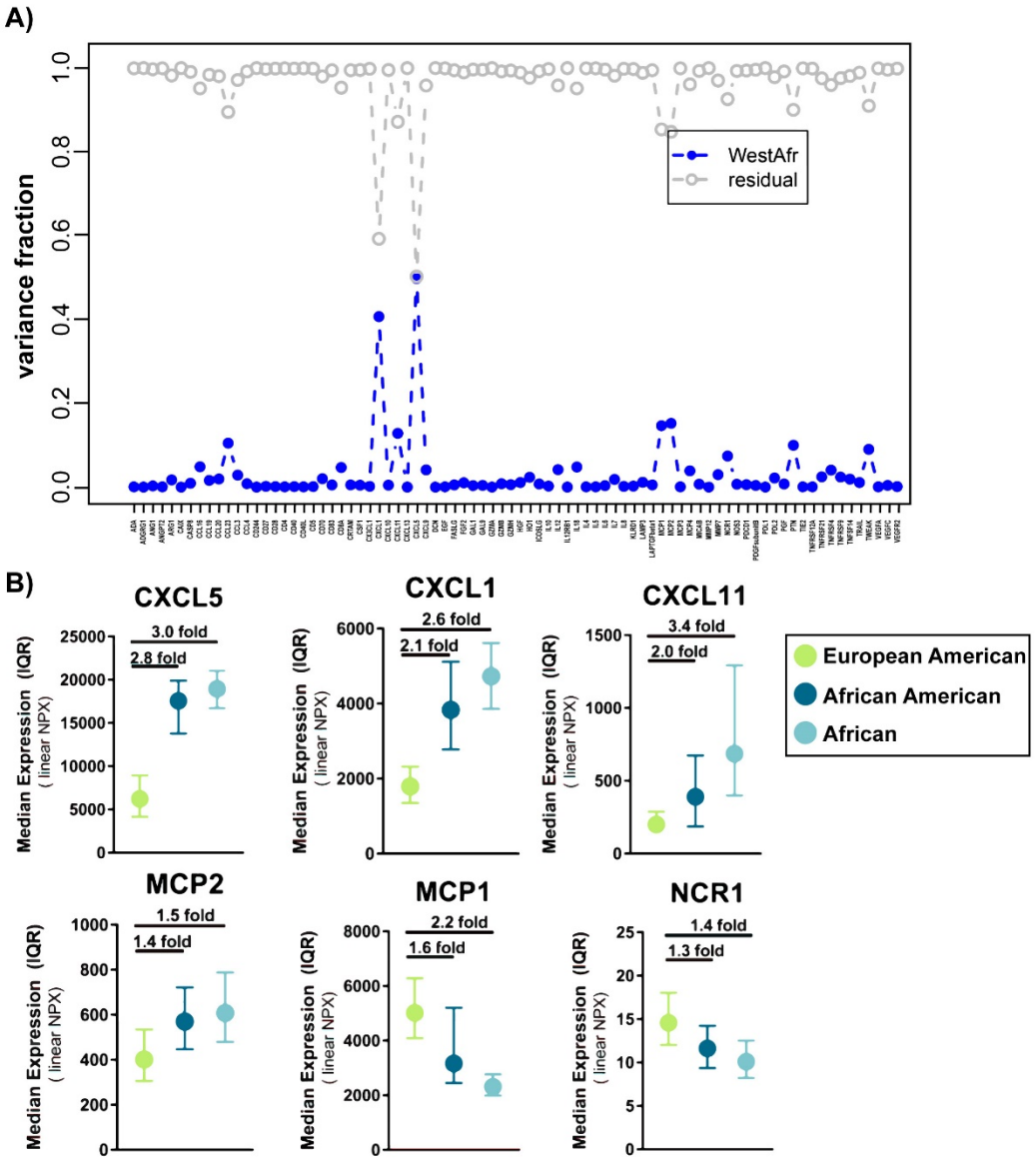


Fig. 3. Immune-oncological proteins and their relationship with West-African ancestry. (A) Variance analysis for the levels of each of the 82 immune-oncological cytokines assessed as a function of genetic estimation of West African admixture among men without prostate cancer within the NCI-Maryland study. 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 other factors other than West-African ancestry. **(B)** The median levels of the top six West-African ancestry correlated immune-oncological proteins were compared between Af, AA, and EA. Error bars represent inter quartile range (IQR). Linearized protein abundances (2^{NPX}) were used to determine median and IQR for each of the proteins.

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: 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 4**). 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 4**). 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 4C 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 5**). Prostate cancer patients with an increased activity of this pathway had the highest risk of death from all causes (**Figure 5A**). 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, although not statistically significant (**Figure 5B**). 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 5C**), indicating a more general predisposition to cancer in patients with a high suppression of tumor immunity score.

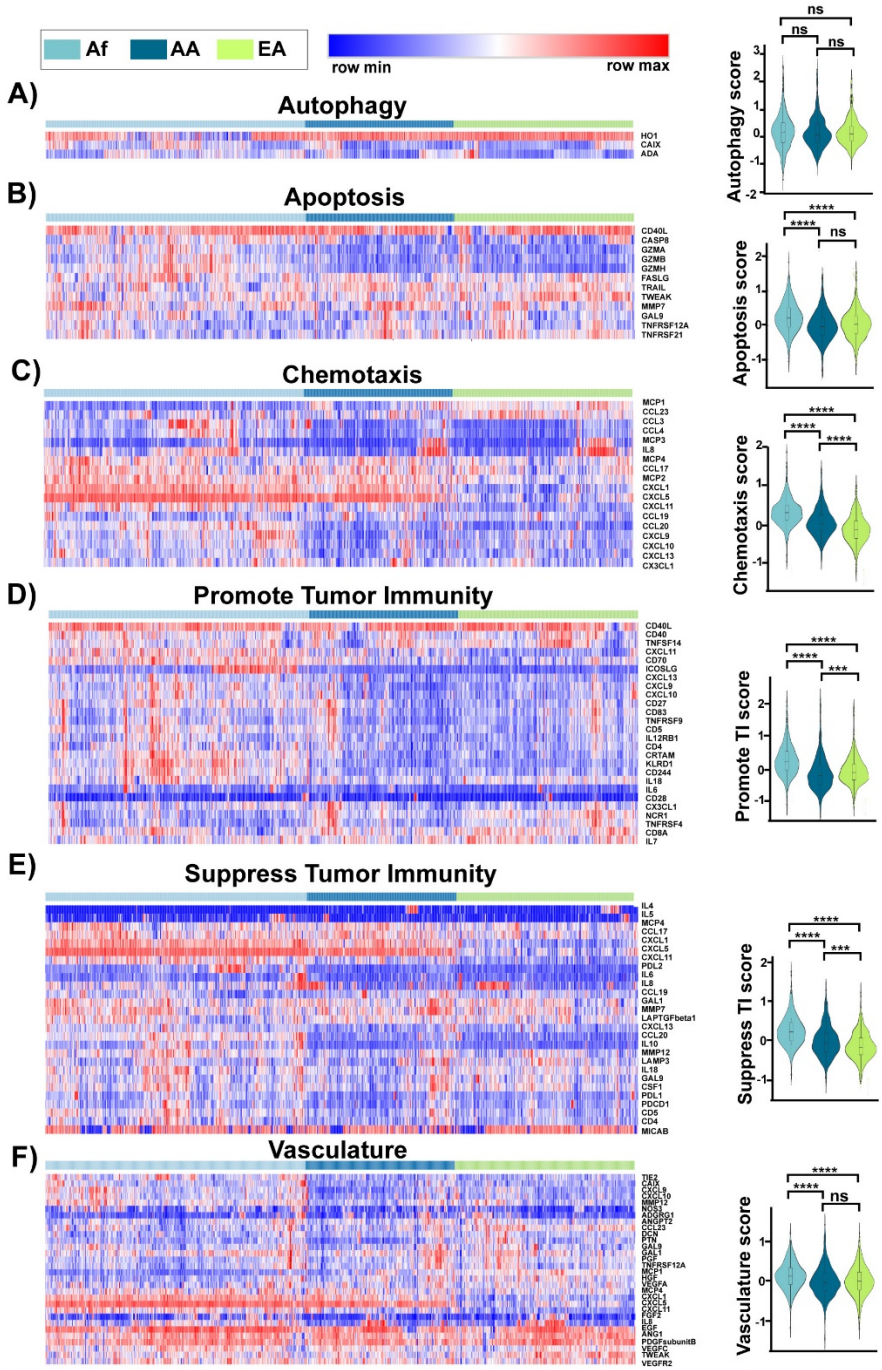


Fig. 4. Population differences in the abundance of proteins driving (A) autophagy, (B) apoptosis, (C) chemotaxis, (D) promotion of tumor immunity, (E) suppression of tumor immunity, and (F) vasculature. Heatmaps showing levels of process/pathway-associated proteins in relationship to population group [Ghanaian (Af), 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 > or = 0.05 (ns).

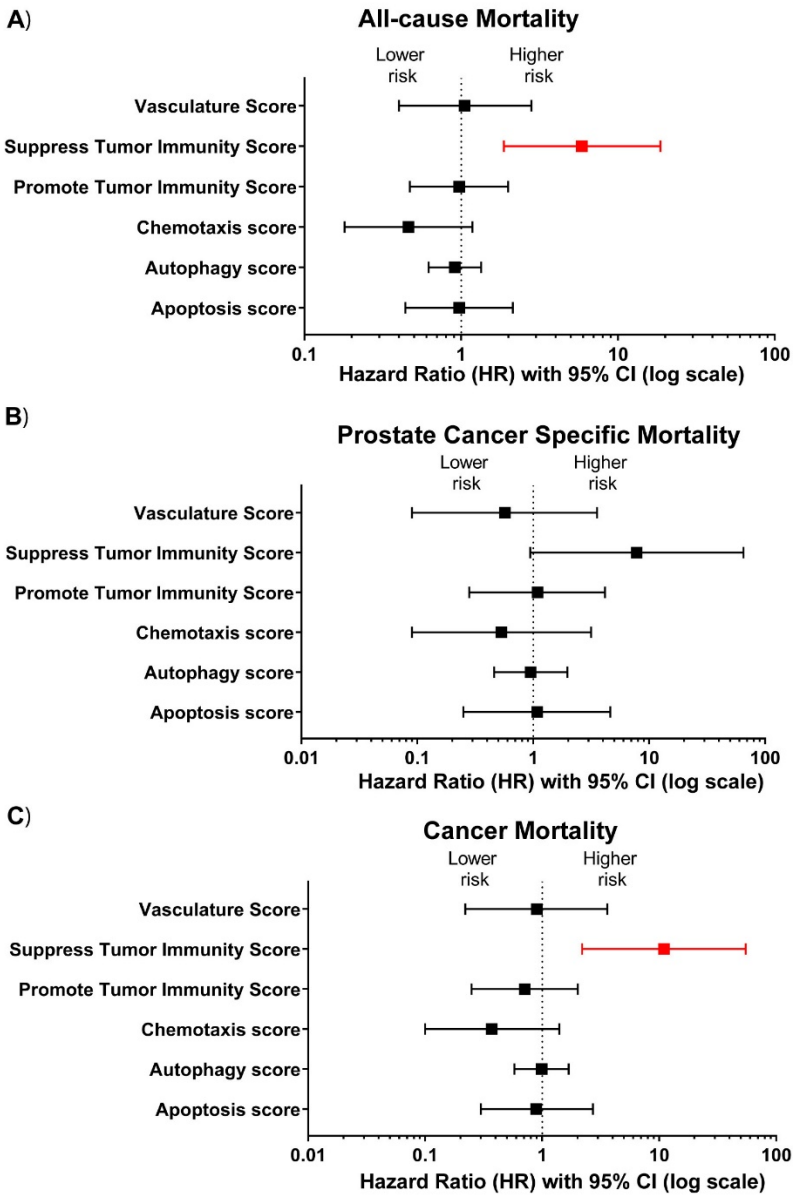


Fig. 5. Suppression of the tumor immunity pathway associates with lethal prostate cancer. We assessed the association of the six pathways defined by the 82 immune-oncology markers with all-cause mortality, prostate cancer-specific mortality or mortality due to any cancer after a prostate cancer diagnosis. 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. We adjusted for the following potential confounding factors: age at study entry (years), body-mass index (BMI, kg/m2), self-reported race (AA/EA), education (high school or less, some college, college, professional school), income (less than $10k, $10-30K, $30-60K, $60-90k, greater than $90k), smoking history (never, former, current), diabetes (no/yes), aspirin use (no/yes), and treatment (0=none, 1=surgery, 2=radiotherapy, 3=hormone, 4=combination). The hazard ratios (HR) 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 6A**) and the weight of the features' contribution to the prediction (**Figure 6B**). 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 (**Figure 7**). 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 6C**). 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 6C**), highlighting the utility of these blood markers for risk stratification of African-American prostate cancer patients.

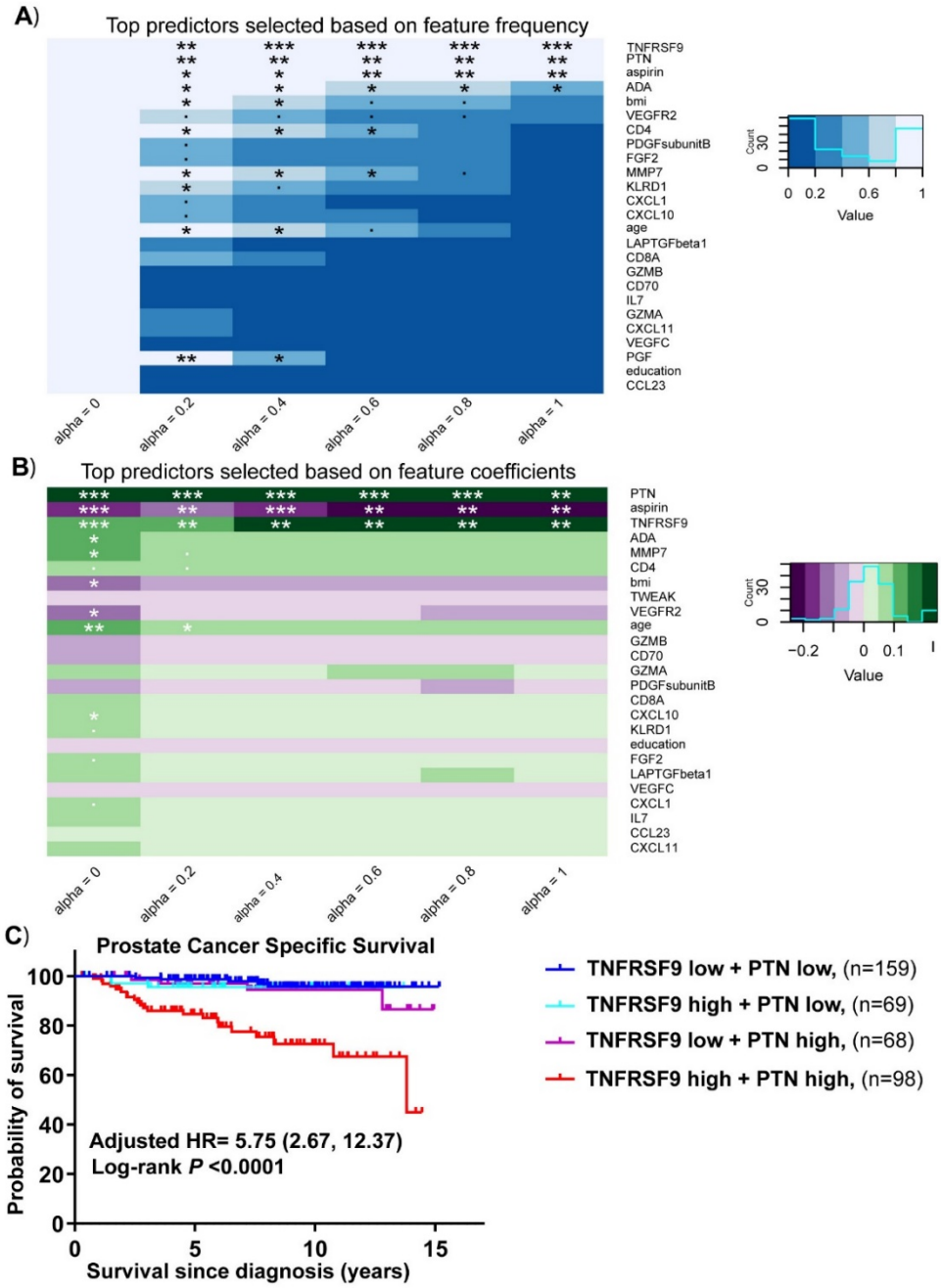


Fig. 6. A signature of two serum markers is predictive of lethal prostate cancer in AA patients. Cross-validated, regularized Cox regression models with different elastic net mixture parameters from ridge (alpha=0) to lasso (alpha=1) were implemented to identify a predictive proteomic signature. **(A)** 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 PTN (pleiotrophin) 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, smoking history, diabetes status, aspirin use, treatment, and income. In **A** & **B,** *P* value significance was coded as <0.001 (***), <0.01 (**), <0.05 (*), and <0.1 (.).

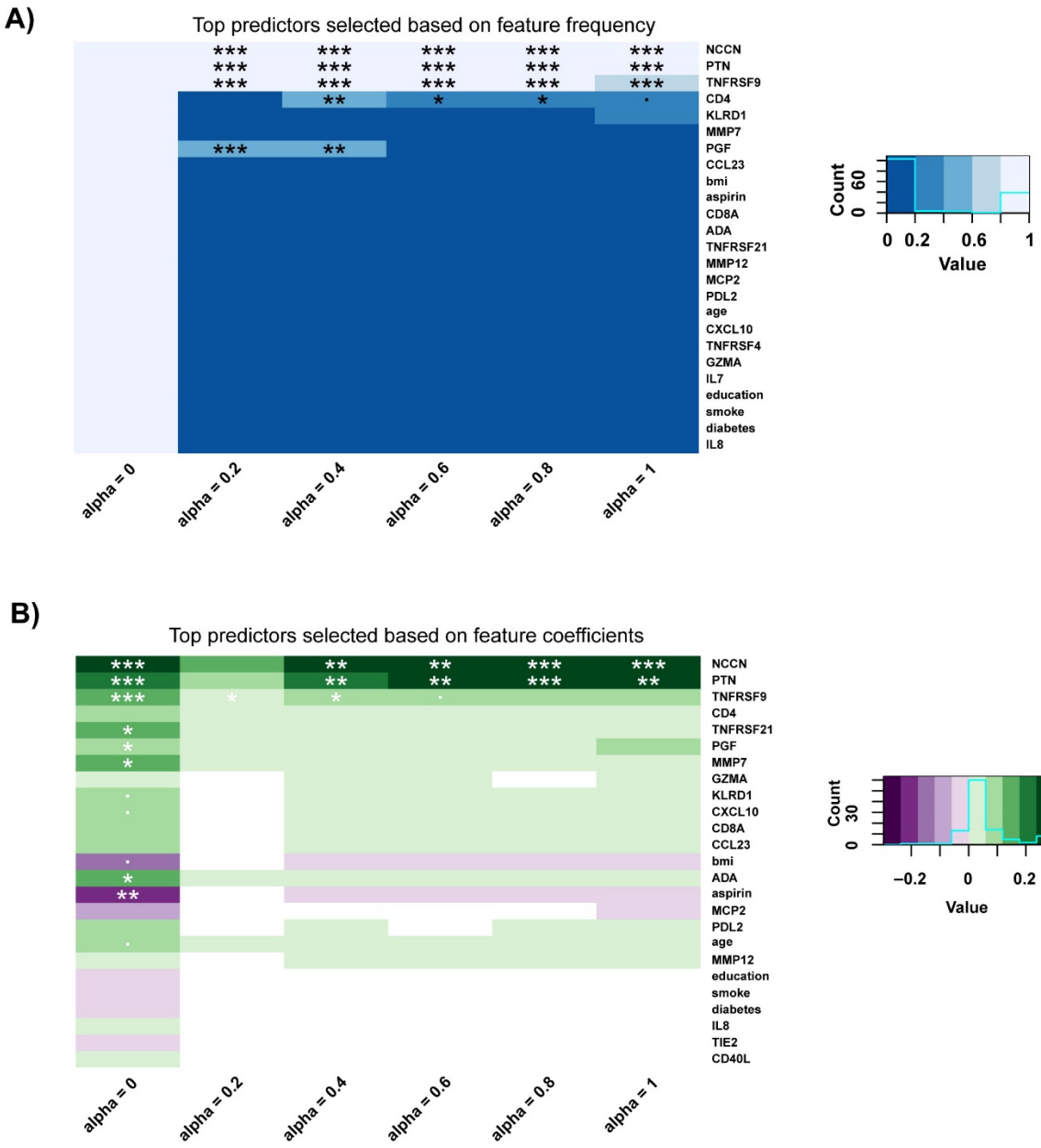


Fig. 7. NCCN and analytes PTN (pleiotrophin) and TNFRSF9/CD137/4-1BB are the most predictive features for lethal prostate cancer in AA patients. Cross-validated, regularized Cox regression models with different elastic net mixture parameters from ridge (alpha=0) to lasso (alpha=1) were implemented to 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. *P* value significance was coded as <0.001 (***), <0.01 (**), <0.05 (*), and <0.1 (.).

Urinary arachidonic acid metabolites study

The tasks described under **Specific Aim 1**, **Major Tasks 1-3**, also included the measurements of five urinary arachidonic acid metabolites (PGD-M, PGE-M, PGI-M, 11dTxB2, TNE) that are surrogates for cyclooxygenase (COX) signaling, a pro-inflammatory and oncogenic signaling pathway (**Figure 8**). These metabolites were only measured in the NCI-Maryland Study, as outlined in the statement of work, because urine was not collected in the NCI-Ghana study. The characteristics of the study population is shown in **Table 4**. We sent a total of 2131 samples to

Table 4. Characterstics of prostate cancer cases and population controls of NCI-MD Study used for the urine study

	Cases[a]			Population Controls		
Demographics	**All (n=977)**	**AA[b] (n=490)**	**EA[c] (n=487)**	**All (n=1,023)**	**AA (n=480)**	**EA (n=543)**
Age[d]						
Median (IQR[e]) in years	64 (11)	63 (10)	65 (11)	64 (12)	64 (10)	66 (13)
BMI						
Mean(SD[f]) in kg/m^2	28.0 (5.1)	28.0 (5.0)	28.0 (5.2)	28.8 (5.2)	29.0 (5.3)	28.6 (5.0)
Education, *N(%)*						
High school or less	353 (36)	227 (46)	126 (26)	243 (24)	138 (29)	105 (19)
Some college	295 (30)	167 (34)	128 (26)	261 (26)	140 (29)	121 (22)
College	173 (18)	58 (12)	115 (24)	256 (25)	103 (21)	153 (28)
Graduate	140 (14)	29 (6)	111 (23)	250 (24)	90 (19)	160 (30)
Did not provide	16 (2)	9 (2)	7 (1)	13 (1)	9 (2)	4 (1)
Baseline Health Factors						
Family history of prostate cancer[g], *N (%)*						
No	746 (76)	346 (71)	400 (82)	726 (71)	299 (62)	427 (79)
Yes	211 (22)	135 (27)	76 (16)	281 (27)	173 (36)	108 (20)
Did not provide	20 (2)	9 (2)	11 (2)	16 (2)	8 (2)	6 (1)
Smoking status[h], *N (%)*						
Current	240 (25)	164 (33)	76 (16)	150 (15)	96 (20)	54 (10)
Former	393 (40)	176 (36)	217 (44)	457 (44)	196 (41)	261 (48)
Never	320 (33)	137 (28)	183 (38)	396 (39)	178 (37)	218 (40)
Did not provide	24 (2)	13 (3)	11 (2)	20 (2)	10 (2)	10 (2)
Stage[i], *N(%)*						
T1	180 (18)	72 (15)	108 (22)			
T2	636 (65)	342 (70)	294 (61)			
T3	75 (8)	25 (5)	50 (10)			
T4	58 (6)	34 (7)	24 (5)			
Missing	28 (3)	17 (3)	11 (2)			
Gleason score, *N (%)*						
≤7	799 (81)	400 (82)	399 (82)			
>7	162 (17)	82 (16)	80 (16)			
Missing	16 (2)	8 (2)	8 (2)			
Disease aggressiveness, *N (%)*						
Nonaggressive disease[j]	722 (74)	368 (75)	354 (73)			
Aggressive disease[k]	240 (25)	114 (23)	126 (26)			
Missing	15 (1)	8 (2)	7 (1)			
PSA						
Median (IQR) in ng/ml	5.4 (3.5)	6.7 (5.1)	4.6 (3.8)			

[a] Cases recruited within 2 years after disease diagnosis with an average interval between diagnosis and enrollment of 6.7 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] Cases with pathologically confirmed T1 or T2 and Gleason score ≤7
[k] Cases with pathologically confirmed T3 or T4 or Gleason score >7
[l] PSA: Prostate specific antigen

the Eicosanoid Core Laboratory at Vanderbilt University to be analyzed by mass spectrometry using assays that have previously been validated at the facility (**Table 5**). The core facility measured these metabolites and then standardized the measurements to urinary creatinine content, which is determined by a separate colorimetric assay. A pilot study that we conducted

Table 5. Summary of Samples Sent for Analysis		
Number of Patient Samples (N)		2000
Number of Duplicates (N)	Pilot Study	22
	Duplicates in Main Study	107
Negative Controls (N)		2
Total of samples sent for analysis (main study + pilot study)		2131

with 22 blinded duplicates indicated that duplicate measurements would have the expected CV of 10-15%. However, we had a much higher variability in our duplicate measurements across the 2131 samples. An investigation revealed that the cause was a high variability in the measurement of the urinary creatinine content, using the colorimetric assay. Having this clarification, the Eicosanoid Core Laboratory re-measured urinary creatinine in all urine samples, using an improved protocol, and provided us with the new data on March 30, 2020. The new measurements showed significantly lower variability in duplicate measurements, in agreement with previous quality control data at this facility and in our pilot study. Yet, we decided to restrict our analysis for research papers to two of the five measured metabolites, prostaglandin E-metabolite (PGE-M) and thromboxane B2 (TXB2), because their measurements showed the best quality control performance whereas the other three metabolites had either many missing data or higher variability in their measurements, or both. Thus, we were not sure about the reliability of these data.

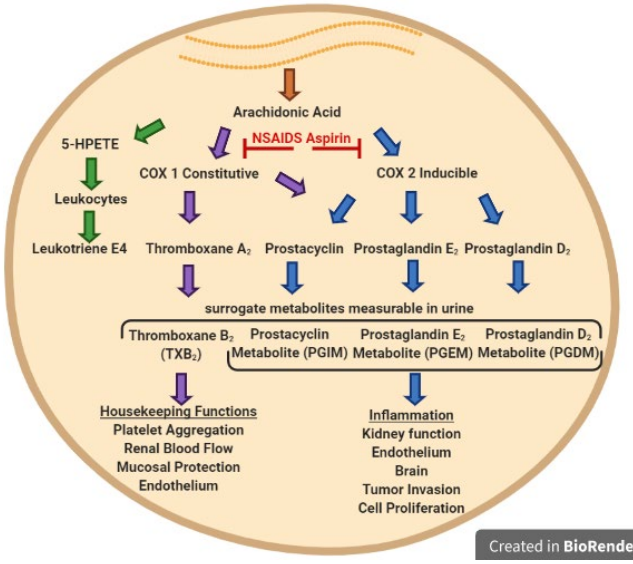
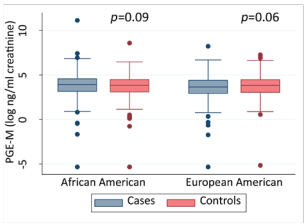


Figure 8. Cyclooxygenase (COX) pathway is one of the key mechanisms driving the association of inflammation and cancer

Dysregulated COX2 signaling and elevated PGE-M, reflecting *in vivo* PGE2 biosynthesis, have been associated with a risk of cancer in many sites including gastric, colon and breast, and breast cancer metastasis to the lung. This pattern is not universally observed, however, and a null association has been reported for ovarian cancer. Aspirin can inhibit PGE-M levels, and urinary PGE-M has been suggested as a biomarker of aspirin effectiveness in the prevention of disease recurrence. Aspirin also exhibits chemo preventive effects against aggressive prostate cancer in high-risk populations and lethal prostate cancer in general. Thus, we hypothesized that elevated urinary PGE-M levels are associated with adverse survival outcomes in men with prostate cancer and that aspirin use may influence these survival outcomes. Analyzing the relationship of PGE-M with prostate cancer, we did not find robust differences in urinary PGE-M

levels between men with prostate cancer (cases) and controls (**Figure 9A**). Additionally, we did not observe an association between elevated PGE-M and prostate cancer or metastatic disease as defined by the NCCN risk score groupings (**Figure 9B-C**). We also could not find an association with prostate cancer-specific mortality (**Figure 9D**). However, we detected a significant association between elevated PGE-M and increased all-cause mortality in African-American men with prostate cancer when they did not take aspirin (**Table 6**). This association

A



B

	Odds of Case Status *N*, (%)								
	All Cases			African American			European American		
PGE-M	Control	Case	OR (95% CI) *	Control	Case	OR (95% CI) *	Control	Case	OR (95% CI) *
≤ Median[a]	499 (50)	482 (51)	Reference	231 (50)	216 (46)	Reference	268 (50)	266 (56)	Reference
> Median	499 (50)	466 (49)	0.86 (0.72–1.04)	234 (50)	256 (54)	1.0 (0.75–1.31)	265 (50)	210 (44)	0.76 (0.59–0.99)

C

NCCN Risk Score	OR (95% CI) *	*p* Value
Low	Reference	
Intermediate	0.96 (0.65–1.43)	0.85
High/Very High	1.13 (0.71–1.81)	0.60
Regional/Metastatic	0.93 (0.44–1.97)	0.85

D

	PGE-M	Alive	Death from PC	Death from Other Cause	HR (95% CI)	HR (95% CI) †	Alive*	Death from PC*	Death from Other Cause*	HR (95% CI) *
All Cases	≤ Median[a]	388 (53)	28 (42)	80 (44)	Reference	Reference	375 (53)	27 (42)	78 (45)	Reference
	> Median	339 (47)	38 (58)	100 (56)	1.47 (0.90–2.41)	1.39 (0.85–2.28)	327 (47)	38 (58)	96 (55)	1.46 (0.86–2.46)
African American	≤ Median[a]	172 (49)	15 (38)	36 (38)	Reference	Reference	165 (49)	15 (38)	35 (37)	Reference
	> Median	178 (51)	25 (63)	60 (63)	1.43 (0.75–2.70)	1.33 (0.70–2.52)	171 (51)	25 (62)	59 (63)	1.49 (0.75–2.98)
European American	≤ Median[a]	216 (57)	13 (50)	44 (52)	Reference	Reference	210 (57)	12 (48)	43 (54)	Reference
	> Median	161 (43)	13 (50)	40 (48)	1.28 (0.58–2.81)	1.16 (0.53–2.56)	156 (43)	13 (52)	37 (46)	1.30 (0.54–3.14)

[a] median cutoff point is 14.22 ng PGE-M per mg creatinine.
*Unconditional Cox regression adjusted for age at study entry, BMI (kg/m2), diabetes (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 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), Gleason score (0 = Gleason ≤ 7 and 1 = Gleason > 7) and stratified by aspirin (no/yes).

Figure 9. (A) Urinary PGE-M levels do not differ between cases and controls in African American and European American men. (B) Urinary PGE-M levels do not associate with prostate cancer (C) High urinary PGE-M does not associate with NCCN risk groups. (D) Urinary PGE-M levels don't associate with prostate cancer-specific mortality among prostate cancer patients.

was not observed in African-American men who had reported aspirin use, suggesting that aspirin may reduce all-cause mortality for African-American men with prostate cancer. The observation is potentially of clinical importance as these results support a role for aspirin as a chemo preventive agent against mortality in men with prostate cancer. However, the hypothesis that the chemo preventive mechanism of action of aspirin is via PGE-M in prostate cancer is not supported by this study, as we did not find that aspirin use inhibited PGE-M

	All Cases			African American				European American		
PGE-M	Alive	Dead	HR (95% CI) *	Alive	Dead	HR (95% CI) *	P hetero-geneity	Alive	Dead	HR (95% CI) *
Aspirin use										
≤ Median	182 (53)	62 (50)	Reference	61 (43)	25 (42)	Reference	0.04	121 (60)	37 (56)	Reference
> Median	162 (47)	63 (50)	0.92 (0.63–1.34)	81 (57)	34 (58)	0.70 (0.40–1.23)		81 (40)	29 (44)	1.05 (0.63–1.75)
Continuous[b]			0.93 (0.81–1.07)			0.85 (0.70–1.02)				1.00 (0.81–1.23)
No aspirin use										
≤ Median	193 (53)	43 (38)	Reference	104 (54)	25 (33)	Reference		89 (54)	18 (46)	Reference
> Median	165 (46)	71 (62)	**1.52 (1.03–2.24)**	90 (46)	50 (67)	**2.04 (1.23–3.37)**		75 (46)	21 (54)	0.99 (0.48–2.07)
Continuous[b]			1.14 (0.96–1.35)			**1.27 (1.03–1.57)**				0.91 (0.67–1.22)

Table 6. Association of urinary PGE-M levels with all-cause mortality among men with prostate cancer who did not report aspirin use.

formation in African-American men with prostate cancer. Hence, our results provide rather little evidence for elevated PGE-M playing a role in prostate cancer.

In the 2020 annual report, we already described preliminary findings of a distinct association between urinary thromboxane B2 (TXB2), a surrogate marker of thromboxane A2 bioavailability, and increased odds of prostate cancer only among the African-American men (OR = 1.50; 95% CI: 1.13 to 2.00) (**Figure 10A**). With TXB2 data distributed into quartiles according to control values and stratified by race, a higher proportion of African-American cases presented with the most elevated (Q4) TXB2 levels compared with controls (**Figure 10B**). In addition, when assigned to NCCN risk score groups, the highest median level of TXB2 associated with prostate cancer patients who had developed metastatic disease (**Figure 10C**). We did not find an association of TXB2 with localized disease, consistent with the role of thromboxane A2 signaling in metastasis.

A

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

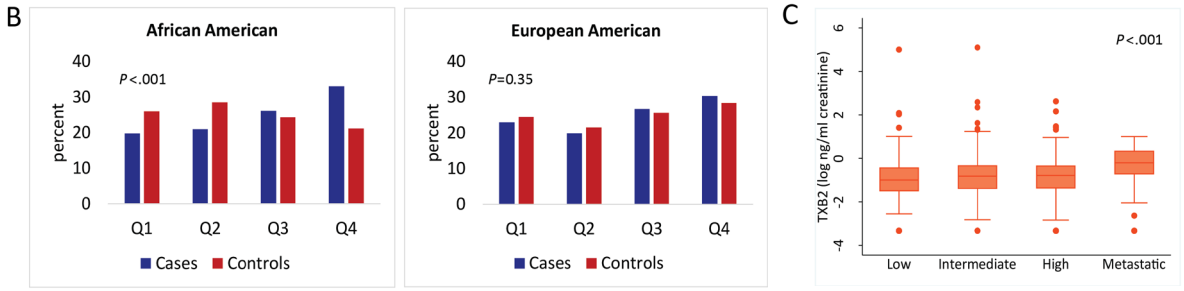


Figure 10. (A) Elevated Urinary 11-dehydrothromboxane B2 (TXB2) levels associate with prostate cancer in African-American men only. (B) A higher proportion of African American cases presented with the most elevated (Q4) TXB2 levels compared with controls. (C) Urinary TXB2 levels across four NCCN risk score categories where elevated TXB2 associates with metastatic disease.

To summarize, these observations suggest a distinct relationship between high TXB2 levels and lethal prostate cancer – a relationship that does not exist for PGE-M. The data suggest that the systemic immune-inflammation environment in African-American men may increase the odds of metastasis rather than changing the biology of the primary tumor – the location where PGE-M is mainly produced - as a driving force of the prostate cancer health disparity. This is of clinical importance as we previously showed that TXB2 formation was inhibited by aspirin use in our study population among both cases and controls. Moreover, the aspirin inhibitory effect tended to be more robust among both African-American cases and controls than European-American cases and controls.

To expand on these findings, Maeve utilized updated National Death Index mortality data for our cohort, to examine the association of urinary thromboxane B2 levels with prostate cancer survival. In agreement with the published literature, African-American men in the NCI-Maryland cohort were more likely to die after a prostate cancer diagnosis than European-American men (all-cause mortality, adjusted HR = 1.59, 95% CI 1.20-2.10) (**Table 7-A**). Moreover, a higher proportion of them died because of prostate cancer (prostate cancer-specific mortality, adjusted HR = 1.71, 95% CI 0.97-3.01) (**Table 7-B**), corroborating that African-American patients are at an increased risk of lethal prostate cancer, as generally reported. To examine the role of thromboxane A2/TXB2 in this survival disparity, Maeve investigated the relationship of urinary TXB2 with survival outcomes in our case population. In the Kaplan-Meier and multivariable-adjusted Cox regression survival analyses, when the data were stratified by self-reported race/ethnicity, high TXB2 was associated with a significantly elevated all-cause mortality in African-American men with prostate cancer only (HR = 1.59, 95% CI 1.06-2.40) but not in European-American men (HR = 1.11, 95% CI 0.70-1.76) (**Figure 11**). Maeve's analysis further identified an association between high TXB2 and lethal prostate cancer in African-American men (HR = 4.74, 95% CI 1.62-13.88), but not in European-American men (HR = 1.12, 95% CI 0.34-3.66) (**Figure 12**).

A **Adjusted Hazard ratios and 95% confidence intervals for all-cause mortality among African American prostate cancer patients - when compared to European American patients - in the NCI-Maryland Prostate Cancer study**

Race/Ethnicity	All Cases			
	Alive, No. (%)	Dead, No. (%)	HR (95% CI)[a]	HR (95% CI)[b]
European American	375 (52.0)	108 (44.3)	Ref	Ref
African American	346 (48.0)	136 (55.7)	1.73 (1.34-2.23)	1.59 (1.20-2.10)
Total[c]	721 (100.0)	244 (100.0)		

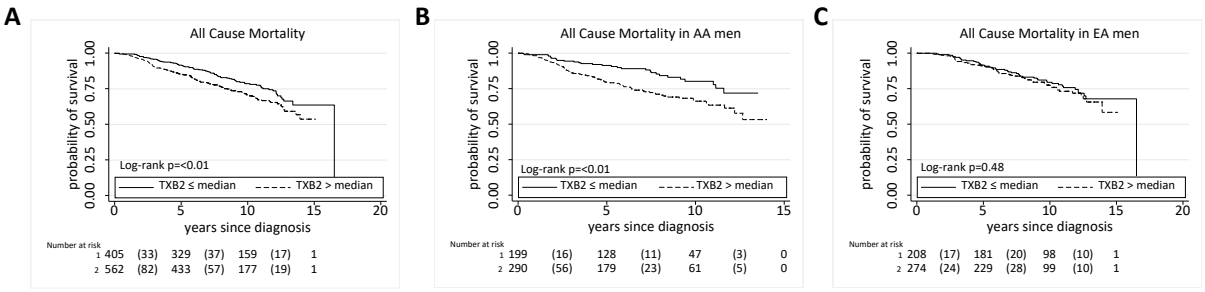
B **Adjusted Hazard ratios and 95% confidence intervals for prostate cancer-specific mortality among African American prostate cancer patients - when compared to European American patients - in the NCI-Maryland Prostate Cancer study**

Race/ethnicity	All Cases			
	Death from other causes, No. (%)	Death from Prostate Cancer, No. (%)	HR (95% CI)[a]	HR (95% CI)[b]
European American	83 (46.4	25 (38.5)	Ref	Ref
African American	96 (53.6)	40 (61.5)	2.15 (1.29-3.56)	1.71 (0.97-3.01)
Total[c]	179 (100.0)	65 (100.0)		

[a]Unconditional Cox regression adjusted for age at study entry. HR = hazard ratio; CI = confidence interval; Ref = reference.
[b]Unconditional Cox regression adjusted for age at study entry, 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), Gleason score (0=Gleason ≤ 7 and 1=Gleason > 7).
[c]Column total sums that differ are due to missing data.

Table 7. African-American men are more likely to die after a prostate cancer diagnosis.

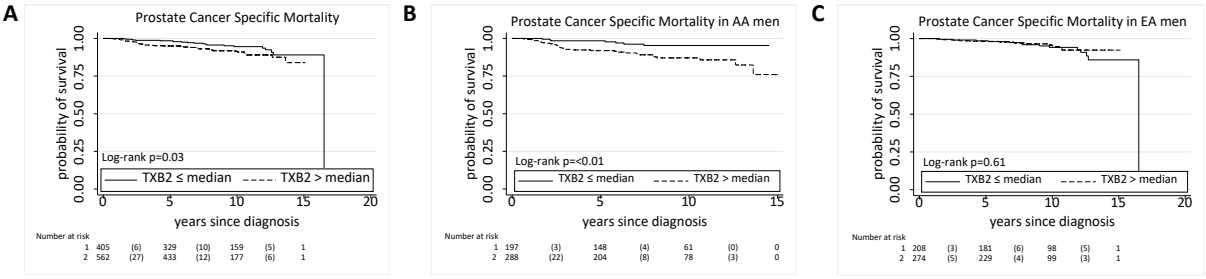
As a final point, Maeve explored the possibility of an interaction between aspirin use and TXB2 on survival. Although a significant interaction was not found in the analysis of all-cause and prostate cancer-specific survival, stratification of cases by aspirin use status (yes/no) revealed disparate outcomes. The association between high TXB2 and all-cause mortality remained significant only in African-American men who did not use aspirin (**Table 8**) but not for aspirin users. Our observations suggest that aspirin may reduce all-cause mortality by decreasing thromboxane A2/TXB2 levels in African-American patients with otherwise significant upregulation of this pro-metastatic COX1/thromboxane A2 signaling pathway.



TXB2	All Cases			African American			European American		
	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]		82 (33.2)	Ref		39 (28.7)	Ref		43 (39.6)	Ref
		158 (66.8)	-		95 (71.3)	-		63 (60.4)	-

[a]Unconditional Cox regression adjusted for age at study entry, body mass index (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 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), 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.

Figure 11. High urinary TXB2 levels associate with increased all-cause mortality among African-American men. Kaplan–Meier survival plots showing association of dichotomized TXB2 levels with all-cause mortality among all cases (A) and after stratification into African-American (AA) (B) and in European-American (EA) men (C). (D) Association of urinary TXB2 levels with all-cause mortality among prostate cancer patients.



TXB2	All Cases			African American			European American		
	Death from PC, No. (%)	Death from Other Cause, No. (%)	HR[a] (95% CI)	Death from PC, No. (%)	Death from Other Cause, No. (%)	HR[a] (95% CI)	Death from PC, No. (%)	Death from Other Cause, No. (%)	HR[a] (95% CI)
≤ Median[b]	17 (28.8)	56 (34.8)	Ref	6 (15.8)	31 (34.1)	Ref	11 (52.3)	25 (35.7)	Ref
> Median	42 (71.2)	105 (65.2)	1.69 (0.88-3.24)	32 (84.2)	60 (65.9)	**4.74 (1.62-13.88)**	10 (47.6)	45 (64.3)	1.12 (0.34-3.66)

[a] Cox regression model 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 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), Gleason score (0=Gleason ≤ 7 and 1=Gleason > 7). HR hazard ratio, SHR subdistribution hazard ratio. CI = confidence interval; OR= odds ratio; Ref = reference.
[b]Median cutoff point is 0.505 ng TXB2 per mg creatinine.

Figure 12. High urinary TXB2 levels associate with increased prostate cancer specific mortality among African American men. Kaplan–Meier survival plots showing association of dichotomized TXB2 levels with all-cause mortality among all cases (A) and after stratification into African-American (AA) (B) and in European-American (EA) men (C). (D) Association of urinary TXB2 levels with prostate cancer specific mortality among prostate cancer patients

TXB2	All Cases			African American			European American			
	Alive	Dead	HR (95% CI) *	Alive	Dead	HR (95% CI) *	Alive	Dead	HR (95% CI) *	P interaction
Aspirin use										0.14
≤ Median	180 (52)	59 (47)	Ref	79 (55)	27 (46)	Ref	101 (50)	32 (48)	Ref	
> Median	167 (48)	67 (53)	1.12 (0.76-1.65)	65 (45)	32 (54)	1.22 (0.71-2.09)	102 (50)	35 (52)	1.00 (0.55-1.83)	
No aspirin use										
≤ Median	133 (37)	23 (20)	Ref	74 (38)	12 (39)	Ref	59 (36)	11 (28)	Ref	
> Median	228 (63)	91 (80)	1.72 (1.03-2.89)	121 (62)	63 (61)	**2.33 (1.14-4.73)**	107 (64)	28 (72)	1.04 (0.45-2.44)	

*Unconditional Cox regression adjusted for age at study entry, BMI (kg/m2), diabetes (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 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), Gleason score (0=Gleason ≤ 7 and 1=Gleason > 7) and stratified by aspirin (no/yes).

Table 8. Association of urinary TXB2 levels with all-cause mortality among men with prostate cancer after stratification by aspirin use.

To summarize, the described investigations identified a novel association between high urinary TXB2 and aggressive prostate cancer as well as adverse survival outcomes for African-American men. While these observations need further validation, they are consistent with our previous findings of an inverse association of aspirin use with lethal prostate cancer in these patients. Our analysis of the COX-derived eicosanoids in this study suggests that aspirin is not mechanistically targeting tumor PGE2 in this high-risk group of men to reduce the disease burden. Instead, the study highlights the potential benefit of aspirin for prevention of lethal prostate cancer through inhibition of thromboxane A2 synthesis in the circulation, directly affecting the metastatic process.

Omega-3 fatty acid study

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 an initial data analysis and started to draft a manuscript. Postdoctoral fellows Brittany Lord (Jenkins) and Tsion Minas have characterized these fatty acids in sera from 1,562 men with prostate cancer and 1,693 controls, spanning Ghanaian, African-American, and European-American men, including determining their relationship with odds of developing prostate cancer and with the measured Olink immune-oncology markers. 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. Unsupervised hierarchical clustering analyses showed aggregation of individual fatty acids by population group, with Ghanaian men showing significantly increased levels of omega-3 fatty acids compared to African-American and European-American men (**Figure 13A**). European-American men had the highest total fatty acid concentrations, as seen in **Figure 13B**, and African-American and European-American men showed significantly higher omega-6 and *trans* fatty acid concentrations when compared to Ghanaian men. A higher omega 6:3 ratio was also observed

among African-American men compared to all other population groups, which is an indicator of poorer dietary health (data not shown).

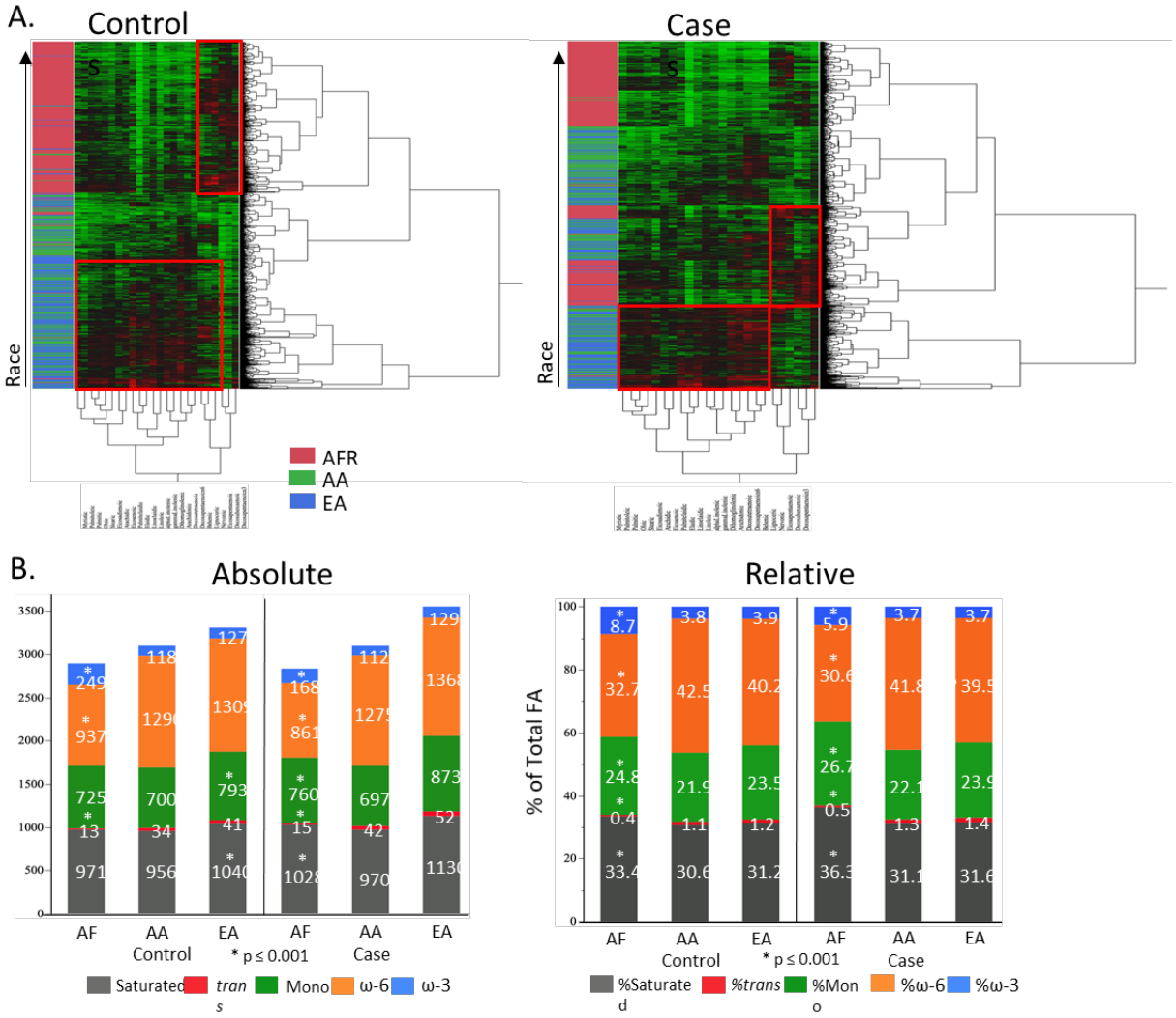


Figure 13. Characterization of absolute and relative circulating fatty acid concentrations individually and by fatty acid groups in the NCI-Maryland and NCI-Ghana cohorts.

Differentially abundant circulating fatty acid levels were further characterized comparing cases with controls across population groups. **Figure 14A** shows three *trans* fatty acids, palmitelaidic, elaidic and linoelaidic acids, were significantly increased in Ghanaian, African-American, and European-American men with prostate cancer as compared to controls. Frequency distributions of these *trans* fatty acids further showed consistently lower relative and absolute concentrations in Ghanaian (African) men (cases and controls) as compared to African-American and European-American men (**Figure 14B**), an expected result given the increased consumption of *trans* fatty acids in the Western diet. Further logistic regression models revealed a significant dose-dependent relationship between increased *trans* fatty acid levels and odds of developing

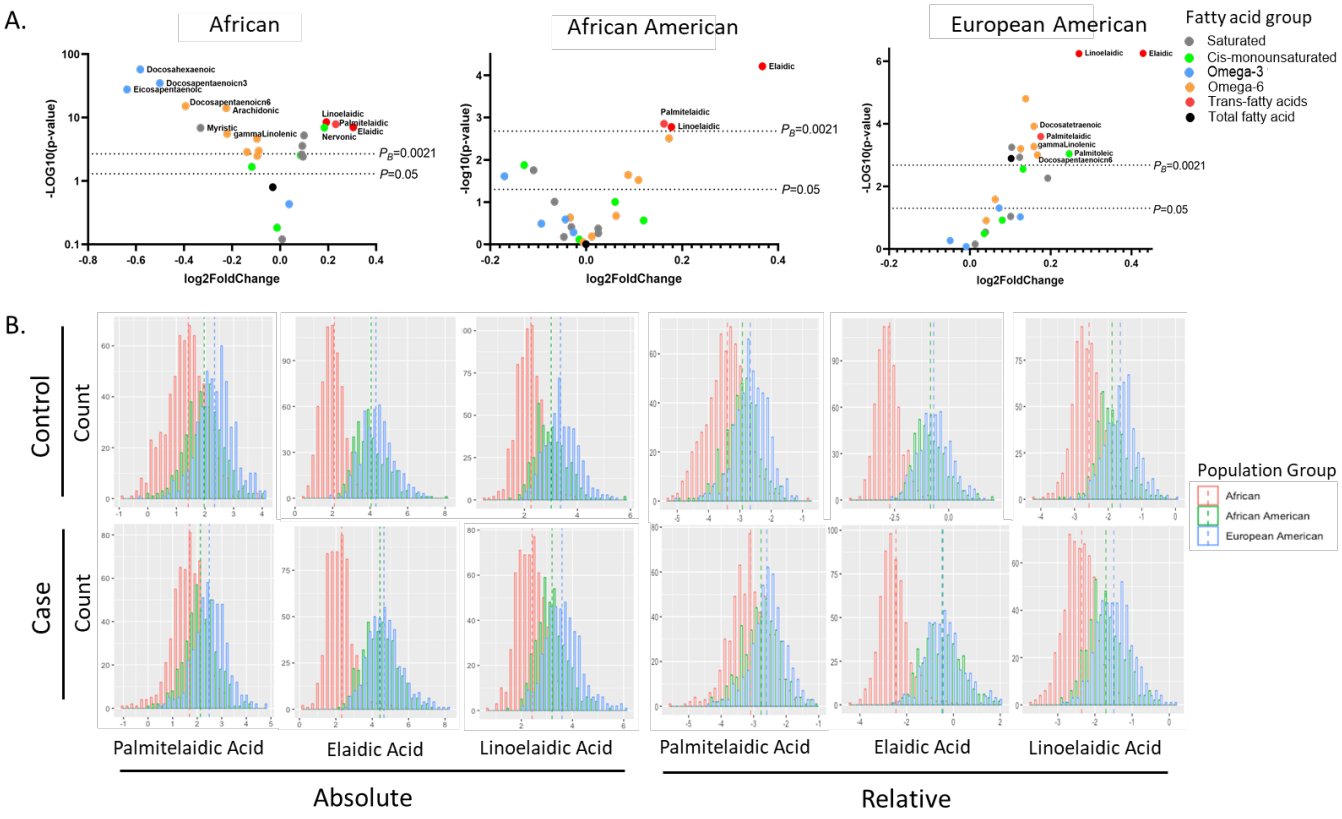


Figure 14. (A) Differentially abundant circulating fatty acids levels in controls versus cases across population groups and (B) frequency distributions of trans fatty acids by population group

prostate cancer in all three population groups (**Table 9**). Interestingly, although Ghanaian men were found to have the overall lowest level of *trans* fatty acids compared to African-American and European-American men, they still experienced significantly increased odds of developing prostate cancer with increasing *trans* fatty acid levels. Elaidic acid was additionally correlated with the NCCN risk score for prostate cancer aggressiveness, where increasing elaidic acid levels were protective against the odds of having a high or very high NCCN risk score for prostate cancer aggressiveness (**Table 10**). While this finding is contrary to what we expected, another similar study also showed that men with the highest circulating *trans* fatty acid levels were 50% less likely to have developed aggressive, high-grade prostate cancer.

Finally, we explored the role of the 82 immune oncological markers in influencing the relationship between fatty acids and prostate cancer and applied a mediation analysis to estimate direct effects of *trans* fatty acids on prostate cancer risk and indirect effects through immune-oncological protein markers. For this analysis, the immune-oncological markers were grouped by pathway (autophagy, apoptosis, others) and the log-transformed absolute *trans* fatty acid levels were used. Mediation analyses were adjusted for age at interview, BMI, education, and race. In the unstratified analysis with all men included, the autophagy ($P < 0.003$, confounding 15.9%) and apoptosis ($P < 0.035$, confounding 10.1%) pathways were found to mediate the relationship between palmitelaidic acid and prostate cancer. Immune-oncology markers-defined pathways

did not influence the relationship of elaidic and linoelaidic acid with prostate cancer. Additional analyses are underway to further assess the role of immune-related proteins as risk mediators by population group, but our current findings point to a previously unexplored oncogenic role of fatty acids and immune-oncological mediators in men of African descent with prostate cancer.

Table 9. Association of trans-fatty acids (elaidic acid, palmitelaidic acid, and linoelaidic acid) with odds of case status

	European-American				African-American				African			
	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												
Low	128 (27)	70 (16)	Ref.		154 (40)	99 (24)	Ref.		219 (33)	143 (24)	Ref.	
Intermediate	159 (34)	133 (30)	1.38 (0.94, 2.03)	0.103	123 (32)	128 (31)	**1.80 (1.23, 2.64)**	**0.002**	219 (33)	169 (29)	**1.55 (1.03, 2.35)**	**0.036**
High	177 (38)	236 (54)	**2.17 (1.51, 3.11)**	**<0.001**	105 (27)	180 (44)	**2.97 (2.04, 4.33)**	**<0.001**	220 (33)	273 (47)	**1.92 (1.30, 2.84)**	**0.001**
Trend				**<0.001**				**<0.001**				**0.001**
Palmitelaidic acid												
Low	109 (23)	75 (17)	Ref.		173 (45)	139 (34)	Ref.		220 (33)	105 (18)	Ref.	
Intermediate	152 (33)	139 (32)	1.32 (0.90, 1.94)	0.157	130 (34)	136 (33)	**1.48 (1.03, 2.11)**	**0.032**	218 (33)	178 (30)	1.51 (0.98, 2.32)	0.062
High	203 (44)	225 (51)	**1.48 (1.03, 2.13)**	**0.032**	79 (21)	132 (32)	**2.60 (1.76, 3.84)**	**<0.001**	220 (33)	302 (52)	**2.26 (1.50, 3.39)**	**<0.001**
Trend				**0.038**				**<0.001**				**<0.001**
Linoelaidic acid												
Low	104 (22)	61 (14)	Ref.		178 (47)	125 (31)	Ref.		219 (33)	153 (26)	Ref.	
Intermediate	164 (35)	129 (29)	1.27 (0.85, 1.90)	0.250	118 (31)	158 (39)	**2.17 (1.52, 3.10)**	**<0.001**	220 (33)	148 (25)	0.97 (0.64, 1.46)	0.868
High	196 (42)	249 (57)	**1.92 (1.31, 2.80)**	**0.001**	86 (23)	124 (30)	**2.35 (1.60, 3.46)**	**<0.001**	219 (33)	284 (49)	**1.52 (1.03, 2.23)**	**0.033**
Trend				**<0.001**				**<0.001**				**0.025**

[a]Logistic regression adjusted for age at study entry, BMI, education, diabetes, smoking history, and aspirin use
High elaidic acid is defined by the median score in the control population (≤ median vs. > median)
Elaidic acid tertile cutoffs for NCI-Maryland cohort were 13.51 and 22.67ug/ml
Elaidic acid tertile cutoffs for NCI-Ghana cohort were 3.47 and 4.98ug/ml
Palmitelaidic acid tertile cutoffs for NCI-Maryland cohort were 3.70 and 5.57 ug/ml
Palmitelaidic acid tertile cutoffs for NCI-Ghana cohort were 2.22 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
OR denotes Odds Ratio; 95% CI denotes 95% confidence interval
NOTE: Bolded data indicate significant associations in the multivariable logistic regression analysis.

Table 10. Association of high trans fatty acids (Elaidic acid, Palmitelaidic acid, or Linoelaidic acid) with National Comprehensive Cancer Network (NCCN) risk score for prostate cancer aggressiveness

NCCN Risk Score	Elaidic acid OR (95% CI)*	Palmitelaidic acid OR (95% CI)*	Linoelaidic acid OR (95% CI)*
Low	Ref	Ref	Ref
Intermediate	**0.65 (0.43, 0.99)**	0.77 (0.52, 1.15)	0.89 (0.60, 1.33)
High/Very High	**0.56 (0.35, 0.89)**	0.77 (0.49, 1.21)	0.82 (0.52, 1.29)
Regional/Metastatic	0.53 (0.26, 1.07)	0.92 (0.45, 1.88)	0.82 (0.40, 1.68)
***P* value for Trend**	**0.017**	0.499	0.395

Note: Bolded data indicate significant associations in the logistic regression
*Logistic regression adjusted for age at study entry, BMI (kg/m2), diabetes
High trans fatty acid level is defined by the median serum concentration in the NCI-Maryland control population (> median vs. ≤ median)

For Specific Aim 2, Major Task 1 describes as subtask 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 completed GWAS genotyping with the Infinium HumanOmni5-Quad BeadChip for a total of 1806 subjects in the study, corresponding to the numbers of 900 cases and 900 controls that we will genotype per our Statement of Work for the award – as reported in 2020. The raw genotyping data were moved to the analysis team at the Cancer Genomics Research Laboratory for quality control including "data cleaning". This subsequent inspection of the data indicated that the raw data were not of optimal quality for about 10% of the samples. Some of them could be re-genotyped, yielding finalized genotyping data for 1670 men (cases: 401 African-American; 397 European-American; controls: 423 African-American, 449 European-American). This milestone finishes the laboratory-based tasks for **Specific Aim 2**. We also received the finalized data file for this cohort with additional imputed data for missing genotypes from the bioinformatics group with the Cancer Genomics Research Laboratory. Per previous arrangement, a postdoctoral fellow in Michael Cook's group, Ebonee Butler - now an Assistant Professor at the University of North Carolina - will conduct a combined analysis of the NCI-

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

Maryland and NCI-Ghana studies, with the main aim to investigate whether germline genetic variants are associated with immune-inflammation markers and prostate cancer, as outlined under **Specific Aim 2**, Major Task 2. GWAS data using the Infinium HumanOmni5-Quad BeadChip have been generated for the NCI-Ghana study (659 cases, 659 controls) and are available to us. Ebonee will be joined by Amy Zhang, a postbaccalaureate fellow who joined the Ambs laboratory in 2020. Amy has acquired skills in the analysis of GWAS datasets and has gained familiarity with the NCI-Maryland and NCI-Ghana GWAS datasets. Amy will assist Ebonee and will conduct her own analysis, proceeding with a quantitative trait locus analysis to identify the genetic and ancestral influence on serum levels of the 24 fatty acids and how these relationships may influence prostate cancer risks. This added analysis was not proposed in the SOW of the grant. There are also plans to incorporate the GWAS data into a study of the viral infection history of men and how this history relates to prostate cancer. This study includes the application of VirScan, which was developed by Stephen Elledge of Harvard Medical, and 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. Amy is 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 prostate cancer or without the disease. This will be done for Ghanaian and US men.

For Specific Aim 3, management of Major Tasks 1 & 2 primarily falls under the responsibility of Dr. Clayton Yates, our co-investigator at Tuskegee University. Yet, the Tuskegee and NCI research teams have been together working on these tasks, in close collaboration.

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), 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

To obtain cores of tumor and adjacent non-cancerous tissue from FFPE tumor blocks, we asked the University of Maryland Department of Pathology for help with this task. 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. We had previously identified the NCI-Leidos Sequencing Core as service provider, however, with further discussions it became uncertain that this facility could sequence RNA obtained from FFPE tissue blocks with RNA degradation. At HudsonAlpha, nevertheless, quality control analysis indicated that most RNA samples would likely fail sequencing. HudsonAlpha later informed us that the extracted DNA may too not work for whole exome sequencing (WES) in their pipeline. Thus, we provided them with additional FFPE tissue cores for RNA and DNA extraction. HudsonAlpha 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 again failed quality control measures. Accordingly, we have WES data for tumor-noncancerous tissue pairs from only 10 Nigerian, 11 African-American, and 14 European-American men at this time. We are working on submitting another WES request with HudsonAlpha to potentially increase the number of sequenced tumor-noncancerous tissue pairs by 7 for Nigerian men, by 34 for African-American men, and by 33 for European-American men. 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 still struggling in obtaining good quality WES data for a similar number of tumors.

Opportunities for training and professional development.

Despite the many challenges because of COVID-19, we continued to provide thorough mentorship and opportunities for training and professional development to our trainees. In the past 12 months, we provided these opportunities to the following fellows in the context of this award: Jason White and Isra Elhussin (both Tuskegee U and mentored by Clayton Yates); Tsion Minas, Maeve Bailey-Whyte, Brittany Lord, Anuoluwapo Ajao, Amy Zhang and Margaret Pichardo (all NCI-associated and mentored by Stefan Ambs and Michael Cook). Initially, many of the tasks to move the research forward provided rather limited opportunity for training and professional development. Those included mostly routine tasks of sample preparation, quality control analysis, and shipment, and included study design tasks for pilot studies and the main study, and project management by the PIs. Due to COVID-19, we still cannot host visitors in our laboratory at the NCI. This restriction will likely continue into 2022. We hosted Jason White from the Yates laboratory at our NCI laboratory for several months in 2019. Jason White is a PhD student and participates in data analysis of the tumor data (genomics and transcriptome analysis) for this DoD grant. Jason is also a project co-manager of the research activities under Specific Aim 3. Jason received mentoring in the analysis of whole exome sequencing data by our Staff Scientist and data science expert, Dr. Wei Tang, and has access to use the NIH Biowulf Cluster for high-performance computing. Jason can use the NIH high-performance computing capabilities remotely when working in the Yates lab. This arrangement will continue for an additional year and currently ends in late 2022. Wei continued to mentor Jason using existing

health disparity-related prostate cancer datasets that were generated at Tuskegee University, investigating driver mutations and mutational signatures with state-of-the-art data science tools. It is the aim of this interaction that Jason becomes an independent data scientist of genomics data and can apply this knowledge to the data generated under this award. He is making great progress and should soon earn his PhD. We have regular Zoom meetings to discuss his progress with the project. Jason has been joined by Isra Elhussin in working on the analysis of data from this award. She is a physician scientist with a minority background seeking to earn her PhD with a concentration in data science. Isra will focus on the analysis of the RNA-seq data. Isra participated in the web-based Clinical and Translational Research summer course offered by the NIH in 2021. I had the opportunity of an additional meeting with her, discussing her career plans as a physician scientist and the career options that she may find at the NIH.

With the data in hand for **Specific Aim 1**, Tsion Minas and Maeve Bailey-Whyte, both postdoctoral fellows at the NCI, have largely completed the analysis of the immune-oncology and urinary eicosanoid markers, respectively, and are at the stage of finishing manuscripts (Tsion) or having them published in peer-reviewed journals (Maeve). Both are teleworking, solely concentrating on data analysis and scientific reports. Maeve is currently collaborating with Brittany Lord, another postdoctoral fellow in our group, on a review article for Trends in Cancer entitled “Immune response and inflammation in cancer health disparities”, highlighting the context for our DoD award and discussing recent findings from this research. Tsion made great progress in mastering the R software for statistical computing and analyzing data for the award. She is closely collaborating with Julian Candia, a Staff Scientist and Senior Data Analyst, who continues to be her mentor in the use of novel approaches of data analysis and visualization of her findings. Tsion has presented her findings covering Aim 1 at an NCI Interlaboratory Seminar and twice at a Laboratory of Human Carcinogenesis (LHC) seminar. Each time she did very well. She functioned as a chairperson for the 3rd NCI Symposium on Cancer Health Disparities (https://ncifrederick.cancer.gov/events/conferences/3rdNCICHD) and gave a short talk entitled “Blood levels of TNFRSF9 and PTN predict lethal prostate cancer among African American men”, showcasing her research findings related to **Specific Aim 1**. Furthermore, Tsion was mentoring Anuoluwapo Ajao, a postbaccalaureate fellow involved with our prostate cancer research. Under Tsion’s mentorship, Anuoluwapo was learning statistical analysis and epidemiological methodologies. Anuoluwapo very recently left for a Fulbright Fellowship, studying risk factors for breast cancer in Nigeria. Tsion’s mentorship and Anuoluwapo’s involvement with the prostate cancer studies and the health disparity research certainly contributed to her success in earning this competitive fellowship. Maeve, an NCI Cancer Prevention Fellow with a Master’s in Public Health degree, greatly advanced in learning methodologies to analyze epidemiological and clinical data, as evidenced by her recent publications. Michael Cook was a great help in advising her with the analysis methodology, leading to new analysis approaches that we did not use previously in our laboratory. Maeve works closely with Tsion and had additional support by a postdoctoral fellow from Michael Cook’s laboratory, Lauren Hurwitz, who is a trained prostate cancer epidemiologist. Lauren also partnered with Wei Tang in the research showing that aspirin use at time of recruitment associates with a reduced prostate cancer mortality among the African-American men in SCCS, again highlighting the many productive interactions between the fellows and the PIs. Like Tsion,

Maeve had the opportunity to serve as a chairperson for the 3rd NCI Symposium on Cancer Health Disparities. Maeve has presented her findings on thromboxane B2 at an NCI Cancer Prevention Fellow seminar and at LHC seminars in 2020 and at an NCI Interlaboratory Seminar in 2021. She also prepared a poster for the AACR conference on the Science of Cancer Health Disparities, October 2-4, 2020. She presented her 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". Maeve received an AACR Scholar-in-Training-Award for her contribution. Her progress has been very impressive. In 2021, Brittany Lord and Amy Zhang joined the group of trainees being involved with research opportunities under the award. Brittany is partnering with Tsion in the investigation of the fatty acid data, making great progress. The very productive partnership 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 are 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. Amy came to our laboratory as pre-doctoral fellow to study the viral infection history of prostate cancer patients. She has been mentored by Wei Tang and became quickly proficient with the R software for statistical computing. She is now using this knowledge to analyze the GWAS data that have been generated for the NCI-Maryland study. She has her own project but also assists Tsion and Brittany by providing an analysis of GWAS data for their fatty acid-related manuscript and is assisting Ebonee with her analysis, currently by linking the GWAS data to the fatty acid and immune-oncology marker data, and by generating a large common database with integrated GWAS data for all projects. Lastly, Margaret Pichardo began to be involved with important work related to this award, starting in 2020. She has been instrumental in establishing a research project related to this award that will collect 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, a multi-year effort, has now been completed. The index contains the following variables: percent (%) households in poverty, % female headed households with dependent children, % households on public assistance, % households earning under $30,000/year, % households with no car, and % males and females unemployed. Margaret is a rising star in cancer health disparity research. She will defend her PhD thesis in epidemiology at Yale in November 2021, while being a resident physician at Howard University in Washington, DC. Per agreement, she will conduct her research using this data starting in December, to finish her research with an anticipated research manuscript in May of 2022. Her research will explore 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. It is Margaret's career plan to become a physician scientist. The research project is aimed to help her achieving this goal.

Dissemination of results 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 is also invited to give 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 has two additional scheduled 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 10-13, 2021. Brittany Lord presented a poster at the 2021 AACR virtual conference on the Science of Cancer Health Disparities and will give 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 rescheduled 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 at invited talks at the NCI Laboratory of Genitourinary Cancer Pathogenesis seminar series and the NIH Academy to support health disparity research. He was also invited to Grand Rounds at the University of Maryland (Frontiers in Oncology) and presented the prostate cancer research related to this grant. Lastly, our research was highlighted 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.

Goals to accomplish during the next reporting period. The continued focus during the next period will be the completion of manuscripts related to **Specific Aims 1 & 2** and getting them published in peer-reviewed journals. Tsion Minas has submitted her revised manuscript to *Nat Communications* that reports a serum proteomics signature that links suppression of tumor immunity to ancestry and lethal prostate cancer. Maeve Bailey-Whyte, Brittany Lord, and Stefan Ambs (authors) have submitted a review article for *Trends in Cancer* that is subject-related to the research of this award. This contribution is now under review. Brittany and Tsion are currently drafting a manuscript that will report the relationship of circulating fatty acids with inflammation markers and prostate cancer in men of African ancestry. Tsion will then concentrate on a final manuscript from the immune-oncology marker study. Ebonee Butler and Amy Zhang will analyze the GWAS data for future publications. Amy has made herself quite familiar with this GWAS data, as she has worked with the initial data, and the analysis should quickly proceed as the finalized dataset is now available to us. We anticipate that this research will generate two manuscripts in 2022, one related to the immune-oncology marker research and the other related to the circulating fatty acids, with both being quantitative trait locus studies, asking how germline genetics and ancestry may influence the abundance levels of circulating immune-oncology marker and fatty acids and their effects on prostate cancer. I also expect that Margaret Pichardo will soon start her analysis with the neighborhood deprivation index. She is aiming for a manuscript in May of 2022, reporting how neighborhood deprivation relates to prostate cancer and immune-oncology marker levels. It is her interest to find out whether neighborhood deprivation induces a prostate cancer-promoting immune-inflammation signature in men. Moreover, as outlined in the report, we will integrate the GWAS dataset into other prostate cancer-related research that is ongoing in the Ambs laboratory. Thus, we are aiming for 5-10 additional manuscripts that will be generated from the existing data. Lastly, we are sharing the GWAS data with Chris Haiman (USC) as the lead PI to integrate those into a meta-analysis of genetic risk factors in men of African ancestry as part of the AAPC consortium. This additional community use of the generated data will lead to co-authorships and the acknowledgement of DoD as the funding source. Lastly, we will move forward the analysis of the genomics data coming from the analysis of prostate tumors, as described under **Specific Aim 3**, Major Task 2. Here, the lead is with the Yates laboratory at Tuskegee University. Although issues remain to obtain whole exome sequencing for many of the prostate tumors, especially from the Nigerian cohort, our partnership should succeed in generating informative gene expression profiles and mutational signatures that we will translate into manuscripts and publications starting in 2022.

4. Impact

The research interest in the immune-inflammation signature in prostate tumors of African-American men 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 interferon signature in these tumors, as we have described it (Tang et al., PMID: 30012562), would suggest that African-American prostate cancer patients may respond better to cancer vaccines targeting the cancerous prostate than European-American men. We believe that the research questions that we pursue under this award will have important implications for immune therapy in African-

American men with otherwise lethal prostate cancer. Drs. Yates and Ambs have spoken with Dr. Sartor. He was a speaker at the 3rd NCI Symposium on Cancer Health Disparities in 2021. Tumors with an interferon-stimulated gene signature are susceptible to inhibition of adenosine deaminase acting on RNA (ADAR1), as was recently discovered. These inhibitors are being developed for clinical use by the pharmaceutical industry and may have therapeutic activity particularly in African American men with prostate cancer as their tumors frequently harbor an interferon signature.

As already described in our 2019 annual report, our research had significant impact on performance measures at both Olink and the Eicosanoid Core Laboratory at Vanderbilt. As such, the project will likely improve knowledge and practices at these two places. The Eicosanoid Core Laboratory changed their methods to measure urinary creatinine as the standard to normalize urinary marker measurements by mass spectrometry. The laboratory manager, Ginger Milne, has been very receptive to our feedback and has been working with us to improve their measurement and analysis pipeline.

5. Changes/Problems

We have no major changes/problems to report for this award period. At this time, all procedures, measurements, and proposed analyses will continue as planned. Yet, we continue to encounter technical problems with obtaining good quality WES data from collected prostate tumors. We are working with HudsonAlpha to resolve this problem. It is our understanding that when RNAseq can be obtained from FFPE tissues, and we succeeded with this task, DNA isolated from the same tissue should yield WES results.

Specific Aim 1, Major Task 1: There was an issue with the variability of duplicate measurements at the Eicosanoid Core Laboratory, Vanderbilt University – as outlined in the 2019 annual report. We had a high variability in our duplicate measurements and initially did not know the cause (since we did not have this issue in our pilot study). An investigation revealed that the cause was a high variability in the measurement of the urinary creatinine content, using a colorimetric assay. Urinary creatinine content is used as the reference to standardize the mass-spectrometry-based measurements of the 5 urinary eicosanoid metabolites – our metabolites of interest. The Eicosanoid Core Laboratory re-measured urinary creatinine in all the 2131 urine samples, using an improved protocol, and provided us with the new data on March 30, 2020. The new measurements showed significantly lower variability in duplicate measurements, in agreement with previous QC data at this facility. The problem was resolved, finishing the laboratory work for this project.

Specific Aim 2, Major Task 1: We encountered a problem with the amount and quality of germline DNA that we sent to the genotyping core facility at the NCI – as mentioned in the 2019 annual report. This core facility - Cancer Genomics Research Laboratory – has high QC requirements on DNA quality to avoid downstream genotyping failure. 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 problem has been resolved, finishing the laboratory work for this project. The NCI Cancer Genomics Research

Laboratory successfully completed GWAS genotyping with the Infinium HumanOmni5-Quad BeadChip for a total of 1670 men (cases: 401 African-American; 397 European-American; controls: 423 African-American, 449 European-American), thus not fully matching the proposed numbers of 900 cases and 900 controls in our Statement of Work for the award. We are 7.2% below this number. The GWAS data for those 1670 men will be used for subsequent analyses.

Specific Aim 3, Major Task 1: Initially, we encountered a quality control issue with the extracted total RNA from FFPE prostate tumor and adjacent non-cancerous tissue cores. It remains technologically challenging to obtain good quality RNA from formalin-fixed human tissue samples that meet the requirement for RNA sequencing. The FFPE prostate tissues from the NCI-Maryland study have been stored 5-15 years which inevitably will lead to degradation of RNA in these tissues. The other tissues have been collected in Nigeria and their collection, fixation, and storage may also have caused degradation. The NCI-Leidos Molecular Histopathology Laboratory was the service provider that extracted total RNA and DNA from about 400 FFPE cores using a previously established protocol that allows further processing of the RNA for RNAseq and DNA for WES. The RNA and DNA samples were then sent to the service provider, HudsonAlpha Institute for Biotechnology, a leader in applied genomics technologies. QC analysis indicated that most RNA samples would likely fail sequencing. HudsonAlpha 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 obtained good quality RNAseq data for 77 prostate tumors from Nigerian men, 50 from African-American men, and 40 from European-American men (n = 167 in total). However, the extracted DNA again failed quality control measures. Accordingly, we have WES data for tumor-noncancerous tissue pairs from only 10 Nigerian, 11 African-American, and 14 European-American men at this time. We are working on submitting another WES request with HudsonAlpha to potentially increase the number of sequenced tumor-noncancerous tissue pairs by 7 for Nigerian men, by 34 for African-American men, and by 33 for European-American men. The poor performance of DNA in the WES pipeline at HudsonAlpha was unexpected. Thus, we are still working on obtaining good quality WES data for most prostate tumors.

Changes to vertebrate animals and select agents do not apply.

6. Products

Five publications:

1. Kiely, M., et al., *Urinary Thromboxane B2 and Lethal Prostate Cancer in African American Men.* J Natl Cancer Inst, 2021.
2. Kiely, M., et al., *Urinary PGE-M in Men with Prostate Cancer.* Cancers (Basel), 2021. **13**(16).
3. Kiely, M. and S. Ambs, *Immune Inflammation Pathways as Therapeutic Targets to Reduce Lethal Prostate Cancer in African American Men.* Cancers (Basel), 2021. **13**(12).
4. Tang, W., et al., *Aspirin Use and Prostate Cancer among African-American Men in the Southern Community Cohort Study.* Cancer Epidemiol Biomarkers Prev, 2021. **30**(3): p. 539-544.

5. Minas, T.Z., et al., *An overview of cancer health disparities: new approaches and insights and why they matter.* Carcinogenesis, 2021. **42**(1): p. 2-13.

Additionally, the research was highlighted by The Geneva Foundation on their website at https://genevausa.org/news/story/reducing-lethal-prostate-cancer-in-men-with-african-descent/

7. Participants and Other Collaborating Organizations

The following individuals have worked on the described tasks in the past 12 months. They are either members of the Ambs laboratory, or co-PIs (Yates and Cooks) or collaboratively worked with the Ambs laboratory on tasks (members of the Yates laboratory and Michael Cook). 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. Preparation of FFPE tissue cores and of extracted DNA for genotyping, obtaining updated patient data and generating the neighborhood deprivation index as well shipments to service providers were major tasks for the Ambs laboratory in 2020. In addition, we began the analysis of research data and submitted the first manuscripts. In 2021, project management to obtain RNAseq and WES data from HudsonAlpha were important tasks. Neighborhood deprivation index data were finalized and linked to all study participants in the NCI-Maryland study. However, most time were spent on data analysis and interpretation, and preparation of manuscripts. 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.

Name	Tsion Minas
Project Role	Postdoctoral Fellow
Researcher Identifier	
Nearest person month worked	8
Contribution to Project	Project manager for the immune-oncology marker and GWAS studies; communication with service providers; aliquoting of serum; DNA extraction and aliquoting for GWAS; development of template for sample analysis including random distribution and blinded duplicates across plates; data collection for the NCI-Maryland study to generate the NCCN risk score for prostate cancer patients; QC analysis for all serum markers; 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

Name	Tiffany Dorsey
Project Role	Laboratory Manager/Microbiologist

Researcher Identifier	
Nearest person month worked	2
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; aliquoting of serum; DNA extraction and aliquoting; managed processing of FFPE tumor tissues including samples from Nigeria: preparation of cores, review by pathologist, RNA and DNA extraction by NCI-Leidos; shipment of biospecimens to Hudson alpha for RNAseq and DNAseq; design of pilot study with Hudson alpha; supervision of Post-baccalaureate fellows
Funding support	NCI intramural program

Name	Maeve Bailey-Whyte
Project Role	NCI Cancer Prevention Fellow
Researcher Identifier	
Nearest person month worked	8
Contribution to Project	Project manager for the urine metabolite study; communication with Eicosanoid Core Laboratory; troubleshooting; aliquoted urine samples; performed pilot study; development of template for sample analysis including random distribution and blinded duplicates across plates; QC analysis for all urine markers; data analysis for research in the urinary eicosanoid marker study and preparation of manuscript(s); preparation of abstracts, posters, and talks
Funding support	NCI intramural program

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 abstracts, posters, and talks
Funding support	NCI intramural program

Name	Margaret Pichardo
Project Role	Volunteer and PhD student in epidemiology at Yale University
Researcher Identifier	
Nearest person month worked	1

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; establishment of database with neighborhood deprivation index for all study participants
Funding support	NCI intramural program and scholarship at Yale

Name	Anuoluwapo Ajao
Project Role	NIH Academy Post-baccalaureate fellow
Researcher Identifier	
Nearest person month worked	1
Contribution to Project	Re-extraction of DNA
Funding support	NCI intramural program

Name	Amy Zhang
Project Role	NIH Academy Post-baccalaureate fellow
Researcher Identifier	
Nearest person month worked	1
Contribution to Project	Analysis of GWAS data; preparation of datasets
Funding support	NCI intramural program

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 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 abstracts, posters, and talks
Funding support	Tuskegee University

Name	Wei Tang
Project Role	Associate Scientist
Researcher Identifier	
Nearest person month worked	2
Contribution to Project	Mentor of Jason White and Isra Elhussin (PhD students from Tuskegee U) and Amy Zhang (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

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

Changes in active other support: We have no changes in the support for the PI or other key personnel to report.

What other organizations were involved as partners? We established a collaboration with SCCS to investigate aspirin use among African-American men as strategy to prevent the development of lethal prostate cancer. We will validate findings from the NCI-Maryland and NCI-Ghana study related this award in SCCS. Furthermore, we have a collaboration with the University of Maryland Medical School, Department of Pathology. The collaborating pathologist took the cores from FFPE tumor blocks, supporting **Specific Aim 3**. We are receiving additional expert advice by Dr. Harris Yfantis, Chief, Anatomic Pathology Section, Department of Pathology and Laboratory Medicine, VAMHCS. He reviewed FFPE prostate tumor tissue blocks, provided Gleason score assessment, and provided guidance for obtaining cores. The pathologists will receive co-authorship on publications related to this work. We will also share part of the generated GWAS 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

This is a collaborative award. The initiating PI, Stefan Ambs, and the Collaborating/Partnering PI, Clayton Yates, will submit separate reports.

9. Appendices

PDF of five peer-reviewed publications (three research reports, two reviews [1-5]), all published in 2021, with acknowledgement of the funding support by DoD award W81XWH-18-1-0588.

1. Kiely, M., et al., *Urinary Thromboxane B2 and Lethal Prostate Cancer in African American Men.* J Natl Cancer Inst, 2021.
2. Kiely, M., et al., *Urinary PGE-M in Men with Prostate Cancer.* Cancers (Basel), 2021. **13**(16).
3. Kiely, M. and S. Ambs, *Immune Inflammation Pathways as Therapeutic Targets to Reduce Lethal Prostate Cancer in African American Men.* Cancers (Basel), 2021. **13**(12).
4. Tang, W., et al., *Aspirin Use and Prostate Cancer among African-American Men in the Southern Community Cohort Study.* Cancer Epidemiol Biomarkers Prev, 2021. **30**(3): p. 539-544.
5. Minas, T.Z., et al., *An overview of cancer health disparities: new approaches and insights and why they matter.* Carcinogenesis, 2021. **42**(1): p. 2-13.

OXFORD

JNCI J Natl Cancer Inst (2021) 00(0): djab129

doi: 10.1093/jnci/djab129
First published online 15 July 2021
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

Received: 9 March 2021; **Revised:** 29 April 2021; **Accepted:** 23 June 2021

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 .

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

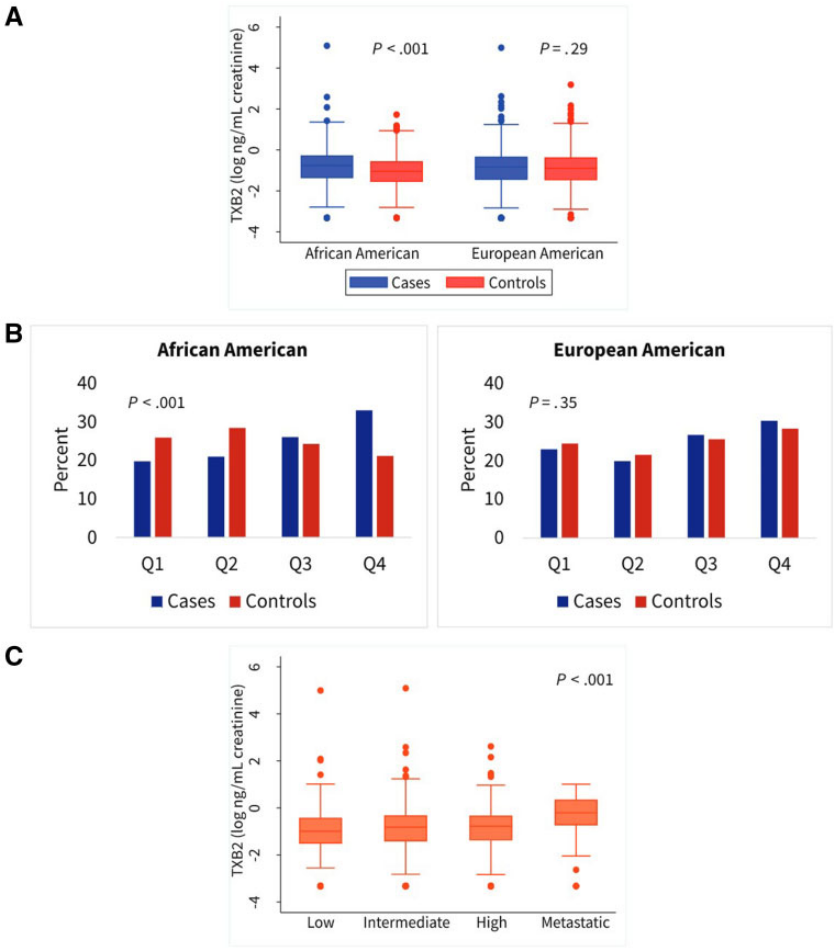

Figure 1. Urinary 11-dehydrothromboxane B2 (TXB2) levels in African American and European American men and their association with prostate cancer and National Comprehensive Cancer Network (NCCN) risk scores. **A)** Urinary TXB2 levels in cases and controls shown as a continuous measure, stratified by 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.

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

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.

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.

Funding

This research was supported by the DoD award W81XWH1810588 (to S.A. and C.Y.) and the Intramural Research Program of the NIH, National Cancer Institute (NCI), Center for Cancer Research and Division of Cancer Epidemiology and Genetics; Maeve Kiely is supported by the NCI Cancer Prevention Fellowship program.

Notes

Role of the funders: No sponsor had any role in the study design, data collection, analysis, interpretation, the writing, and decision to submit the manuscript.

Disclosures: None of the contributing authors have any conflicts of interest or financial ties to disclose.

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.

Acknowledgements: We would like to thank personnel at the University of Maryland and the Baltimore Veterans Administration Hospital for their contributions with the recruitment of participants. We would also like to thank the participants who contributed their time and biospecimens to make the NCI-Maryland study possible.

Data Availability

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

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Urinary PGE-M in Men with Prostate Cancer

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Simple Summary: Elevated levels of urinary prostaglandin E metabolite (PGE-M), a marker of inflammation, have previously been associated with cancer incidence and metastasis. Studies investigating PGE-M in prostate cancer are lacking even though chronic inflammation is a candidate risk factor for the disease. We investigated the association of PGE-M with lethal prostate cancer. We measured PGE-M in the urine of men with prostate cancer and in men without prostate cancer (population controls). Our participants included African American and European American men. Because African American men die more frequently from prostate cancer than European American men, we investigated whether high PGE-M may contribute to the increased mortality among African American prostate cancer patients. We did not observe a relationship between PGE-M and prostate cancer aggressiveness or prostate cancer-specific mortality in our study population, neither in the combined cohort nor in the race/ethnicity stratified analysis. Interestingly, however, we observed a significant relationship between high PGE-M and all-cause mortality in African American men with prostate cancer. Yet, there was no association between high PGE-M and all-cause mortality when these men were regular aspirin users.

Abstract: Urinary PGE-M is a stable metabolite of prostaglandin E2 (PGE2). PGE2 is a product of the inflammatory COX signaling pathway and has been associated with cancer incidence and metastasis. Its synthesis can be inhibited by aspirin. We investigated the association of PGE-M with lethal prostate cancer in a case–control study of African American (AA) and European American men. We measured urinary PGE-M using mass-spectrometry. Samples were obtained from 977 cases and 1022 controls at the time of recruitment. We applied multivariable logistic and Cox regression modeling to examine associations of PGE-M with prostate cancer and participant survival. Median survival follow-up was 8.4 years, with 246 deaths among cases. Self-reported aspirin use over the past 5 years was assessed with a questionnaire. Race/ethnicity was self-reported. Urinary PGE-M levels did not differ between men with prostate cancer and population-based controls. We observed no association between PGE-M and aggressive disease nor prostate-cancer-specific survival. However, we observed a statistically significant association between higher (>median) PGE-M and all-cause mortality in AA cases who did not regularly use aspirin (HR = 2.04, 95% CI 1.23–3.37). Among cases who reported using aspirin, there was no association. Our study does not support a meaningful association between urinary PGE-M and prostate cancer. Moreover, PGE-M levels were not associated with aggressive prostate cancer. However, the observed association between elevated PGE-M and all-cause mortality in AA non-aspirin users reinforces the potential benefit of aspirin to reduce mortality among AA men with prostate cancer.

Citation: 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.; et al. Urinary PGE-M in Men with Prostate Cancer. *Cancers* **2021**, *13*, 4073. https://doi.org/10.3390/cancers13164073

Academic Editor: Mary F McMullin

Received: 8 July 2021
Accepted: 10 August 2021
Published: 13 August 2021

Keywords: prostate cancer; aspirin; health disparity; prostaglandin E metabolite; cyclooxygenase; inflammation

1. Introduction

Chronic inflammation has been implicated in prostate cancer etiology and progression [1–3]. The pro-inflammatory cyclooxygenase (COX) pathway, where arachidonic acid is converted to bioactive prostaglandins and eicosanoids via the COX-1 and COX-2 enzymes, is linked to elevated systemic inflammation [4]. Upregulated expression of the COX-2 enzyme in cancer cells has been associated with metastatic prostate cancer [5–7], but the clinical significance of COX-2 inhibition for the treatment of prostate cancer remains uncertain [8].

Prostaglandin E2 (PGE2) is the most abundant prostaglandin synthesized by the COX pathway. In colon cancer, this prostaglandin is the key mediator of the oncogenic effects of COX-2 [9]. PGE2 promotes cancer cell survival and metastasis through stem cell expansion and inhibition of the p53 pathway in colorectal cancer [10,11]. Endogenous PGE2 production can be estimated by measuring its major metabolite, urinary PGE-M (11α-hydroxy-9,15-dioxo-2,3,4,5-tetranor-prostane-1,20-dioic acid) [12,13]. Elevated PGE-M has been associated with a risk of cancer in many sites, including colorectal cancer [14], postmenopausal breast cancer [15], pancreatic cancer [16], and gastric cancer [17]. This pattern is not universally described, however, and a null association has been reported for ovarian cancer [18]. Additionally, the use of the COX-2-specific inhibitor, celecoxib, did not improve survival in prostate cancer, as reported in the STAMPEDE trial [8]. Elevated PGE-M has been associated with metastasis of breast cancer to the lung [4] and colorectal metastasis in mouse models [10,14]. To date, there has been a lack of studies investigating the relationship between PGE-M and prostate cancer despite strong rationale, with chronic inflammation being linked to the disease as a candidate risk factor [2,19].

The anti-inflammatory drug aspirin is now recommended by the US Preventative Services Task Force for the prevention of colorectal cancer [20,21]. Aspirin can inhibit PGE-M levels, and the ASPIRED trial reports utility for measuring urinary PGE-M as a biomarker of aspirin effectiveness in the prevention of disease recurrence [22]. Aspirin has also shown chemopreventive effects against aggressive prostate cancer in high-risk populations [23,24] and lethal prostate cancer in general [25,26].

With strong prior evidence suggesting inflammation as a risk factor of aggressive prostate cancer, this study aimed to identify if elevated urinary PGE-M levels are associated with adverse survival outcomes in men with prostate cancer. Furthermore, we aimed to identify if aspirin use may influence these survival outcomes.

2. Materials and Methods

2.1. Study Population

The NCI–Maryland prostate cancer case–control study has been previously described [24,27]. The study was initiated to test the primary hypothesis that environmental exposures and ancestry-related factors contribute to the excessive prostate cancer burden among African American (AA) men. Prior to interview, all subjects 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 STROBE guidelines for the reporting of observational studies. See Supplementary Methods for exclusion/inclusion criteria, questionnaire, and biospecimen information.

2.2. Laboratory Assay for Urinary PGE-M Measurement

Urinary 11a-hydroxy-9,15-dioxo-2,3,4,5-tetranor-prostane-1,20-dioic acid (PGE-M) was measured by the Eicosanoid Core Laboratory at Vanderbilt University Medical Center (Nashville, TN, USA). See Supplementary Methods for more details about this assay and its quality control performance. Measurement of urinary PGE-M has been established as a reliable reflection of circulating prostaglandin E2 [28].

2.3. 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 one pill per week for 2 months during the past 5 years", with responses no, yes, or do not know.

2.4. Statistical Analysis

Data analysis was performed using the Stata/SE 16.0 statistical software package (StataCorp). All statistical tests were two-sided. An association was considered statistically significant with $p < 0.05$. For analysis, we assessed PGE-M as either a continuous measure or assigned PGE-M values to quartiles (Q1–Q4, Q1 being the lowest, Q4 being the highest) and median (≤median/>median) with cutoff points determined using the distribution of PGE-M values among all controls. PGE-M data analyzed as a continuous measure were log2 transformed. The non-parametric Mann–Whitney test was used to determine differences in PGE-M levels across cases and controls. 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 [29]. We condensed these risk groups into 4 categories (low, intermediate, high/very high, and regional/metastatic).

Unconditional logistic regression models were used to calculate adjusted odds ratios (OR) and 95% confidence intervals (CI) to assess the association of PGE-M with either use of aspirin in cases and controls, case status, or the NCCN risk score in cases. We adjusted for the following potential confounding factors: age at study entry, body mass index (BMI), diabetes, aspirin use, education, family history of prostate cancer, self-reported race, smoking history, treatment, disease stage, and Gleason score (see Supplementary Methods for more information). To test for heterogeneity of odds ratios, we applied the Breslow–Day test. To test for statistical interactions, we applied the multivariable logistic regression model with and without the interaction term and examined significance with the likelihood ratio test. A $p < 0.05$ was considered as statistical evidence for effect modification.

We applied the Cox regression model to estimate adjusted hazard ratios (HR) and 95% confidence intervals (CI) for all-cause mortality and prostate cancer-specific mortality. Median survival follow-up for cases 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 European American (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 Supplementary Methods). We calculated survival for cases from date of diagnosis to either date of death or to the censor date of 31 December 2018. We confirmed non-violation of the proportionality assumption based on the goodness-of-fit test using Schoenfeld residuals. 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 PGE-M levels.

examine differences in all-cause and prostate cancer-specific mortality according to PGE-M levels.

3. Results

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

Demographic characteristics of the enrolled subjects are shown in Table S1, together 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/ethnicity was self-reported as part of the eligibility screener and within the questionnaire.

3.2. Urinary PGE-M Levels Do Not Differ between Men with Prostate Cancer and Population-Based Controls

We measured urinary PGE-M in samples that were obtained from cases and 1020 controls at time of recruitment. We then investigated whether urinary PGE-M differed between men with and without prostate cancer. Stratified by self-reported race, we did not observe consistent differences between cases and controls for the AA and EA men (Figure 1).

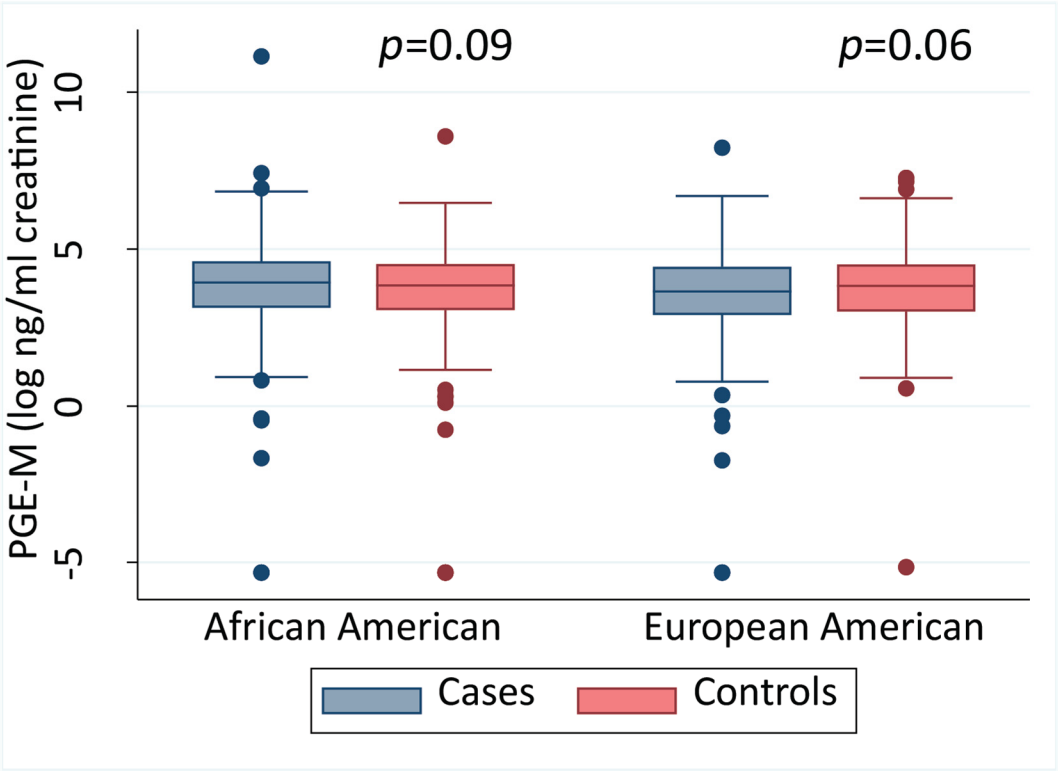

Figure 1. Urinary PGE-M levels in African American and European American men. Urinary PGE-M levels in cases and controls stratified into African American and European American men shown as a continuous measure, stratified by race. A two-sided Mann–Whitney test was applied for statistical significance testing. The error bars represent the 95% CI.

3.3. High PGE-M is Associated with Moderately Decreased Odds of Prostate Cancer in EA Men in the Multivariable Analysis

We used unconditional logistic regression to further analyze the relationship of PGE-M with a prostate cancer diagnosis and estimated the odds of having prostate cancer when PGE-M is elevated. The adjusted OR for prostate cancer was 0.86 (95% CI 0.72–1.04) for men with high (>median) PGE-Mcompared to low PGE-M (≤median) (Table 1), i.e., no statistically significant association of PGE-M levels with case status in the combined cohort. Because the COX signaling pathway may affect AA and EA prostate cancer patients differently, we stratified the analysis by race/ethnicity. Here, a moderate but

statistically significant inverse association between high PGE-M and having prostate cancer was observed among EA men (OR = 0.76, 95% CI 0.59–0.99) but not AA men (OR = 1.00, 95% CI 0.75–1.31). Heterogeneity of the odds ratios was further demonstrated using the Breslow–Day test (p = 0.03). Our finding for EA men was confirmed when PGE-M was treated as a continuous variable (OR = 0.88, 95% CI 0.79–0.98). This observation would suggest a moderate protective rather than a deleterious effect of high PGE-M in association with prostate cancer development in EA men.

Table 1. Association of urinary PGE-M levels with prostate cancer.

	Odds of Case Status *N*, (%)										
	All Cases			African American			European American				
PGE-M	Control	Case	OR (95% CI) *	Control	Case	OR (95% CI) *	Control	Case	OR (95% CI) *	P Heterogeneity	P Interaction
≤Median [a]	499 (50)	482 (51)	Reference	231 (50)	216 (46)	Reference	268 (50)	266 (56)	Reference	0.03	0.04
>Median	499 (50)	466 (49)	0.86 (0.72–1.04)	234 (50)	256 (54)	1.0 (0.75–1.31)	265 (50)	210 (44)	**0.76 (0.59–0.99)**		
Continuous [b]			0.96 (0.89–1.03)			1.03 (0.93–1.14)			**0.88 (0.79–0.98)**		

Note: column total sums (*N*, %) that differ are due to missing data. Bolded data indicate significant associations in the logistic regression analysis. [a] Median cutoff point is 14.22 ng PGE-M per mg creatinine. [b] PGE-M as a continuous, log2 transformed variable. * Unconditional logistic regression adjusted for age at study entry, BMI (kg/m^2), 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 stratified analysis), smoking history (never, former, current).

Elevated PGE-M has previously been associated with lung and colon cancer metastasis [4,14], so we investigated the association between PGE-M, aggressive disease, and metastasis. We assigned men with prostate cancer into NCCN risk groups as described under Methods and shown in Table S2. There was no association between high PGE-M (>median vs. ≤median) and the risk score classification for localized disease and metastatic disease, indicating that urinary PGE-M levels do not define intrinsic prostate cancer aggressiveness (Table 2).

Table 2. Association of high urinary PGE-M with national comprehensive cancer network risk (NCCN) score for metastatic prostate cancer.

NCCN Risk Score	OR (95% CI) *	*p* Value
Low	Reference	
Intermediate	0.96 (0.65–1.43)	0.85
High/Very High	1.13 (0.71–1.81)	0.60
Regional/Metastatic	0.93 (0.44–1.97)	0.85

Note: * Unconditional logistic regression adjusted for age at study entry, BMI (kg/m^2), 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, smoking history (never, former, current), treatment (0 = none, 1 = surgery, 2 = radiation, 3 = hormone, 4 = combination).

3.4. PGE-M and Prostate Cancer Mortality

We next examined if there is an association between PGE-M and survival outcomes in our case population. As of the end of 2018, there have been 246 deaths in our case population, of whom 27% had a prostate cancer as the recorded primary cause of death (n = 66).

Applying a multivariable-adjusted Cox regression model (Table 3), we report no significant association between PGE-M levels and all-cause mortality among men with prostate cancer in the unstratified analysis when PGE-M levels were coded as both a dichotomized (≤median compared to >median) and continuous measure. The observation remained consistent even when our analysis was further stratified by self-reported race. Prostate cancer-specific survival is a key outcome measure for prostate cancer patients.

Using a multivariable Cox regression model, we found that high PGE-M was not associated with lethal prostate cancer, although the number of prostate cancer deaths was limited (Table 4) (Figure S1 for unadjusted Kaplan–Meier graphs). Similar relationships were observed when we used continuous PGE-M data in the survival analysis (Table 4).

Table 3. Association of urinary PGE-M levels with all-cause mortality among prostate cancer patients.

Title	PGE-M	Alive	Dead	HR (95% CI)	HR (95% CI) †	HR (95% CI) *
All Cases	≤Median [a]	376 (53)	105 (44)	Reference	Reference	Reference
	>Median	329 (47)	135 (56)	**1.43 (1.11–1.84)**	**1.35 (1.05–1.74)**	1.16 (0.89–1.52)
	Continuous [b]			**1.12 (1.02–1.24)**	1.09 (0.99–1.21)	1.00 (0.90–1.12)
African American	≤Median [a]	165 (49)	50 (37)	Reference	Reference	Reference
	>Median	171 (51)	84 (63)	**1.47 (1.04–2.08)**	1.39 (0.98–1.96)	1.30 (0.91–1.87)
	Continuous [b]			1.09 (0.96–1.23)	1.07 (0.94–1.23)	1.02 (0.88–1.17)
European American	≤Median [a]	211 (57)	55 (52)	Reference	Reference	Reference
	>Median	158 (43)	51 (48)	1.26 (0.86–1.84)	1.14 (0.78–1.67)	0.97 (0.64–1.47)
	Continuous [b]			1.12 (0.96–1.32)	1.05 (0.89–1.24)	0.95 (0.80–1.13)

Note: column total sums (*N*, %) that differ are due to missing data. Bolded data indicate significant associations in the Cox regression analysis. [a] Median cutoff point is 14.22 ng PGE-M per mg creatinine. [b] PGE-M as a continuous, log2 transformed variable. † Unconditional Cox regression adjusted for age at study entry. * Unconditional Cox regression adjusted for age at study entry, BMI (kg/m^2), 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 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, and 4 = stage IV), Gleason score (0 = Gleason ≤ 7 and 1 = Gleason > 7).

Table 4. Association of urinary PGE-M levels with prostate cancer-specific mortality among prostate cancer patients.

Title	PGE-M	Alive	Death from PC	Death from Other Cause	HR (95% CI)	HR (95% CI) †	Alive *	Death from PC *	Death from Other Cause *	HR (95% CI) *
All Cases	≤Median [a]	388 (53)	28 (42)	80 (44)	Reference	Reference	375 (53)	27 (42)	78 (45)	Reference
	>Median	339 (47)	38 (58)	100 (56)	1.47 (0.90–2.41)	1.39 (0.85–2.28)	327 (47)	38 (58)	96 (55)	1.46 (0.86–2.46)
	Continuous [b]				**1.24 (1.03–1.50)**	**1.23 (1.01–1.49)**				1.21 (0.97–1.50)
African American	≤Median [a]	172 (49)	15 (38)	36 (38)	Reference	Reference	165 (49)	15 (38)	35 (37)	Reference
	>Median	178 (51)	25 (63)	60 (63)	1.43 (0.75–2.70)	1.33 (0.70–2.52)	171 (51)	25 (62)	59 (63)	1.49 (0.75–2.98)
	Continuous [b]				1.18 (0.94–1.48)	1.17 (0.92–1.50)				1.13 (0.85–1.48)
European American	≤Median [a]	216 (57)	13 (50)	44 (52)	Reference	Reference	210 (57)	12 (48)	43 (54)	Reference
	>Median	161 (43)	13 (50)	40 (48)	1.28 (0.58–2.81)	1.16 (0.53–2.56)	156 (43)	13 (52)	37 (46)	1.30 (0.54–3.14)
	Continuous [b]				1.27 (0.91–1.78)	1.20 (0.85–1.70)				1.43 (0.95–2.15)

Note: column total sums (*N*, %) that differ are due to missing data. Bolded data indicate significant associations in the Cox regression analysis. [a] Median cutoff point is 14.22 ng PGE-M per mg creatinine. [b] PGE-M as a continuous, log2 transformed variable. † Unconditional Cox regression adjusted for age at study entry. * Unconditional Cox regression adjusted for age at study entry, BMI (kg/m^2), 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 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, and 4 = stage IV), Gleason score (0 = Gleason ≤ 7 and 1 = Gleason > 7).

3.5. Aspirin Use Attenuates the Association between Elevated PGE-M and All-Cause Mortality in Prostate Cancer Patients

Aspirin has been shown to inhibit PGE-M biosynthesis [22], so we explored the possibility of an interaction between aspirin use and PGE-M on survival. No such interaction was found in the analysis of all-cause survival (p = 0.11) (Table 5) but stratification of cases by aspirin use status (yes/no) revealed disparate outcomes. There was a statistically significant association between high PGE-M and all-cause survival in the cases who reported no aspirin use. For the cases who reported using aspirin, there was no significant

relationship (HR = 0.92 95% CI 0.63–1.34). However, when stratified by self-reported race, the association between high PGE-M and all-cause mortality remained significant only in AA men who did not use aspirin (HR = 2.04, 95% CI 1.23–3.37) (Table 5). This finding is mirrored in the analysis of PGE-M as a continuous variable with a positive association with all-cause mortality for non-aspirin users (HR = 1.27 95% CI 1.03–1.57) and no association with all-cause mortality of aspirin users (HR = 0.85 95% 0.70–1.02).

Table 5. Association of urinary PGE-M levels with all-cause mortality among men with prostate cancer after stratification by aspirin use.

Title	All Cases			African American				European American				
PGE-M	**Alive**	**Dead**	**HR (95% CI) ***	**Alive**	**Dead**	**HR (95% CI) ***	**P Hetero-geneity**	**Alive**	**Dead**	**HR (95% CI) ***	**P Inter-action #**	**P Inter-action †**
Aspirin use												
≤Median	182 (53)	62 (50)	Reference	61 (43)	25 (42)	Reference	0.04	121 (60)	37 (56)	Reference	0.11	0.38
>Median	162 (47)	63 (50)	0.92 (0.63–1.34)	81 (57)	34 (58)	0.70 (0.40–1.23)		81 (40)	29 (44)	1.05 (0.63–1.75)		
Continuous [b]			0.93 (0.81–1.07)			0.85 (0.70–1.02)				1.00 (0.81–1.23)		
No aspirin use												
≤Median	193 (53)	43 (38)	Reference	104 (54)	25 (33)	Reference		89 (54)	18 (46)	Reference		
>Median	165 (46)	71 (62)	**1.52 (1.03–2.24)**	90 (46)	50 (67)	**2.04 (1.23–3.37)**		75 (46)	21 (54)	0.99 (0.48–2.07)		
Continuous [b]			1.14 (0.96–1.35)			**1.27 (1.03–1.57)**				0.91 (0.67–1.22)		

Note: Bolded data indicate significant associations in the Cox regression analysis. [a] median cutoff point is 14.22 ng PGE-M per mg creatinine. [b] PGE-M as a continuous, log2 transformed variable. * Unconditional Cox regression adjusted for age at study entry, BMI (kg/m^2), diabetes (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 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), Gleason score (0 = Gleason ≤ 7 and 1 = Gleason > 7) and stratified by aspirin (no/yes). # Test for interaction between level of PGE-M and aspirin use. † Test for interaction between self-reported race and aspirin use.

4. Discussion

In this retrospective case–control study, we report no difference in urinary PGE-M levels between cases and controls. We observed no association between elevated PGE-M and metastatic disease nor prostate-cancer-specific mortality. Of note, however, we report a significant association between elevated PGE-M and increased all-cause mortality in AA men with prostate cancer when they did not take aspirin. This association was not observed in AA men who had reported aspirin use, suggesting that aspirin may reduce all-cause mortality for AA men with prostate cancer. This is potentially of clinical importance as these results support a role for aspirin as a chemopreventive agent against mortality in men with prostate cancer. However, the hypothesis that the chemopreventive mechanism of action of aspirin is via PGE-M in prostate cancer is not supported by this study, as we did not find that aspirin use inhibited PGE-M formation in AA men with prostate cancer (Table S3).

Dysregulated COX signaling and PGE2 production have been observed across many tumor types [6,30–33]. Elevated PGE-M, reflecting in vivo PGE2 biosynthesis, has been associated with cancers of the colon [14,22,34], pancreas [16], stomach [17,35], post-menopausal breast [15,36], and lung [4]. However, this relationship was not observed in ovarian cancer [18], and our results now indicate very little evidence for elevated PGE-M playing a role in prostate cancer.

The finding of moderately decreased odds of prostate cancer in EA men with elevated PGE-M was unexpected given what is already known about elevated PGE-M being associated with cancer development at other organ sites such as the colon (summarized in Table S4). We do not have an answer as to why this may be happening, but what it does suggest is that COX signaling may be working differently in prostate cancer when

compared to other cancer sites, with PGE2 signaling not being an oncogenic driver of the disease.

Chronic inflammation has been described as a prostate cancer risk factor that is associated with aggressive disease [2,3]. Epidemiological studies have reported protective effects of aspirin against aggressive disease and adverse outcomes in high-risk groups with prostate cancer [23,24]. Identifying PGE-M as a novel marker of aggressive disease would have importance for high-risk groups such as men of African descent who experience a disproportionately high burden of prostate cancer lethality. However, we did not find evidence for such a mechanism.

The lack of a robust PGE-M inhibition in both cases and controls who reported aspirin use in our study was surprising. Many studies have now demonstrated how aspirin use, at various doses, lowers urinary PGE-M levels [34,37]. However, Drew et al. reported approximately 20–25% of participants in the ASPIRED study who were randomized to 81 or 325 mg/day of aspirin experienced no inhibition or even an increase in PGE-M from baseline despite demonstrating a strong inhibition of urinary thromboxane B2 [22]. This finding is consistent with our data, where in a previous study, using the same cohort of participants, we reported strong thromboxane B2 inhibition in aspirin users across both cases and controls [38]. This suggests that the participants in our study are not non-responders to aspirin, but rather, aspirin use mechanistically is not targeting PGE-M in these men. It may also suggest that higher doses of aspirin are required in some men to achieve significant levels of PGE-M inhibition. Our lack of dose information prevents us from further investigating this phenomenon.

Our study has certain limitations. First, the case–control study design is retrospective, so it remains unknown if elevated PGE-M is protective against prostate cancer development in EA men. Second, we lack aspirin dose and dose compliance information, which prevents us from presenting data on the importance of aspirin dose in preventing all-cause mortality in AA men. However, the ASPIRED and AFPPS trials observed similar levels of PGE-M inhibition when either 81 mg/day or 325 mg/day doses were administered [22,37]. We also lack detailed data on the frequency of aspirin use, which would potentially provide more accurate estimates of PGE-M inhibition following aspirin use.

A major strength of this case–control study is the participant diversity, with very similar numbers of AA and EA participants. With 50% of cases and 47% of controls self-reporting as AA, we have a unique opportunity to examine biological factors that may promote cancer differently between population groups.

5. Conclusions

In summary, we evaluated associations between PGE-M levels and prostate cancer in a case–control study. We found no association between urinary PGE-M and aggressive prostate cancer. An association between elevated PGE-M and all-cause mortality in non-aspirin AA users reinforces the potential benefit of aspirin for the prevention of lethal prostate cancer, but the evidence does not support the mechanism of action to be via PGE-M inhibition. A prospective study is needed to confirm these findings.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/cancers13164073/s1, Supplementary Methods, Table S1: Characteristics of cases and population controls in the NCI–Maryland Prostate Cancer Case–Control Study, Table S2: Men with prostate cancer by urinary PGE-M level and national comprehensive cancer network risk score, Table S3: Association of regular aspirin use with urinary PGEM levels in cases and controls, Table S4: Association of PGE-M and cancer risk from published literature, Figure S1: High urinary PGE-M levels are not significantly associated with increased prostate cancer-specific mortality.

Author Contributions: M.K.: conceptualization; investigation; data curation; methodology; formal analysis; writing—original draft; writing—review and editing, G.L.M.: methodology, T.Z.M.: conceptualization; writing—review and editing, T.H.D.: data curation; project administration, W.T.: supervision; writing—review and editing, C.J.S.: writing—review and editing, F.B.: project administration, C.A.L.: methodology; writing—review and editing, C.Y.: writing—review and editing,

M.B.C.: methodology; writing—review and editing, S.A.; conceptualization; methodology; funding acquisition; resources; writing—review and editing; supervision. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the DoD award W81XWH1810588 (to S.A. and C.Y.) and the Intramural Research Program of the NIH, National Cancer Institute (NCI), Center for Cancer Research and Division of Cancer Epidemiology and Genetics; Maeve Kiely is supported by the NCI Cancer Prevention Fellowship program.

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Review Board of NCI (protocol #05-C-N021) and the University of Maryland (protocol #0298229).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data underlying this article will be shared upon reasonable request to the corresponding author.

Acknowledgments: We would like to thank the personnel at the University of Maryland and the Baltimore Veterans Administration Hospital for their contributions to the recruitment of subjects. We would also like to thank the subjects who contributed their time and biospecimens to make the NCI–Maryland study possible. Graphical abstract was created with BioRender.com.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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cancers

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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

Citation: Kiely, M.; Ambs, S. Immune Inflammation Pathways as Therapeutic Targets to Reduce Lethal Prostate Cancer in African American Men. *Cancers* **2021**, *13*, 2874. https://doi.org/10.3390/cancers13122874

Academic Editors: W. Martin Kast and Sharon Glynn

Received: 7 May 2021
Accepted: 7 June 2021
Published: 9 June 2021

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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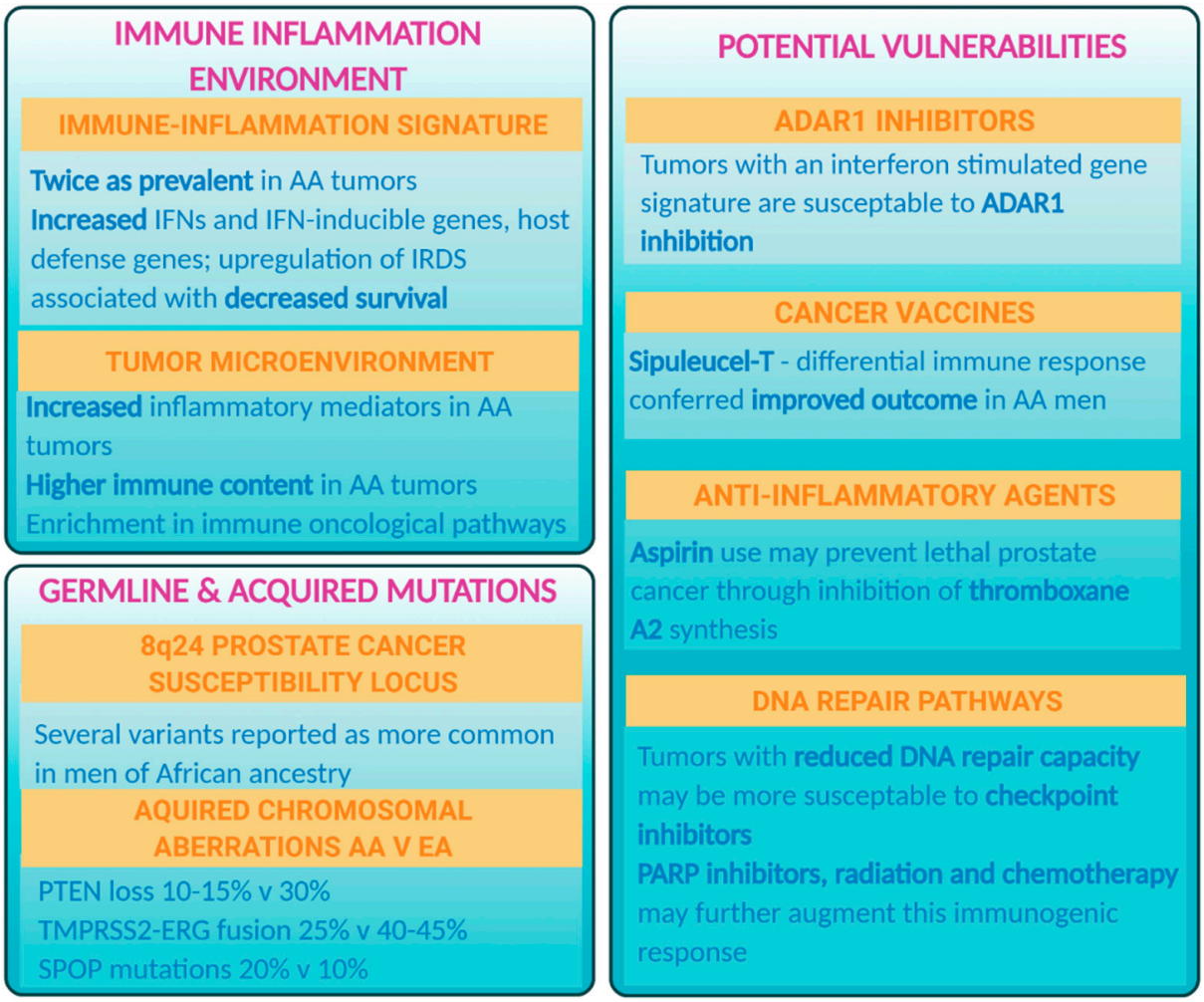

Figure 1. Key differences in the tumor immune–inflammation environment and the mutational spectrum between African American and European American men with prostate cancer. These differences open up potential vulnerabilities which preliminary studies have indicated could be exploited with treatment options,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

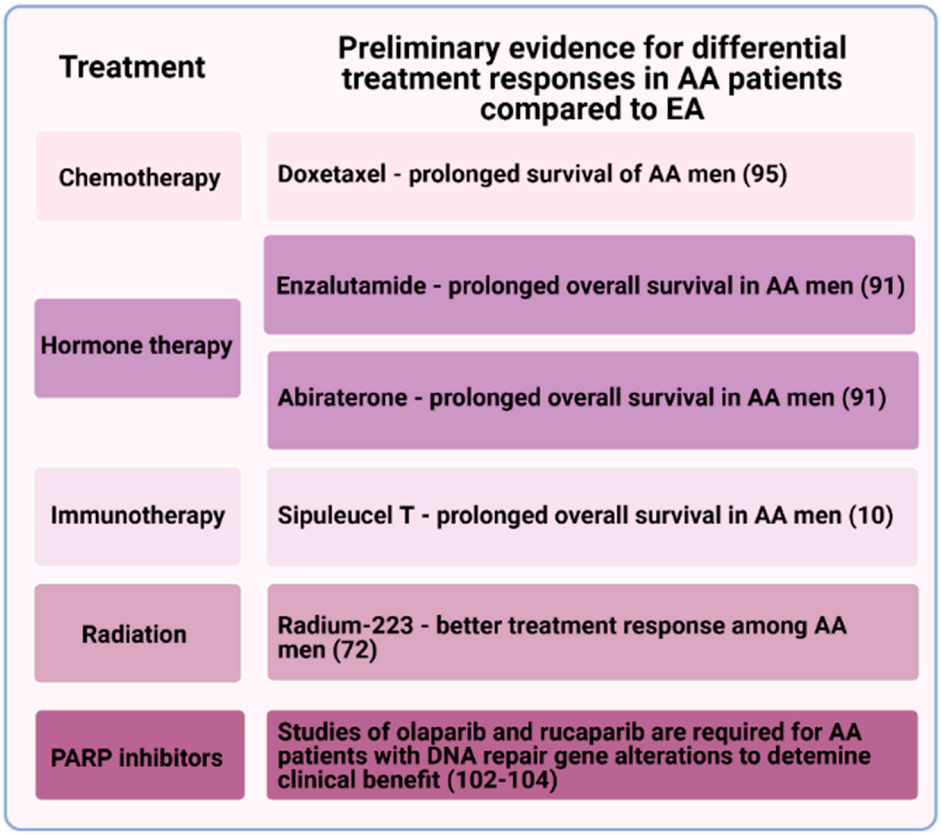

Figure 2. Preliminary evidence for certain treatment response differences between African American (AA) and European American (EA) patients with metastatic castration-resistant prostate cancer (mCRPC). Current approved treatment modalities for mCRPC, how clinical responses may differ between the two 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%–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.

Author Contributions: Conceptualization, M.K. and S.A.; writing—original draft preparation, M.K. and S.A.; writing—review and editing, M.K. and S.A.; funding acquisition, M.K. and S.A. Both authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by the Intramural Research Program of the NIH, National Cancer Institute (NCI), Center for Cancer Research and the DoD award W81XWH1810588 (to S.A.); Maeve Kiely is supported by the NCI Cancer Prevention Fellowship program.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The study is a literature review and did not analyze any data.

Conflicts of Interest: The authors declare no conflict of interest.

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Published OnlineFirst December 8, 2020; DOI: 10.1158/1055-9965.EPI-19-0792

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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Cancer Epidemiol Biomarkers Prev 2021;30:539–44

doi: 10.1158/1055-9965.EPI-19-0792

Published OnlineFirst December 8, 2020; DOI: 10.1158/1055-9965.EPI-19-0792

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–

Published OnlineFirst December 8, 2020; DOI: 10.1158/1055-9965.EPI-19-0792

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

Published OnlineFirst December 8, 2020; DOI: 10.1158/1055-9965.EPI-19-0792

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.

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

W.J. Blot reports grants from NIH during the conduct of the study. No disclosures were reported by the other authors.

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.

Acknowledgments

This research was supported by the Intramural Research Program of the NIH, NCI, Center for Cancer Research (ZIA BC 010499, ZIA BC 010624, and ZIA BC 010887, to S. Ambs); Department of Defense award W81XWH1810588 (to S. Ambs); the NCI Cancer Prevention Fellowship program (to L. M. Hurwitz); R01CA092447, NCI/NIH (to W.J. Blot); U01 CA202979, NCI/NIH (to W.J. Blot); and The Southern Community Cohort Study (SCCS). SCCS data collection was performed by the Survey and Biospecimen Shared Resource, which is supported, in part, by the Vanderbilt-Ingram Cancer Center (P30 CA68485). The authors thank the SCCS Publications Committee for reviewing the article and helpful comments.

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Received July 9, 2020; revised October 13, 2020; accepted December 4, 2020; published first December 8, 2020.

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Published OnlineFirst December 8, 2020; DOI: 10.1158/1055-9965.EPI-19-0792

Cancer Epidemiology, Biomarkers & Prevention

AACR American Association for Cancer Research

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

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Cancer Epidemiol Biomarkers Prev 2021;30:539-544. Published OnlineFirst December 8, 2020.

Carcinogenesis, 2021, Vol. 42, No. 1, 2–13

doi:10.1093/carcin/bgaa121
Advance Access publication November 13, 2020
40th Anniversary Review Article

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

Received: October 13, 2020; **Revised:** November 1, 2020; **Accepted:** November 6, 2020

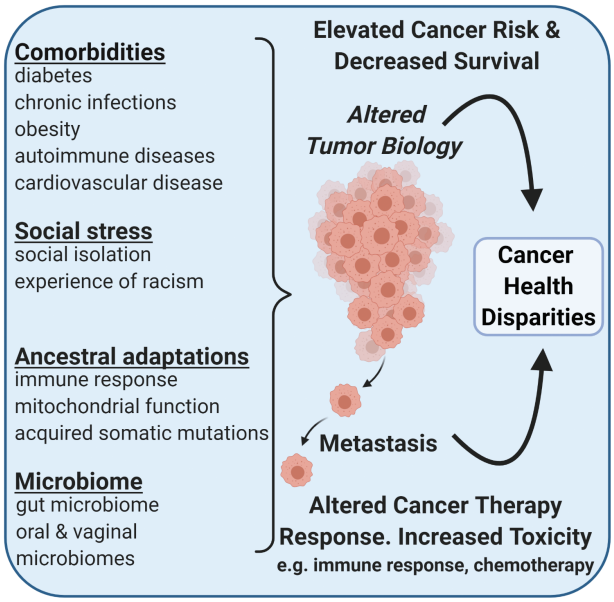

Figure 1. Comorbid chronic diseases, stress exposure, population differences in immune response, and dysbiosis are factors that contribute to cancer health disparities.

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.

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

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

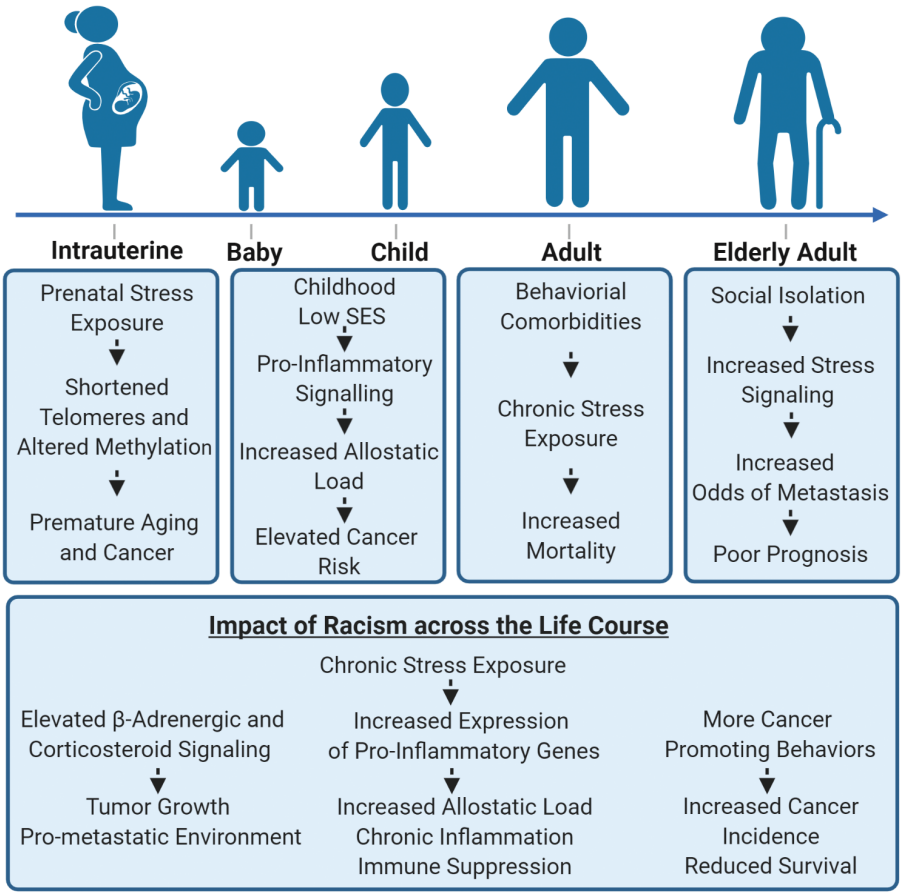

Figure 2. Stress exposure over the life course and its potential impact on socially deprived and minority populations.

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

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).

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).

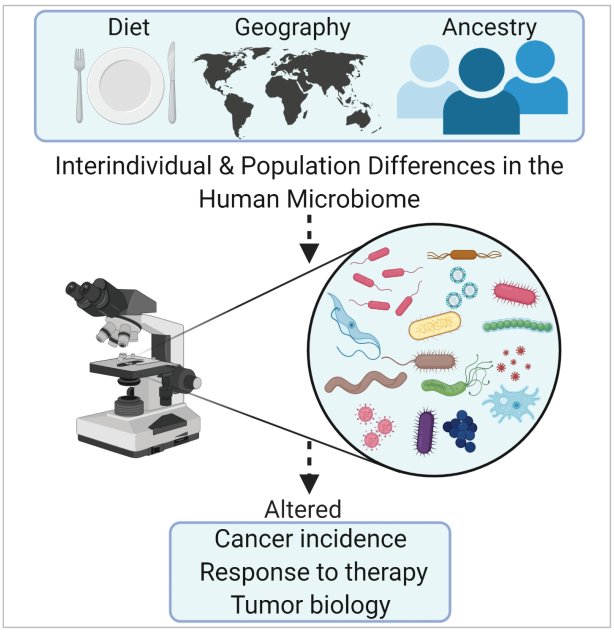

Figure 3. Diet, geographic location, and ethnicity strongly associate with the diversity of the gut microbiome and may increase the risk of dysbiosis, a cancer risk factor.

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

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.

Acknowledgements

Maeve Bailey-Whyte is an NCI Cancer Prevention Fellow funded by the NCI Division of Cancer Prevention. Anuoluwapo Ajao is a Post-baccalaureate Fellow sponsored by the NIH Academy Fellowship Program and by a National Institute of Minority Health and Health Disparities Fellowship. Figures were generated using the software BioRender.

Funding

Intramural Research Program of the National Institutes of Health (NIH), National Cancer Institute (NCI), Center for Cancer Research (ZIA BC 010499, ZIA BC 010624, and ZIA BC 010887); U.S. Department of Defense award (W81XWH1810588), and National Institute of Minority Health and Health Disparities.
Conflict of interest statement: None declared.

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