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"
PRINCIPAL INVESTIGATOR:	Dr. Stefan Ambs
CONTRACTING ORGANIZATION:	The Geneva Foundation 917 Pacific Ave #600 Tacoma, WA 98402
REPORT DATE:	October 29, 2020
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 29, 2020	Annual Report	30 September 2019-29 September 2020

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)
Dr. Stefan Ambs

ambss@mail.nih.gov

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

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

Table of Contents

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. 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 collaboration with our Co-PI, Dr. Clayton Yates at Tuskegee University, we will determine the prevalence and origin of an immune-inflammation signature in tumors of men of African and European ancestry.

2. Keywords

African-American, Africa, ancestry, biomarker, case control study, chromatin, cyclooxygenase, disease progression, DNA, genetic variation, genomics, immunity, inflammation, mutation, RNA, risk factor, omega-3 fatty acid, tumor biology, transcriptome, urine.

3. Accomplishments

We continued our research addressing all aims, tasks, and subtasks of the award, but were negatively impacted by the COVID-19 pandemic and the closures that resulted from it. The impact of COVID-19 on tasks was as follows: NCI laboratories and service providers like University of Maryland contractor for NCI-Maryland Prostate Cancer study, NCI-Leidos (RNA and DNA extractions) and the NCI Cancer Genomics Research Laboratory (GWAS genotyping) were closed from Friday, March 20, into July 2020, when these facilities started to resume services. These closures affected data collection for participants in the NCI-Maryland Prostate Cancer study and two projects. For **Specific Aim 2**, they delayed completion of Major Task 2 (GWAS genotyping). For **Specific Aim 3**, they delayed completion of Major Task 1 & 2 (RNAseq and whole exome sequencing). In addition, several team members are working on reduced schedules (Tsion Minas, Maeve Bailey-Whyte, Tiffany Dorsey) as they have young kids and day care has not been available to them. Despite these obstacles, we made great progress with the work and research, and achieved several milestones, and started to work on research manuscripts.

Progress in the reporting period.

During the past 24 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 these 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 metabolites), 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 (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. Most recently (April 2020), we received from Olink the immune-oncology marker data for 70 Nigerian prostate cancer patients and 170 Nigerian men without prostate cancer (controls). 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 has been resolved (see Changes/Problems, page 22).

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 a few of the immune-inflammation markers (5-10), 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. However, the markers in question cannot be re-measured and will be excluded from analysis.

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 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, have begun to analyze them for research manuscripts. 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. As an initial step – to examine whether the abundance pattern of the 82 immune-oncology markers is specific to population groups – Tsion performed an unsupervised hierarchical cluster analysis, using only data from the healthy volunteers to exclude confounding by a prostate cancer diagnosis, and found that serum

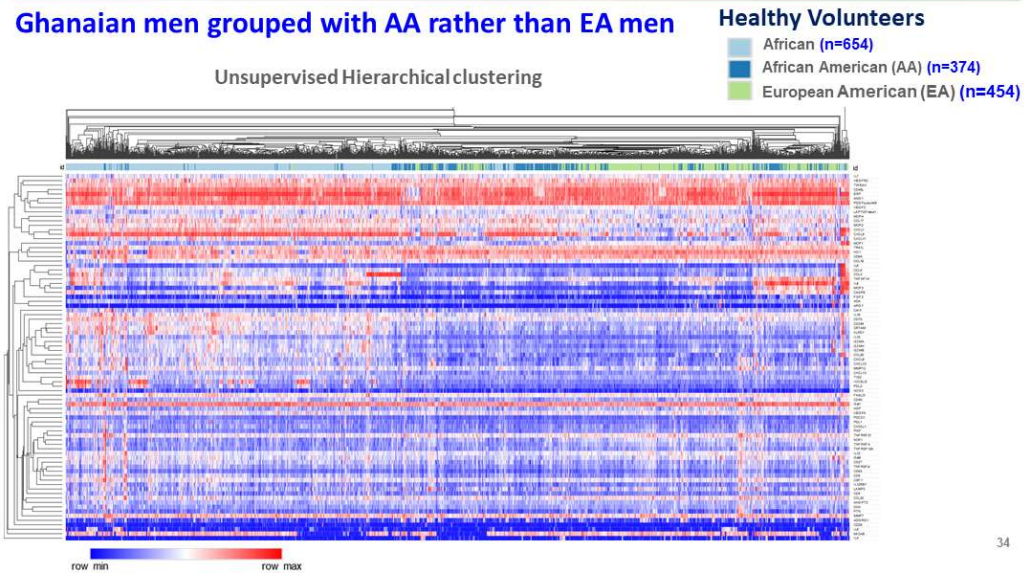

Figure 1

levels of these markers clustered by population group (Figure 1). Ghanaian men grouped closer with African-American than European-American men. The finding is consistent with our hypothesis that a unique systemic immune-inflammation signature might exist in men of African ancestry. Next, Tsion explored if these differences in immune-oncology marker expression may relate to West African ancestry. We could ask this question because participants in the NCI-Maryland study were previously typed for West African, Native American, and European ancestry as part of a collaboration with Rick Kittles' laboratory, now at the City of Hope, using a panel of 104 ancestry-informative markers. In her analysis - again focusing on the healthy volunteer population in the study – Tsion found that blood levels of some chemokines strongly correlated with the degree of West African ancestry among these men (Figure 2), suggesting a potential ancestral influence on the immune response, consistent with published data (PMID: 27768889) and our study hypothesis. We will further investigate this relationship between immune-oncology marker levels and genetic ancestry with the analysis of the genotyping data from the HumanOmni5-Quad BeadChip, using a genome-wide association study approach, with access to 4 million genetic markers, as described under Specific Aim 2.

To better understand the functional implication of the immune-oncology markers, Tsion grouped the markers into 6 pathways, namely 1. apoptosis, 2. autophagy, 3. chemotaxis, 4. promotion of tumor immunity, 5. suppression of tumor immunity, and 6. vasculature and tissue remodeling, following Olink classification, and assigned z-scores of pathway activity to each man in the study. She then investigated the association of pathway activity with survival of prostate cancer patients in the NCI-Maryland study - as survival data are not available for the NCI-Ghana cohort. These investigations showed that the increased activity of one pathway, suppression of tumor immunity, was significantly associated with decreased overall survival among men with prostate

cancer (HR = 5.9, 95% CI 1.46 to 23.9) , but not among men without the disease - controls (Figure 3).

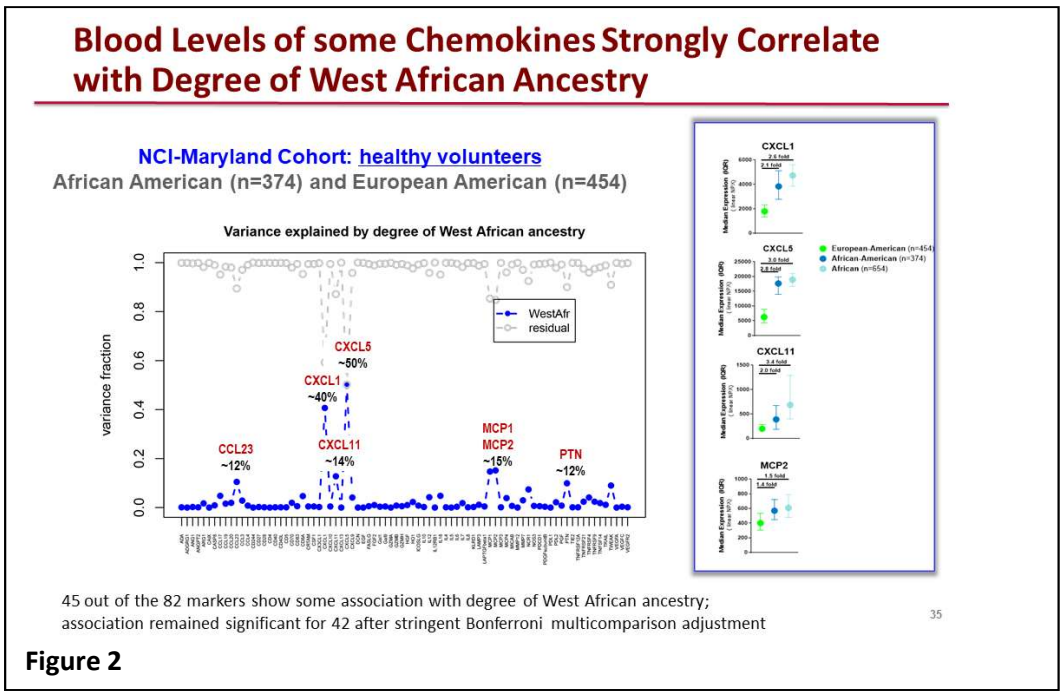

Figure 2

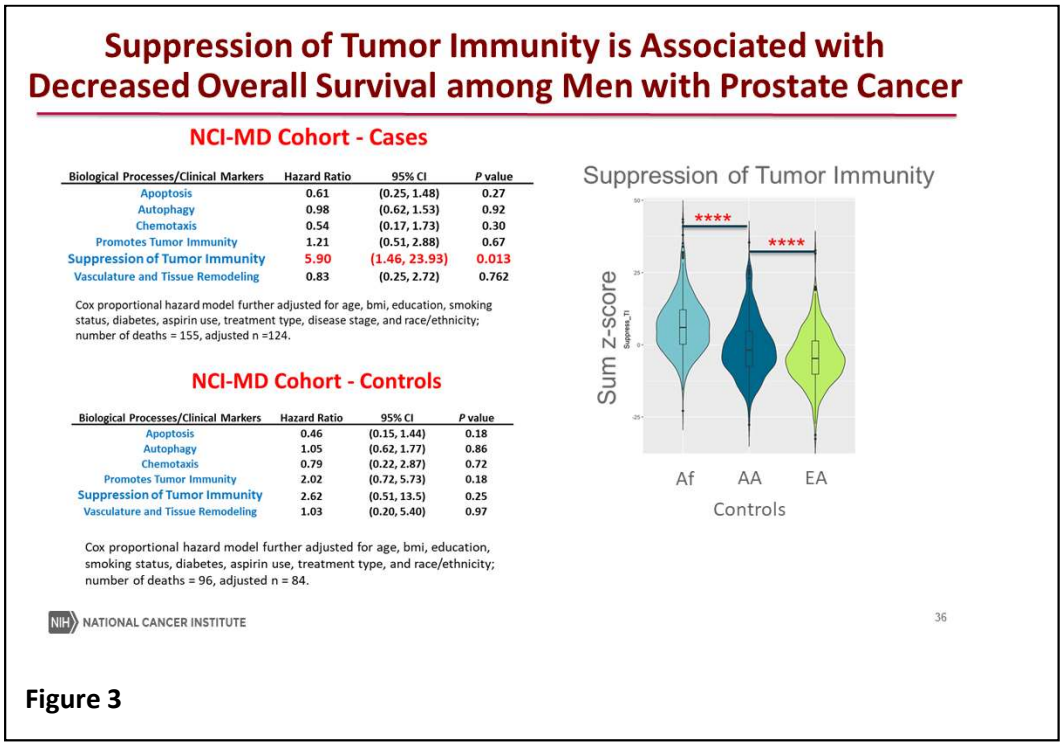

Figure 3

Notably, activation of this pathway was associated with African descent as well, with the highest activation scores in Ghanaian men, followed by African-American men, and yielding the lowest scores in European-American men – as shown for healthy volunteers in the three population groups (Figure 3, figure to the right). Suppression of tumor immunity also associated with decreased cancer survival

among the prostate cancer patients (HR = 17.3, 95% CI 2.58 to 116.4), an analysis that included all reported cancers in this patient cohort – not just prostate cancer, and showed an association with an increased likelihood of a prostate cancer-specific death (HR = 5.77, 95% CI 0.38 to 86.9), however, this association was not statistically significant because of the low number of prostate cancer-specific deaths in the adjusted analysis (n = 36) (Figure 4). The latter analysis will be repeated with the now updated mortality data from the National Death Index database.

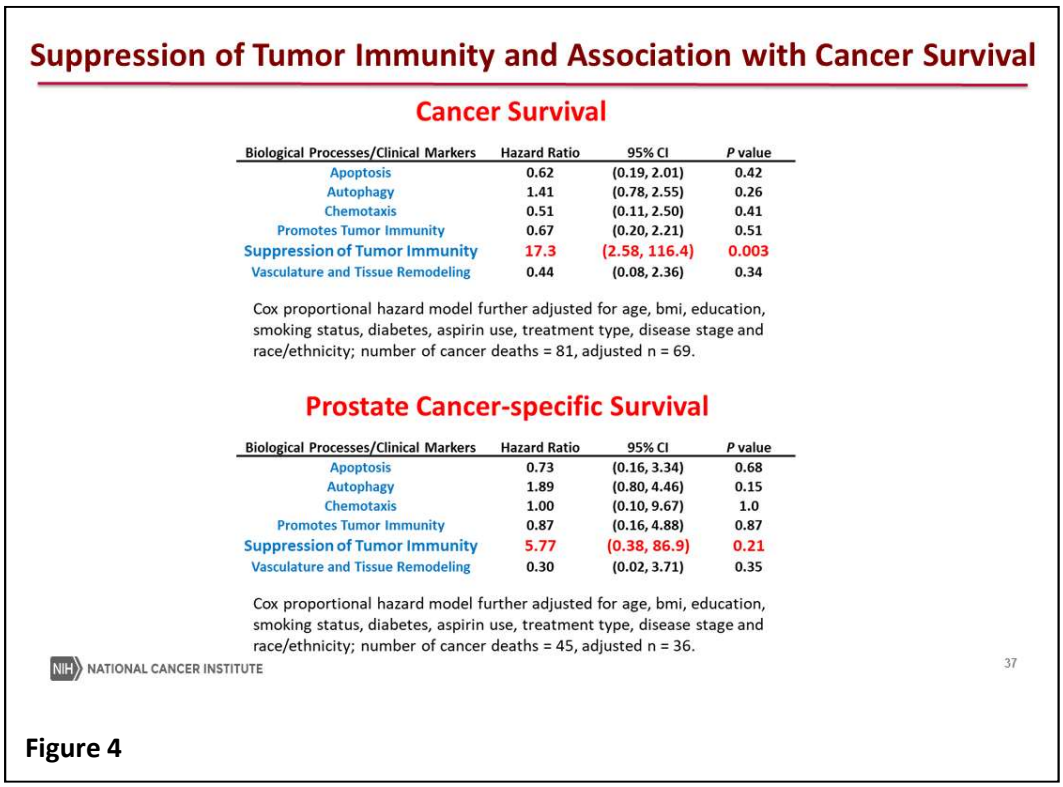

Figure 4

Lastly, Tsion's analysis revealed an significant association of the suppression of tumor immunity pathway with higher odds of presenting with metastatic prostate cancer in the NCI-Maryland study (adjusted OR = 3.04, 95% CI: 1.09 to 8.53 for patients with the highest pathway activation scores - top 25%; Figure 5), further indicating that increased suppression of tumor immunity may predispose to lethal prostate cancer. In this analysis, prostate cancer patients with the lowest pathway activation scores (quartile 1, lowest 25%) were set as reference. Tsion's findings are consistent with our hypothesis that a distinct immune-inflammation signature exists in men of African ancestry that includes suppression of tumor immunity and may increase the risk of lethal disease among these men. Tsion is currently working on a manuscript to submit these important findings to a peer-reviewed journal.

Suppression of Tumor Immunity Associates with Increased Odds of Metastatic Prostate Cancer

	Localized Prostate Cancer[a] *N*(%)	Regional or Metastatic Prostate Cancer[b] *N* (%)	Univariable OR (95% CI)	Multivariable[c] OR (95% CI)	*P*-value
Chemotaxis score					
Quartile 1	242 (32)	10 (20)	Ref.	Ref.	
Quartile 2	244 (32)	14 (29)	1.39 (0.61, 3.19)	1.15 (0.48, 2.77)	0.759
Quartile 3	171 (23)	11 (22)	1.56 (0.65, 3.75)	1.33 (0.51, 3.48)	0.564
Quartile 4	99 (13)	14 (29)	**3.42 (1.47, 7.96)**	2.48 (0.89, 6.89)	0.081
			P_{trend} **0.006**	P_{trend} 0.095	
Suppression of TI score					
Quartile 1	241 (32)	9 (18)	Ref.	Ref.	
Quartile 2	227 (30)	15 (31)	1.77 (0.76, 4.12)	1.71 (0.69, 4.27)	0.247
Quartile 3	171 (23)	10 (20)	1.57 (0.62, 3.94)	1.36 (0.49, 3.78)	0.559
Quartile 4	117 (15)	15 (31)	**3.43 (1.46, 8.08)**	**3.04 (1.09, 8.53)**	**0.034**
			P_{trend} **0.009**	P_{trend} 0.073	

[a]Localized prostate cancer cases wi per 2019 NCCN guideline
[b]Regional prostate cancer cases with lymphnode involvement or metastatic prostate cancer cases with distant metastasis per 2019 NCCN guideline
[c]Unconditional logistic regression adjusted for body mass index at study enrollment (BMI, kg/m^2), age at study entry, education (high school or less, some college, college, professional school), smoking history (never, former, current), aspirin use (regular user, yes/no), diabetes (yes/no), annual household income (less than $10k, $10-30k, $30-60k, $60-90k, more than $90k) and race

OR denotes Odds Ratio; 95% CI denotes 95% confidence interval

NIH NATIONAL CANCER INSTITUTE

38

Figure 5

The tasks described under **Specific Aim 1**, included the measurement of five urinary metabolites (PGD-M, PGE-M, PGI-M, 11dTxB2, TNE) that are surrogates for cyclooxygenase signaling, a pro-inflammatory and oncogenic signaling pathway (Figure 6). 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 (Table 5) to the Eicosanoid Core Laboratory at

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

Vanderbilt University to be analyzed by mass spectrometry using assays that have previously been validated at the facility. 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 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 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 the colorimetric assay. Having this clarification, the Eicosanoid Core Laboratory re-measured urinary creatinine in all 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 quality control data at this facility and in our pilot study.

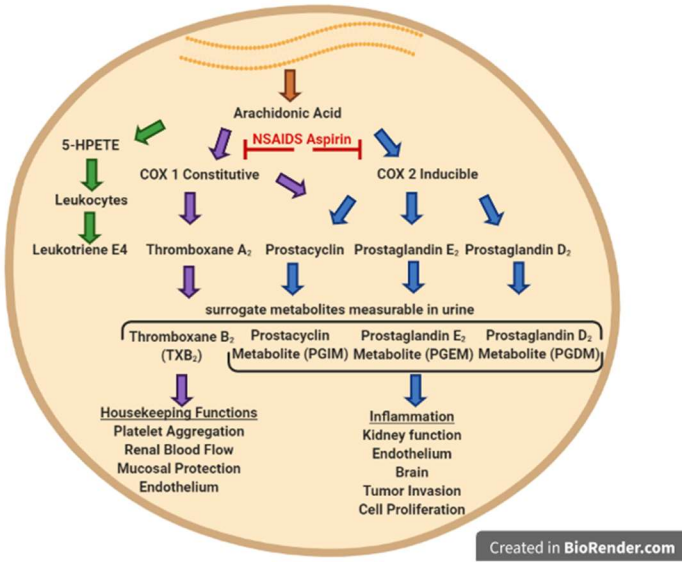

Figure 6: Cyclooxygenase pathway is a principle mechanism driving the association of inflammation and cancer

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

Maeve Bailey-Whyte has begun to analyze the data, initially focusing on the thromboxane A2 signaling pathway as this pathway has recently been linked to increased cancer metastasis through its effect on platelet function and aggregation (*Lucotti et al., JCI 2019*; PMID: 30907747). This paper also showed that aspirin can inhibit this process. Levels of urinary thromboxane B2 (TBX2), a surrogate marker of thromboxane A2 bioavailability, were indeed greatly inhibited by aspirin use in the study population among men with prostate cancer - cases (Figure 7) and men without prostate cancer - controls (Figure 8), yet more so among the African-American (AA) than European-American (EA) men, as shown by the reduced fraction of men with the highest urinary TBX2 levels (top 25%) among aspirin users. The data suggest that aspirin use may have a stronger protective effect in African-American men.

TXB2 Formation is Inhibited by Aspirin Use in Cases

Aspirin Use in AA Cases

TXB2 Quartile	Aspirin Use (%) No	Yes	Total
Quartile 1 (low)	37	63	100
Quartile 2	54	46	100
Quartile 3	59	41	100
Quartile 4 (high)	71	29	100
Total	58	42	100

chi score p=<0.000

Aspirin Use in EA Cases

TXB2 Quartile	Aspirin Use (%) No	Yes	Total
Quartile 1	34	66	100
Quartile 2	35	65	100
Quartile 3	43	57	100
Quartile 4	56	44	100
Total	43	57	100

chi score p=0.001

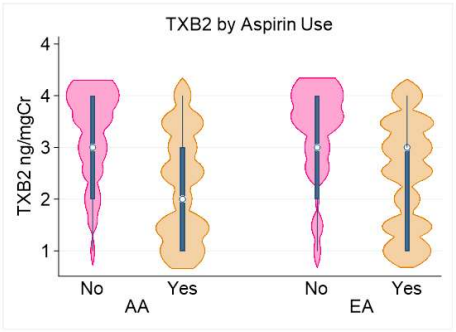

Figure 7: Thromboxane B2 formation is inhibited by aspirin use in men with prostate cancer

TXB2 Formation is Inhibited by Aspirin Use in Controls

Aspirin Use in Controls AA

TXB2 Quartile	Aspirin Use (%) No	Yes	Total
Quartile 1	38	69	100
Quartile 2	58	56	100
Quartile 3	68	41	100
Quartile 4	69	31	100
Total	50	50	100

chi score p=<0.000

Aspirin Use in Controls EA

TXB2 Quartile	Aspirin Use (%) No	Yes	Total
Quartile 1	26	74	100
Quartile 2	25	75	100
Quartile 3	46	54	100
Quartile 4	52	48	100
Total	38	62	100

chi score p=<0.000

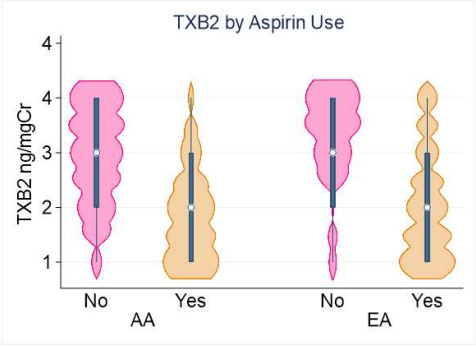

Figure 8: Thromboxane B2 formation is inhibited by aspirin use in men without prostate cancer

Consistent with these findings, Maeve observed that high urinary thromboxane B2 levels are

associated with increased odds of prostate cancer only among the African-American men (OR 1.58; 95% CI: 1.04 to 2.39; Figure 9). In addition, when prostate cancer patients were categorized by their NCCN risk score for the disease (https://www.nccn.org/patients/guidelines/content/PDF/prostate-patient.pdf), prostate cancer patients with urinary thromboxane B2 levels in the highest quartile (top 25%) were at a greatly increased risk of presenting with metastatic prostate cancer (OR 7.96; 95% CI 1.65 to 38.4; Figure 10). These findings are consistent with the recent observation from animal studies that high thromboxane A2 increases the risk of metastasis. Maeve will now examine the association of urinary thromboxane B2 levels with overall and prostate cancer-specific survival in the NCI-Maryland study. We very recently received updated National Death Index mortality data for our study - through 2018. With this analysis included, Maeve will write up her first manuscript while continuing to examine the other four urinary metabolites (PGD-M, PGE-M, PGI-M, TNE).

Association of High TXB2 with Prostate Cancer among African-American Men

	Total			
Level of TXB2	Control N (%)	Case N (%)	OR (95% CI)	p-value
Q1	251 (25)	204 (21)	Ref	
Q2	249 (25)	193 (20)	0.99 (0.75-1.30)	0.9412
Q3	251 (25)	251 (26)	1.18 (0.90-1.54)	0.2254
Q4	249 (25)	303 (32)	1.25 (0.95-1.64)	0.0991
	1000	951	p trend (0.0324)	

	AA				EA			
Level of TXB2	Control N (%)	Case N (%)	OR (95% CI)	p-value	Control N (%)	Case N (%)	OR (95% CI)	p-value
Q1	122 (26)	95 (20)	Ref		129 (24)	109 (23)	Ref	
Q2	132 (28)	98 (21)	1.00 (0.66-1.50)	0.9874	117 (22)	95 (20)	0.98 (0.67-1.44)	0.9326
Q3	114 (24)	125 (26)	1.31 (0.87-1.96)	0.1968	137 (26)	126 (26)	1.08 (0.75-1.55)	0.6729
Q4	99 (21)	157 (33)	**1.58 (1.04-2.39)**	**0.0322**	150 (28)	146 (31)	1.05 (0.73-1.51)	0.7857
	467	475	p trend (0.0097)		533	476	p trend 0.6931	

*Unconditional logistic regression adjusted for age at study entry, BMI(kg/m2), diabetes(yes/no), 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), socioeconomic status (1=Very Good, 2= Good, 3= Fair, 4= Poor), aspirin use (yes/no)

Men with high TXB2 (highest 25%) are more likely to have Metastasis than Men with low TXB2 Levels (lowest 25%)

NCCN Risk Score	OR (95% CI)	*P value*
Low	1.31 (0.72-2.4)	0.38
Favorable Intermediate	1.42 (0.71-2.82)	0.32
Unfavorable Intermediate	1.39 (0.64-3.01)	0.41
High	0.73 (0.20-2.63)	0.63
Regional	1.83 (0.32-10.47)	0.5
Metastatic	**7.96 (1.65-38.43)**	**0.01**
	P_{trend} = 0.012	

Odds of having a particular NCCN Risk Score, with low TXB2 levels (lowest 25%) as reference

Figure 10

For Specific Aim 2, Major Task 1 describes as subtask the preparation of DNA samples and shipment to the genotyping samples 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 the genotyping core facility at the NCI – as mentioned in the 2019 annual report. 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 has now completed GWAS genotyping with the Infinium HumanOmni5-Quad BeadChip for a total of 1806 subjects in the study (cases: 431 African-American; 413 European-American; controls: 458 African-American, 504 European-American), corresponding to the numbers of 900 cases and 900 controls that we will genotype per our Statement of Work for the award. This milestone finishes the laboratory-based tasks for **Specific Aim 2**. The raw genotyping data have been moved to the Analysis Team at the Cancer Genomics Research Laboratory for clustering and quality control including "data cleaning". When this task is completed, the genotyping data will be transferred to Drs. Wei Tang and Michael Cook. Per previous arrangement, a postdoctoral fellow in Michael Cook's group will start with a combined analysis of the NCI-Maryland and NCI-Ghana study, with the main aim to assess whether germline genetic variants are associated with immune-inflammation markers and prostate cancer, as outlined under **Specific Aim 2**, Major Task 2.

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

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 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, quality control analysis indicated that most RNA samples would likely fail sequencing. However, Hudson Alpha has a proprietary RNA extraction protocol for FFPE tissues. Thus, we provided them with additional FFPE tissue cores and will now examine if extraction of RNA with this protocol will yield an RNA quality that allows untargeted, large-scale sequencing. If these attempts would fail, we may consider a targeted sequencing approach that does not require the same stringent RNA quality. Alternatively, we may consider quantitation of gene expression using Nanostring technology with predeveloped assays that work with degraded RNA. However, the latter methods would not allow a whole transcriptome-wide assessment of gene expression in our samples. Aside the issues with RNA sequencing, we will – as the next step – proceed with whole exome sequencing of the isolated genomic DNA. We do not anticipate similar issues as DNA is a much more inert macromolecule than RNA and anticipate having whole exome sequencing data for 175 tissue pairs by the end of 2020.

DNA for whole exome sequencing:
175 tissue pairs (tumor and adjacent non-cancerous tissue): 61 from Nigerian, 58 from African-American, and 56 from European-American men.

Remaining FFPE cores for extraction of RNA at HudsonAlpha: 86 from Nigerian, 45 from African-American, and 47 from European-American men. Total: 178.

Opportunities for training and professional development.

Despite the challenges with COVID-19 including the closures of laboratories from Friday, March 20 into July 2020 we continued to provide opportunities to trainees. In the past 12 months, we provided these opportunities to the following fellows: Jason White (Tuskegee U); Tsion Minas, Maeve Bailey-Whyte, Anuoluwapo Ajao, and Margaret Pichardo (all NCI-associated). Yet, many of the tasks that have been completed so far provided only limited opportunity for training and professional development. Those included mostly routine tasks of sample preparation, QC 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 cannot host visitors at the NCI at this time, and this restriction will likely continue into 2021. We hosted Jason White from the Yates laboratory at our NCI laboratory for several months in 2019. Jason White is a PhD student and will participate in data analysis of the tumor data (genomics and transcriptome analysis) for this DoD grant. 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. In the past 12 months, Wei continued to mentor Jason using an existing prostate cancer dataset that was 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 is ready to earn his PhD. We have regular

Zoom meetings to discuss his progress with the project.

With the completion of data collection for **Specific Aim 1**, Tsion Minas and Maeve Bailey-Whyte, both postdoctoral fellows at the NCI, have started the analysis of the immune-oncology and urinary eicosanoid markers, respectively, and are at the stage of writing up their first manuscripts. Both are teleworking, solely focusing on data analysis and scientific reports. Tsion is more and more mastering the R software for statistical computing and is closely collaborating with Julian Candia, a Staff Scientist and Senior Data Analyst in Dr. Xin Wang's laboratory at the NCI, in the use of novel approaches of data analysis and visualization of her findings. She is making great progress. In addition, Tsion regularly consults with Michael Cook about her analysis approaches. Tsion has presented her findings covering Aim 1 at an NCI Interlaboratory Seminar and more recently at a Laboratory of Human Carcinogenesis (LHC) seminar. Both times she did very well. She was selected a chairperson for the 3rd NCI Symposium on Cancer Health Disparities, which was scheduled to take place April 16-17, 2020, but is now rescheduled due to COVID-19 for May 25-26, 2021. Furthermore, Tsion is mentoring Anuoluwapo Ajao, a PostBac in our laboratory involved with prostate cancer research, who wants to participate in epidemiological research in Nigeria as a Fulbright Research Fellow. Under Tsion's mentorship, Anuoluwapo is learning statistical analysis and epidemiological methodologies. Maeve, an NCI Cancer Prevention Fellow with a Master's in Public Health (MPH) degree, is still learning methodologies to analyze epidemiological and clinical data. Here, she is making great progress and recently introduced to our laboratory the assessment of competing risks in a survival analysis, an important analysis tool in prostate cancer research. Maeve works closely with Tsion and has additional support by a postdoctoral fellow from Michael Cook's laboratory, Lauren Hurwitz, who is a trained prostate cancer epidemiologist. Maeve has presented her findings on thromboxane B2 at an NCI Cancer Prevention Fellow seminar and is scheduled to give an upcoming presentation at an LHC seminar in late 2020 and an NCI Interlaboratory Seminar in 2021. 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". Maeve received an AACR Scholar-in-Training-Award for her contribution. Her progress is very impressive. Lastly, Margaret Pichardo began to be involved with important work related to this award. 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 is currently performed. Margaret is a rising star in cancer health disparity research. She completed her PhD in epidemiology at Yale in 2020 while being a resident physician at Howard University in Washington, DC. She already has published one research paper with our group as a volunteer (PMID: 29784730). I have no doubt that her involvement will lead to additional publications covered under this award, focusing on 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.

Dissemination of results to communities of interest. Tsion Minas gave her first data presentation at the AORTIC 2019 conference in Maputo, Mozambique, on November 6, 2019, 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 an LHC seminar. 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". Maeve has also presented her findings in an NCI Cancer Prevention Fellow seminar and is scheduled to give an LHC seminar in late 2020 and NCI Interlaboratory Seminar in 2021, covering findings from this grant. Stefan Ambs was an invited speaker at the 3rd NCI Symposium on Cancer Health Disparities, which was scheduled to take place April 16-17, 2020 but is now rescheduled for May 25-26, 2021. His presentation will focus on results from this grant. He was also scheduled as a speaker at 13th National Symposium on Prostate Cancer at Clark Atlanta University and the 6th Biennial Science of Global Prostate Cancer Disparities in Black men Conference in Barcelona, Spain. Both presentations would have focused on results from this grant. New dates for these conferences have not been set. 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. Future efforts of dissemination will focus on completing manuscripts and getting them published in peer-reviewed journals.

Goals to accomplish during the next reporting period. A major focus during the next period will be the completion of manuscripts related to **Specific Aim 1** and getting them published in peer-reviewed journals. Tsion Minas and Maeve Bailey-Whyte will likely be supported by a third postdoctoral fellow, Brittany Jenkins, who will join our laboratory in January 2021. Brittany is an NCI Cancer Prevention Fellow with an MPH but will have to get experienced with data analysis. Per discussion, she will work with Tsion for mentorship but will have her own project, likely the analysis of the available omega-3 fatty acid data and their relationship with inflammation and prostate cancer in men of African ancestry. We also expect that Margaret Pichardo will soon start her analysis with the neighborhood deprivation index. This work covers the NCI-Maryland Prostate Cancer Study, but not the NCI-Ghana study, and should lead to manuscripts within a year. Margaret has previously worked with data from the NCI-Maryland study, thus is already familiar with the dataset. Furthermore, genotyping for the genome-wide association study has been successfully completed in early October. This finishes the laboratory-based tasks for **Specific Aim 2**. The data have been moved to the Analysis Team at the Cancer Genomics Research Laboratory for clustering and quality control including "data cleaning". When this is completed, the genotyping data will be transferred to Wei Tang and Michael Cook. Per previous arrangement, a postdoctoral fellow in Michael Cook's group will start with a combined analysis of the NCI-Maryland and NCI-Ghana study, with the main aim to assess whether germline genetic variants are associated with the immune-oncology markers and prostate cancer, as outlined under **Specific Aim 2**, Major Task 2. Genotyping data for the NCI-Ghana Prostate study exist already. There are two postdoctoral fellows in Michael Cook's laboratory who can do this analysis, Ebonee Butler and Lauren Hurwitz. Lastly, we should be

able to start the analysis of the genomics data coming from the analysis of prostate tumors. Although issues remain with the RNA quality, whole exome sequencing should proceed as planned and generate the mutational data by the end of 2020, as described under **Specific Aim 3**, Major Task 2.

Besides pursuing these milestones, the trainees will continue to submit abstracts at conferences related to cancer health disparities and prostate cancer.

4. Impact

There is "Nothing to report" currently. However, 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), interest in the immune-inflammation signature in prostate tumors of African-American men has surged. 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 will be a speaker at the 3rd NCI Symposium on Cancer Health Disparities in 2021.

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. We encountered minor technical problems in the previous reporting period that were resolved. We are still working on improving the quality of extracted RNA from FFPE prostate tumor cores.

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 has been resolved, finishing the laboratory work for this project. Milestone achieved.

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 NCI Cancer Genomics Research Laboratory has now completed GWAS genotyping with the Infinium HumanOmni5-Quad BeadChip for a total of 1806 subjects in the study, matching the numbers of 900 cases and 900 controls in our Statement of Work for the award. The problem has been resolved, finishing the laboratory work for this project. Milestone achieved.

Specific Aim 3, Major Task 1: We are encountering 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 extracted total RNA and DNA from about 400 cores using a previously established protocol that allows further processing of the RNA for RNA sequencing and DNA for whole exome sequencing. The RNA and DNA samples were 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. We provided the service provider with additional FFPE tissue cores and will now examine if extraction of RNA with this protocol will yield RNA quality that allows untargeted, large-scale sequencing. If these attempts would fail, we may consider a targeted sequencing approach that does not require the same stringent RNA quality. Alternatively, we may consider quantitation of gene expression using Nanostring technology with predeveloped assays that work with degraded RNA. However, the latter methods would not allow a whole transcriptome-wide assessment of gene expression in our samples.

Changes to vertebrate animals and select agents do not apply.

6. Products

Tsion Minas gave a presentation at the AORTIC 2019 conference in Maputo, Mozambique, on November 6, 2019. Her talk presented first findings under this award and was entitled “Distinct circulating immune-oncological markers in men of African descent”.

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”.

The Ambs group submitted an invited review entitled "New Approaches to Cancer Health Disparity Research and Why They Matter" with Tsion Zewdu Minas, Maeve Kiely (Bailey-Whyte), Anuoluwapo Ajao, and Stefan Ambs as authors to the journal Carcinogenesis as the 40th Anniversary contribution, and credited the DoD award W81XWH-18-1-0588 for funding support. The manuscript is currently under review with the journal. We also submitted a revised manuscript entitled "Aspirin Use and Prostate Cancer among African-American Men in the Southern Community Cohort Study" to the journal Cancer Epidemiology Biomarkers and Prevention, with Wei Tang, Jay H. Fowke, Lauren M. Hurwitz, Mark D. Steinwandel, William J. Blot, and Stefan Ambs as authors. We credited the DoD award W81XWH-18-1-0588 for funding support because the research findings are interrelated with the work in this award, asking whether the anti-inflammatory drug, aspirin, can reduce prostate cancer mortality among men of African ancestry. We found it can. Furthermore, Tsion Minas and Maeve Bailey-Whyte are working on manuscripts from research described under **Specific Aim 1** of the award. The manuscripts will be finalized with the updated National Death Index data that we just received for the survival analysis in the manuscripts. We expect to have two manuscripts submitted by the end of this year. Tsion will report differences in immune-oncology marker expression between men of African and European ancestry and how these differences affect immune function and survival of prostate cancer patients. Maeve Bailey-Whyte will report the association of urinary thromboxane B2, a surrogate metabolite for thromboxane A2 availability, with prostate cancer and metastatic disease in African-American men.

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 serum samples and FFPE tissue cores, obtaining updated patient data and generating the neighborhood deprivation index as well as extraction of RNA and DNA from biospecimens for shipment to the service providers were the major tasks for the Ambs laboratory. In addition, we began the analysis of data for research and have begun drafting manuscripts.

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 study and preparation of manuscript(s)
Funding support	NCI intramural program

Name	Tiffany Dorsey
Project Role	Laboratory Manager/Microbiologist
Researcher Identifier	
Nearest person month worked	6
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	6
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)
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	2
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	Jason White
Project Role	PhD student
Researcher Identifier	
Nearest person month worked	2
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 (with mentorship by Wei Tang)
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 (PhD student from Tuskegee U)
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 have established a collaboration with the University of Maryland Medical School, Department of Pathology, to have a collaborating pathologist taking the cores from FFPE tumor blocks, supporting **Specific Aim 3**. This collaboration includes our laboratory, the Co-PI Clayton Yates, and the Department of Pathology at the University of Maryland. We have received 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. Dr. Yfantis 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. Quad Chart also attached.

9. Appendices

PDF of submitted manuscripts with acknowledgement the funding support by DoD award W81XWH-18-1-0588.

OXFORD UNIVERSITY PRESS | Carcinogenesis

New Approaches to Cancer Health Disparity Research and Why They Matter

Journal:	*Carcinogenesis*
Manuscript ID	CARCIN-2020-00468
Manuscript Type:	Review
Date Submitted by the Author:	13-Oct-2020
Complete List of Authors:	Minas, Tsion; National Cancer Institute, Center for Cancer Research Baily-Whyte, M; National Cancer Institute, Laboratory of Human Carcinogenesis Ajao, Anuoluwapo; National Cancer Institute, Laboratory of Human Carcinogenesis Ambs, Stefan; National Cancer Institute, Laboratory of Human Carcinogenesis;
Keywords:	Health disparity, cancer, risk factor, tumor biology, genetics, stress, comorbidity, microbiome, survival

New Approaches to Cancer Health Disparity Research and Why They Matter

Tsion Zewdu Minas, Maeve Kiely, Anuoluwapo Ajao, and Stefan Ambs

Laboratory of Human Carcinogenesis, Center of Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA

Running title: Cancer and Health disparity

Key words: Health disparity, cancer, risk factor, tumor biology, genetics, stress, comorbidity, microbiome, and survival

Conflict of interest statement: None declared.

Acknowledgement of funding: This research was supported by the Intramural Research Program of the NIH, National Cancer Institute (NCI), Center for Cancer Research (ZIA BC 010499, ZIA BC 010624, and ZIA BC 010887), DoD award W81XWH1810588, and funding support by the National Institute of Minority Health and Health Disparities.

Address all correspondence to: Stefan Ambs, Laboratory of Human Carcinogenesis, National Cancer Institute, Bldg. 37/Room 3050B, Bethesda, MD 20892-4258, Phone 240-760-6836; Email: ambss@mail.nih.gov

Abstract (word count: 190)

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 to 50% of all pre-mature deaths might not occur if all segments of the U.S. population would experience the

death rates of college graduates (5). Socioeconomic status is 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 cancer risk (10,11).

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 (12). 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 (13-15). 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 diets in East Asia, the incidence difference has narrowed. Notably, prostate cancer is the leading cause of cancer death among men in sub-Saharan Africa and the Caribbean (16), 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 (17-20). Cervical cancer is a major cause of cancer deaths among women in sub-Saharan Africa and South-East Asia because of human papillomavirus (HPV) 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 *Helicobacter 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.

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 (21,22). Disparities in liver cancer occur across U.S. states and race/ethnic groups (23,24). 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 (1,25,26). 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 (27). 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 (23). 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 (21,28). Reasons of why these specific cancer disparities exist have been extensively reviewed (29-35). 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 (36,37). African Americans have an excess risk of developing early onset cancer, which is reminiscent of disease presentation in Africa (38); 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 (39). 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 (2,28). Barriers still exist and current lung cancer screening guidelines may often exclude African American smokers at increased risk of lung cancer (40). 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 (41).

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 (42,43). The importance of marital status suggests that social isolation and stress may contribute to racial/ethnic disparities.

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 (44). 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 (45). 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 (46). 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 (47). 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 (44). To end with, there are also cancer health disparities related to sex 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 (48).

Impact of Affordable Care Act on cancer health disparities. 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 (49), 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 (50). 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 (51). 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 (52-54). Americans living in areas of greater deprivation and rurality still have lower rates of recommended cancer screening than others (55). With the Affordable Care Act, however, colorectal cancer screening uptake seems to have increased, albeit modestly (56), yet race/ethnic disparities persist (57). 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 (52,53,58). 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 (54,59). Still, race/ethnic disparities remain (59), and Medicaid expansion may not have lowered the disparity in breast cancer mortality between African American and European American women (60). 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 (SEER) 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 (61). 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 (25). 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 (62) and cancer patients (63).

Diabetes, hyperinsulinemia, and obesity are closely related comorbid conditions. They are all cancer risk factors (64,65). 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 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 (64,66). Diabetes-related advanced glycation end products have been linked to a cancer health disparity (67). Diabetes is thought to promote cancer development and progression through insulin and insulin-like growth factor signaling, oxidative stress, and excessive inflammation (68). This comorbidity is excessively high among African Americans and in the Hispanic/Latino community (25,69). Insulin resistance and the metabolic syndrome have been found to contribute to disparities in breast cancer outcomes between African American and European American women (70,71). Diabetes also increases the risk of pancreatic cancer in African American and Hispanic/Latino (72), 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 (73). Accordingly, clinical trial participation of U.S. minorities remains low (74,75), 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 (76,77). Cancer patients with a comorbidity are generally less likely to receive curative treatment than those without the comorbidity (78). 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 (79). Posttraumatic stress because of a cancer diagnosis may disproportionally affect minority populations (80). Social adversity in early life can lead to decreased glucocorticoid and increased pro-inflammatory signaling in humans (81). Intrauterine stress exposures associate with a shortened telomere length in young adulthood (82), 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 (83,84). In breast tumors, social isolation may lead to reprogramming of tumor biology (85,86). 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 (87). Cancer patients have higher rates of depression than most Americans (88). Major depression affects about 5% to 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 (89-91). They are cancer risk factors and have been linked to elevated concentrations of circulating pro-inflammatory cytokines and chemokines (87-89).

Stress exposures and depression transduce their biological effects through the hypothalamic-pituitary-adrenal axis. This signaling pathway is characterized by 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 (92). Chronic stress influences tumor biology through two major pathways involving catecholamines (adrenaline, noradrenaline) and glucocorticoids (93). Socially isolated ovarian cancer patients were found to have elevated tumor noradrenaline levels (94). In mouse models of ovarian and breast cancer, chronic stress promotes invasive tumor growth and metastasis in a β-adrenergic signaling-dependent manner (95-97). Here, catecholamines activate β-adrenergic signaling in cancer cells and tumor-associated macrophages (92,96), 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 (98) and a decrease in chronic depression may slow metastasis in breast cancer patients (99). In other studies, social stress was

found to up-regulate inflammatory gene expression in monocytes through β-adrenergic signaling (100). Likewise, African Americans with exposure to racial discrimination showed up-regulation of these genes (101).

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 (43). 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 (102). β-blocker use after a disease diagnosis reduces disease recurrence and improves survival of breast cancer patients (103), while regular users of the β-blocker, propranolol, are less likely to develop advanced breast cancer and have a reduced breast cancer-specific mortality (104). Beta-blocker use has been associated with improved recurrence-free survival in triple-negative breast cancer as well (105). 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 (106). 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 (107). 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 (108). Recent observations have shown that population differences in genetic ancestry can contribute to population differences in cancer susceptibility (17,18,109-111). Genetic ancestry and natural selection are underlying causes of population differences in immune response to pathogens (112,113). Those differences may relate to cancer (35,114). Relationships of ancestry with expression levels of inflammatory cytokines are evident in human populations (115,116). These differences may contribute to lung cancer disparities (117,118). Two studies investigated gene expression variations between subjects of European and West African ancestry using lymphoblastoid cell lines (119,120) 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 cancer, because of their effect on the transcriptome (111,121).

One mechanism by which ancestry-related factors affect cancer outcomes is by inducing an adverse tumor biology (122). Research has now documented that tumors from patients of either African, Asian, or European descent show notable differences in acquired somatic

mutations (123). 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 (124,125). 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 (125). 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 (126). 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 (127,128). The latter is consistent with many previous reports (38,129). 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 (130). 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 (31,131-133). 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 (29). 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 (134,135) 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 (135). 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 (136). 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 (137), another study discovered the distinct occurrence of *PTPRT* and *JAK2* mutations in lung adenocarcinomas among African Americans and their association with increased STAT3 signaling (138).

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 (139,140). Localized prostate cancer contains few recurrent mutations in oncogenes (e.g., ETS gene fusions) or tumor suppressor genes (141,142). Instead, prostate tumors are characterized by recurrent 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 (143,144). 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 (140,145-147), 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%) (147). By contrast, this gene is mutated at <10% in European-ancestry populations. Comparing African American (AA) with European American (EA) patients in TCGA, significant differences were observed in the frequency of *TMPRSS2-ERG* fusions (29.3% AA vs. 39.6% EA), *SPOP* mutations (20.3% AA vs. 10% EA), and *PTEN* deletions (11.5% AA vs. 30.2% EA), consistent with other studies in the United States and Africa (140,148-150). The application of whole genome sequencing to the disease in African men, currently performed on only few tumors (151), 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 (151,152).

As a key discovery of the study of prostate tumors in African American men, *Wallace et al.* were the first to describe a prevalent immune-inflammation signature in prostate tumors of African American patients (153), followed by others (154). This finding has been validated in TCGA (124). 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 (155,156). 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 (156). Yet, these tumors may have an improved response to immunotherapies, and specifically to cancer vaccines, and perhaps ADAR1 inhibitors (157). 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 (158). 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 (159). The finding is consistent with a similar observation in a previous study (160) 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 (124); 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 (121,155). The precise origin of the

signature remains poorly understood and may include an infection history in the context of the interferon-λ4 ΔG genotype (161), dietary factors (162), or changes to the epigenome, manifesting in the re-activation of endogenous retroviral sequences (163,164). We described up-regulation of *HERV-K* retroviral sequences in African American prostate cancer patients (163). In addition, a pro-inflammatory diet that associates with high-grade prostate cancer is more commonly consumed by African American than European American men (162).

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 (165). Recruitment of tumor-associated macrophages is elevated in breast tumors of African and African-American women, as described by us and others (166-169). Moreover, *Martin et al.* observed an increased microvessel density in these tumors (166). An elevated tumor vascularization in African-American breast cancer patients was confirmed by *Lindner et al.* (170). Tumor angiogenesis correlates with breast cancer metastasis and poor survival (171). 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 (128). 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 (116,122).

Microbiome and cancer health disparities: impact of geography, ethnicity, and genetics on the human microbiome composition. The gut microbiome affects human health (172,173). A dysbiosis can increase cancer risk and modify the cancer therapy response (174-177). Diet and

genetics shape the gut microbiome (178-180) 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 (181,182). Hence, there is evidence that a dysbiosis can be a cause of cancer (175). An altered microbiome and the accumulation of microbiome-derived metabolites have been reported for various human cancers (183-185). Alterations to the human microbiome can induce an aggressive tumor biology (186), linking the microbiome to cancer survival outcomes.

Geographic location and ethnicity strongly associate with the diversity of the gut microbiome (187,188) although geography (e.g., rural vs. urban) usually confers a larger effect than ethnicity (189,190). Dissimilarities in the gut microbiota among ethnic groups with a shared environment have been reported, as shown for Amsterdam, a city in the Netherlands (188). 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 (190). 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 (191), which is reminiscent of findings from migration studies that immigrants tend to acquire cancer rates of their new home country within two generations (14,192).

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 (193,194). 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 (195). 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 (196). 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 (197). Abundance of these bacteria have previously been linked to diet (198) 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.

References

1. Siegel, R.L., *et al.* (2020) Cancer statistics, 2020. *CA Cancer J Clin*, **70**, 7-30.
2. Zeng, C., *et al.* (2015) Disparities by Race, Age, and Sex in the Improvement of Survival for Major Cancers: Results From the National Cancer Institute Surveillance, Epidemiology, and End Results (SEER) Program in the United States, 1990 to 2010. *JAMA Oncol*, **1**, 88-96.
3. Henley, S.J., *et al.* (2018) Rural Cancer Control: Bridging the Chasm in Geographic Health Inequity. *Cancer Epidemiol Biomarkers Prev*, **27**, 1248-1251.
4. Singh, G.K., *et al.* (2014) Widening rural-urban disparities in life expectancy, U.S., 1969-2009. *Am J Prev Med*, **46**, e19-29.
5. Jemal, A., *et al.* (2008) Mortality from leading causes by education and race in the United States, 2001. *Am J Prev Med*, **34**, 1-8.
6. Siegel, R.L., *et al.* (2018) An assessment of progress in cancer control. *CA Cancer J Clin*, **68**, 329-339.
7. Kish, J.K., *et al.* (2014) Racial and ethnic disparities in cancer survival by neighborhood socioeconomic status in Surveillance, Epidemiology, and End Results (SEER) Registries. *J Natl Cancer Inst Monogr*, **2014**, 236-43.
8. Singh, G.K., *et al.* (2017) Socioeconomic and Racial/Ethnic Disparities in Cancer Mortality, Incidence, and Survival in the United States, 1950-2014: Over Six Decades of Changing Patterns and Widening Inequalities. *J Environ Public Health*, **2017**, 2819372.
9. Cheng, I., *et al.* (2009) Socioeconomic status and prostate cancer incidence and mortality rates among the diverse population of California. *Cancer Causes Control*, **20**, 1431-40.
10. Powell-Wiley, T.M., *et al.* (2020) The relationship between neighborhood socioeconomic deprivation and telomere length: The 1999-2002 National Health and Nutrition Examination Survey. *SSM Popul Health*, **10**, 100517.
11. Tsai, C.W., *et al.* (2020) Leukocyte telomere length is associated with aggressive prostate cancer in localized African American prostate cancer patients. *Carcinogenesis*, **41**, 1213-1218.
12. Bray, F., *et al.* (2018) Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*, **68**, 394-424.
13. Buell, P. (1973) Changing incidence of breast cancer in Japanese-American women. *J Natl.Cancer Inst.*, **51**, 1479-1483.
14. Thomas, D.B., *et al.* (1987) Cancer in first and second generation Americans. *Cancer Res.*, **47**, 5771-5776.
15. Ziegler, R.G., *et al.* (1993) Migration patterns and breast cancer risk in Asian-American women. *J Natl Cancer Inst*, **85**, 1819-1827.
16. Ragin, C., *et al.* (2017) Cancer in populations of African Ancestry: studies of the African Caribbean Cancer Consortium. *Cancer Causes Control*, **28**, 1173-1176.
17. Freedman, M.L., *et al.* (2006) Admixture mapping identifies 8q24 as a prostate cancer risk locus in African-American men. *Proc.Natl.Acad.Sci.U.S.A*, **103**, 14068-14073.
18. Lachance, J., *et al.* (2018) Genetic Hitchhiking and Population Bottlenecks Contribute to Prostate Cancer Disparities in Men of African Descent. *Cancer Res*, **78**, 2432-2443.

19. Maruthappu, M., *et al.* (2015) Incidence of prostate and urological cancers in England by ethnic group, 2001-2007: a descriptive study. *BMC Cancer*, **15**, 753.
20. Petersen, D.C., *et al.* (2019) African KhoeSan ancestry linked to high-risk prostate cancer. *BMC Med Genomics*, **12**, 82.
21. Wallace, T.A., *et al.* (2011) Interactions among genes, tumor biology and the environment in cancer health disparities: examining the evidence on a national and global scale. *Carcinogenesis*, **32**, 1107-1121.
22. Nguyen, V.K., *et al.* (2020) A comprehensive analysis of racial disparities in chemical biomarker concentrations in United States women, 1999-2014. *Environ Int*, **137**, 105496.
23. Islami, F., *et al.* (2017) Disparities in liver cancer occurrence in the United States by race/ethnicity and state. *CA Cancer J Clin*, **67**, 273-289.
24. El-Serag, H.B., *et al.* (2020) Texas Has the Highest Hepatocellular Carcinoma Incidence Rates in the USA. *Dig Dis Sci*. doi: 10.1007/s10620-020-06231-4.
25. Daw, J. (2017) Contribution of Four Comorbid Conditions to Racial/Ethnic Disparities in Mortality Risk. *Am J Prev Med*, **52**, S95-S102.
26. Melkonian, S.C., *et al.* (2019) Disparities in Cancer Incidence and Trends among American Indians and Alaska Natives in the United States, 2010-2015. *Cancer Epidemiol Biomarkers Prev*, **28**, 1604-1611.
27. Trinh, Q.D., *et al.* (2015) Cancer-specific mortality of Asian Americans diagnosed with cancer: a nationwide population-based assessment. *J Natl Cancer Inst*, **107**, djv054.
28. DeSantis, C.E., *et al.* (2019) Cancer statistics for African Americans, 2019. *CA Cancer J Clin*, **69**, 211-233.
29. Ryan, B.M. (2018) Lung cancer health disparities. *Carcinogenesis*, **39**, 741-751.
30. Augustus, G.J., *et al.* (2018) Colorectal Cancer Disparity in African Americans: Risk Factors and Carcinogenic Mechanisms. *Am J Pathol*, **188**, 291-303.
31. Ashktorab, H., *et al.* (2017) Racial Disparity in Gastrointestinal Cancer Risk. *Gastroenterology*, **153**, 910-923.
32. Daly, B., *et al.* (2015) A perfect storm: How tumor biology, genomics, and health care delivery patterns collide to create a racial survival disparity in breast cancer and proposed interventions for change. *CA Cancer J Clin*, **65**, 221-38.
33. Rebbeck, T.R., *et al.* (2013) Global patterns of prostate cancer incidence, aggressiveness, and mortality in men of african descent. *Prostate Cancer*, **2013**, 560857.
34. McGinley, K.F., *et al.* (2016) Prostate cancer in men of African origin. *Nat Rev Urol*, **13**, 99-107.
35. Smith, C.J., *et al.* (2018) Biological determinants of health disparities in multiple myeloma. *Blood Cancer J*, **8**, 85.
36. Medhanie, G.A., *et al.* (2017) Cancer incidence profile in sub-Saharan African-born blacks in the United States: Similarities and differences with US-born non-Hispanic blacks. *Cancer*, **123**, 3116-3124.
37. Pinheiro, P.S., *et al.* (2020) Cancer mortality among US blacks: Variability between African Americans, Afro-Caribbeans, and Africans. *Cancer Epidemiol*, **66**, 101709.
38. Huo, D., *et al.* (2009) Population differences in breast cancer: survey in indigenous African women reveals over-representation of triple-negative breast cancer. *J.Clin Oncol.*, **27**, 4515-4521.

39. Robbins, H.A., *et al.* (2015) Age at cancer diagnosis for blacks compared with whites in the United States. *J Natl Cancer Inst*, **107**, dju489.
40. Aldrich, M.C., *et al.* (2019) Evaluation of USPSTF Lung Cancer Screening Guidelines Among African American Adult Smokers. *JAMA Oncol*., **5**, 1318-1324.
41. Butler, E.N., *et al.* (2020) Fatal prostate cancer incidence trends in the United States and England by race, stage, and treatment. *Br J Cancer*, **123**, 487-494.
42. Silber, J.H., *et al.* (2013) Characteristics associated with differences in survival among black and white women with breast cancer. *JAMA*, **310**, 389-397.
43. Ellis, L., *et al.* (2018) Racial and Ethnic Disparities in Cancer Survival: The Contribution of Tumor, Sociodemographic, Institutional, and Neighborhood Characteristics. *J Clin Oncol*, **36**, 25-33.
44. Mauvais-Jarvis, F., *et al.* (2020) Sex and gender: modifiers of health, disease, and medicine. *Lancet*, **396**, 565-582.
45. Li, C.H., *et al.* (2018) Sex Differences in Cancer Driver Genes and Biomarkers. *Cancer Res*, **78**, 5527-5537.
46. Ma, W.L., *et al.* (2014) Androgen receptor roles in hepatocellular carcinoma, fatty liver, cirrhosis and hepatitis. *Endocr Relat Cancer*, **21**, R165-82.
47. Conforti, F., *et al.* (2018) Cancer immunotherapy efficacy and patients' sex: a systematic review and meta-analysis. *Lancet Oncol*, **19**, 737-746.
48. Islami, F., *et al.* (2017) International trends in anal cancer incidence rates. *Int J Epidemiol*, **46**, 924-938.
49. Dess, R.T., *et al.* (2019) Association of Black Race With Prostate Cancer-Specific and Other-Cause Mortality. *JAMA Oncol*, **5**, 975-983.
50. Aghdam, N., *et al.* (2020) Ethnicity and insurance status predict metastatic disease presentation in prostate, breast, and non-small cell lung cancer. *Cancer Med*, **9**, 5362-5380.
51. Zhao, J., *et al.* (2020) The Affordable Care Act and access to care across the cancer control continuum: A review at 10 years. *CA Cancer J Clin*, **70**, 165-181.
52. Han, X., *et al.* (2018) Comparison of Insurance Status and Diagnosis Stage Among Patients With Newly Diagnosed Cancer Before vs After Implementation of the Patient Protection and Affordable Care Act. *JAMA Oncol*, **4**, 1713-1720.
53. Takvorian, S.U., *et al.* (2020) Association of Medicaid Expansion Under the Affordable Care Act With Insurance Status, Cancer Stage, and Timely Treatment Among Patients With Breast, Colon, and Lung Cancer. *JAMA Netw Open*, **3**, e1921653.
54. Han, X., *et al.* (2020) Changes in Noninsurance and Care Unaffordability Among Cancer Survivors Following the Affordable Care Act. *J Natl Cancer Inst*, **112**, 688-697.
55. Kurani, S.S., *et al.* (2020) Association of Neighborhood Measures of Social Determinants of Health With Breast, Cervical, and Colorectal Cancer Screening Rates in the US Midwest. *JAMA Netw Open*, **3**, e200618.
56. Xu, M.R., *et al.* (2020) Impact of the Affordable Care Act on Colorectal Cancer Outcomes: A Systematic Review. *Am J Prev Med*, **58**, 596-603.
57. May, F.P., *et al.* (2020) Disparities in Colorectal Cancer Screening in the United States Before and After Implementation of the Affordable Care Act. *Clin Gastroenterol Hepatol*, **18**, 1796-1804 e2.

58. Kim, U.*, et al.* (2020) The effect of Medicaid expansion among adults from low-income communities on stage at diagnosis in those with screening-amenable cancers. *Cancer*.
59. Crocker, A.B.*, et al.* (2019) Expansion coverage and preferential utilization of cancer surgery among racial and ethnic minorities and low-income groups. *Surgery*, **166**, 386-391.
60. Semprini, J.*, et al.* (2020) Evaluating the Effect of Medicaid Expansion on Black/White Breast Cancer Mortality Disparities: A Difference-in-Difference Analysis. *JCO Glob Oncol*, **6**, 1178-1183.
61. Renzi, C.*, et al.* (2019) Comorbid chronic diseases and cancer diagnosis: disease-specific effects and underlying mechanisms. *Nat Rev Clin Oncol*, **16**, 746-761.
62. Price-Haywood, E.G.*, et al.* (2020) Hospitalization and Mortality among Black Patients and White Patients with Covid-19. *N Engl J Med*, **382**, 2534-2543.
63. Mehta, V.*, et al.* (2020) Case Fatality Rate of Cancer Patients with COVID-19 in a New York Hospital System. *Cancer Discov*, **10**, 935-941.
64. Klil-Drori, A.J.*, et al.* (2017) Cancer, obesity, diabetes, and antidiabetic drugs: is the fog clearing? *Nat Rev Clin Oncol*, **14**, 85-99.
65. Gallagher, E.J.*, et al.* (2020) Hyperinsulinaemia in cancer. *Nat Rev Cancer*. doi: 10.1038/s41568-020-0295-5
66. Tsilidis, K.K.*, et al.* (2015) Type 2 diabetes and cancer: umbrella review of meta-analyses of observational studies. *BMJ*, **350**, g7607.
67. Foster, D.*, et al.* (2014) AGE metabolites: a biomarker linked to cancer disparity? *Cancer Epidemiol.Biomarkers Prev.*, **23**, 2186-2191.
68. Giovannucci, E.*, et al.* (2010) Diabetes and cancer: a consensus report. *Diabetes Care*, **33**, 1674-85.
69. Geiss, L.S.*, et al.* (2014) Prevalence and incidence trends for diagnosed diabetes among adults aged 20 to 79 years, United States, 1980-2012. *JAMA*, **312**, 1218-26.
70. Tammemagi, C.M.*, et al.* (2005) Comorbidity and survival disparities among black and white patients with breast cancer. *JAMA*, **294**, 1765-1772.
71. Gallagher, E.J.*, et al.* (2020) Insulin resistance contributes to racial disparities in breast cancer prognosis in US women. *Breast Cancer Res*, **22**, 40.
72. Setiawan, V.W.*, et al.* (2019) Pancreatic Cancer Following Incident Diabetes in African Americans and Latinos: The Multiethnic Cohort. *J Natl Cancer Inst*, **111**, 27-33.
73. Unger, J.M.*, et al.* (2019) Association of Patient Comorbid Conditions With Cancer Clinical Trial Participation. *JAMA Oncol*, **5**, 326-333.
74. Duma, N.*, et al.* (2018) Representation of Minorities and Women in Oncology Clinical Trials: Review of the Past 14 Years. *J Oncol Pract*, **14**, e1-e10.
75. Loree, J.M.*, et al.* (2019) Disparity of Race Reporting and Representation in Clinical Trials Leading to Cancer Drug Approvals From 2008 to 2018. *JAMA Oncol*, e191870.
76. Berndt, S.I.*, et al.* (2007) Disparities in treatment and outcome for renal cell cancer among older black and white patients. *J.Clin Oncol.*, **25**, 3589-3595.
77. Lee, L.*, et al.* (2011) Impact of comorbidity on chemotherapy use and outcomes in solid tumors: a systematic review. *J Clin Oncol*, **29**, 106-17.
78. Sarfati, D.*, et al.* (2016) The impact of comorbidity on cancer and its treatment. *CA Cancer J Clin*, **66**, 337-50.

79. Juarez, P.D., *et al.* (2014) The public health exposome: a population-based, exposure science approach to health disparities research. *Int J Environ Res Public Health*, **11**, 12866-95.
80. Vin-Raviv, N., *et al.* (2013) Racial disparities in posttraumatic stress after diagnosis of localized breast cancer: the BQUAL study. *J Natl Cancer Inst*, **105**, 563-72.
81. Miller, G.E., *et al.* (2009) Low early-life social class leaves a biological residue manifested by decreased glucocorticoid and increased proinflammatory signaling. *Proc.Natl.Acad.Sci.U.S.A*, **106**, 14716-14721.
82. Entringer, S., *et al.* (2011) Stress exposure in intrauterine life is associated with shorter telomere length in young adulthood. *Proc.Natl.Acad.Sci.U.S.A*, **108**, E513-E518.
83. Taylor, T.R., *et al.* (2007) Racial discrimination and breast cancer incidence in US Black women: the Black Women's Health Study. *Am J Epidemiol.*, **166**, 46-54.
84. Shariff-Marco, S., *et al.* (2010) Racial/ethnic differences in self-reported racism and its association with cancer-related health behaviors. *Am.J.Public Health*, **100**, 364-374.
85. Williams, J.B., *et al.* (2009) A model of gene-environment interaction reveals altered mammary gland gene expression and increased tumor growth following social isolation. *Cancer Prev Res (Phila)*, **2**, 850-61.
86. Volden, P.A., *et al.* (2013) Chronic social isolation is associated with metabolic gene expression changes specific to mammary adipose tissue. *Cancer Prev Res (Phila)*, **6**, 634-45.
87. Bortolato, B., *et al.* (2017) Depression in cancer: The many biobehavioral pathways driving tumor progression. *Cancer Treat Rev*, **52**, 58-70.
88. Currier, M.B., *et al.* (2014) Depression as a risk factor for cancer: from pathophysiological advances to treatment implications. *Annu Rev Med*, **65**, 203-21.
89. Chida, Y., *et al.* (2008) Do stress-related psychosocial factors contribute to cancer incidence and survival? *Nat.Clin Pract.Oncol.*, **5**, 466-475.
90. Satin, J.R., *et al.* (2009) Depression as a predictor of disease progression and mortality in cancer patients: a meta-analysis. *Cancer*, **115**, 5349-61.
91. Machado, M.O., *et al.* (2018) The association of depression and all-cause and cause-specific mortality: an umbrella review of systematic reviews and meta-analyses. *BMC Med*, **16**, 112.
92. Cole, S.W., *et al.* (2015) Sympathetic nervous system regulation of the tumour microenvironment. *Nat Rev Cancer*, **15**, 563-72.
93. Antoni, M.H., *et al.* (2006) The influence of bio-behavioural factors on tumour biology: pathways and mechanisms. *Nat.Rev.Cancer*, **6**, 240-248.
94. Lutgendorf, S.K., *et al.* (2011) Social isolation is associated with elevated tumor norepinephrine in ovarian carcinoma patients. *Brain Behav.Immun.*, **25**, 250-255.
95. Thaker, P.H., *et al.* (2006) Chronic stress promotes tumor growth and angiogenesis in a mouse model of ovarian carcinoma. *Nat.Med.*, **12**, 939-944.
96. Sloan, E.K., *et al.* (2010) The sympathetic nervous system induces a metastatic switch in primary breast cancer. *Cancer Res*, **70**, 7042-52.
97. Le, C.P., *et al.* (2016) Chronic stress in mice remodels lymph vasculature to promote tumour cell dissemination. *Nat Commun*, **7**, 10634.

98. Bower, J.E., *et al.* (2018) Prometastatic Molecular Profiles in Breast Tumors From Socially Isolated Women. *JNCI Cancer Spectr*, **2**, pky029.
99. Giese-Davis, J., *et al.* (2011) Decrease in depression symptoms is associated with longer survival in patients with metastatic breast cancer: a secondary analysis. *J Clin Oncol*, **29**, 413-20.
100. Powell, N.D., *et al.* (2013) Social stress up-regulates inflammatory gene expression in the leukocyte transcriptome via beta-adrenergic induction of myelopoiesis. *Proc Natl Acad Sci U S A*, **110**, 16574-9.
101. Thames, A.D., *et al.* (2019) Experienced discrimination and racial differences in leukocyte gene expression. *Psychoneuroendocrinology*, **106**, 277-283.
102. Kurozumi, S., *et al.* (2019) beta2-Adrenergic receptor expression is associated with biomarkers of tumor immunity and predicts poor prognosis in estrogen receptor-negative breast cancer. *Breast Cancer Res Treat*, **177**, 603-610.
103. Powe, D.G., *et al.* (2010) Beta-blocker drug therapy reduces secondary cancer formation in breast cancer and improves cancer specific survival. *Oncotarget.*, **1**, 628-638.
104. Barron, T.I., *et al.* (2011) Beta blockers and breast cancer mortality: a population- based study. *J.Clin Oncol.*, **29**, 2635-2644.
105. Melhem-Bertrandt, A., *et al.* (2011) Beta-blocker use is associated with improved relapse-free survival in patients with triple-negative breast cancer. *J.Clin Oncol.*, **29**, 2645-2652.
106. Kinlock, B.L., *et al.* (2017) Prevalence and Correlates of Major Depressive Symptoms among Black Men with Prostate Cancer. *Ethn Dis*, **27**, 429-436.
107. Lu, D., *et al.* (2016) Stress-Related Signaling Pathways in Lethal and Nonlethal Prostate Cancer. *Clin Cancer Res*, **22**, 765-772.
108. Piyarathna, D.W.B., *et al.* (2019) ERR1 and PGC1alpha associated mitochondrial alterations correlate with pan-cancer disparity in African Americans. *J Clin Invest*, **129**, 2351-2356.
109. Fejerman, L., *et al.* (2008) Genetic ancestry and risk of breast cancer among U.S. Latinas. *Cancer Res*, **68**, 9723-9728.
110. Fejerman, L., *et al.* (2010) European ancestry is positively associated with breast cancer risk in Mexican women. *Cancer Epidemiol.Biomarkers Prev.*, **19**, 1074-1082.
111. Oak, N., *et al.* (2020) Ancestry-specific predisposing germline variants in cancer. *Genome Med*, **12**, 51.
112. Nedelec, Y., *et al.* (2016) Genetic Ancestry and Natural Selection Drive Population Differences in Immune Responses to Pathogens. *Cell*, **167**, 657-669 e21.
113. Barreiro, L.B., *et al.* (2020) Evolutionary and population (epi)genetics of immunity to infection. *Hum Genet*, **139**, 723-732.
114. Koshiol, J., *et al.* (2011) Racial differences in chronic immune stimulatory conditions and risk of non-Hodgkin's lymphoma in veterans from the United States. *J Clin Oncol*, **29**, 378-85.
115. Coe, C.L., *et al.* (2011) Population differences in proinflammatory biology: Japanese have healthier profiles than Americans. *Brain Behav Immun*, **25**, 494-502.

116. Yao, S., *et al.* (2018) Genetic ancestry and population differences in levels of inflammatory cytokines in women: Role for evolutionary selection and environmental factors. *PLoS Genet*, **14**, e1007368.
117. Pine, S.R., *et al.* (2016) Differential Serum Cytokine Levels and Risk of Lung Cancer Between African and European Americans. *Cancer Epidemiol Biomarkers Prev*, **25**, 488-97.
118. Meaney, C.L., *et al.* (2019) Circulating Inflammation Proteins Associated With Lung Cancer in African Americans. *J Thorac Oncol*, **14**, 1192-1203.
119. Storey, J.D., *et al.* (2007) Gene-expression variation within and among human populations. *Am.J.Hum.Genet.*, **80**, 502-509.
120. Zhang, W., *et al.* (2008) Evaluation of genetic variation contributing to differences in gene expression between populations. *Am.J.Hum.Genet.*, **82**, 631-640.
121. Prokunina-Olsson, L., *et al.* (2013) A variant upstream of IFNL3 (IL28B) creating a new interferon gene IFNL4 is associated with impaired clearance of hepatitis C virus. *Nat.Genet.*, **45**, 164-171.
122. Jenkins, B.D., *et al.* (2019) Atypical Chemokine Receptor 1 (DARC/ACKR1) in Breast Tumors Is Associated with Survival, Circulating Chemokines, Tumor-Infiltrating Immune Cells, and African Ancestry. *Cancer Epidemiol Biomarkers Prev*, **28**, 690-700.
123. Tan, D.S., *et al.* (2016) Cancer Genomics: Diversity and Disparity Across Ethnicity and Geography. *J Clin Oncol*, **34**, 91-101.
124. Yuan, J., *et al.* (2018) Integrated Analysis of Genetic Ancestry and Genomic Alterations across Cancers. *Cancer Cell*, **34**, 549-560 e9.
125. Carrot-Zhang, J., *et al.* (2020) Comprehensive Analysis of Genetic Ancestry and Its Molecular Correlates in Cancer. *Cancer Cell*, **37**, 639-654 e6.
126. Yeh, C.H., *et al.* (2018) FBXW7: a critical tumor suppressor of human cancers. *Mol Cancer*, **17**, 115.
127. Keenan, T., *et al.* (2015) Comparison of the Genomic Landscape Between Primary Breast Cancer in African American Versus White Women and the Association of Racial Differences With Tumor Recurrence. *J Clin Oncol*, **33**, 3621-7.
128. Pitt, J.J., *et al.* (2018) Characterization of Nigerian breast cancer reveals prevalent homologous recombination deficiency and aggressive molecular features. *Nat Commun*, **9**, 4181.
129. Carey, L.A., *et al.* (2006) Race, breast cancer subtypes, and survival in the Carolina Breast Cancer Study. *JAMA*, **295**, 2492-2502.
130. Craig, D.W., *et al.* (2013) Genome and transcriptome sequencing in prospective metastatic triple-negative breast cancer uncovers therapeutic vulnerabilities. *Mol.Cancer Ther.*, **12**, 104-116.
131. Guda, K., *et al.* (2015) Novel recurrently mutated genes in African American colon cancers. *Proc Natl Acad Sci U S A*, **112**, 1149-54.
132. Yoon, H.H., *et al.* (2015) Racial Differences in BRAF/KRAS Mutation Rates and Survival in Stage III Colon Cancer Patients. *J Natl Cancer Inst*, **107**, djv186.
133. Xicola, R.M., *et al.* (2018) Lack of APC somatic mutation is associated with early-onset colorectal cancer in African Americans. *Carcinogenesis*, **39**, 1331-1341.

134. Shigematsu, H., *et al.* (2005) Clinical and biological features associated with epidermal growth factor receptor gene mutations in lung cancers. *J Natl.Cancer Inst.*, **97**, 339-346.
135. Chen, J., *et al.* (2020) Genomic landscape of lung adenocarcinoma in East Asians. *Nat Genet*, **52**, 177-186.
136. Soo, R.A., *et al.* (2011) Ethnic differences in survival outcome in patients with advanced stage non-small cell lung cancer: results of a meta-analysis of randomized controlled trials. *J Thorac Oncol*, **6**, 1030-8.
137. Campbell, J.D., *et al.* (2017) Comparison of Prevalence and Types of Mutations in Lung Cancers Among Black and White Populations. *JAMA Oncol*, **3**, 801-809.
138. Mitchell, K.A., *et al.* (2019) Recurrent PTPRT/JAK2 mutations in lung adenocarcinoma among African Americans. *Nat Commun*, **10**, 5735.
139. Attard, G., *et al.* (2016) Prostate cancer. *Lancet*, **387**, 70-82.
140. Faisal, F.A., *et al.* (2016) Racial Variations in Prostate Cancer Molecular Subtypes and Androgen Receptor Signaling Reflect Anatomic Tumor Location. *Eur Urol*, **70**, 14-7.
141. Berger, M.F., *et al.* (2011) The genomic complexity of primary human prostate cancer. *Nature*, **470**, 214-220.
142. Fraser, M., *et al.* (2017) Genomic hallmarks of localized, non-indolent prostate cancer. *Nature*, **541**, 359-364.
143. Zhao, S., *et al.* (2017) Epigenome-Wide Tumor DNA Methylation Profiling Identifies Novel Prognostic Biomarkers of Metastatic-Lethal Progression in Men Diagnosed with Clinically Localized Prostate Cancer. *Clin Cancer Res*, **23**, 311-319.
144. Mundbjerg, K., *et al.* (2017) Identifying aggressive prostate cancer foci using a DNA methylation classifier. *Genome Biol*, **18**, 3.
145. Magi-Galluzzi, C., *et al.* (2011) TMPRSS2-ERG gene fusion prevalence and class are significantly different in prostate cancer of Caucasian, African-American and Japanese patients. *Prostate*, **71**, 489-497.
146. Rosen, P., *et al.* (2012) Clinical potential of the ERG oncoprotein in prostate cancer. *Nat.Rev.Urol.*, **9**, 131-137.
147. Li, J., *et al.* (2020) A genomic and epigenomic atlas of prostate cancer in Asian populations. *Nature*, **580**, 93-99.
148. Khani, F., *et al.* (2014) Evidence for molecular differences in prostate cancer between African American and Caucasian men. *Clin Cancer Res*, **20**, 4925-34.
149. Blackburn, J., *et al.* (2019) TMPRSS2-ERG fusions linked to prostate cancer racial health disparities: A focus on Africa. *Prostate*, **79**, 1191-1196.
150. Koga, Y., *et al.* (2020) Genomic Profiling of Prostate Cancers from Men with African and European Ancestry. *Clin Cancer Res*, **26**, 4651-4660.
151. Jaratlerdsiri, W., *et al.* (2018) Whole-Genome Sequencing Reveals Elevated Tumor Mutational Burden and Initiating Driver Mutations in African Men with Treatment-Naive, High-Risk Prostate Cancer. *Cancer Res*, **78**, 6736-6746.
152. Petrovics, G., *et al.* (2015) A novel genomic alteration of LSAMP associates with aggressive prostate cancer in African American men. *EBioMedicine*, **2**, 1957-64.
153. Wallace, T.A., *et al.* (2008) Tumor immunobiological differences in prostate cancer between African-American and European-American men. *Cancer Res.*, **68**, 927-936.

154. Reams, R.R., *et al.* (2009) Microarray comparison of prostate tumor gene expression in African-American and Caucasian American males: a pilot project study. *Infect.Agent.Cancer*, **4 Suppl 1**, S3.
155. Tang, W., *et al.* (2018) IFNL4-DeltaG Allele Is Associated with an Interferon Signature in Tumors and Survival of African-American Men with Prostate Cancer. *Clin Cancer Res*, **24**, 5471-5481.
156. Weichselbaum, R.R., *et al.* (2008) An interferon-related gene signature for DNA damage resistance is a predictive marker for chemotherapy and radiation for breast cancer. *Proc.Natl.Acad.Sci.U.S.A*, **105**, 18490-18495.
157. Gannon, H.S., *et al.* (2018) Identification of ADAR1 adenosine deaminase dependency in a subset of cancer cells. *Nat Commun*, **9**, 5450.
158. Sartor, O., *et al.* (2020) Survival of African-American and Caucasian men after sipuleucel-T immunotherapy: outcomes from the PROCEED registry. *Prostate Cancer Prostatic Dis*, **23**, 517-526.
159. Smith, C.J., *et al.* (2017) Aspirin Use Reduces the Risk of Aggressive Prostate Cancer and Disease Recurrence in African-American Men. *Cancer Epidemiol Biomarkers Prev*, **26**, 845-853.
160. Osborn, V.W., *et al.* (2016) Impact of aspirin on clinical outcomes for African American men with prostate cancer undergoing radiation. *Tumori*, **102**, 65-70.
161. Minas, T.Z., *et al.* (2018) IFNL4-DeltaG is associated with prostate cancer among men at increased risk of sexually transmitted infections. *Commun Biol*, **1**, 191.
162. Vidal, A.C., *et al.* (2019) Dietary inflammatory index (DII) and risk of prostate cancer in a case-control study among Black and White US Veteran men. *Prostate Cancer Prostatic Dis*, **22**, 580-587.
163. Wallace, T.A., *et al.* (2014) Elevated HERV-K mRNA expression in PBMC is associated with a prostate cancer diagnosis particularly in older men and smokers. *Carcinogenesis*, **35**, 2074-2083.
164. Chiappinelli, K.B., *et al.* (2015) Inhibiting DNA Methylation Causes an Interferon Response in Cancer via dsRNA Including Endogenous Retroviruses. *Cell*, **162**, 974-86.
165. Lehmann, B.D., *et al.* (2011) Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. *J.Clin Invest*, **121**, 2750-2767.
166. Martin, D.N., *et al.* (2009) Differences in the Tumor Microenvironment between African-American and European-American Breast Cancer Patients. *PLoS.ONE.*, **4**, e4531.
167. Mukhtar, R.A., *et al.* (2011) Elevated PCNA+ tumor-associated macrophages in breast cancer are associated with early recurrence and non-Caucasian ethnicity. *Breast Cancer Res Treat.*, **130**, 635-644.
168. Adisa, C.A., *et al.* (2012) Biology of breast cancer in Nigerian women: a pilot study. *Ann.Afr.Med.*, **11**, 169-175.
169. Sawe, R.T., *et al.* (2016) Aggressive breast cancer in western Kenya has early onset, high proliferation, and immune cell infiltration. *BMC Cancer*, **16**, 204.
170. Lindner, R., *et al.* (2013) Molecular phenotypes in triple negative breast cancer from african american patients suggest targets for therapy. *PLoS.ONE.*, **8**, e71915.

171. Weidner, N. (1995) Intratumor microvessel density as a prognostic factor in cancer. *Am.J Pathol.*, **147**, 9-19.
172. Zackular, J.P.*, et al.* (2013) The gut microbiome modulates colon tumorigenesis. *mBio*, **4**, e00692-13.
173. Zhernakova, D.V.*, et al.* (2018) Individual variations in cardiovascular-disease-related protein levels are driven by genetics and gut microbiome. *Nat Genet*, **50**, 1524-1532.
174. O'Keefe, S.J. (2016) Diet, microorganisms and their metabolites, and colon cancer. *Nat Rev Gastroenterol Hepatol*, **13**, 691-706.
175. Scott, A.J.*, et al.* (2019) International Cancer Microbiome Consortium consensus statement on the role of the human microbiome in carcinogenesis. *Gut*, **68**, 1624-1632.
176. Iida, N.*, et al.* (2013) Commensal bacteria control cancer response to therapy by modulating the tumor microenvironment. *Science*, **342**, 967-70.
177. Routy, B.*, et al.* (2018) Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors. *Science*, **359**, 91-97.
178. David, L.A.*, et al.* (2014) Diet rapidly and reproducibly alters the human gut microbiome. *Nature*, **505**, 559-63.
179. Goodrich, J.K.*, et al.* (2014) Human genetics shape the gut microbiome. *Cell*, **159**, 789-99.
180. Schulz, M.D.*, et al.* (2014) High-fat-diet-mediated dysbiosis promotes intestinal carcinogenesis independently of obesity. *Nature*, **514**, 508-12.
181. Yoshimoto, S.*, et al.* (2013) Obesity-induced gut microbial metabolite promotes liver cancer through senescence secretome. *Nature*, **499**, 97-101.
182. Forslund, K.*, et al.* (2015) Disentangling type 2 diabetes and metformin treatment signatures in the human gut microbiota. *Nature*, **528**, 262-266.
183. Kostic, A.D.*, et al.* (2013) Fusobacterium nucleatum potentiates intestinal tumorigenesis and modulates the tumor-immune microenvironment. *Cell Host Microbe*, **14**, 207-15.
184. Greathouse, K.L.*, et al.* (2018) Interaction between the microbiome and TP53 in human lung cancer. *Genome Biol*, **19**, 123.
185. Tang, W.*, et al.* (2019) Liver- and Microbiome-derived Bile Acids Accumulate in Human Breast Tumors and Inhibit Growth and Improve Patient Survival. *Clin Cancer Res*, **25**, 5972-5983.
186. Zitvogel, L.*, et al.* (2015) Cancer and the gut microbiota: an unexpected link. *Sci Transl Med*, **7**, 271ps1.
187. Yatsunenko, T.*, et al.* (2012) Human gut microbiome viewed across age and geography. *Nature*, **486**, 222-7.
188. Deschasaux, M.*, et al.* (2018) Depicting the composition of gut microbiota in a population with varied ethnic origins but shared geography. *Nat Med*, **24**, 1526-1531.
189. He, Y.*, et al.* (2018) Regional variation limits applications of healthy gut microbiome reference ranges and disease models. *Nat Med*, **24**, 1532-1535.
190. Brooks, A.W.*, et al.* (2018) Gut microbiota diversity across ethnicities in the United States. *PLoS Biol*, **16**, e2006842.
191. Vangay, P.*, et al.* (2018) US Immigration Westernizes the Human Gut Microbiome. *Cell*, **175**, 962-972 e10.

192. Maskarinec, G.*, et al.* (2004) The effect of migration on cancer incidence among Japanese in Hawaii. *Ethn Dis*, **14**, 431-9.
193. Fettweis, J.M.*, et al.* (2014) Differences in vaginal microbiome in African American women versus women of European ancestry. *Microbiology (Reading)*, **160**, 2272-2282.
194. Yang, Y.*, et al.* (2019) Racial Differences in the Oral Microbiome: Data from Low-Income Populations of African Ancestry and European Ancestry. *mSystems*, **4**.
195. Feng, Y.*, et al.* (2019) Metagenomic analysis reveals a rich bacterial content in high-risk prostate tumors from African men. *Prostate*, **79**, 1731-1738.
196. Thompson, K.J.*, et al.* (2017) A comprehensive analysis of breast cancer microbiota and host gene expression. *PLoS One*, **12**, e0188873.
197. Yazici, C.*, et al.* (2017) Race-dependent association of sulfidogenic bacteria with colorectal cancer. *Gut*, **66**, 1983-1994.
198. O'Keefe, S.J.*, et al.* (2015) Fat, fibre and cancer risk in African Americans and rural Africans. *Nat Commun*, **6**, 6342.

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 Disparity Fellowship. Figures were generated using the software BioRender.

Figure legends

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

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

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.

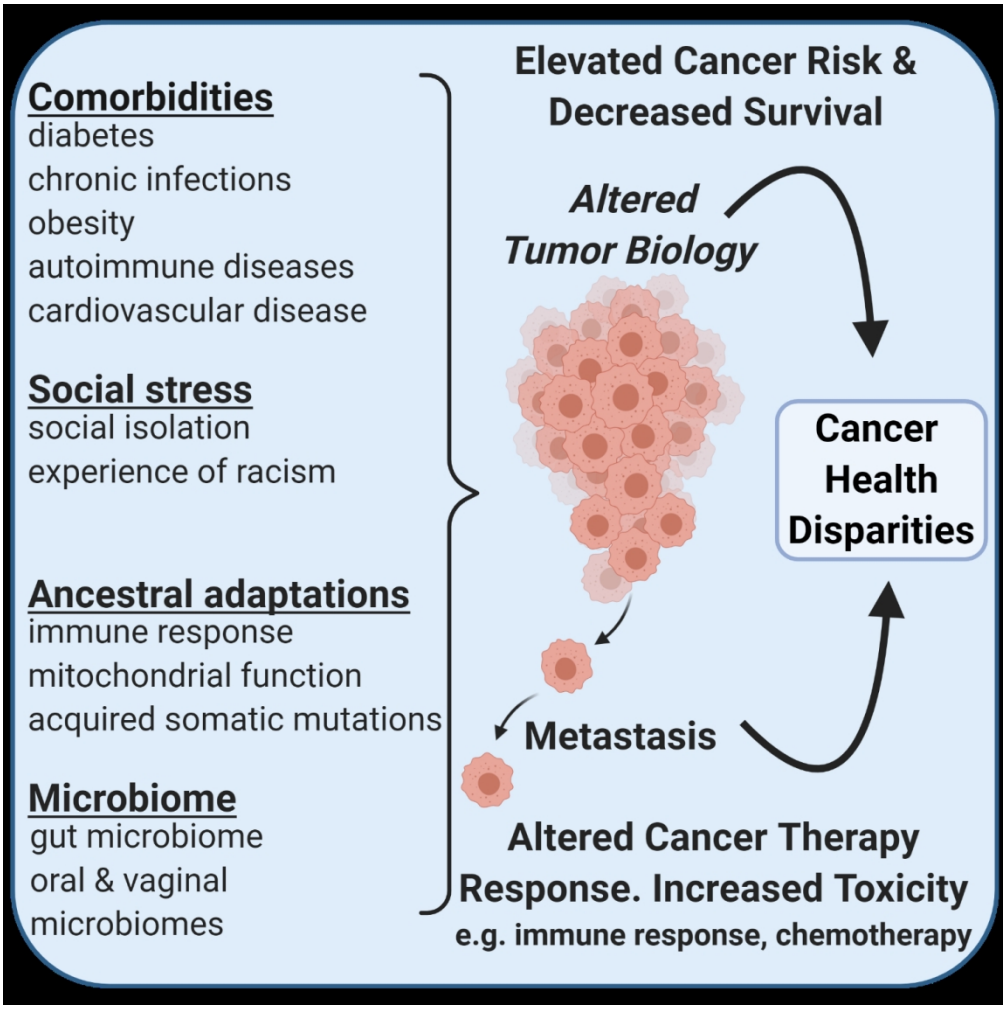

Figure 1. Comorbid chronic diseases, stress exposure, population differences in immune response, and dysbiosis are factors that contribute to cancer health disparities.

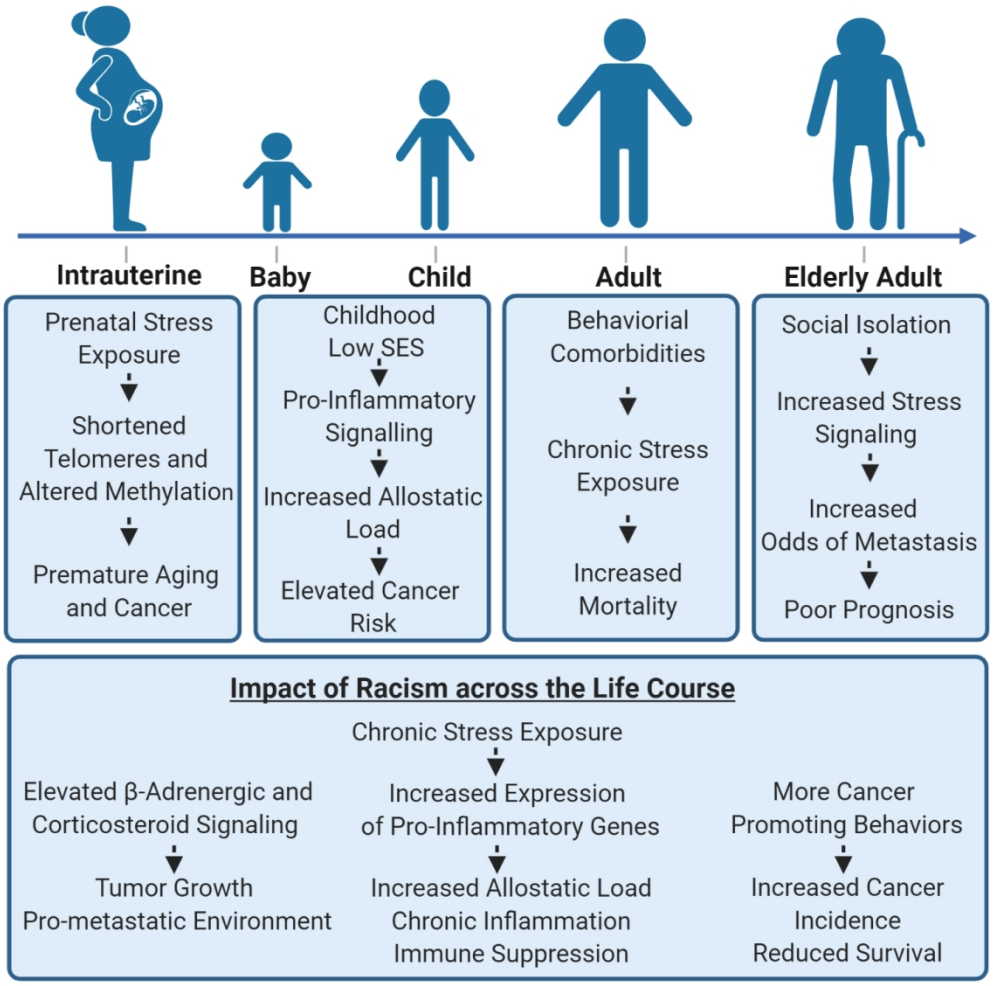

Figure 2. Stress exposure over the life course and its potential impact on socially deprived and minority populations.

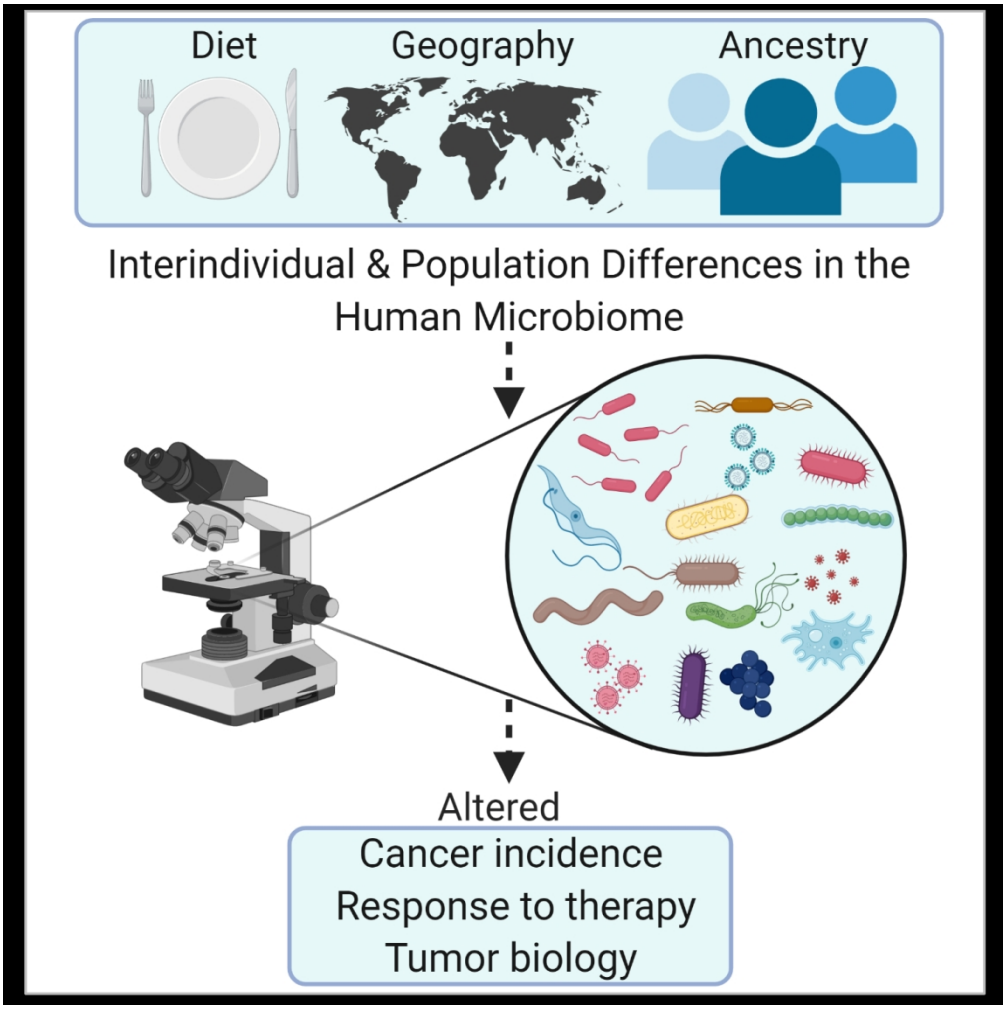

Figure 3. Diet, geographic location and ethnicity strongly associate with the diversity of the gut microbiome and may increase the risk of dysbiosis, a cancer risk factor.

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

Wei Tang, Ph.D.[1], Jay H. Fowke, Ph.D.[2], Lauren M. Hurwitz, Ph.D.[3], Mark D. Steinwandel, BBA[4], William J. Blot, Ph.D.[4,5], and Stefan Ambs, Ph.D.[1]

[1] Laboratory of Human Carcinogenesis, Center for Cancer Research, National Cancer Institute (NCI), National Institutes of Health (NIH), Bethesda, MD, USA
[2] Department of Preventive Medicine, University of Tennessee Health Science Center, Memphis, TN, USA
[3] Metabolic Epidemiology Branch, Division of Cancer Epidemiology and Genetics, NCI, NIH, Rockville, MD, USA
[4]International Epidemiology Institute, Rockville, MD, USA
[5] Vanderbilt Epidemiology Center, Vanderbilt University School of Medicine, Nashville, TN, USA

Key words: Prostate cancer, aspirin, African American, health disparity, Southern Community Cohort Study

Abbreviations: SCCS, Southern Community Cohort Study; HR, hazard ratio; OR, odds ratio; CI, confidence interval; PSA, prostate-specific antigen; BMI, body mass index; BPH, benign prostate hyperplasia; NSAID, non-steroidal anti-inflammatory drug

Financial Support: This research was supported by the Intramural Research Program of the NIH, National Cancer Institute (NCI), Center for Cancer Research; the NCI Cancer Prevention Fellowship program; DoD award W81XWH1810588 (to S.A.); R01CA092447, NCI/NIH (to W.J.B.); U01 CA202979, NCI/NIH, to 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).

Address all correspondence to: Stefan Ambs, Laboratory of Human Carcinogenesis, National Cancer Institute, Bldg.37/Room 3050B, Bethesda, MD 20892-4258, Phone 240-760-6836; Email: ambss@mail.nih.gov

Abstract (word count: 249)

Background: The association of aspirin use with prostate cancer has been investigated but few studies have 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), a large prospective cohort study that investigates causes of cancer health disparities.

Methods: SCCS recruited 22,426 African American men between 2002 and 2009. During follow-up, 1058 men developed prostate cancer including 103 prostate cancer-specific deaths. Aspirin use was assessed at time of 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, 5486 men (25.1%) reported taking any aspirin and 2634 men (12.1%) reported regular strength aspirin use. Aspirin use at enrollment was not associated with prostate cancer development but was suggestively associated with a reduced prostate cancer mortality [adjusted hazard ratio (HR) = 0.66, 95% confidence interval (CI): 0.39 to 1.14 for any aspirin use; HR = 0.41, 95% CI: 0.17 to 1.00 for regular strength aspirin use].

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

Impact: Prospective data from SCCS suggest that aspirin use may help prevent prostate cancer mortality 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 previously described 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

Southern Community Cohort Study. 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 aged 40 to 79 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) TNM system for clinical stage, abbreviated as I - IV. We defined aggressive prostate cancer as Stage IV or N1 or M1 by AJCC, or Gleason score ≥ 8 (high grade). Cohort member deaths were identified through annual linkages with both the Social Security Administration and the National Death Index (NDI). For the current study, SCCS state cancer registries reporting was completed through 12/31/2016 and NDI reporting through 12/31/2018. We examined the SCCS dataset for all self-reported African American men (n = 22,426). Of these men, 1058 developed prostate cancer. During the study follow-up, a total of 6627 deaths occurred

including 103 prostate cancer-related deaths as defined by International Classification of Diseases 10th criteria using NDI data.

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 one 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. # years was calculated. (iii) When you took this regularly, what is the average number of pills you took per week? # pills was calculated. 97.4% of the participants (21,851 out of 22,426) answered the questions on aspirin use at enrollment. We created aspirin use categories based on 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, non-users of aspirin were men who did not report any aspirin use. The aspirin category was further categorized as either "taken more than 7 pills per week" or "taken 7 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 hazard ratios (HR) for disease risk and disease-specific mortality. Our exposures of interest were any aspirin use and regular strength aspirin. Each exposure variable was separately compared with non-users. 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, graduate degree), household income (<$15,000, $15,000-25,000, >$25,000-50,000, >$50,000-100,000, >$100,000), family history of prostate cancer including father and brother (yes/no), smoking status (current, former, never), diabetes (yes/no), body mass index (BMI, as six cateogories <18.5, 18.5-25, 25-30, 30-35, 35-40, >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 non-steroidal anti-inflammatory drug (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 versus III/IV), by Gleason score [≤ 7 (low/medium grade) versus ≥ 8 (high grade)], and by disease aggressiveness [T4/N1/M1/high grade]. 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. Because PSA levels were measured in a subset of men at baseline, we compared these PSA levels between aspirin users and non-users 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. Deaths from causes other than prostate cancer were censored. Subdistribution hazard ratios were calculated using Fine and Grey regression to examine

the impact of aspirin use on the cumulative probability of prostate cancer 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 $P < 0.05$.

Results

Demographics and clinicopathological features. The study recruited 22,426 African American men of whom 1058 developed prostate cancer on follow up. At enrollment, 5486 men (25.1%) reported taking any aspirin and 2634 men (12.1%) reported taking regular strength aspirin. Characteristics of the African American men by aspirin use are shown in **Table 1**. The mean age at enrollment was 50.8 (S.D.= 8.0) years, with a median follow-up time of 12 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 (29.4 vs. 27.2 for any aspirin; 28.9 vs. 27.2 for regular strength aspirin), a history of diabetes (33.8% vs. 12.9% for any aspirin; 27.6% vs. 12.9% for regular strength aspirin), BPH (9.4% vs. 3.8% for any aspirin; 7.2% vs. 3.8% for regular strength aspirin), or family history of prostate cancer (6.0% vs. 4.3% for any aspirin; 5.9% vs. 4.3%). Among men who were diagnosed with prostate cancer, 11.7% with disease stage information presented with an advanced stage disease (TNM III/IV) and 16.5% with an aggressive disease (T4/N1/M1/high grade).

Aspirin use and prostate cancer risk. In the multivariable-adjusted Cox regression analysis, any aspirin use at enrollment was not associated with prostate cancer risk (adjusted HR 1.07, 95% CI 0.92-1.25, **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, HR 1.07, 95% CI 0.91-1.26 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, 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. Aspirin use at enrollment tentatively associated with a reduced prostate cancer mortality [adjusted hazard ratio (HR) = 0.66, 95% confidence interval (CI): 0.39 to 1.14 for any aspirin use; HR = 0.41, 95% CI: 0.17 to 1.00 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 sub-distribution HRs 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 (1,2,24,25). Using the NCI-Maryland Prostate Cancer Case-Control Study, we previously reported 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 into 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 reduced prostate cancer mortality. This protective effect may mainly relate to use of regular strength aspirin. Our observations are plausible as recent mechanistic observations and epidemiologic data showed that aspirin could have promising effect on reducing metastasis and cancer mortality (26,27).

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. 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 (28-30) and disease mortality and survival (18-20). Yet, data for men of African ancestry remain sparse. *Hurwitz et al.* investigated the relationship of aspirin use with prostate cancer in the Atherosclerosis Risk in Communities Study, a prospective study that included 5060 European American men and 1534 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 the current study.

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 (31). Treating prostate cancer patients with celecoxib, a selective cyclooxygenase-2 (COX2) inhibitor, did not confer a survival benefit in the STAMPEDE trial (32). Aspirin is thought to have cancer preventive activity by inhibiting the same pathway and the production of prostaglandins. Yet, in contrast to COX2-specific inhibitors, aspirin irreversibly inhibits both COX1 & 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 (26), yet comparable data for prostate cancer and bone metastasis are still missing. Nevertheless, 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 et al.* (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 (33,34). Lastly, 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 US population, which is primarily for prevention of cardiovascular disease. Its usage increases among the elderly (35). A recent survey reported an estimated use of about 50% among U.S. adults aged 45-75 years (36). The observation indicates

that aspirin use can be under-reported 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 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 (37) whereas our any aspirin use group comprised additional users of low-dose aspirin (about 50%). Accordingly, we found that the protective effect of aspirin on prostate cancer mortality seemed to be somewhat stronger among regular strength aspirin than any aspirin users, consistent with a dose effect, however, the confidence intervals largely overlapped and there was no significant difference between the two groups.

There are several limitations to our study. Firstly, 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 U.S. and follows recommendations by primary care physicians (36). Secondly, we did not receive information on disease characteristics for all men with prostate cancer. Accordingly, we had missing data on disease stage for 26% and on Gleason score for 30% of the patients, limiting our ability to analyze the relationship between aspirin use and advanced disease in this study. Thirdly, studies reported that aspirin leads to lower blood PSA, leading to a potential under-estimate of disease occurrence among aspirin users in the prostate cancer risk analysis (38). 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 Figure 1**). Forthly, 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 non-users (39) 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. Lastly, 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. Aspirin for prevention should be further evaluated as an opportunity to decrease lethal prostate cancer in these men.

Acknowledgments

We would like to thank the SCCS Publications Committee for reviewing the manuscript and helpful comments. The authors have no conflicts of interest related to the work to disclose.

Data Availability

The data underlying this article were obtained through a data access agreement with the Southern Community Cohort Study [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 Southern Community Cohort Study and can be obtained from the corresponding author upon request.

References

1. Powell IJ. Epidemiology and pathophysiology of prostate cancer in African-American men. J Urol **2007**;177(2):444-9.
2. Rebbeck TR, Devesa SS, Chang BL, Bunker CH, Cheng I, Cooney K*, et al.* Global patterns of prostate cancer incidence, aggressiveness, and mortality in men of african descent. Prostate Cancer **2013**;2013:560857.
3. Wallace TA, Martin DN, Ambs S. Interactions among genes, tumor biology and the environment in cancer health disparities: examining the evidence on a national and global scale. Carcinogenesis **2011**;32:1107-21.
4. Zeigler-Johnson CM, Rennert H, Mittal RD, Jalloh M, Sachdeva R, Malkowicz SB*, et al.* Evaluation of prostate cancer characteristics in four populations worldwide. Can J Urol **2008**;15:4056-64.
5. Ragin CC, Taioli E, McFarlane-Anderson N, Avery G, Bennett F, Bovell-Benjamin A*, et al.* African-Caribbean cancer consortium for the study of viral, genetic and environmental cancer risk factors. Infect Agent Cancer **2007**;2:17.
6. Odedina FT, Akinremi TO, Chinegwundoh F, Roberts R, Yu D, Reams RR*, et al.* Prostate cancer disparities in Black men of African descent: a comparative literature review of prostate cancer burden among Black men in the United States, Caribbean, United Kingdom, and West Africa. Infect Agent Cancer **2009**;4 Suppl 1:S2.
7. Wallace TA, Prueitt RL, Yi M, Howe TM, Gillespie JW, Yfantis HG*, et al.* Tumor immunobiological differences in prostate cancer between African-American and European-American men. Cancer Res **2008**;68(3):927-36.
8. Reams RR, Agrawal D, Davis MB, Yoder S, Odedina FT, Kumar N*, et al.* Microarray comparison of prostate tumor gene expression in African-American and Caucasian American males: a pilot project study. Infect Agent Cancer **2009**;4 Suppl 1:S3.
9. Powell IJ, Dyson G, Land S, Ruterbusch J, Bock CH, Lenk S*, et al.* Genes associated with prostate cancer are differentially expressed in African American and European American men. Cancer Epidemiol Biomarkers Prev **2013**;22(5):891-7.
10. Hardiman G, Savage SJ, Hazard ES, Wilson RC, Courtney SM, Smith MT*, et al.* Systems analysis of the prostate transcriptome in African-American men compared with European-American men. Pharmacogenomics **2016**;17(10):1129-43 doi 10.2217/pgs-2016-0025.
11. Tang W, Wallace TA, Yi M, Magi-Galluzzi C, Dorsey TH, Onabajo OO*, et al.* IFNL4-DeltaG Allele Is Associated with an Interferon Signature in Tumors and Survival of African-American Men with Prostate Cancer. Clin Cancer Res **2018**;24(21):5471-81 doi 10.1158/1078-0432.Ccr-18-1060.
12. Yuan J, Kensler KH, Hu Z, Zhang Y, Zhang T, Jiang J*, et al.* Integrative comparison of the genomic and transcriptomic landscape between prostate cancer patients of predominantly African or European genetic ancestry. PLoS Genet **2020**;16(2):e1008641 doi 10.1371/journal.pgen.1008641.
13. Smith CJ, Dorsey TH, Tang W, Jordan SV, Loffredo CA, Ambs S. Aspirin Use Reduces the Risk of Aggressive Prostate Cancer and Disease Recurrence in African-American Men. Cancer Epidemiol Biomarkers Prev **2017**;26(6):845-53 doi 10.1158/1055-9965.EPI-16-1027.

14. Mahmud SM, Franco EL, Aprikian AG. Use of nonsteroidal anti-inflammatory drugs and prostate cancer risk: a meta-analysis. Int J Cancer **2010**;127(7):1680-91 doi 10.1002/ijc.25186.
15. Huang TB, Yan Y, Guo ZF, Zhang XL, Liu H, Geng J*, et al.* Aspirin use and the risk of prostate cancer: a meta-analysis of 24 epidemiologic studies. Int Urol Nephrol **2014**;46(9):1715-28 doi 10.1007/s11255-014-0703-4.
16. Jacobs EJ, Thun MJ, Bain EB, Rodriguez C, Henley SJ, Calle EE. A large cohort study of long-term daily use of adult-strength aspirin and cancer incidence. J Natl Cancer Inst **2007**;99(8):608-15 doi 10.1093/jnci/djk132.
17. Sauer CM, Myran DT, Costentin CE, Zwisler G, Safder T, Papatheodorou S*, et al.* Effect of long term aspirin use on the incidence of prostate cancer: A systematic review and meta-analysis. Crit Rev Oncol Hematol **2018**;132:66-75 doi 10.1016/j.critrevonc.2018.09.013.
18. Liu Y, Chen JQ, Xie L, Wang J, Li T, He Y*, et al.* Effect of aspirin and other non-steroidal anti-inflammatory drugs on prostate cancer incidence and mortality: a systematic review and meta-analysis. BMC Med **2014**;12:55 doi 10.1186/1741-7015-12-55.
19. Hurwitz LM, Joshu CE, Barber JR, Prizment AE, Vitolins MZ, Jones MR*, et al.* Aspirin and Non-Aspirin NSAID Use and Prostate Cancer Incidence, Mortality, and Case Fatality in the Atherosclerosis Risk in Communities Study. Cancer Epidemiol Biomarkers Prev **2019**;28(3):563-9 doi 10.1158/1055-9965.EPI-18-0965.
20. Downer MK, Allard CB, Preston MA, Gaziano JM, Stampfer MJ, Mucci LA*, et al.* Regular Aspirin Use and the Risk of Lethal Prostate Cancer in the Physicians' Health Study. Eur Urol **2017**;72(5):821-7 doi 10.1016/j.eururo.2017.01.044.
21. Zhou CK, Daugherty SE, Liao LM, Freedman ND, Abnet CC, Pfeiffer R*, et al.* Do Aspirin and Other NSAIDs Confer a Survival Benefit in Men Diagnosed with Prostate Cancer? A Pooled Analysis of NIH-AARP and PLCO Cohorts. Cancer Prev Res (Phila) **2017**;10(7):410-20 doi 10.1158/1940-6207.CAPR-17-0033.
22. Signorello LB, Hargreaves MK, Blot WJ. The Southern Community Cohort Study: investigating health disparities. J Health Care Poor Underserved **2010**;21(1 Suppl):26-37 doi 10.1353/hpu.0.0245.
23. Moses KA, Zhao Z, Bi Y, Acquaye J, Holmes A, Blot WJ*, et al.* The impact of sociodemographic factors and PSA screening among low-income Black and White men: data from the Southern Community Cohort Study. Prostate Cancer Prostatic Dis **2017**;20(4):424-9 doi 10.1038/pcan.2017.32.
24. Martin DN, Starks AM, Ambs S. Biological determinants of health disparities in prostate cancer. Curr Opin Oncol **2013**;25(3):235-41.
25. Smith CJ, Minas TZ, Ambs S. Analysis of Tumor Biology to Advance Cancer Health Disparity Research. Am J Pathol **2018**;188(2):304-16 doi 10.1016/j.ajpath.2017.06.019.
26. Lucotti S, Cerutti C, Soyer M, Gil-Bernabe AM, Gomes AL, Allen PD*, et al.* Aspirin blocks formation of metastatic intravascular niches by inhibiting platelet-derived COX-1/thromboxane A2. J Clin Invest **2019**;129(5):1845-62 doi 10.1172/JCI121985.
27. Rothwell PM, Wilson M, Price JF, Belch JF, Meade TW, Mehta Z. Effect of daily aspirin on risk of cancer metastasis: a study of incident cancers during randomised controlled trials. Lancet **2012**;379(9826):1591-601 doi 10.1016/S0140-6736(12)60209-8.

28. Vidal AC, Howard LE, Moreira DM, Castro-Santamaria R, Andriole GL, Freedland SJ. Aspirin, NSAIDs, and risk of prostate cancer: results from the REDUCE study. Clin Cancer Res **2015**;21(4):756-62 doi 10.1158/1078-0432.CCR-14-2235.
29. Shebl FM, Sakoda LC, Black A, Koshiol J, Andriole GL, Grubb R*, et al.* Aspirin but not ibuprofen use is associated with reduced risk of prostate cancer: a PLCO study. Br J Cancer **2012**;107(1):207-14 doi 10.1038/bjc.2012.227.
30. Dhillon PK, Kenfield SA, Stampfer MJ, Giovannucci EL. Long-term aspirin use and the risk of total, high-grade, regionally advanced and lethal prostate cancer in a prospective cohort of health professionals, 1988-2006. Int J Cancer **2011**;128(10):2444-52 doi 10.1002/ijc.25811.
31. Schlesinger M. Role of platelets and platelet receptors in cancer metastasis. J Hematol Oncol **2018**;11(1):125 doi 10.1186/s13045-018-0669-2.
32. Mason MD, Clarke NW, James ND, Dearnaley DP, Spears MR, Ritchie AWS*, et al.* Adding Celecoxib With or Without Zoledronic Acid for Hormone-Naïve Prostate Cancer: Long-Term Survival Results From an Adaptive, Multiarm, Multistage, Platform, Randomized Controlled Trial. J Clin Oncol **2017**;35(14):1530-41 doi 10.1200/jco.2016.69.0677.
33. Arita M, Clish CB, Serhan CN. The contributions of aspirin and microbial oxygenase to the biosynthesis of anti-inflammatory resolvins: novel oxygenase products from omega-3 polyunsaturated fatty acids. Biochem Biophys Res Commun **2005**;338(1):149-57 doi 10.1016/j.bbrc.2005.07.181.
34. Spite M, Serhan CN. Novel lipid mediators promote resolution of acute inflammation: impact of aspirin and statins. Circ Res **2010**;107(10):1170-84 doi 10.1161/CIRCRESAHA.110.223883.
35. Stuntz M, Bernstein B. Recent trends in the prevalence of low-dose aspirin use for primary and secondary prevention of cardiovascular disease in the United States, 2012-2015. Prev Med Rep **2017**;5:183-6 doi 10.1016/j.pmedr.2016.12.023.
36. Williams CD, Chan AT, Elman MR, Kristensen AH, Miser WF, Pignone MP*, et al.* Aspirin use among adults in the U.S.: results of a national survey. Am J Prev Med **2015**;48(5):501-8 doi 10.1016/j.amepre.2014.11.005.
37. Bosetti C, Santucci C, Gallus S, Martinetti M, La Vecchia C. Aspirin and the risk of colorectal and other digestive tract cancers: an updated meta-analysis through 2019. Ann Oncol **2020**;31(5):558-68 doi 10.1016/j.annonc.2020.02.012.
38. Fowke JH, Motley SS, Smith JA, Jr., Cookson MS, Concepcion R, Chang SS*, et al.* Association of nonsteroidal anti-inflammatory drugs, prostate specific antigen and prostate volume. J Urol **2009**;181(5):2064-70 doi 10.1016/j.juro.2009.01.031.
39. Downer MK, Allard CB, Preston MA, Wilson KM, Kenfield SA, Chan JM*, et al.* Aspirin Use and Lethal Prostate Cancer in the Health Professionals Follow-up Study. Eur Urol Oncol **2019**;2(2):126-34 doi 10.1016/j.euo.2018.07.002.

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

	No Aspirin		Any Aspirin		Regular strength Aspirin		Any aspirin vs no aspirin	Regular strength aspirin vs no aspirin
	(n)	(%)	(n)	(%)	(n)	(%)	*P value*	*P value*
Total	16365	74.9[a]	5486	25.1	2634	12.1		
Age median (IQR)	48	10.0	53	13.0	52	12.0	< 0.001	< 0.001
BMI mean (SD)	27.2	5.8	29.4	6.3	28.9	6.2	< 0.001	< 0.001
Education								
Less High	5526	33.8[b]	1823	33.2	913	34.7		
High school	6903	42.2	2028	37.0	991	37.6		
College	2751	16.8	992	18.1	471	17.9		
More than College	1085	6.6	548	10.0	227	8.6	< 0.001	< 0.001
HH income								
< 15k	10138	61.9	2900	52.9	1477	56.1		
15K-25K	3453	21.1	1176	21.4	539	20.5		
25K-50	1840	11.2	855	15.6	386	14.7		
50K-100K	620	3.8	381	6.9	156	5.9		
> 100k	153	0.9	113	2.1	38	1.4	< 0.001	< 0.001
Family History of Prostate Cancer								
No	15606	95.4	5123	93.4	2460	93.4		
Yes	703	4.3	331	6.0	156	5.9	< 0.001	< 0.001
Tobacco use								
Current	10017	61.2	2457	44.8	1343	51.0		
Former	2814	17.2	1640	29.9	701	26.6		
Never	3475	21.2	1335	24.3	559	21.2	< 0.001	< 0.001
Diabetes								
No	14242	87.0	3629	66.2	1906	72.4		

Yes	2112	12.9	1853	33.8	725	27.5	< 0.001	< 0.001
BPH								
No	15689	95.9	4936	90.0	2432	92.3		
Yes	627	3.8	516	9.4	189	7.2	< 0.001	< 0.001
PSA screening								
No	9076	55.5	2067	37.7	1180	44.8		
Yes	6402	39.1	3109	56.7	1299	49.3	< 0.001	< 0.001
DRE screening								
No	7611	46.5	1760	32.1	978	37.1		
Yes	8666	53.0	3681	67.1	1636	62.1	< 0.001	< 0.001
Disease characteristics								
TNM Stage (clinical)								
I	65	9.4	32	9.6	19	14.0		
II	355	51.4	182	54.8	71	52.2		
III	34	4.9	17	5.1	6	4.4		
IV	56	8.1	13	3.9	3	2.2	0.09	0.05
Gleason								
<=7	404	58.6	181	54.5	76	55.9		
>=8	86	12.5	44	13.3	16	11.8	0.59	1.00
Aggressive Disease								
Yes	116	16.8	53	16.0	19	14.0		
No	574	92.3	279	84.0	117	86.0	0.80	0.49

Abbreviations: IQR, interquartile range; SD, standard deviation; BMI, body mass index; HH, household income; BPH, benign prostate hyperplasia; PSA, prostate cancer-specific antigen

[a] percent of all men in study (n = 21851); [b] percent of men within aspirin group [no aspirin (n = 16365), any aspirin (n = 5486), regular strength aspirin (n = 2634)] for all listed variables. Missing data not included in percentages

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 value	HR (95% CI)[b]	P value
Overall						
No use	690	162857	ref.		ref.	
Any use	332	50057	1.11 (0.97-1.27)	0.14	1.07 (0.92-1.25)	0.40
Regular strength	136	25091	1.01 (0.84-1.21)	0.95	0.97 (0.78-1.19)	0.75
Less than daily	54	10934	1.00 (0.75-1.32)	0.97	1.03 (0.77-1.39)	0.84
Daily and more	269	37850	1.13 (0.98-1.31)	0.09	1.07 (0.91-1.26)	0.42
≤3 years	189	30283	1.09 (0.92-1.28)	0.31	1.07 (0.89-1.28)	0.48
>3 years	135	18417	1.12 (0.93-1.36)	0.24	1.05 (0.84-1.30)	0.68
TNM stage I/II						
No use	420	162857	ref.		ref.	
Any use	214	50057	1.12 (0.94-1.32)	0.21	1.03 (0.85-1.26)	0.74
Regular strength	90	25091	1.06 (0.84-1.34)	0.61	0.98 (0.75-1.28)	0.88
TNM stage III/IV						
No use	90	162857	ref.		ref.	
Any use	30	50057	0.83 (0.54-1.28)	0.40	1.00 (0.63-1.60)	0.98
Regular strength	9	25091	0.54 (0.27-1.07)	0.08	0.70 (0.34-1.41)	0.31
Gleason ≤ 7						
No use	404	162857	ref.		ref.	
Any use	181	50057	1.08 (0.90-1.29)	0.41	0.95 (0.77-1.17)	0.64
Regular strength	76	25091	0.98 (0.77-1.26)	0.88	0.90 (0.68-1.20)	0.48
Gleason ≥ 8						
No use	86	162857				

Any use	44	50057	1.15 (0.79-1.68)	0.46	1.36 (0.89-2.08)	0.15
Regular strength	16	25091	0.93 (0.54-1.59)	0.78	0.95 (0.51-1.75)	0.87
Aggressive						
No use	116	162857	ref.		ref.	
Any use	53	50057	1.04 (0.74-1.46)	0.80	1.17 (0.80-1.71)	0.42
Regular strength	19	25091	0.82 (0.51-1.34)	0.44	0.84 (0.49-1.46)	0.54

Abbreviations: PY, Person-Years; HR, hazard ratio; CI, confidence interval; TNM, tumor-node-metastasis

[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, NSAIDs other than aspirin

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	2368439	ref.		ref.		ref.	
Any aspirin use	25	744964	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	369317	0.36 (0.16-0.84)	0.02	0.41 (0.17-1.00)	0.05	0.43 (0.16-1.13)	0.09

Abbreviations: PY, Person-Years; HR, hazard ratio; CI, confidence interval; SHR, sub-distribution hazard ratio

[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, NSAIDs other than aspirin