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14. ABSTRACT

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

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

15. SUBJECT TERMS
Oncology, Cancer, Prostate Cancer

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1. Introduction

Men of African descent experience a disproportionately high prostate cancer mortality. We and others have shown that prostate tumors in African-Americans harbor a distinct immune-inflammation signature. Low-grade inflammation has been described as a prostate cancer risk factor that is associated with aggressive disease. We also reported that regular aspirin use reduces the risk of aggressive prostate cancer and disease recurrence in these men. 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 Stefan Ambs at , 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.

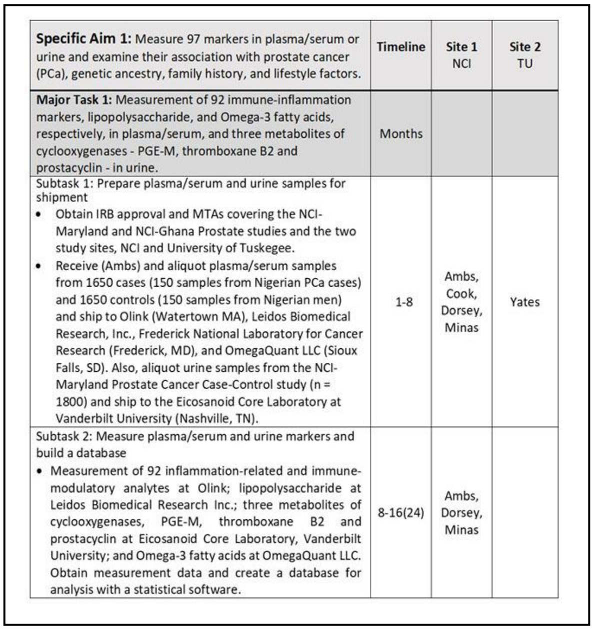
3. Accomplishments

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.

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.



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	

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

		Cases[a]				Population Controls		
Demographics		All (n=846)	AA[b] (n=407)	EA[c] (n=439)		All (n=846)	AA (n=382)	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.
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.

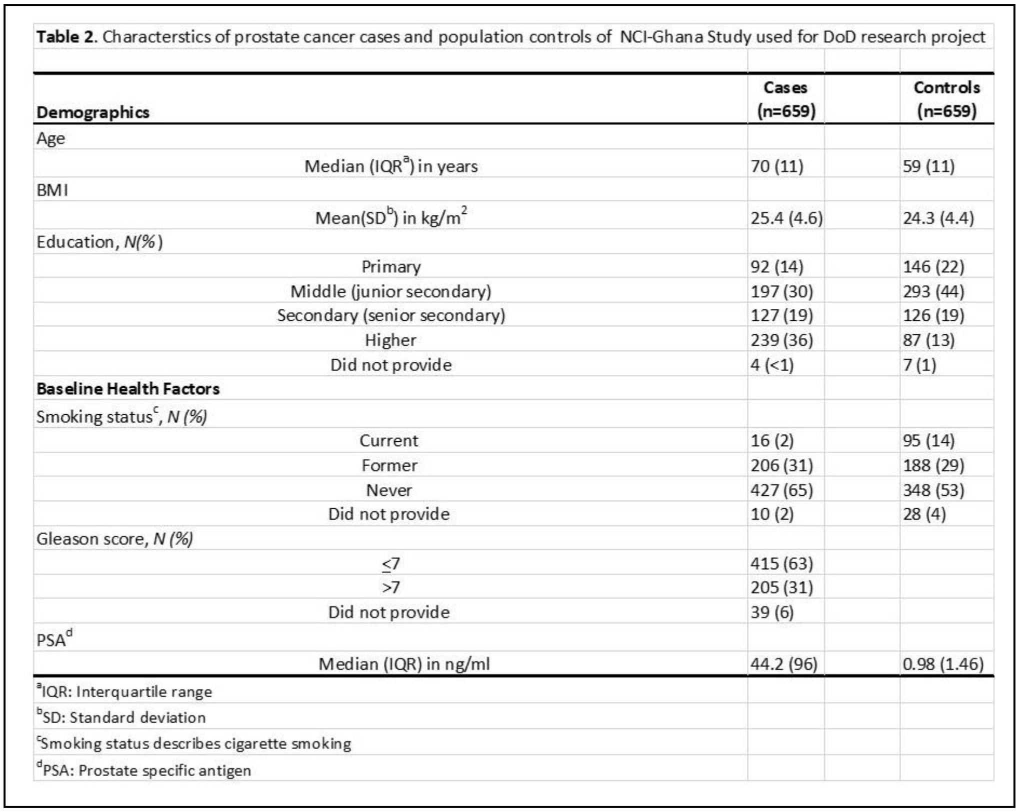


Table 2. Characterstics of prostate cancer cases and population controls of NCI-Ghana Study used for DoD research project

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

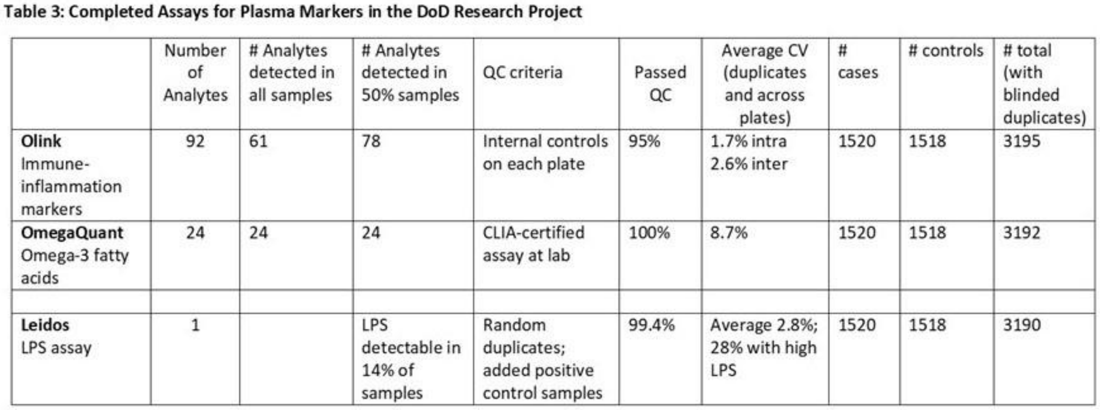
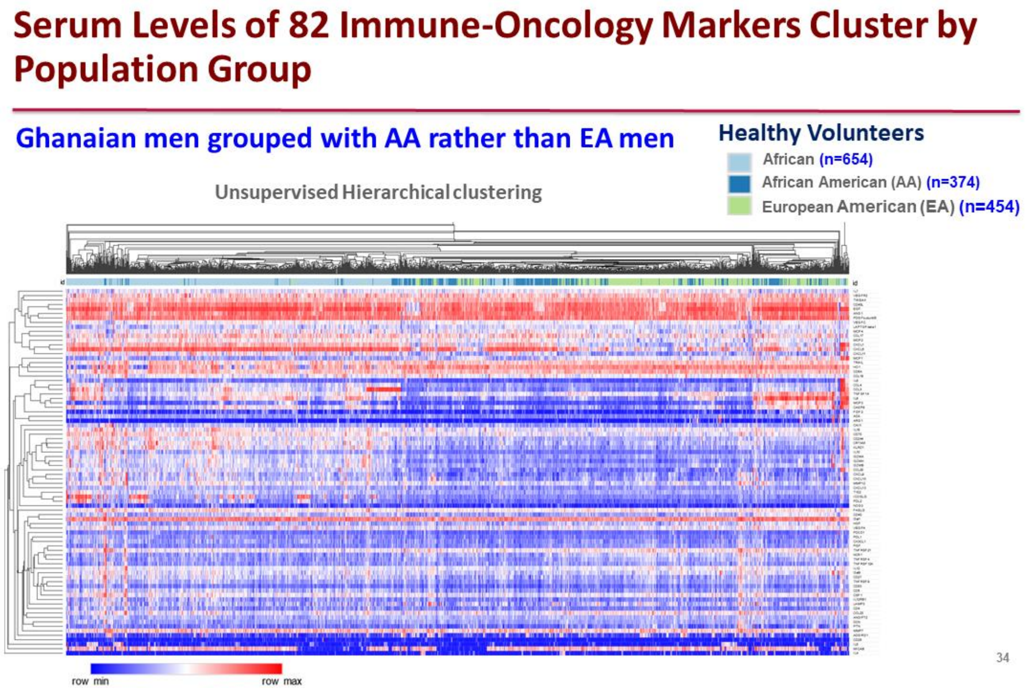


Table 3: Completed Assays for Plasma Markers in the DoD Research Project

	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



Serum Levels of 82 Immune-Oncology Markers Cluster by Population Group

Ghanaian men grouped with AA rather than EA men

Healthy Volunteers

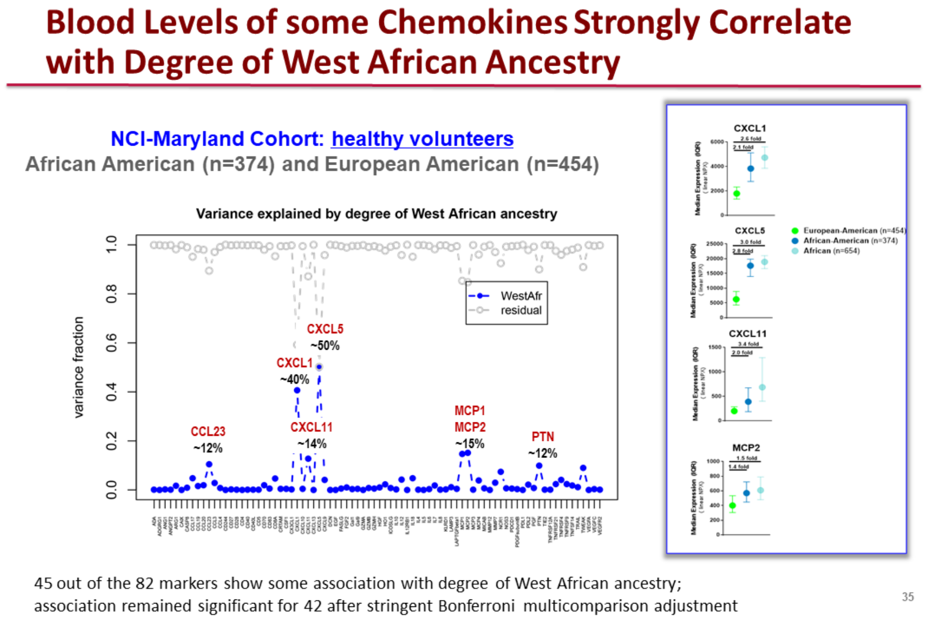
- African (n=654)
- African American (AA) (n=374)
- European American (EA) (n=454)

Unsupervised Hierarchical clustering

Figure 1

found that serum 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).



Blood Levels of some Chemokines Strongly Correlate with Degree of West African Ancestry

NCI-Maryland Cohort: healthy volunteers

African American (n=374) and European American (n=454)

45 out of the 82 markers show some association with degree of West African ancestry; association remained significant for 42 after stringent Bonferroni multicomparison adjustment

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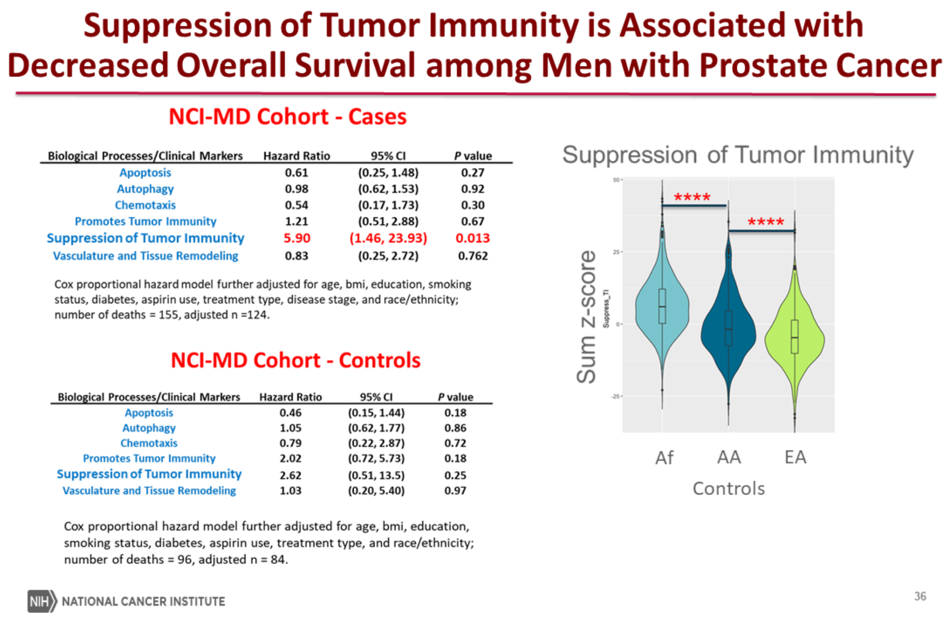
Figure 2



Suppression of Tumor Immunity is Associated with Decreased Overall Survival among Men with Prostate Cancer

NCI-MD Cohort - Cases

Biological Processes/Clinical Markers	Hazard Ratio	95% CI	*P* value
Apoptosis	0.61	(0.25, 1.48)	0.27
Autophagy	0.98	(0.62, 1.53)	0.92
Chemotaxis	0.54	(0.17, 1.73)	0.30
Promotes Tumor Immunity	1.21	(0.51, 2.88)	0.67
Suppression of Tumor Immunity	5.90	(1.46, 23.93)	0.013
Vasculature and Tissue Remodeling	0.83	(0.25, 2.72)	0.762

Cox proportional hazard model further adjusted for age, bmi, education, smoking status, diabetes, aspirin use, treatment type, disease stage, and race/ethnicity; number of deaths = 155, adjusted n =124.

NCI-MD Cohort - Controls

Biological Processes/Clinical Markers	Hazard Ratio	95% CI	*P* value
Apoptosis	0.46	(0.15, 1.44)	0.18
Autophagy	1.05	(0.62, 1.77)	0.86
Chemotaxis	0.79	(0.22, 2.87)	0.72
Promotes Tumor Immunity	2.02	(0.72, 5.73)	0.18
Suppression of Tumor Immunity	2.62	(0.51, 13.5)	0.25
Vasculature and Tissue Remodeling	1.03	(0.20, 5.40)	0.97

Cox proportional hazard model further adjusted for age, bmi, education, smoking status, diabetes, aspirin use, treatment type, and race/ethnicity; number of deaths = 96, adjusted n = 84.

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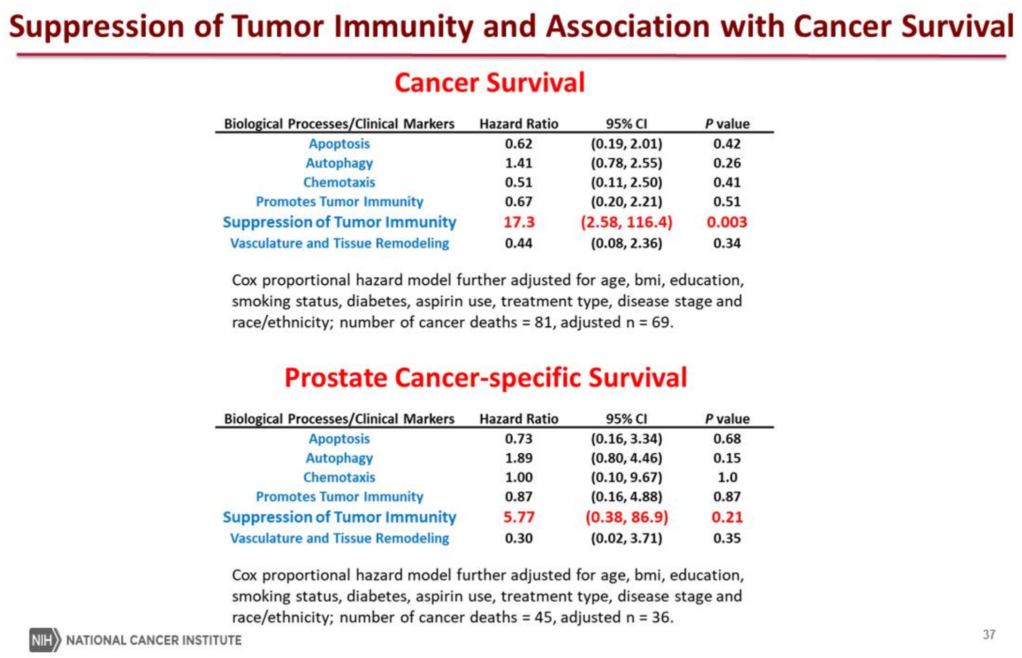
Figure 3

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.



Suppression of Tumor Immunity and Association with Cancer Survival

Cancer Survival

Biological Processes/Clinical Markers	Hazard Ratio	95% CI	*P* value
Apoptosis	0.62	(0.19, 2.01)	0.42
Autophagy	1.41	(0.78, 2.55)	0.26
Chemotaxis	0.51	(0.11, 2.50)	0.41
Promotes Tumor Immunity	0.67	(0.20, 2.21)	0.51
Suppression of Tumor Immunity	**17.3**	**(2.58, 116.4)**	**0.003**
Vasculature and Tissue Remodeling	0.44	(0.08, 2.36)	0.34

Cox proportional hazard model further adjusted for age, bmi, education, smoking status, diabetes, aspirin use, treatment type, disease stage and race/ethnicity; number of cancer deaths = 81, adjusted n = 69.

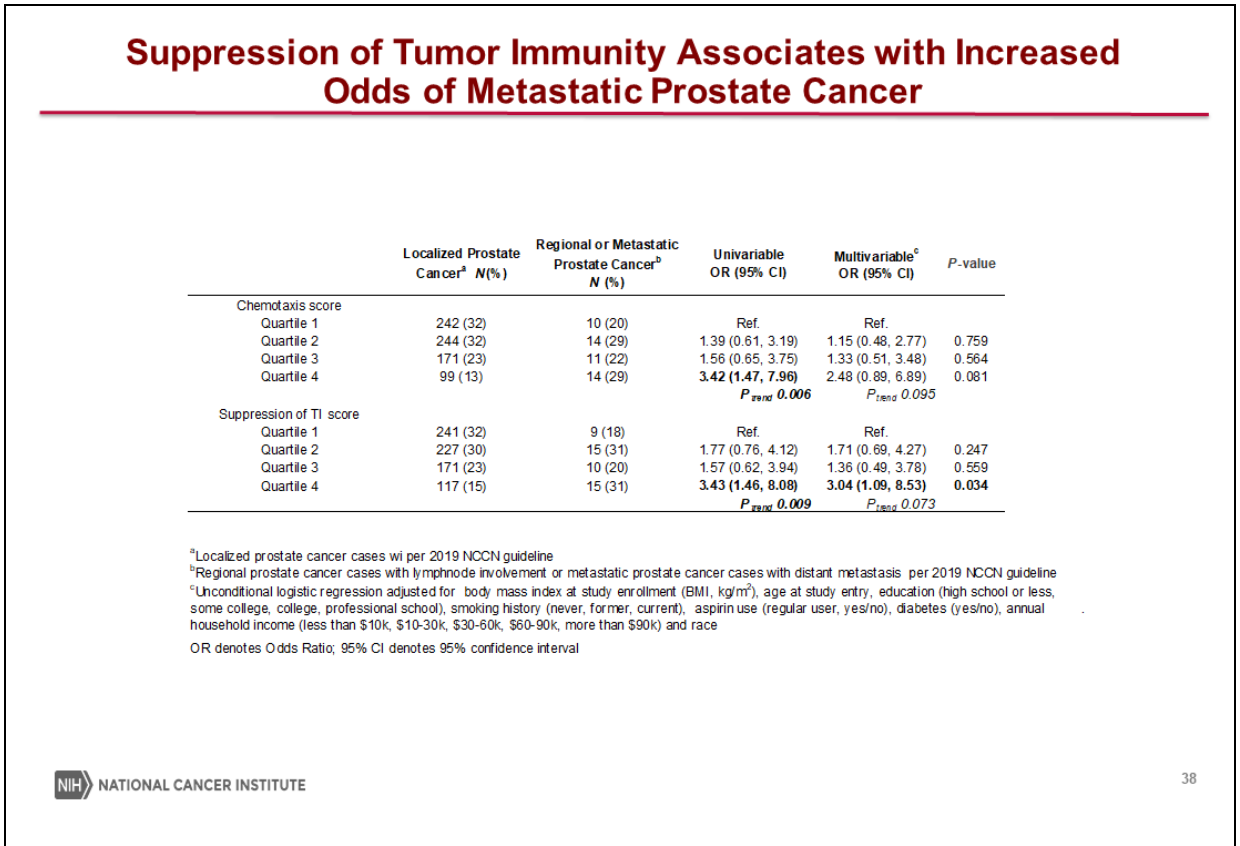
Prostate Cancer-specific Survival

Biological Processes/Clinical Markers	Hazard Ratio	95% CI	*P* value
Apoptosis	0.73	(0.16, 3.34)	0.68
Autophagy	1.89	(0.80, 4.46)	0.15
Chemotaxis	1.00	(0.10, 9.67)	1.0
Promotes Tumor Immunity	0.87	(0.16, 4.88)	0.87
Suppression of Tumor Immunity	**5.77**	**(0.38, 86.9)**	**0.21**
Vasculature and Tissue Remodeling	0.30	(0.02, 3.71)	0.35

Cox proportional hazard model further adjusted for age, bmi, education, smoking status, diabetes, aspirin use, treatment type, disease stage and race/ethnicity; number of cancer deaths = 45, adjusted n = 36.

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

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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 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

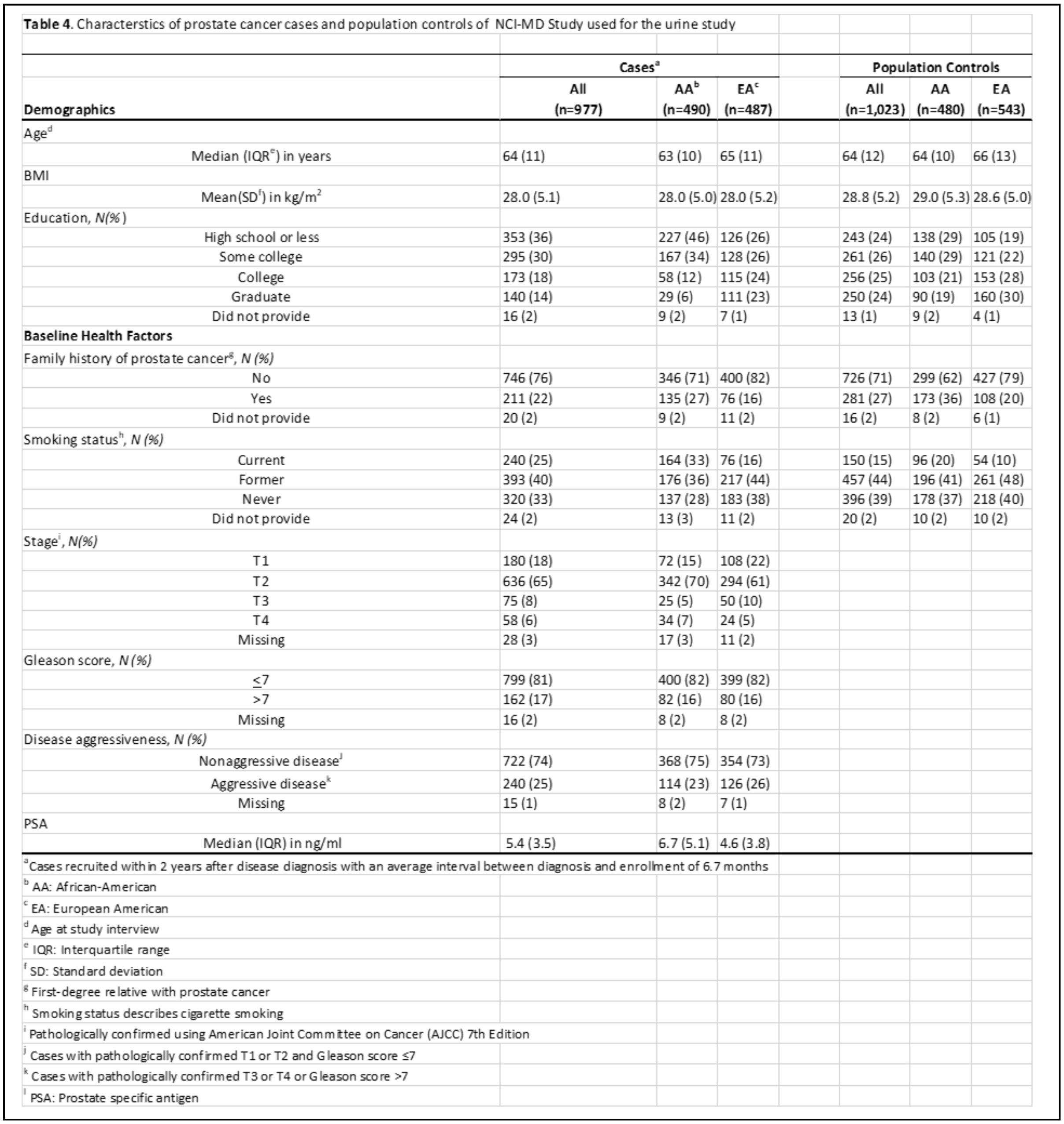


Table 4. Characterstics of prostate cancer cases and population controls of NCI-MD Study used for the urine study

	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

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.

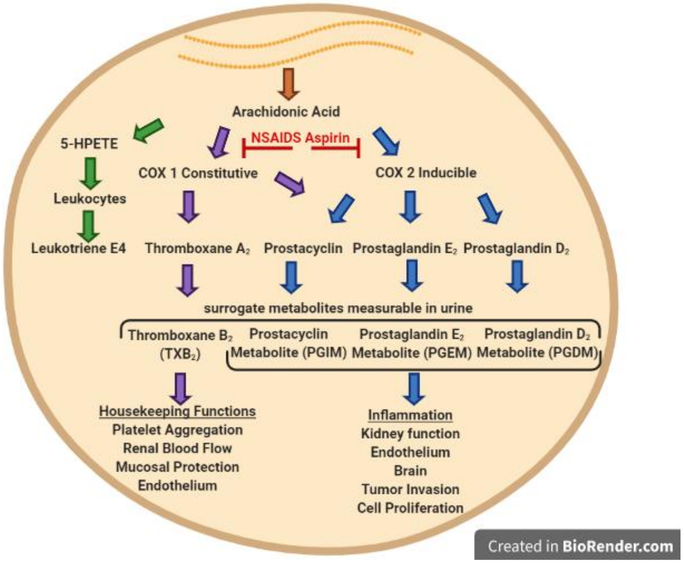
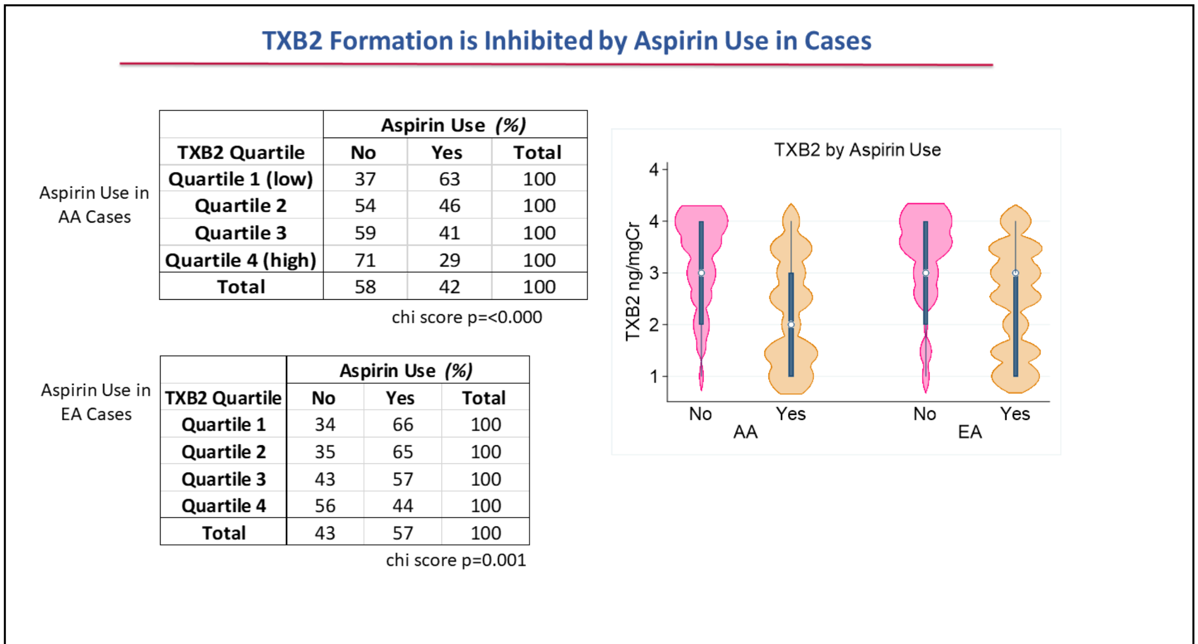


Figure 6: Cyclooxygenase pathway is a principle mechanism driving the association of inflammation and cancer

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

	Aspirin Use *(%)*		
TXB2 Quartile	**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

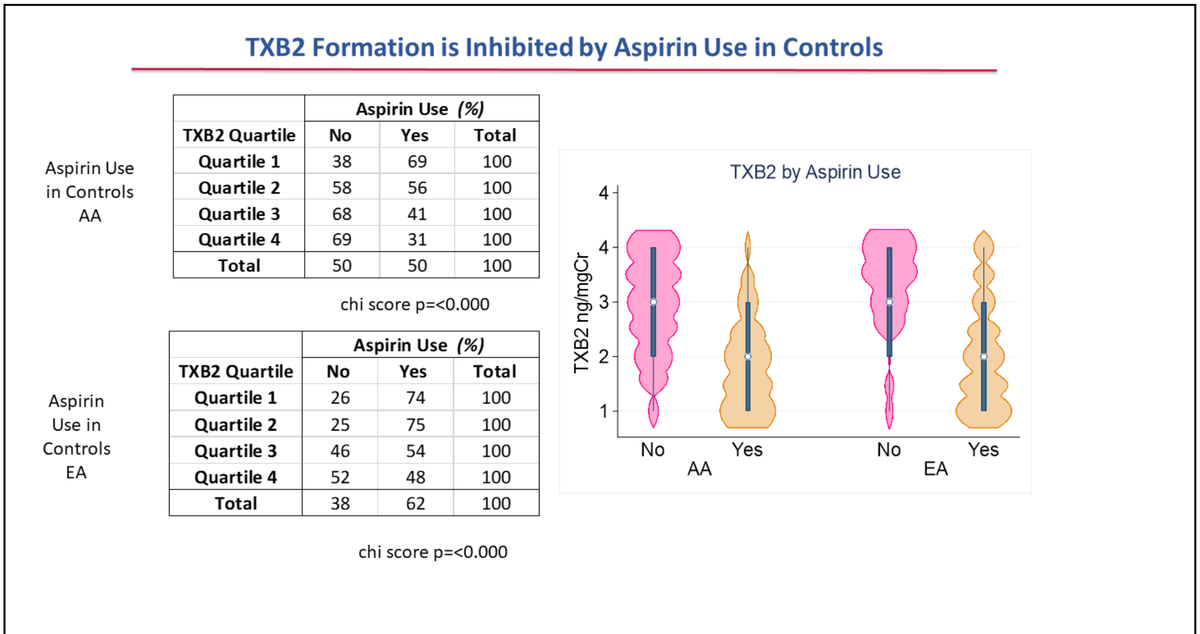
chi score p=<0.000

Aspirin Use in EA Cases

	Aspirin Use *(%)*		
TXB2 Quartile	**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

	Aspirin Use *(%)*		
TXB2 Quartile	**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

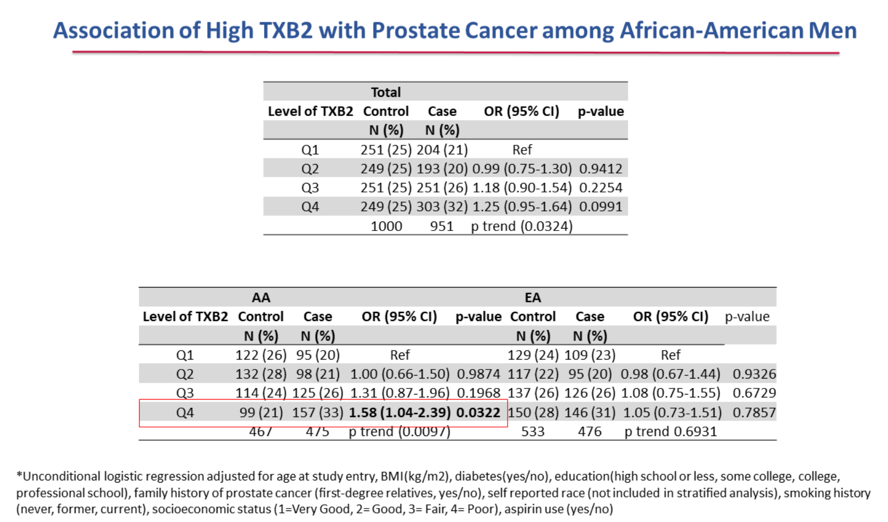
Aspirin Use in Controls EA

	Aspirin Use *(%)*		
TXB2 Quartile	**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).



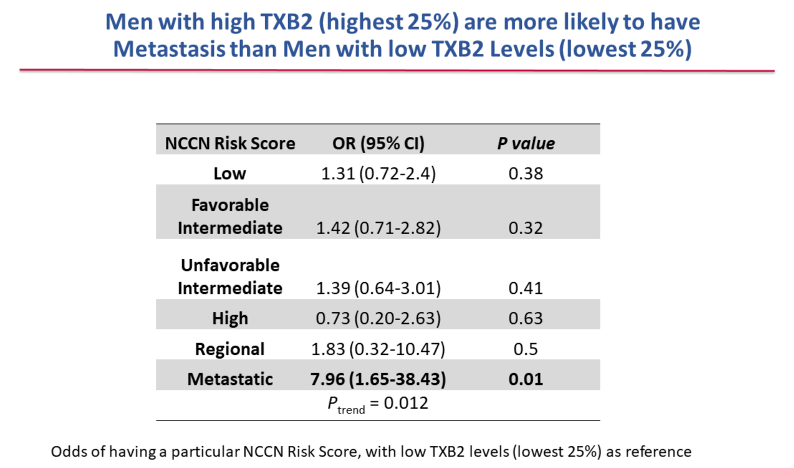
Association of High TXB2 with Prostate Cancer among African-American Men

	Total			
Level of TXB2	**Control**	**Case**	**OR (95% CI)**	**p-value**
	N (%)	**N (%)**		
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**	**Case**	**OR (95% CI)**	**p-value**	**Control**	**Case**	**OR (95% CI)**	p-value
	N (%)	**N (%)**			**N (%)**	**N (%)**		
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)

Figure 9



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

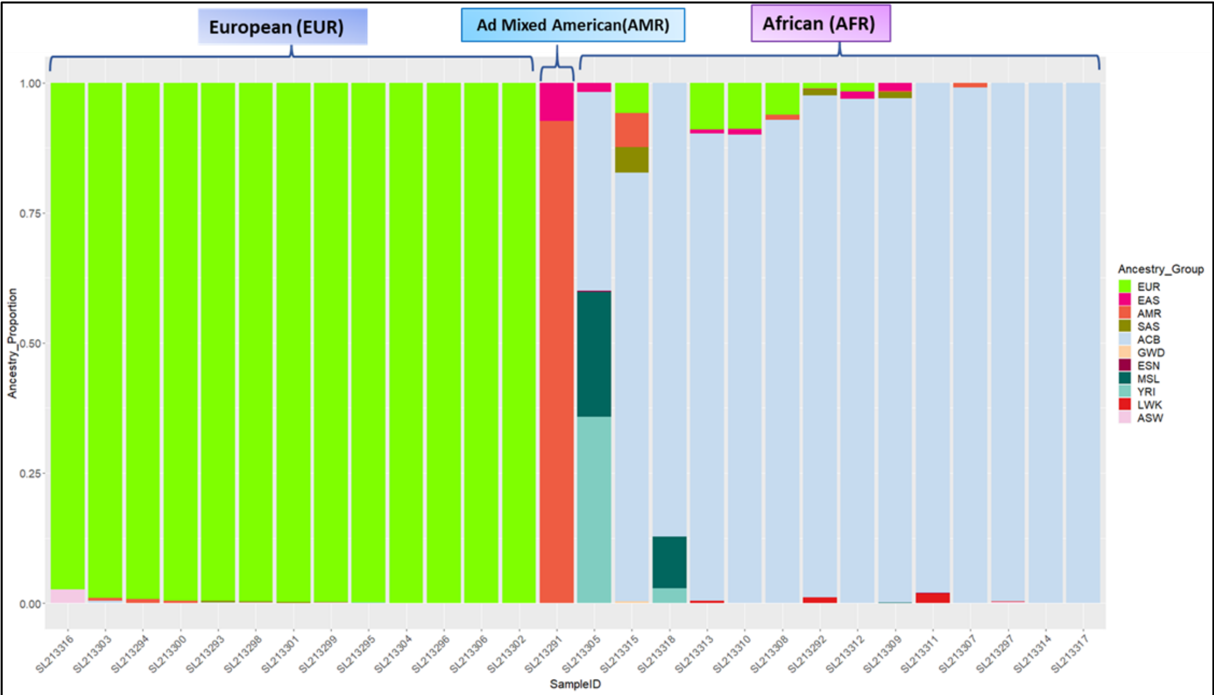
To obtain cores of tumor and adjacent non-cancerous tissue from FFPE tumor blocks, we asked the University of Maryland Department of Pathology for help with this task. Under a contract, the Department of Pathology assigned a pathologist who identified areas in the tissue blocks that could be processed 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.

Validation of Inflammatory signature in AA PCa

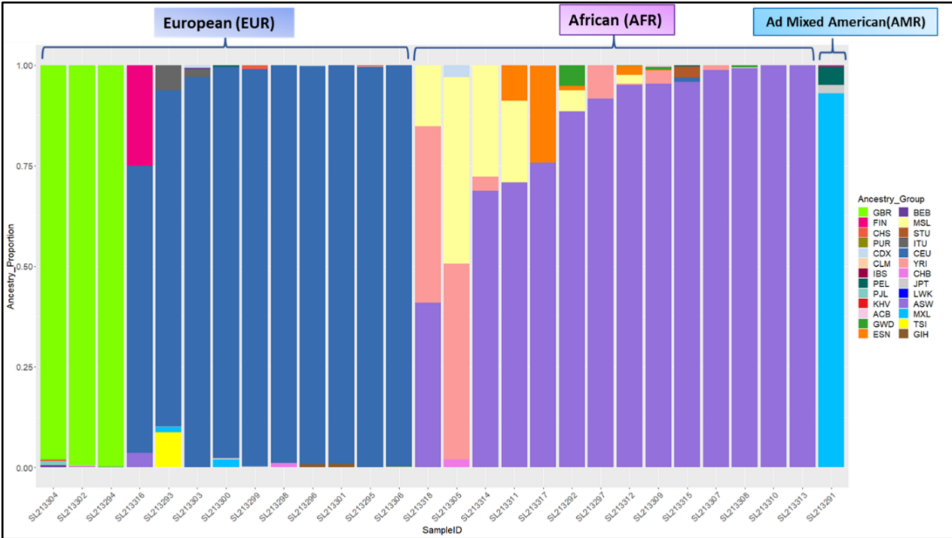
Due to COVID-19 delays in sequencing, we focused on analyzing our current set of RNA-sequenced tissue. 15 patients were self-reported as African American, and 13 patients were self-reported as European American (n=28). RNA sequencing analysis was performed for RNA isolated from macro-dissected FFPE. Prostate tumors, in AA men compared to EA men, were observed to undergo an earlier transformation from latent into the clinical disease, to be more aggressive at diagnosis, and to confer an increased risk of death. Due to this high risk of developing aggressive PCa, self-reported race (SRR) was verified by using patient's allele frequency based on the 1000 Genomes Project (Admixture). Our lab recently pioneered ancestry analysis pipeline from RNA sequencing data. This analysis is based on 11 population algorithms that further showed that the fourteen patients who self-reported as AA, also have a predominate African ancestry specifically from African Caribbean's subpopulation (ACB) **(Figure 11)**.



Symbol	SuperPopulation
AFR	African
EUR	European
SAS	South Asian
EAS	East Asian
AMR	Ad Mixed American

Symbol	SubPopulation	SuperPopulation
ACB	African Caribbeans in Barbados	AFR
GWD	Gambian in Western Divisions in the Gambia	AFR
ESN	Esan in Nigeria	AFR
MSL	Mende in Sierra Leone	AFR
YRI	Yoruba in Ibadan, Nigeria	AFR
LWK	Luhya in Webuye, Kenya	AFR
ASW	Americans of African Ancestry in SW USA	AFR

Figure 11 Global Ancestry Analysis of macro- dissected PCa dataset

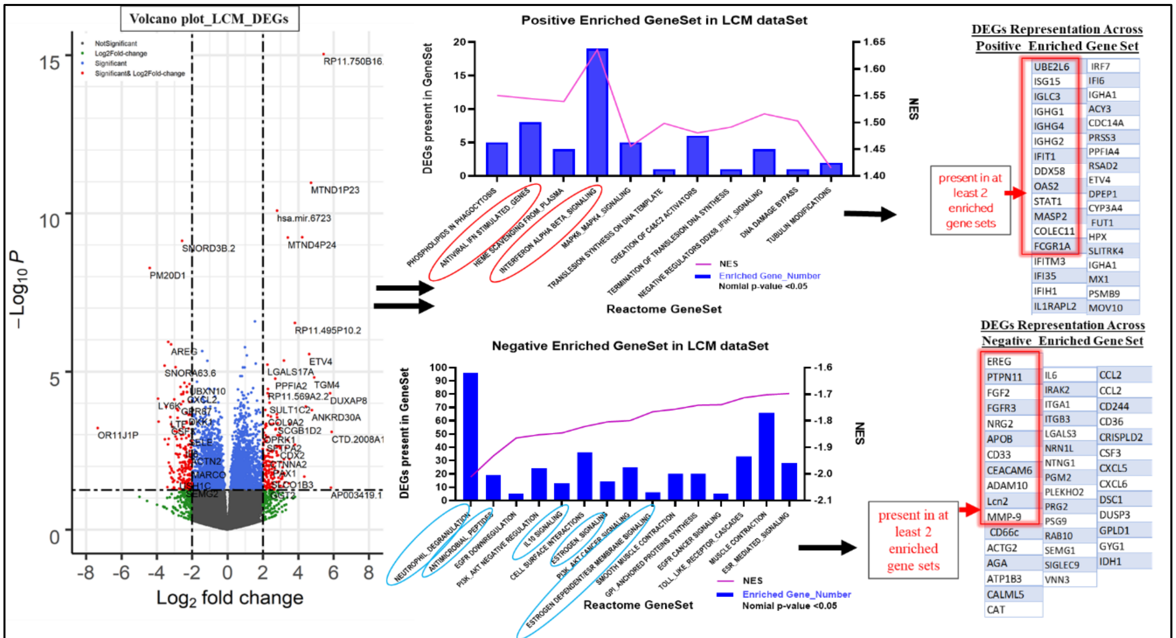


Symbol	SubPopulation	SuperPopulation
ACB	African Caribbeans in Barbados	AFR
GWD	Gambian in Western Divisions in the Gambia	AFR
ESN	Esan in Nigeria	AFR
MSL	Mende in Sierra Leone	AFR
YRI	Yoruba in Ibadan, Nigeria	AFR
LWK	Luhya in Webuye, Kenya	AFR
ASW	Americans of African Ancestry in SW USA	AFR
CEU	Utah Residents (CEPH) with Northern and Western E	EUR
TSI	Toscani in Italia	EUR
GBR	British in England and Scotland	EUR
IBS	Iberian Population in Spain	EUR
FIN	Finnish in Finland	EUR
PUR	Puerto Ricans from Puerto Rico	AMR
CLM	Colombians from Medellin, Colombia	AMR
PEL	Peruvians from Lima, Peru	AMR
BEB	Bengali from Bangladesh	SAS
STU	Sri Lankan Tamil from the UK	SAS
ITU	Indian Telugu from the UK	SAS
PJL	Punjabi from Lahore, Pakistan	SAS
GIH	Gujarati Indian from Houston, Texas	SAS
CDX	Chinese Dai in Xishuangbanna, China	EAS
CHS	Southern Han Chinese	EAS
CHB	Han Chinese in Beijing, China	EAS
JPT	Japanese in Tokyo, Japan	EAS
KHV	Kinh in Ho Chi Minh City, Vietnam	EAS

Figure 12 Global Ancestry with additional ancestry populations.

Interestingly when we assessed a broader population set, fourteen patients have African descent from African ancestry, actually have Southwest USA (ASW) **(Figure 12)**. One patient who self-reported as AA was actually Ad Mixed American (AMR) and was removed from downstream analysis.

All raw reads have passed quality control and then trimmed using Trimmomatic. The trimmed reads were aligned to the GRCh38 genome using STAR aligner with a duplication rate of less than 0.2 %. Then gene-expression level was measured from STAR counts using Ensembl gene annotation with acceptable median global gene expression (**data not shown)**.



DGEs analysis was performed using DESeq2 package in R based on the race. DGEs were sorted by logFC and used for GSEA to rank the enriched geneSet and their genes.

Figure 13 Analysis of macro- dissected Yates-PCa Dataset

Since patients were stratified by ancestry we performed DESeq2/EdgeR analysis which showed that 801 genes (44 genes with LogFC > 2, 76 genes with LogFC < 2) were differentially expressed in AA (p-value <0.05) as shown in the Volcano plot **(Figure 13).**

GSEA software was used to rank DESeq2-DGEs. Our analysis showed that interferon-inducible gene sets (ISG15, IFT1, STAT1) were positively enriched in AA patients (NES +ve, p-value ≤ 0.05) (**Figure 13)**. Previous studies from that Ambs lab demonstrated that ISG15, IFT1, and STAT1 are IFN- gamma-regulated genes and consistently present in AA PCa (Reams et al., 2009; Tiffany A Wallace et al., 2011; Tiffany A. Wallace et al., 2008) which may lead to aggressive and treatment-resistant disease (Budhwani et al., 2018). However, we now add that neutrophil degranulation and Interleukins gene sets (IL8, CXCL8, KRTs, IL6, CXCL6) were negatively enriched in AA patients (NES -ve, p-value ≤0.05) as well. (**Figure 13)**. These enriched gene sets may further support that overall hypothesis of this proposal that immune inflammation signatures play an

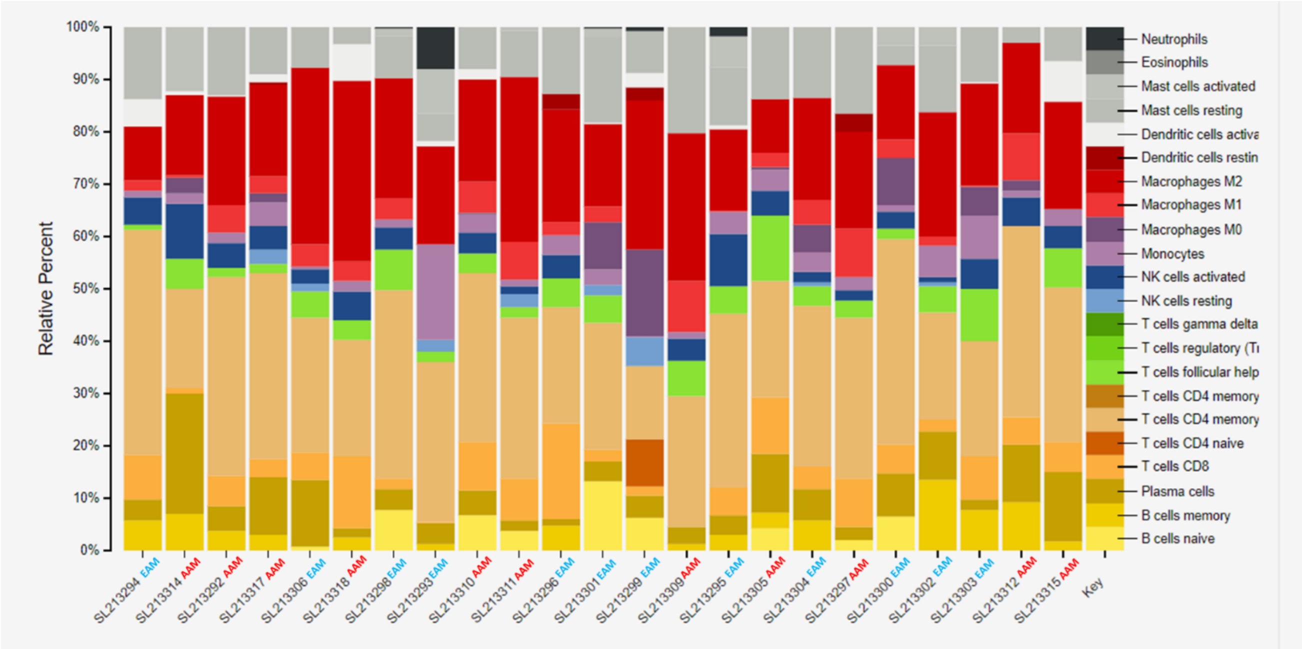
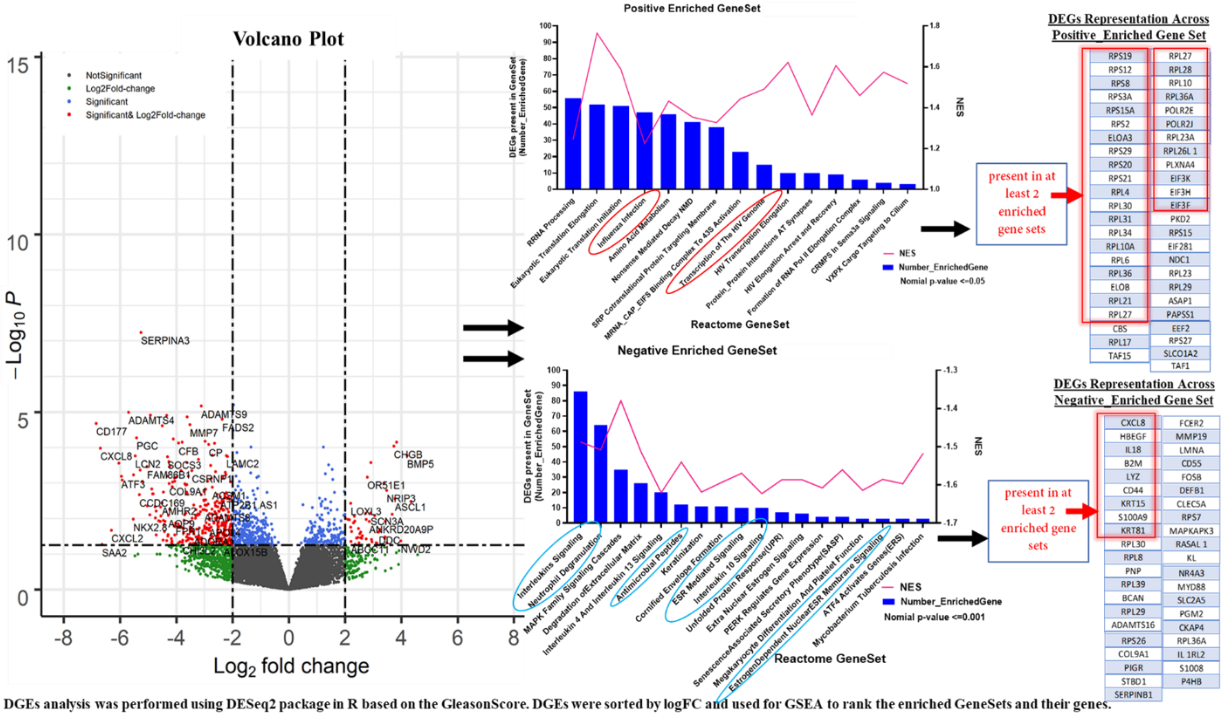


Figure 14. CIBERSORT Analysis of Yates dataset

important role in driving aggressive prostate cancer in AA. In further support, gene ontology (GO (BP)) function in EdgeR package showed that immune-related signaling pathways were enriched in AA men (**data not shown).**

To further delineate the individual immune cell types that possibility contributes this this inflammatory signature in AA men we performed CIBERSORT analysis, which provides an estimation of the abundances of immune cell types members in a mixed cell population. Interesting African American patients have a higher density of M2 macrophages and T cells (CD4 memory resting cells) compared to EA ancestry patients **(Figure 14)**.

To validate our dataset results, we obtained an additional 12 AA patients dataset (Howard University) and 27 patients total 10 self-reported as AA and 17 self-reported as EA (Medical University of South Carolina) who self-reported as African Americans was used. In the Howard university dataset, DEGs analysis and subsequence GSEA ranking validated that immune inflammation pathways such as Influenza Infection and HIV Infection were positively enriched (p-value ≤ 0.05) in this patient population **(Figure 15)**. At the same time, Interleukins signaling, and Neutrophil Degranulation were negatively enriched (p-value ≤ 0.001) **(Figure 15)**. CIBERSORT analysis showed increase abundances of T cell (CD4 memory resting cells) and B cell naïve in this cohorts **(Figure 16)**. Interesting DESeq2 analysis of the MUSC data based on the race were performed as described above demonstrated that AA related immune inflammation pathways such as gamma-alpha 1 signaling were positively enriched in AA (p-value ≤ 0.05) (**Figure 17**). CIBERSORT analysis showed an increase abundances of T cell (CD4 memory resting cells) and Mast cells resting in AA cohorts **(Figure 18)**.



DGEs analysis was performed using DESeq2 package in R based on the GleasonScore. DGEs were sorted by logFC and used for GSEA to rank the enriched GeneSets and their genes.

Figure 15 Analysis of Howard University Dataset

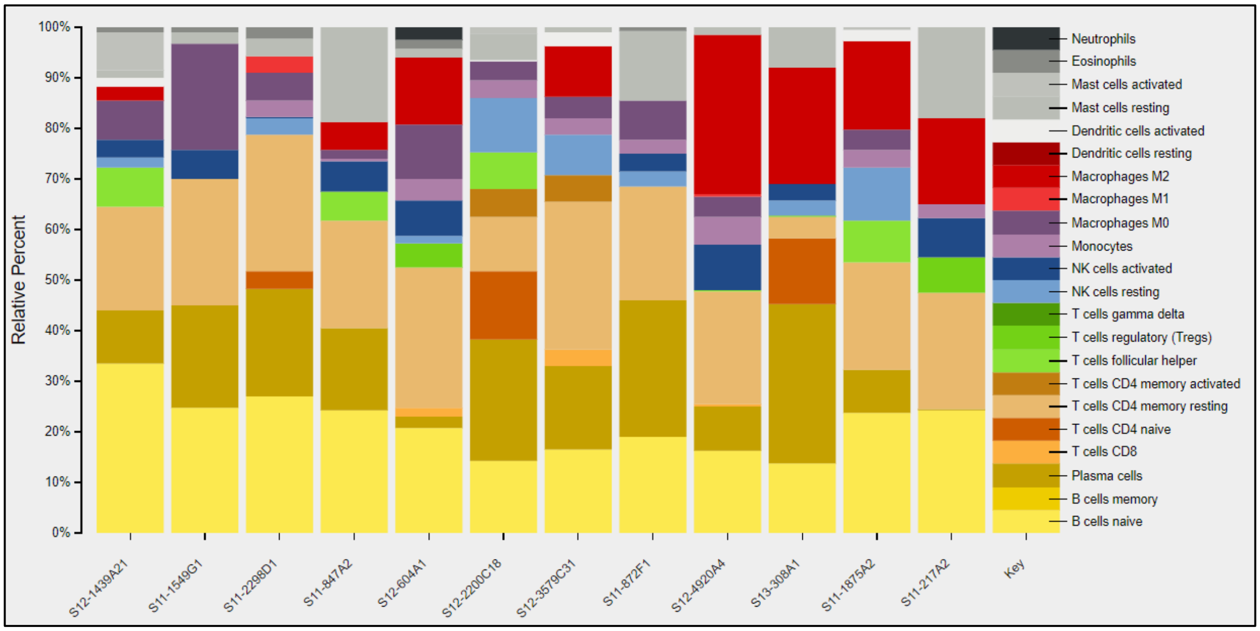
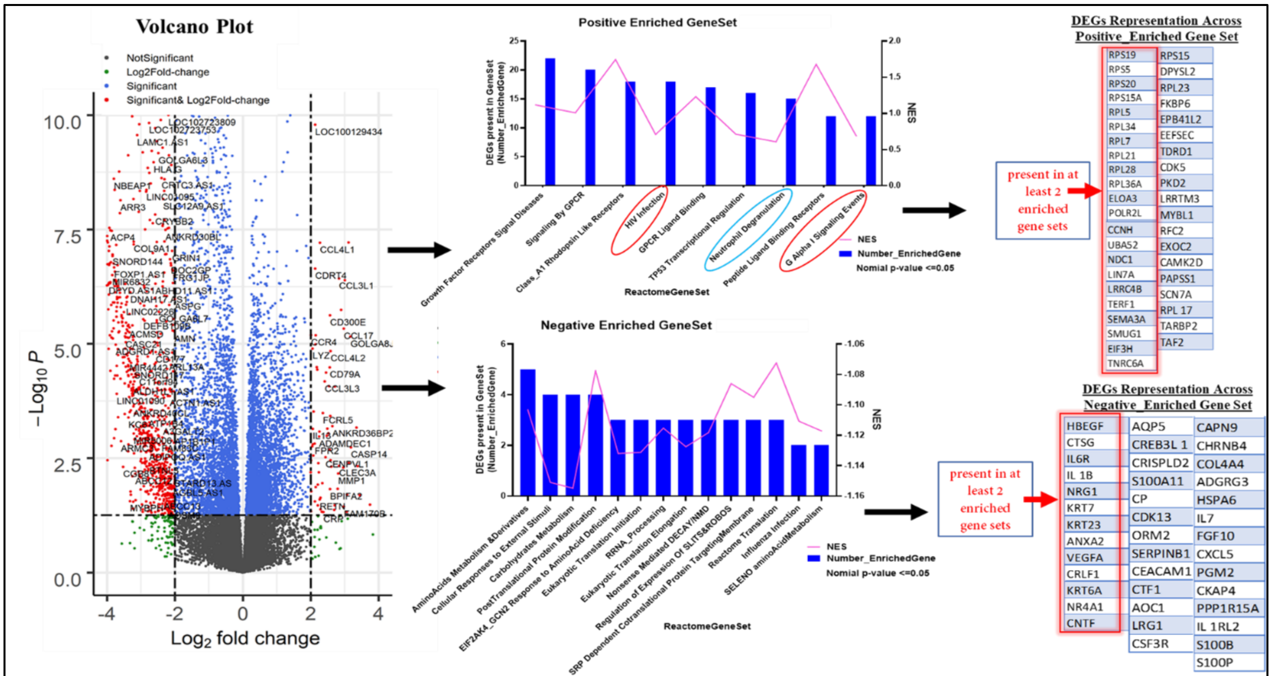


Figure 16 CIBERSORT Analysis of Howard University Dataset



DGEs analysis was performed using DESeq2 package in R based on the Race. DGEs were sorted by logFC and used for GSEA to rank the enriched GeneSets and their genes.

Figure 17 Analysis of the Medical College of South Carolina PCa dataset

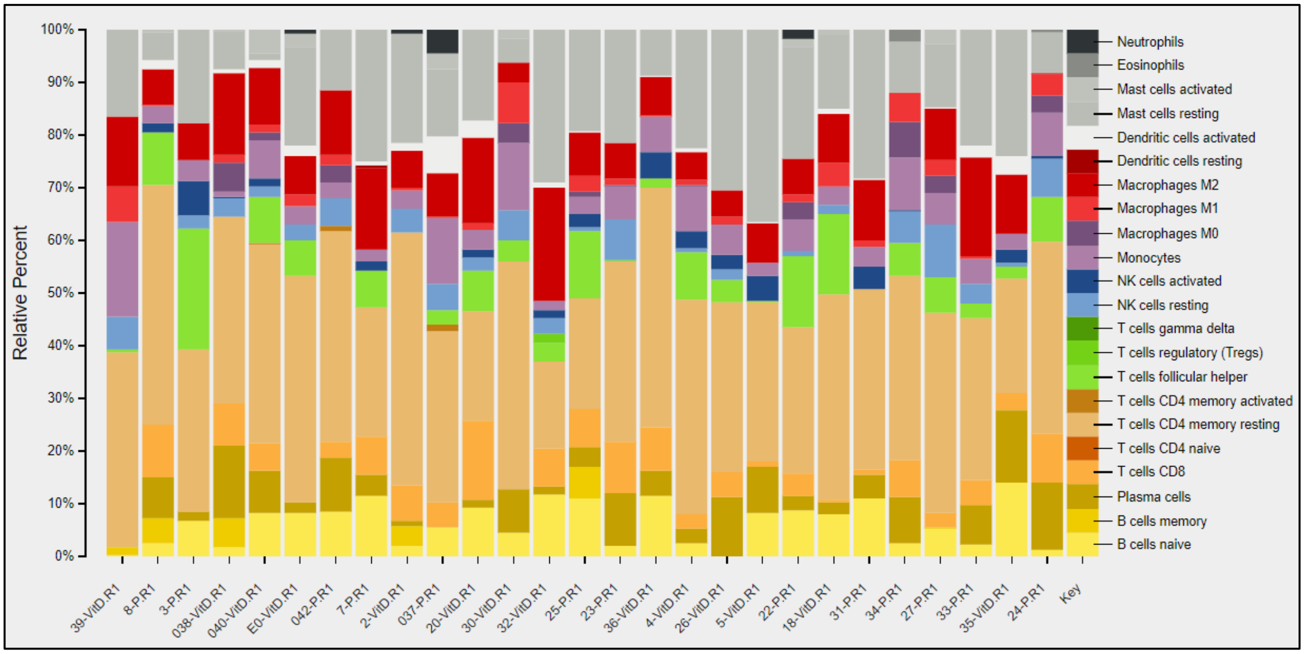


Figure 18 CIBERSORT Analysis of the Medical College of South Carolina PCa dataset

In summary, since we are delayed in sequencing this data analysis serves that we have confidence in our analysis pipeline for the sequencing results. Additionally, this data will be used as external validation of our cohort in comparison with AA and native African patients

Nigerian PCa Tumor Whole Exome Sequencing Pilot Analysis

Since whole exome sequencing (WES) in Nigeran tumors has not been previously done by any lab, we conducted a pilot analysis of 45 Nigerian treatment naive PCa FFPE samples as a proof of principle.

Library preparation was performed using Agilent SureSelectXT Human All Exon V6 r2 Exome Kit (Agilent Technologies, Santa Clara, CA, USA). Sequencing (2x150bp) was performed on either an Illumina HiSeq4000 or on an Illumina NextSeq500 (Illumina, San Diego, CA, USA), to a target of 100 (± 10) million raw data reads per each sample library. Following sequencing, raw fastq files were transferred to the NIH Biowulf supercomputing cluster and analyzed using the CCBR Whole Exome pipeline (https://github.com/CCBR/Pipeliner). Reads were trimmed using Trimmomatic v0.33 and mapped to the hs37d5 version of the human reference genome (ftp://ftp.1000genomes.ebi.ac.uk/vol1/ftp/technical/reference/phase2_reference_assembly_sequence/hs37d5.fa.gz) using BWA-MEM v07.17. BAM files were processed using Samtools v1.8 (http://www.htslib.org/) and Picard v1 (http://broadinstitute.github.io/picard/) was used to mark duplicates. GATK v3.8 was used to perform indel realignment and base recalibration.

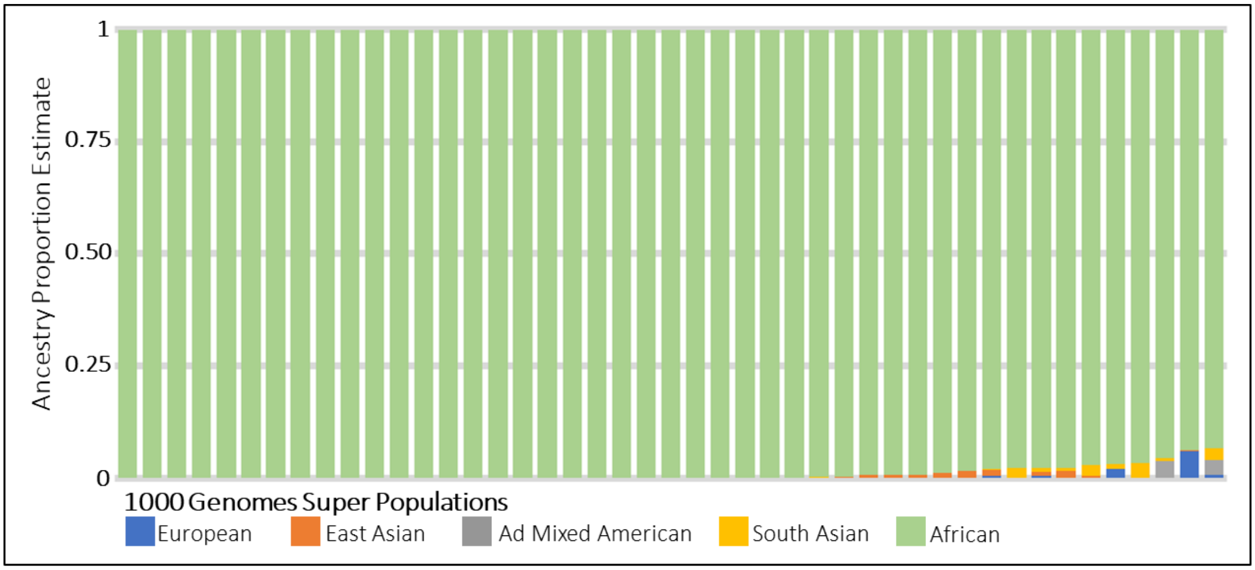


Figure 19 Global Ancestry Analysis of Nigerian PCa Tumor Dataset

Prior to variant analysis, ancestry proportions were estimated using Admixture, based on reference populations from the 1000 Genomes Project phase 3 superpopulations **(Figure 19)**. All patients showed a > 93% African ancestry, however, to further compare the ancestry proportions with European and African American patients, we performed principle component analysis (PCA) using EA and AA PCa patients from the TCGA. Not surprisingly, African American men clustered more closely with Nigerian men, compared to European patients (**Figure 19B**).

Variant calling was completed across three tools, MuTect2, MuTect v1.1.7 and VarDict v1.6.0. Variants were annotated using Annovar v2019Oct24 and filtered as follows: 1) Exclusion of silent and non-protein coding mutations, 2) Variant allele frequency ≥ 10%, 3) Called in two out of three variant callers, 4) dbNSFP Genome Aggregation Database (gnomAD) African exome allele frequencies < 0.001 and 5) Identified as pathogenic in ClinVar. Within this patient population, known pathogenic mutations occurred in 86% of tumors. Calcium Voltage-Gated Channel Auxiliary [CACNA2D2] showed the highest mutation rate at 29% **(Figure 20)**. In addition, eight other genes (Titin [TTN], Spectrin Repeat Containing Nuclear Envelop Protein 1 [SYNE1], Tumor Protein P53 [TP53], ADAM Metallopeptidase with Thrombospondin Type 1 Motif 2 [ADAMTS2], ALMS1 Centrosome and Basal Body Associated Protein [ALMS1], BRCA1 DNA Repair Associated [BRCA1], Plectin [PLEC] and Ryanodine Receptor 1 [RYR1]) harbored known pathogenic mutations in at least 11% of tumors.

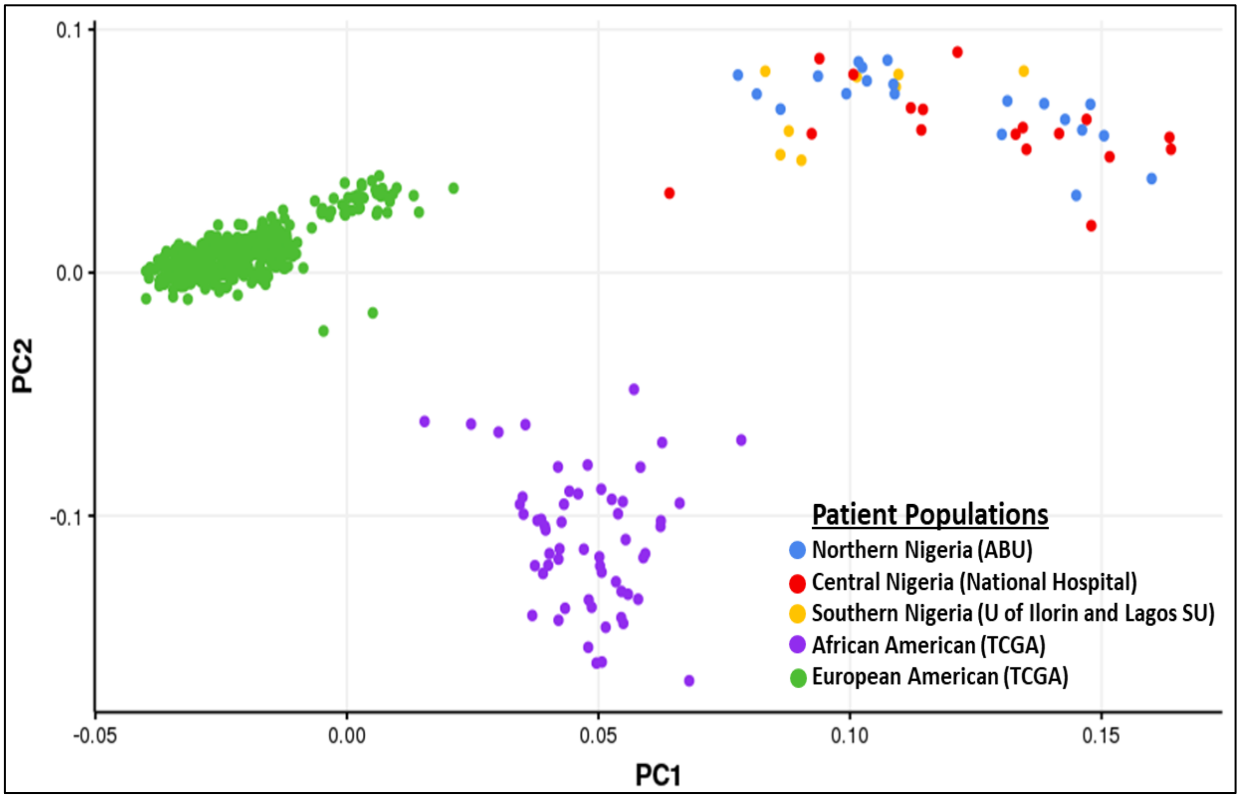


Figure 19B. PCA analysis of European, African American, and Nigerian PCa patients based on Ancestry Estimates

Comparison of cohort mutations and validated COSMIC Signatures **(Figure 21)** showed Nigerian PCa mutation patterns share similarities (cosign similarities > 0.652) with COSMIC Single Base Substitution Signatures 1 and 6. These similarities indicate Nigerian PCa contains a substitution bias toward C>T and T>C transitions. This mutation pattern is linked to increased tumor aggression due to a disruption in DNA repair and DNA methylation.

This pilot analysis is the largest (n=45) whole exome sequencing study of native African PCa tumors to date. Nigerian PCa tumors appear to harbor several novel mutations in PCa related genes. The genes appear to create a mutational signature that suggests a disruption in DNA repair and DNA methylation patterns. Although additional validation is necessary, the data produced by this study provide a strong analysis pipeline that can be used once the HudsonAlpha sequencing is completed.

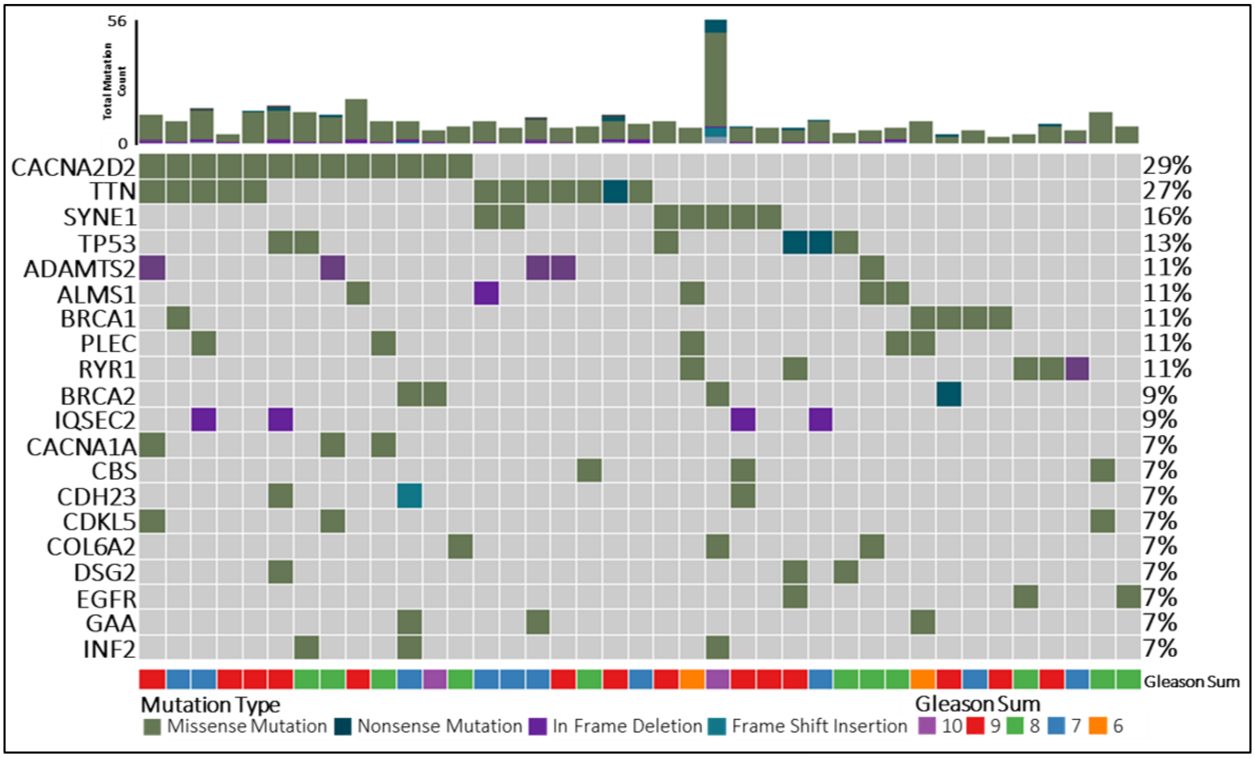


Figure 20 Known Pathogenic Mutations in Nigerian PCa Cohort

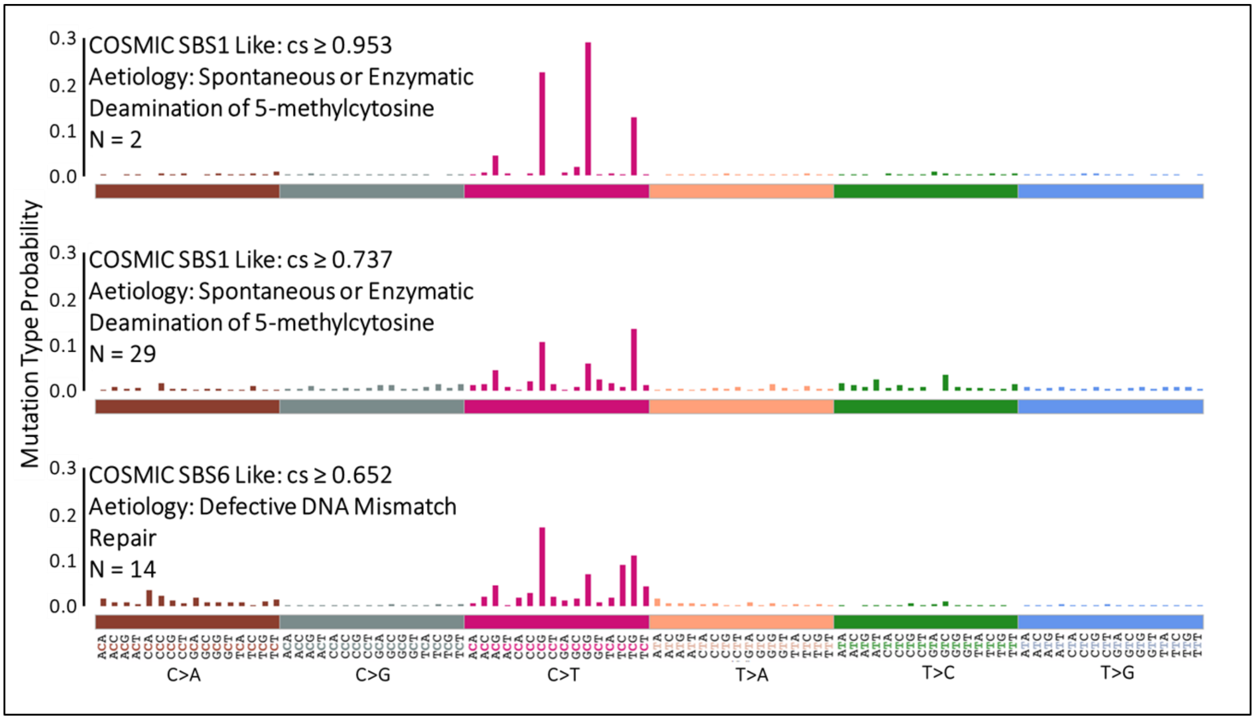


Figure 21 COSMIC Signature Enrichment within Nigerian PCa Cohort

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.

Dr. Isra Elhussin was added to this project in the Yates lab to assist with analysis of the upcoming large amount of data from the ongoing WES, RNA, and ATAC sequencing. Dr. Elhusssin attended several Bioinformatics online Workshops, Hand-on Training , SCREP program, and the NIAID Python Programming for Scientists Series- Hand on Training (Total of ten Webinar/hand on training for two hour). She also attended the following webinars below:

1- NIH-Variant Analysis Bioinformatics workshop (total of 65 hours)
2- UAB COVID-19 Hackathon - Informatics Institute (total of 38 hours)
3- SCREP/ Hands-on Training, Public Health Informatics Workshop (total of 55 hours)
4- SCREP/ Hands-on Training, Bioinformatics Workshop (total of 65 hours)
5- AMIA 2020 Student Design training- Challenge (COVID-19 Dashboard design) total of 32 hours
6- MDI- RNA sequence workshop (total of 43 hours)
7- NIH_Jumpstart Clinical Data Access to COVID-19 Research Workshop (total of 7 hours)
8- UAB-CCTS-NIH-COVID-19 collaborator (NC3) Workshop
9- Nigeria-Diaspora Research Summit
10- 27th Annual Prostate Cancer Foundation Scientific Retreat
11- A number of Educational Virtual Webinars such as:
    a. High-Risk/ Triple negative Breast Cancer and African Ancestry Webinar by Lisa Newman
    b. John Carpten talk -A Research Agenda for Genomic Discoveries in People of African Ancestry
    c. African Cancer Research Webinar Series.
    d. Novel Approaches for Assessing Molecular Heterogeneity in Cancer Webinar
    e. UAB Informatics Institute PowerTalk
    f. Tissue Transcriptomics Data Analysis in Partek Flow

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.

Jason White was awarded an AACR Scholar-in-Training Award to attend and present a Hot Topics short talk and poster at the 12th AACR conference on the Science of Cancer Health Disparities in Racial/Ethnic Minorities and the Medically Underserved for his abstract entitled "Using Whole Exome Sequencing of Archived FFPE Tissue to Characterize the Mutational Landscape of Prostate Cancer in Nigerian Men". Isra Elhussin as abstract accepted for poster presentation on November 4th at the "2020 Virtual Biomedical Research Symposium for Underrepresented Scholars at the Icahn School of Medicine at Mount Sinai." Dr. Yates gave an invited oral presentation at AACR 2020 Cancer Health Disparities virtual meeting which included the validation of inflammatory signature in AA PCa. Dr. Yates was also an invited seminar speaker and guest lecturer in class PHA 6935: Current Topics on Health Disparities (virtually), both offered at University of Florida CARER2 minority health center. Dr. Yates also presented these findings at University of North Carolina (UNC) Lineberger Cancer Center seminar series. This was well attended by investigators at UNC and North Carolina Central University. Dr. Yates was further an invited speaker at the Medical College of South Carolina Disparities Research and Methods (DREAM) seminar series.

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.

A second hurdle during this period was that we found a significant discordance between Gleason Grade obtained from Nigerian samples. This resulted in a significant delay as all the samples had to be re-scored by US pathologist. As a result of this significant finding, and it’s potential impact to the clinical management of prostate cancer patients in Nigeria, we have convened a pathology workshop to review each slide with our US pathologist and this corresponding Nigerian pathologist. While this is not completely within the scope of the project we fill its prudent to increase the clinical capacity in our Nigerian collaborators.

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.

Dr. Yates gave an invited oral presentation at AACR 2020 Cancer Health Disparities virtual meeting which included the validation of inflammatory signature in AA PCa. There is a manuscript in preparation on this data. Based on the previous WES analysis of Nigerian men presented in year 1 that demonstrated a methylation-based signature, Dr. Yates received an 150K additional funding from NIH/NCI to perform DNA methylation sequencing on this same patient cohort being sequenced. The sequencing will be performed after the WES and RNA sequencing occurs.

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, and DNASeq
Funding support	Tuskegee University

Name	Isra Elhussin
Project Role	Graduate Trainee
Researcher Identifier	
Nearest person month worked	8 months
Contribution to Project	Analysis of RNA sequencing in AA prostate cancer validation dataset to confirm inflammation signature.
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.

9. Appendices

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