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Dr. Clayton Yates

cyates@tuskegee.edu

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Tuskegee University
1200 West Montgomery Rd
Tuskegee AL 36088

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14. ABSTRACT

Objective: To explore the genotypic and phenotypic characteristics of African American men that exhibit system/chronic inflammation.

Impact: This study explores 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

Although Specific Aim was the most severely delayed, we have made progress during this cycle. Despite these obstacles, we made great progress with the work and research, and achieved several milestones, and finalized the first manuscript and are currently working on analysis for the second manuscript. Furthermore, we were able to present these finding in a number of conference and open forums.

Accomplishments in the reporting period.

For Specific Aim 3, management of Major Tasks 1 & 2 primarily falls under the responsibility of Dr. Clayton Yates, at Tuskegee University, although the Tuskegee and NCI research teams have been working on these tasks in close collaboration.
To obtain cores of tumor and adjacent non-cancerous tissue from FFPE tumor blocks, we contracted the University of Maryland Department of Pathology. They processed cores from their Maryland cohort as well as the Nigerian samples. into cores of tumor tissue and paired adjacent non-cancerous tissue for RNA and DNA extraction. Many of the Nigerian cases did not present with tumor, therefore we underwent several rounds of submitting tumor blocks.
As stated in the previous report, ledios 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

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

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.

Admixture Analysis of Initial Nigerian Cohort

Race has been recognized as a poor group classifier when linking genetic variation and disease causation[45]; moreover, self-reported race can obscure genetic variation due to misunderstandings about family heritage, cultural influences and/or other societal factors[46]. To ensure that our NG and TCGA cohort comparisons were not skewed by bias within self-reported race, we quantified the individual genetic admixture within each patient sample. To accomplish this, germline SNVs were compared to 1000 genomes super populations (African, European, South Asian, East Asian and Ad Mixed American) and ancestry proportion estimates were calculated (Figure 1A). NG patients showed an average genetic ancestry of 99.1% African. The genetic ancestry of TCGA AA patients was predominantly a mixture of African (50.2%-99.99%) and European admixture (1%-43%). To reduce the impact of this admixture variance on our analysis, we only selected TCGA AA patients with ≥70% African ancestry (n=50). TCGA EA patients showed minimal admixture, with >98.3% European ancestry. Interestingly, nine patients self-identified as EA possessed ≤45% European ancestry. Four of the nine patients were majority (>50%) Ad Mixed American, two were majority East Asian, two were majority African and one was predominantly (45%) European with 35% Ad Mixed American and 16% African admixture. To obtain an EA comparison group, we sorted (high to low) the cohort by European ancestry proportion and selected the top 50 TCGA EA patients. After admixture estimation and sample selection, PCA plots were used to visualize the relationships between each cohort and the five 1000 Genomes superpopulations (Figure 1B). As expected, NG and TCGA EA cohorts clustered with their ancestral 1000 genomes superpopulations. The TCGA AA cohort clustered with the African superpopulation; thus, these patients were used in subsequent analyses.

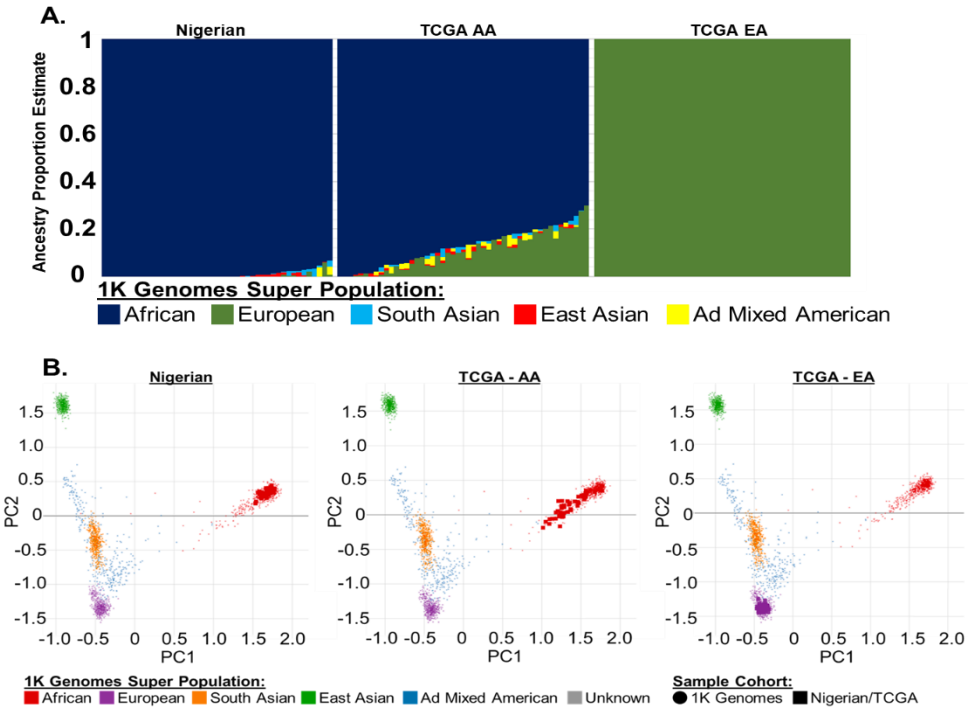

Figure 1 Genetic Admixture Analysis. A. Admixture v1.3.0 was used to estimate ancestry proportions, based on reference populations from the 1000 Genomes Project phase 3 superpopulations. Rare variants (i.e., <5% across all phase 3 1000 genomes), all INDELs and any SNPs that were not biallelic were removed prior to analysis. Samples within the CaPTC cohort had an average African proportion of 99.1%. TCGA Samples (n=50) with >70% African ancestry were classified as African American. 402 TCGA samples contained >60% European admixture. Those samples were sorted by European proportion and the top 50 samples were classified as European American and utilized in this study. B. Principal Component Analysis of Nigerian and TCGA Cohorts. Germline variants within Nigerian and TCGA cohorts were compared to phase 3 1000 Genomes superpopulations using principal component analysis. Nigerian samples strongly clustered with the African superpopulation. African Americans also clustered with the African superpopulation, while European samples strongly clustered with the European superpopulation.

Germline Mutations in Nigerian Men

The NG cohort exhibited 31 known, non-benign, germline variants. Four genes [BRCA1 (100%), BARD1 (45%), BRCA2 (27%) and PMS2 (18%)] were altered in at least two samples (Figure 2). These genes also showed top mutation frequencies within both TCGA cohorts (Figure 2). Across 111 germline variants, the African American TCGA cohort reflected a pattern [BRCA1 (68%), BARD1 (34%), BRCA2 (28%) and PMS2 (16%)] similar to NG samples. 126 germline variants were present in the European American TCGA cohort. Interestingly, disaggregating mutation frequencies down to specific variants revealed both ancestry linked and Nigerian specific germline variant patterns. BRCA1 shows increasing mutation frequency as African admixture increases (Figure 2). That pattern is driven by three variants [rs799917, rs16941 and rs16942] (Figure 3). The frequency of rs799917 is increased in men of African ancestry, while rs16941 and rs16942 are decreased. In esophageal squamous cell carcinoma, the BRCA1_rs799917 SNP has been shown to inhibit mir-638 mediated regulation of BRCA1; thus, reducing BRCA1 expression and increasing cancer cell proliferation[47]. This variant has also been linked to an increased risk of gastric, lung and triple negative breast cancer[48-50]. BRCA1_rs16941 and BRCA1_rs16942 are variants of unknown significance (VUS). BARD1 germline variant patterns appear to be specific to Nigerian men. Compared to AA and EA cohorts, rs2070096 is decreased, while rs2070094 is increased. Intriguingly, the BARD1_rs2070094 SNP resides within the BARD1 binding domain of BRCA1 and may provide a protective function that enhances DNA repair by enhancing BARD1/BRCA1 binding stability[51]. BARD1_rs2070096 is a VUS. BRCA2 germline variants display both ancestry linked and Nigerian specific patterns. rs11571831 is only present in men of African ancestry and rs766173 is increased in Nigerian men. Both BRCA2 variants are classified as VUS.

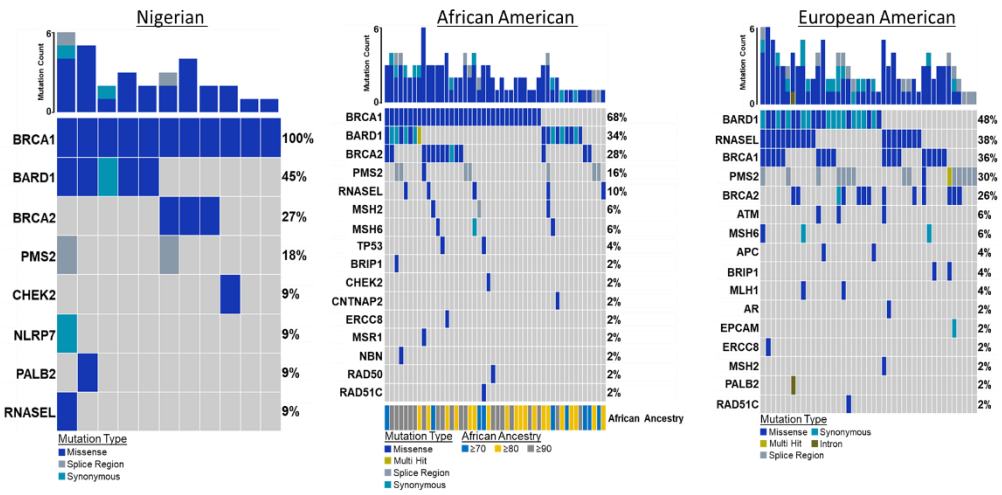

Figure 2: Nigerian PCa Germline Variant Oncoplot. A) Nigerian, B.) African American and C) European American germline variants detected across 11 normal samples were filtered against known ClinVar cancer variants. In the African American cohort, 8 genes showed germline mutations in at least 2 samples. In the European American cohort, 10 genes showed germline mutations in at least 2 samples

To further validate these variants, germline variants within Nigerian normal samples were compared to those within Nigerian tumor samples (Data no shown). Nigerian tumor samples not only possessed comparable rates of gene level mutation [BRCA1 (100%), BARD1 (41%) and BRCA2 (18%)] but also showed comparable variant frequencies. Although most variants observed in NG PCa were identified as variants of unknown significance US (Figure 3), and the differing frequencies, compared to TCGA, suggest future investigations to characterize the full mutational spectrum in patients with predominate African Ancestry.

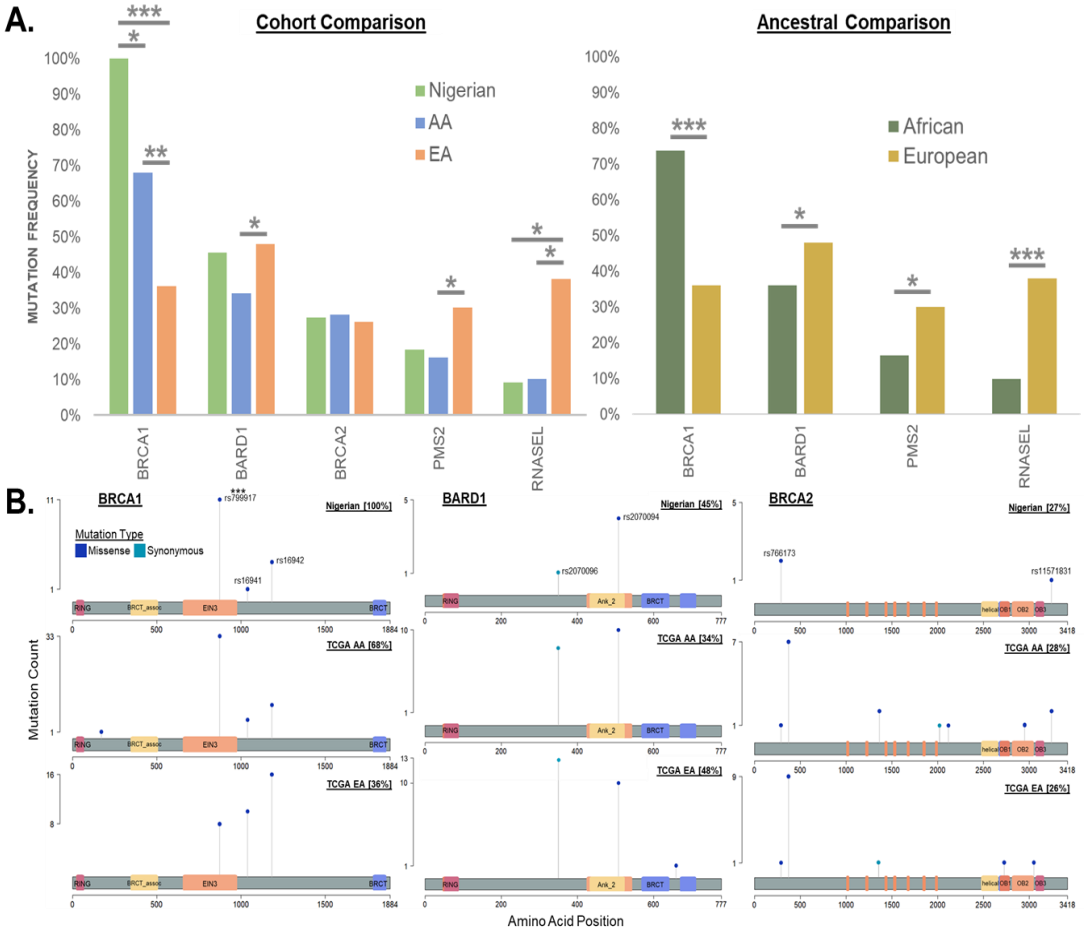

Figure 3 Nigerian PCa Cohort Germline Mutation Comparison to TCGA PCa Cohorts. A) Nigerian and African American men show a statistically significant ($p \leq 0.001$ and $p \leq 0.01$, respectively) increase in BRCA1 germline mutations when compared to European men. Additionally, Nigerian men also show a statistically significant ($p \leq 0.036$) increase in BRCA1 mutations when compared to AA men. BARD1 is mutated at a higher rate in EA men ($p \leq 0.048$). BRCA2 shows no significant difference in cohort mutation rates. **B)** Using lollipop plots to disaggregate mutation rates down to specific variants reveals finer variation in cohort patterns. The ancestry linked pattern of BRCA1 is driven by rs799917, which is statistically ($p \leq 0.001$) increased in men of African ancestry. rs16941 and rs16942 are increased in European men but that increase is not statistically significant. BARD1 germline variants show no significant difference in variant rates; however, the patterns appear to be specific to Nigerian men. Compared to AA and EA cohorts, rs2070096 is decreased, while rs2070094 is increased. BRCA2 germline variants display no statistically different variant rates, but both ancestry linked and Nigerian specific patterns are discernable. rs11571831 is only present in men of African ancestry and rs766173 is increased in Nigerian Men. P values produced via two-sided Fisher's exact test group wise comparison.

Somatic Mutation in Nigerian Prostate Tumors

Somatic variant analysis of tumor-only sequencing data involves multiple nontrivial steps that are distinct from the analysis of matched tumor and normal sequencing. Therefore, we used an established pipeline that incorporated a panel of normal samples. We used an unmatched NG normal sample to filter out Nigerian specific germline variants[32,52], reducing the unique Nigerian variants by 70.8% from 2,506,254 to 730,285 variants (data not shown). Within the TCGA cohorts we used each sample's patient matched normal, which produced 11,208 unique AA variants and 15,191 unique EA variants. Since the NG cohort contained so many somatic variants, we employed two filtering regimes, one for variants within known PCa associated genes (as identified in ClinVar) and one for variants within genes not associated with PCa. We identified 905 variants across 25 genes known to be associated with PCa, and 156 variants across 51 novel PCa genes. Using the same approach, we identified 15,854 variants in the TCGA AA cohort and 21,957 variants in the TCGA EA cohort. Consistent with other sequencing studies[53], our study showed the same racial mutation patterns for SPOP, ATM, TP53 and PIK3CA. TCGA cohorts did not show recurrent gene mutations in genes not associated with PCa. Our dual filtering approach allowed us to independently filter each set of variants across the NG cohort without over-filtering variants within known PCa associated genes and to identify high confidence variants in novel PCa associated genes.

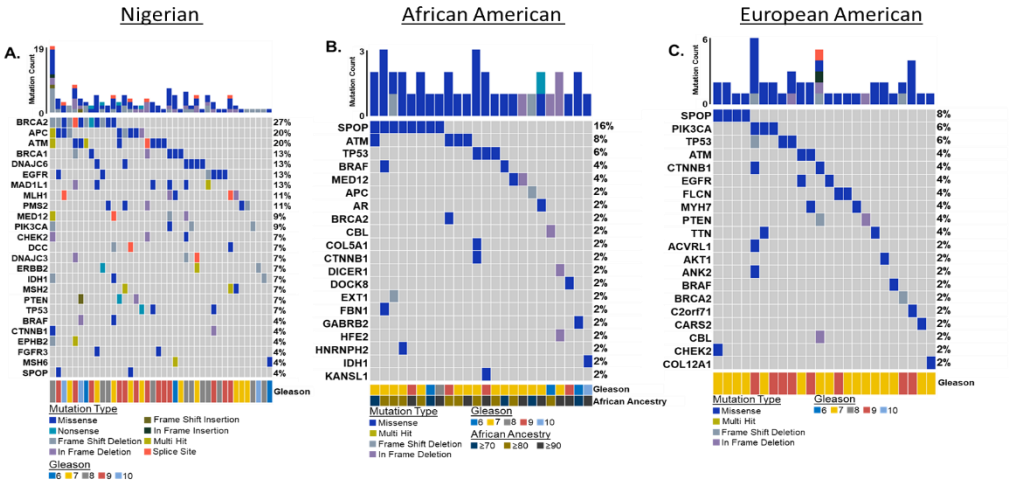

Figure 4 Nigerian PCa Somatic Variants within Known PCa Associated Genes and Functional Clustering. Variant calling within the Nigerian cohort (n=45) produced 1,168,250 variants. **A)** 25 genes known to be associated with PCa harbored variants in at least 2 tumor samples. The most frequently mutated of these included BRCA2 (BRCA2 DNA Repair Associated) – 27%, APC (APC Regulator of WNT Signaling Pathway) – 20%, ATM (ATM Serine/Threonine Kinase) – 20%, BRCA1 (BRCA1 DNA Repair Associated) – 13% and DNAJC6 (DnaJ Heat Shock Protein Family (Hsp40) Member C6) – 13%. **B), C) TCGA** PCa samples (n = 50 African American and n = 50 European American) were downloaded through dbGAP and analyzed for genetic variants

133 somatic variants were present in 26 PCa associated genes within the NG cohort. Nine genes [BRCA2 (27%), APC (20%), ATM (20%), BRCA1 (13%), DNAJC6 (13%), EGFR (13%), MAD1L1 (13%), MLH1 (11%) and PMS2 (11%)] showed mutation frequencies above 10% (Figure 7A). Interestingly, 53% of NG tumors showed mutations in genes (BRCA2, ATM, BRCA1, CHEK2, TP53 and MSH6) associated with genome integrity (Figure 4). The TCGA AA and EA cohorts harbored 67 and 73 somatic variants, respectively. Across both cohorts, fifteen genes were mutated in at least 2 samples [SPOP, ATM, TP53, BRAF, MED12,PIK3CA, CTNNB1, EGFR, FLCN, MYH7, PTEN and TTN] (Figure 4). SPOP and ATM were the most frequently mutated genes in AA tumor and were mutated two times more compared to EA tumors. Comparison of the mutation frequencies between TCGA cohorts did not show any statistically significant increases; however, AA tumors did show a statistically significant increase in SPOP mutations when compared to NG tumors (Figure 4). BRCA2, APC and BRCA1 showed statistically significant increases in the NG cohort. Though not statistically significant, ATM had the highest mutation frequency associated with

Increasing African ancestry, more specifically TCGA EA had an ATM mutation frequency of 4% but TCGA AA and NG cohorts had increased rates of 8% and 20%, respectively. Somatic mutations for Nigerian and African American were distributed across the amino acid sequence of the most mutated genes. None of the variants were shared across or within cohorts. (Figure 5).

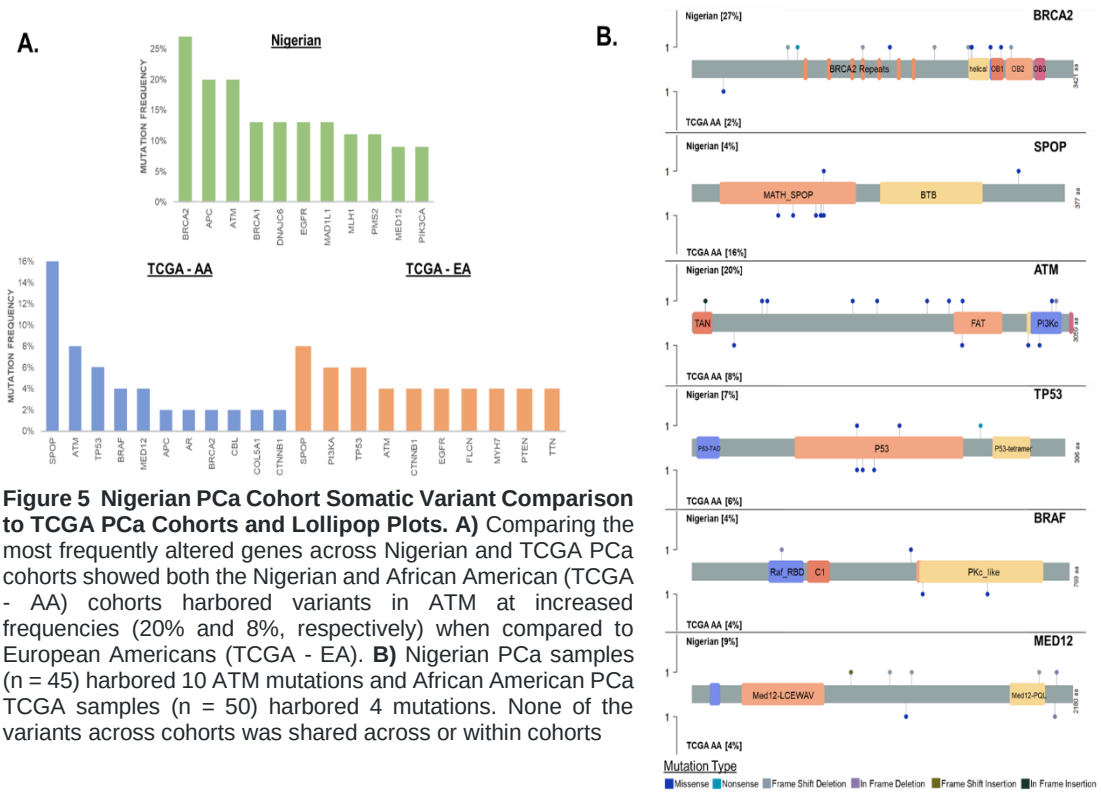

Figure 5 Nigerian PCa Cohort Somatic Variant Comparison to TCGA PCa Cohorts and Lollipop Plots. A) Comparing the most frequently altered genes across Nigerian and TCGA PCa cohorts showed both the Nigerian and African American (TCGA - AA) cohorts harbored variants in ATM at increased frequencies (20% and 8%, respectively) when compared to European Americans (TCGA - EA). **B)** Nigerian PCa samples (n = 45) harbored 10 ATM mutations and African American PCa TCGA samples (n = 50) harbored 4 mutations. None of the variants across cohorts was shared across or within cohorts

Next, we sought to investigate the overall mutational patterns within each cohort to understand global somatic events. The NG cohort shared similarities (cosign similarities ≥ 0.734) with COSMIC signatures 5 and 6 (Figure 6). Five NG cohort samples had a mutational pattern similar (cosign similarity ≥ 0.734) to COSMIC 5. Forty cohort samples were similar (cosign similarity ≥ 0.796) to COSMIC 6. TCGA AA mutational patterns share similarities with COSMIC 1 and 5 (cosign similarities ≥ 0.645), while TCGA EA tumors share similarities with COSMIC 1, 3 and 4 (cosign similarities ≥ 0.481) (Data not Shown). Within the COSMIC database, mutational signatures 1, 5 and 6 are the signatures most often observed in prostate cancer.

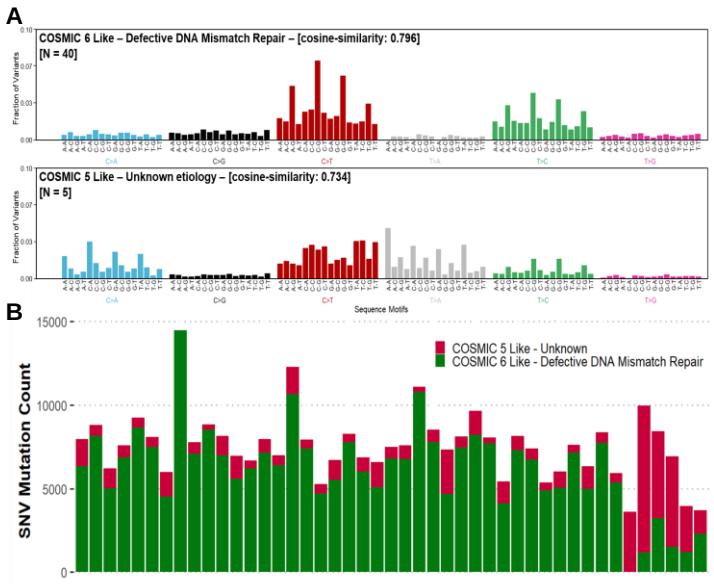

Figure 6 Nigerian PCa Somatic Variant COSMIC Signature Analysis. **A**. Single nucleotide variations in the Nigerian PCa cohort were compared to known cancer related mutation signatures within the Catalogue of Somatic Mutations in Cancer (COSMIC). 89% of Nigerian PCa mutation patterns are similar (cosign similarity ≥ 0.796) with COSMIC signatures 6. **B.** The remaining 11% are more like COSMIC 5.

Finally, to determine the possible mechanism associated with tumorigenesis in Nigerian PCa tumors, mutated genes present in at least two Nigerian patients were analysed for Functional Gene Ontology Enrichment. Not surprisingly, Nigerian tumors showed significant ($q < 0.001$) GO and functional enrichment in mismatch repair and non-homologous repair deficiency (HRD) pathways (Figure 7). Additional enriched pathways included PD-1 checkpoint, thyroid hormone signalling, FOXO signalling, ErB2 signalling, adherens junctions, proteoglycan and sphingolipids.

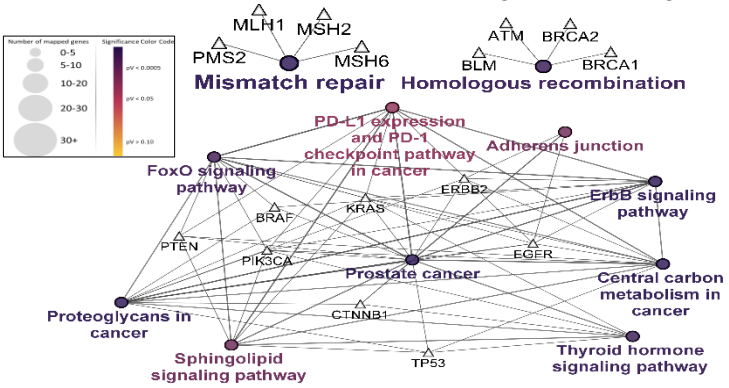

Figure 7: Functional Gene Ontology Enrichment of Nigerian Somatic Variants. Mutated genes (n=83) present in at least two Nigerian PCa tumor samples (n=45) were imported into Cytoscape to assess functional gene ontology

Matched Tumor/Normal WES and Whole Transcriptome Analysis

In addition to the WES we next sought to perform a genome-wide sequencing (Whole-Exome Sequences (WES) and RNA sequences). To overcome the difficulties with tumor only samples, we performed laser capture micro dissected for tumor and normal areas in matched Nigerian, African American, and European American samples. A total of (n= 185) patients obtained from three cohorts with treatment-naive PCa who self-reported race (SRR) as 45 AA, 93 West African (Nigerian), and 47 EA men. DNA and RNA was extracted using a propriety dual DNA/RNA extraction at Hudson Alpha to enhance RNA quality, which is an industry wide problem which extracting from FFPE samples. Unfortunately, due to delays

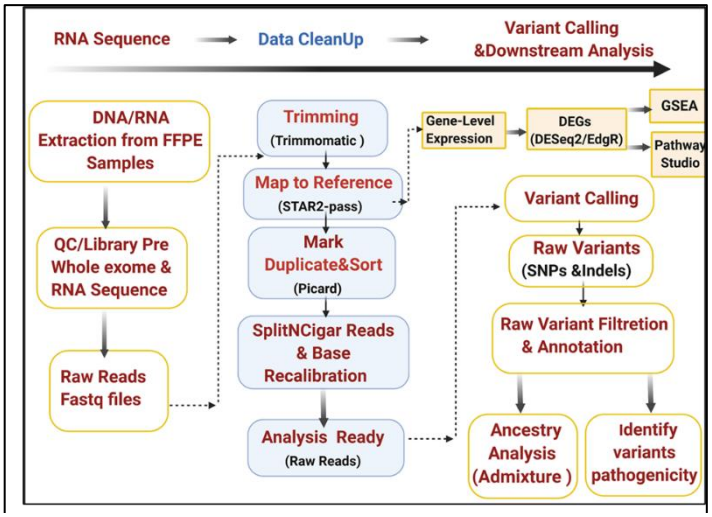

Figure 8 Bioinformatic workflow to determine Ancestry and differentially expressed genes between cohorts

Table1:

Patient characteristics, stratified by race (AAM=African American Men/EAM=European American Men/ Nigerian)**, Pathology stage** (T2/T3), and PSA

	AAM (N=44)	EAM (N=46)	Missing (N=4)	Nigerian (N=77)
Age (years)				
Mean (SD)	56.6 (7.42)	58.2 (5.78)	NA (NA)	NA (NA)
Median [Min, Max]	56.5 [42.0, 74.0]	58.5 [46.0, 69.0]	NA [NA, NA]	NA [NA, NA]
Missing	0 (0%)	0 (0%)	4 (100%)	77 (100%)
Gleason Score				
Mean (SD)	6.91 (0.709)	6.70 (0.695)	NA (NA)	7.92 (1.00)
Median [Min, Max]	7.00 [6.00, 9.00]	7.00 [6.00, 9.00]	NA [NA, NA]	8.00 [6.00, 10.0]
Missing	0 (0%)	0 (0%)	4 (100%)	1 (1.3%)
Pathology Stage				
Missing	27 (61.4%)	40 (87.0%)	4 (100%)	77 (100%)
* pT2	13 (29.5%)	3 (6.5%)	0 (0%)	0 (0%)
** pT3	4 (9.1%)	3 (6.5%)	0 (0%)	0 (0%)
PSA (ng/mL)				
Mean (SD)	9.78 (9.07)	6.61 (10.6)	NA (NA)	NA (NA)
Median [Min, Max]	6.90 [1.91, 48.0]	4.96 [0.460, 75.0]	NA [NA, NA]	NA [NA, NA]
Missing	0 (0%)	0 (0%)	4 (100%)	77 (100%)
Stage				
I	0 (0%)	2 (4.3%)	0 (0%)	0 (0%)
IIA	2 (4.5%)	4 (8.7%)	0 (0%)	0 (0%)
IIB	13 (29.5%)	22 (47.8%)	0 (0%)	0 (0%)
III	11 (25.0%)	11 (23.9%)	0 (0%)	0 (0%)
IV	1 (2.3%)	1 (2.2%)	0 (0%)	0 (0%)
Missing	17 (38.6%)	6 (13.0%)	4 (100%)	77 (100%)

***pT2 (T2/T2b/T2c)**
****pT3 (T3/T3b/T3c)**

in sequencing and poor quality method from these samples, a large number of the WES samples did not pass QC, and therefore were not sequenced. Fortunately, we discovered this at the QC stage and, thus we still have additional samples and funds to perform DNA only extraction from these identical samples. This work is ongoing, and we expect it to be completed within the no-cost extension year.

However we did receive, quality transcriptome analysis and thus we have begun preliminary analysis of these samples. Whole RNA sequencing analysis was performed for RNA isolated from (n= 171) FFPE samples of treatment naive prostate cancer patients who self-reported their race as 44 AAM, 46 EAM, 77 Nigerian and 4 patients their SRR was not reported.

Our lab recently pioneered the ancestry analysis pipeline from RNA sequencing data that has been validated with genotyping ancestry as shown in (Figure 8). Therefore, to verify the self-reported race, we used ADMIXTURE to generate a quantitative estimate of each individual ancestral composition. The raw reads from RNA sequencing were aligned to the GRCh38 genome using STAR aligner (latest version). Then the gene-level expression was measured from STAR counts using Ensembl gene annotation. The resulting datasets were analyzed for differential gene expression using EdgR/DESeq2 and enriched pathways based on the patient's ancestry/race using GSEA and EdgR gene ontology function. Eight Nigerian samples were removed from downstream analysis due to their lower median global gene expression and/or due to their batch effect bias. We filtered the dataset, and we corrected the batch effect. Furthermore, we conduct a descriptive statistical analysis of the study population; patients were stratified by race/Ancestry and pathology stage.

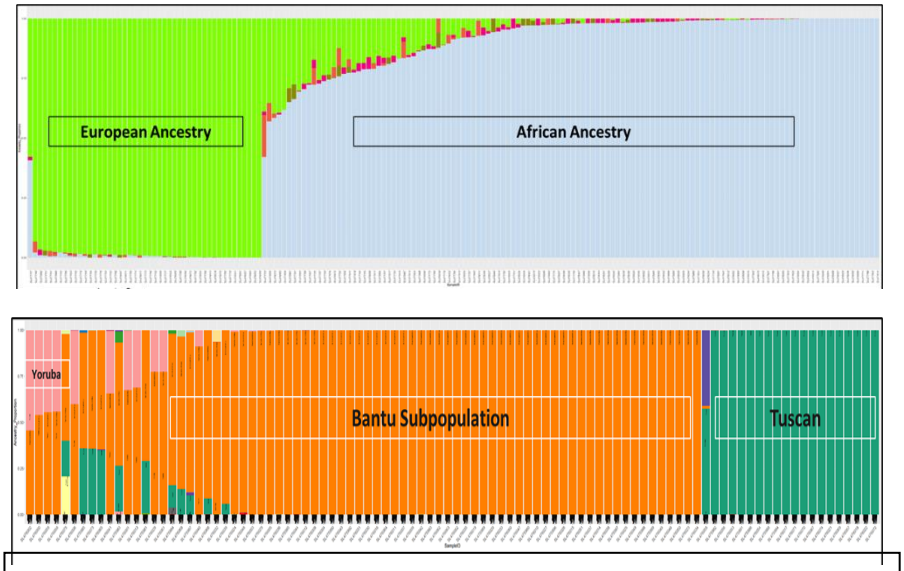

Figure 9. Super and Sup-opulation analysis. We employed a somatic pipeline for Variant-calling, to detect SNV, indels, CNV and SV). We will process the normal samples through our germline pipeline, modeled after GATK's best practices. From the germline profiles, we used estimate genetic ancestry, using the tool ADMIXTURE [21], which allows, with the right set of ancestry-informed markers to estimate subpopulations and differentiate Western African and European subpopulations

Out of 185, we have 25 samples paired WES (normal tumor paired) match with whole transcriptome data. To determine the association of mutations and gene expression, we will measure the percentage of qualified genetic ancestry using SNVs from the matching paired WES and RNA sequences samples. However, as we mentioned above, we have submitted additional samples for DNA only extraction to increase the number of paired WES/ RNA samples particularly in Nigerian patients. Thus, we will only present our initial findings from RNA whole transcriptome data.

Descriptive statistical analysis of the study population was conducted and stratified by SRR. Our results show that patients with higher African Ancestry are diagnosed at a younger age and higher pathology stage (pT2 & pT3). Men of African Ancestry also presented with higher PSA at diagnosis compared to patients with European Ancestry (Table1). Furthermore, the majority of patients in our cohort that self-reported as AAM or Nigerian, their Super population Global Ancestry (validated with Genotype Ancestry) assigned to African Ancestry (Ancestry proportion > 70%) (Figure 2A).

Although the Subpopulation analysis of the rest of the samples is still ongoing , we were able to determine that samples with West African Ancestry , was assigned either to Bantu subpopulation in Sub-Saharan area (western central Africa) and/or Yoruba (Nigeria) subpopulation (Figure 2B).

Utilizing Ancestry to categorize patient samples into predominately African or European, we performed differential gene expression (DEG) analyses to determine unique gene(s)/gene signatures. As previously reported by Stefan Ambs lab, we found that gene sets such as interferon-gamma, complement cascade, and FCGR activation (JAK1, HLA-DRA, IRF1, AIFT1, IFITM1, FCGR) are positively enriched (p-value 0.05), while gene sets such as metabolism and Diabetes (ATF4, CD69, KLF9) are negatively enriched (p-value 0.05) in African Ancestry men Figure 3 A&B respectively.

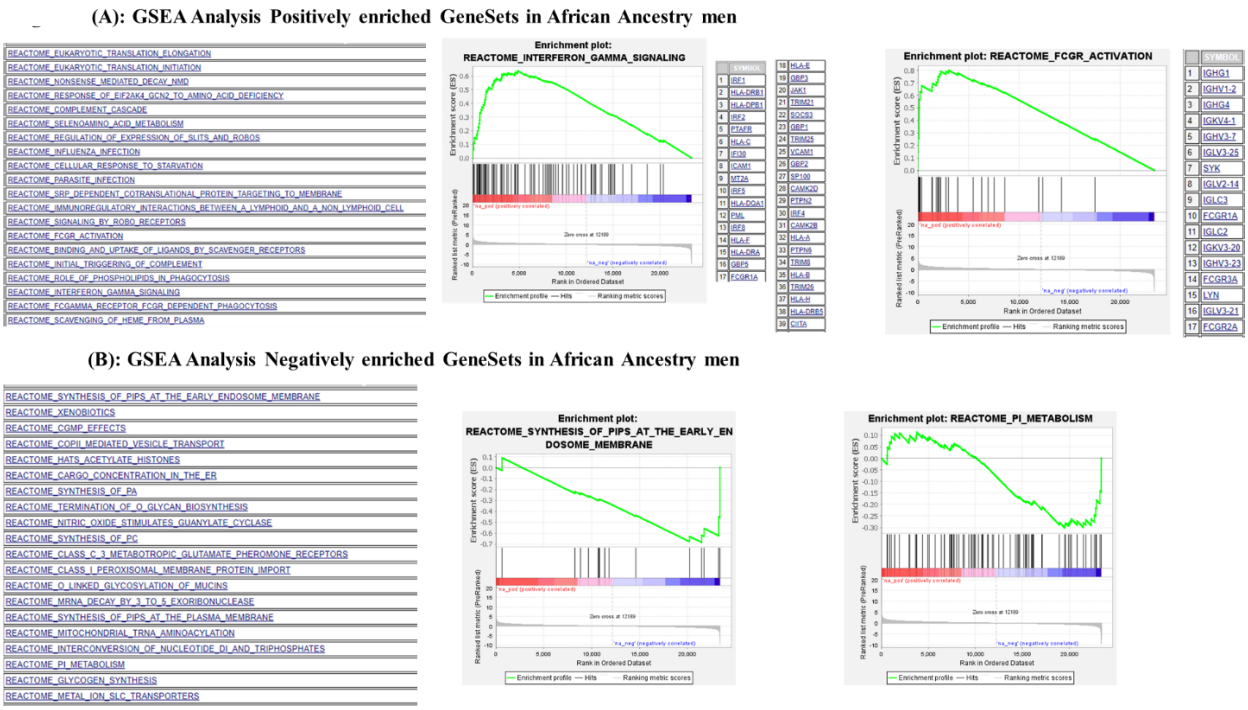

Figure 10. Gene Set Enrichment Associations (GSEA). The top differentially expressed gene were analyzed for pathway and gene set enrichment.

However, whether this increased inflammation in men of African Ancestry is associated with dysregulation of immune cell populations has not been reported. Interestingly, global analysis of immune cell subpopulations, revealed that T cell activation markers such as CD3E, IL6, CD69, CD8A&B, and CD4 are downregulated in men of African Ancestry, while T- cell exhaustion markers such as LAG3, KLRG1, CD274 (PD-L1), and CD160 are upregulated in patients with high African Ancestry compared to European Ancestry patients Figure 4 A&B respectively. This also appears to be ancestry associated as we observed that Nigerian men exhibit this T-cell exhaustion signature more than African American prostate cancer patients.

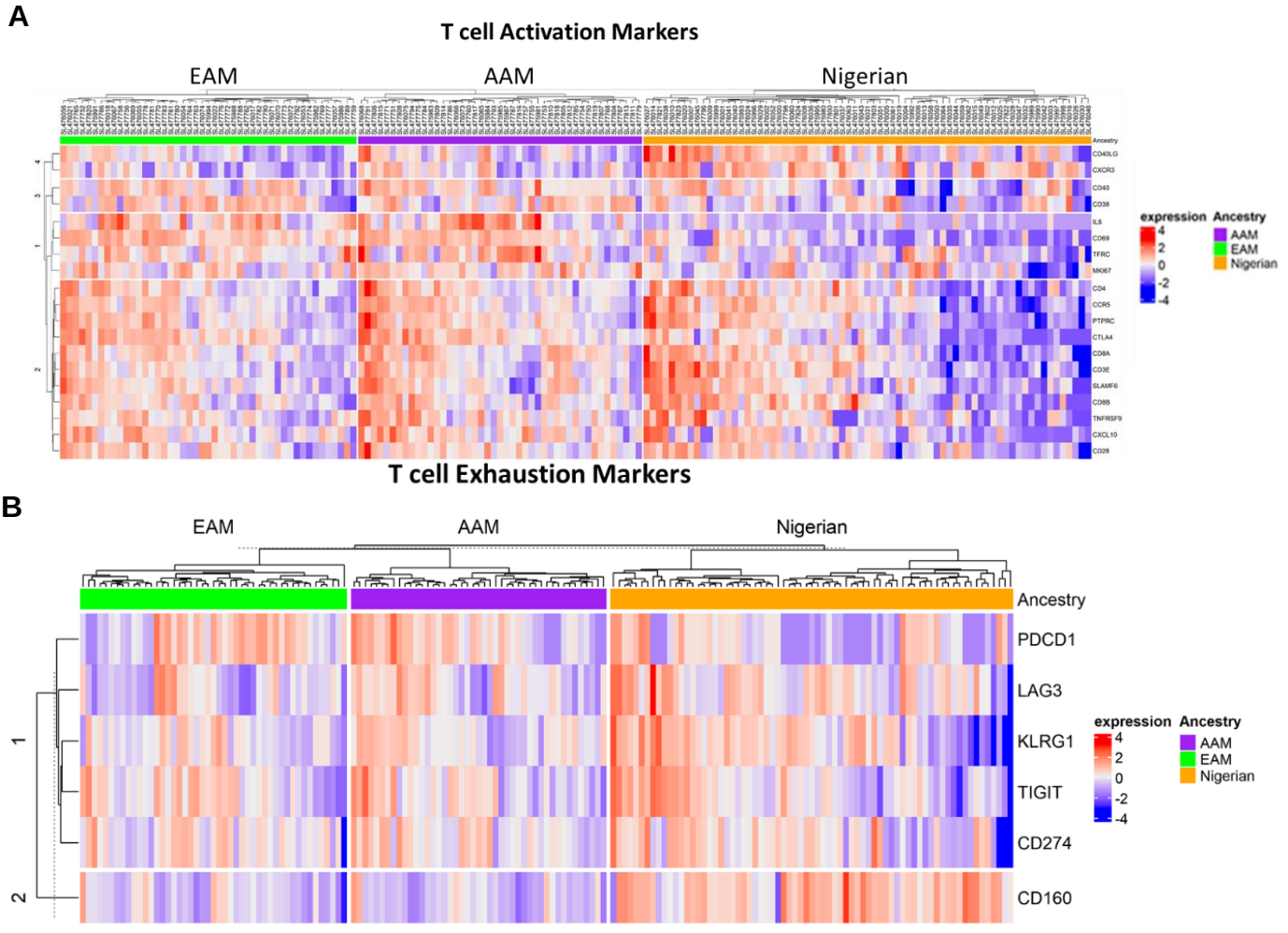

Figure 11. Enrichment of T-Cell Exhaustion Markers in Nigerian Prostate Tumors A. Differential Expression of Enriched T- cell activation markers was determined using supervised hieratical clustering of patients by SSR and Ancestry. B. Differential Expression of Enriched T-cell Exhaustion markers was determined using supervised hieratical clustering of patients by SSR and Ancestry.

IMPACT
Opportunities for training and professional development.

During this period, 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

Dissemination of results to communities of interest.

Isra Elhussin was awarded an AACR Scholar-in-Training Award to attend and present a Hot Topics short talk and poster at the AACR conference". Moreover, Isra was awarded "The Cancer Biology Training Consortium (CABTRAC), travel award, 2021" where she presented our latest Ancestry based analysis. Additionally, Isra nominated to serve as Associate member in the AACR-Associate Member Council (AACR-AMC) during the period during 2022-2025.

Dr. Yates gave an invited oral presentations at Mount Saini, University of Southern California (USC), Duke University. Yale Medical University (Urology Grand Rounds), Society for Basic Urology Research (SBUR), and Amgen (scheduled for Nov 15, 2021). Dr. Yates also gave a lay presentation to prostate cancer survivor organization "Georgia Prostate Cancer Coalition" in Gwinnett County GA. The inclusion of ancestry prostate cancer was well received by the group

of men.

Goals to accomplish during the next reporting period. A major focus during this no-cost extension period will be the completion of manuscripts related to **Specific Aim 3**. The first manuscript is now being reviewed by the coauthors and planned to be submitted before the end of 2021. Additionally, we are awaiting the WES sequencing data from Hudson to increase the number patients with pair DNA/RNA analysis. However if this does not occur we will move forward with analysis of RNA sequencing results recently obtained. Additionally, Dr. Elhussin was awarded a mini-grant from Nanostring for gene expression analysis, and thus additional samples will be analyzed to verify the T-cell signature observed in our AA and Nigerian prostate samples.

Besides pursuing these milestones, the Jason White and Isra Elhussin have both submitted abstracts to the 2022 AACR Annual Meeting, which I am a co-chair. They will also continue to submit abstracts at conferences related to cancer health disparities and prostate cancer.

Publications

1. Maeve Kiely, Ginger L. Milne, Tsion Zewdu Minas, Tiffany H. Dorsey, Wei Tang, Cheryl Jacobs Smith, Francine Baker, Christopher A. Loffredo, Clayton Yates, Michael B. Cook, and Stefan Ambs "High Thromboxane B2 Associates with Lethal Prostate Cancer in African American Men and Inversely Correlates with Aspirin Use" J Natl Cancer Inst . 2021 Jul 15;djab129. doi: 10.1093/jnci/djab129
2. Kiely, Maeve, Ginger L. Milne, Tsion Z. Minas, Tiffany H. Dorsey, Wei Tang, Cheryl J. Smith, Francine Baker, Christopher A. Loffredo, Clayton Yates, Michael B. Cook, and Stefan Ambs. 2021. "Urinary PGE-M in Men with Prostate Cancer" Cancers 13, no. 16: 4073. doi.org/10.3390/cancers13164073
3. Tsion Minas, Julián Candia, Tiffany Dorsey, Francine Baker, Wei Tang, Maeve Kiely, Cheryl Smith, Symone Jordan, Obadi Obadi, Anuoluwapo Ajao, Yao Tettey, Richard Biritwum, Andrew Adjei, James Mensah, Robert Hoover, Frank Jenkins, Rick Kittles, Ann Hsing, Xin Wang, Christopher Loffredo, Clayton Yates, Michael Cook, Stefan Ambs "Serum proteomics links suppression of tumor immunity to ancestry and lethal prostate cancer", 13 July 2021, PREPRINT (Version 1) available at Research Square [https://doi.org/10.21203/rs.3.rs-668276/v1]
4. Prostate cancer: Immune-inflammation signature in men of African ancestry Isra A. Elhussin, Jason A. White, Tamaro S. Hudson, Moray J. Campbell, Chanita Hughes-Halbert, Stefan Ambs and Clayton Yates Cancer Res July 1 2021 (81) (13 Supplement) 2196; **DOI:** 10.1158/1538-7445.AM2021-2196

4. Impact

The impact of our findings to-date are that we have a identified ancestry specific mutation, and gene mutations specifically in men of West African Ancestry. These findings will be significant

to larger cancer community including prostate cancers. Furthermore, this information could be useful to pharmaceutical companies that are interesting in patient stratification. We have already begun initial conversations with pharma companies

5. Changes/Problems

Specific Aim 3, Major Task 1: The major challenges we have encountered this cycle was the lack of quality DNA from the laser captured micro dissected samples. According to multiple sequencing companies DNA quality is normally robust, and thus we chose Hudson Alpha's proprietary RNA isolation enrichment protocol. Fortunately, we have obtained quality RNA sequencing, however during the no-cost extension period we will re-sequence the matching DNA for these patients.

Changes to vertebrate animals and select agents do not apply.

6. Products

7. Participants and Other Collaborating Organizations

The following individuals have worked on the described tasks in the past 12 months. 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.

Name	Jason White
Project Role	PhD student- Lab manager
Researcher Identifier	
Nearest person month worked	3
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
Funding support	Tuskegee University/RCMI

Name	Balasubramanyam Karanam
Project Role	Assistant Professor
Researcher Identifier	
Nearest person month worked	2
Contribution to Project	Key personnel for the immunohistochemistry and lead researcher of immune marker analysis in prostate tumors; since we will have established Akoya CODEX system at Tuskegee University the biospecimens will be stained using this system

Funding support	Tuskegee University

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	12 months
Contribution to Project	Analysis of RNA sequencing in AA and Nigerian, as well as Ancestry validation
Funding support	Tuskegee University

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. Both will submit separate reports.

9. Appendices

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