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13. SUPPLEMENTARY NOTES		

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 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 systemic low-grade inflammation is a prostate cancer risk factor in men of African descent and correlates with West African ancestry, genetic susceptibility, 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 3 was the most severely delayed, however, we made significant progress during this cycle. With the whole exome data obtained period, we published a manuscript in Cancer Research Communication with an organized AACR release and research spotlight at the 2022 AACR Cancer Health Disparities. This included a written and video interview and platform presentation at this meeting as well. In addition, we have successfully completed our multi-omic sequencing approach by adding RNA and DNA methylation sequencing on the US and Nigerian prostate cancer tumors that currently have whole exome data. Lastly, we were able to perform single-cell sequencing on a subset of these tumors. In summary, all of the sequencing was completed during the last cycle and we expect to spend this final period analyzing the data and preparing additional manuscripts.

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 Biotechnology, a leader in applied genomics technologies. We had previously identified the NCI-Leidos Sequencing Core as a 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 to sequence. However, Hudson

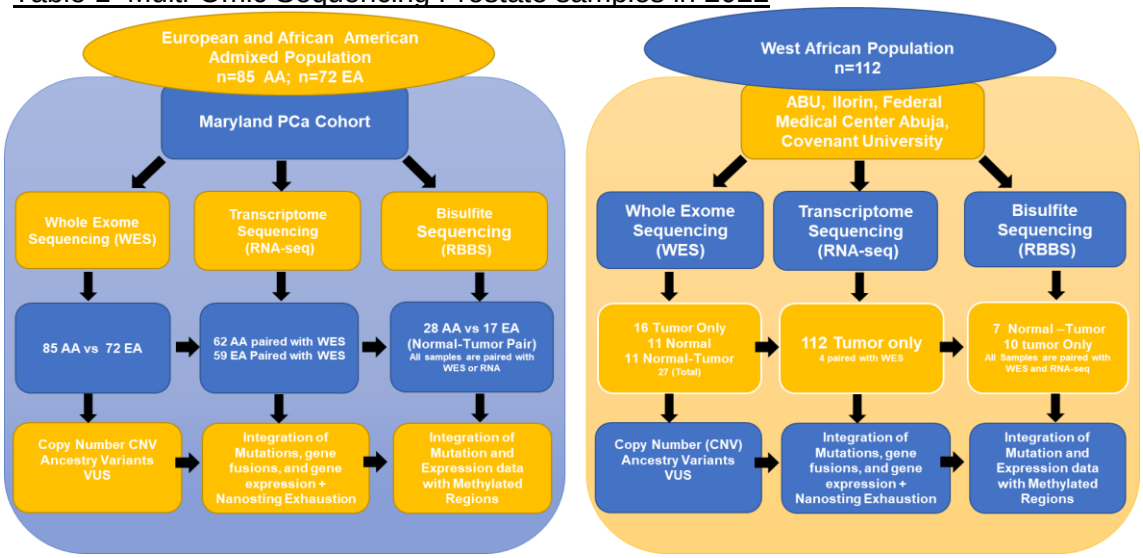
Specific Aim 3: Determine the prevalence of an immune-inflammation signature in prostate tumors of men of European and African ancestry, and evaluate how this signature relates to other gene expression patterns, genomic alterations, and chromatin structure in these tumors, and to patient characteristics.		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

Alpha has a proprietary RNA extraction protocol for FFPE tissues, and we were now able to report the following samples have been successfully sequenced for both DNA (whole exome) and RNA (Whole

transcriptome sequencing). Protocols for successful ATAC sequencing from FFPE samples are not available and thus for the samples that were successfully sequenced for whole exome and RNA sequencing, we sent for **Reduced Representation Bisulfite Sequencing (RRBS),** which is an enhanced bisulfite-based sequencing method utilized to detect up 7 – 8 million unique CpG sites, covering nearly all CpG islands, gene promoters, and most genetic regulatory elements, gene bodies, and repeated DNA sequences. The majority of studies in the literature use only 450K or 850K Ilumina array technology. Thus, we are not aware of any RRBS DNA methylation sequencing EA, AA, and definitely not in Nigerian PCa tumors. Furthermore, this is the only racial and ethnic cohort that has multi-omic sequencing. The methylation sequencing is expected to be returned in Jan 2023. The analysis will begin immediately upon receipt of the data.

Since ATAC-seq was not likely to be successful in the FFPE samples based on advice from Hudson Alpha, we obtained an additional 12 PCa (3EA and 9AA) frozen samples to perform 10X multi-ome sequencing which includes RNA-seq and ATAC-seq on individual cells. The sequencing was completed on 12/15/22. The analysis for this set has not begun due to the holiday however the analysis will begin in Jan 2023.

Table 1 Multi-Omic Sequencing Prostate samples in 2022



Results from Matched Tumor/Normal WES and Whole Transcriptome Analysis

In the previous cycle we confirmed that Nigerian PCa patients demonstrated similar inflammatory-immune profile as African American men with prostate cancer based on transcriptome sequencing. We are currently determining if this signature is associated with translocations to finalize for publication submission. However withing this funding period we primarily focused on integrating the WES and RNA sequencing data to determine the if there are mutational drivers of this inflammatory signature.

Since it is known that ancestral factors may drive aggressive disease, it has been shown that allele frequencies of genetic variants in immune-related genes can markedly differ amongst population groups (Nédélec et al., 2016; C. J. Smith et al., 2018). Therefore, we asked if African ancestry could drive aggressive prostate cancer and leads to genetic alterations with the upregulation of unique signatures in men of African descent. To verify the self-reported race, we used ADMIXTURE to generate a quantitative estimate of each individual ancestral composition (patient's allele frequency) based on the 1000 Genomes Project and Human Genome Diversity Project (HGDP). In our cohort, individuals who self-reported as AAM, were ancestry assigned to African Ancestry (Ancestry proportion > 70%) with either Bantu subpopulation in the Sub-Saharan area (western central Africa) and/or Yoruba (Nigeria) subpopulation [Figure 1, B, C, D]. Then we analyzed the transcriptomic data to reveal the Ancestry-associated transcriptomic signatures. The gene-

level expression from STAR counts using Ensembl gene annotation and the output data were analyzed for differential expressed genes (DEGs) and identified the enriched gene set. There were 300 DEGs in the AFR Ancestry cohort compared to the EUR Ancestry counterpart [Figure 2, A] and the immune signaling pathways were enriched in AFR Ancestry patients [Figure 2, B]. Furthermore, the Interferon signaling genes were highly correlated with AFR Ancestry and, the immune cell abundance (immune score) was significantly higher in the African Ancestry patients as shown in [Figure14] C and D respectively.
We took this further to illustrate the genomic alterations that drive these unique transcriptomic signatures. Somatic mutations analysis (tumor-only-WES), reveals that PCa Hot Spot (SPOP & FOXA1) are within the Top 30 somatic mutations as shown in [Figure 3, A]. SPOP mutation on the top mutation (20%) in African Ancestry patients vs. 10% in European Ancestry counterparts [Figure 3, B, and C, respectively]. SPOP mutation is associated with AFR Ancestry patients, and this association is statistically significant in TCGA data [Figure 3, D &E]. The distribution of the vast majority of the SPOP residues/variants is located within the MATH domain of the SPOP gene. African and European Ancestry have several SPOP variants that are unique to the Ancestry such as Y87C and D130N are linked to AFR Ancestry in our cohort, while Y87D are related to EUR Ancestry. To confirm and validate the association of SPOP residues with AFR/EUR ancestry, we ran Local ancestry to infer the estimation of the regional ancestral origin of chromosomal segments. We have selected three patients, one with dominant global AFR Ancestry as shown in [Figure 4, C] has 100% AFR-local Ancestry at the SPOP genetic loci on chromosome 17. The other with dominant global EUR Ancestry as well as 100% EUR-local Ancestry on chromosome 17 (SPOP genetic loci) [Figure 4, D]. The third patient with Admixed Global Ancestry showed that chromosome 17 at SPOP genetic loci is lined to 100% AFR Ancestry [Figure 4, E]. This means that even within the Admixed population these unique SPOP residues is linked to 100% AFR Ancestry.

Previous studies showed the SPOP mutation and ERG fusion status are mutually exclusive (Abeshouse et al., 2015; Barbieri et al., 2013). Our data showed that ERG-Fusion Negative represents 78% of the AFR Ancestry vs. 55 % of EUR Ancestry, and the SPOP mut patients are mutually exclusive with ERG Fusion status. SPOP mutant transcriptomic signatures change with the Ancestry spectrum and ERG fusion status as shown in [**Figure 5, B**]. The immune-inflammation signaling, for instance, the immune-regulatory and chemokine receptors signaling as well as Interferon Gamma & Alpha-Beta Signaling and the exhaustion geneSet (PD-1 signaling), are significantly (p-values; 0.0001) positively enriched in AFR Ancestry with SPOP mutant tumors compared to EUR Ancestry counterpart [**Figure 5, C**]. Interestingly, SPOP direct substrates are highly correlated with AFR Ancestry, including PD-L1 (CD274) and AR.

Since our data showed that Interferon Gamma signaling (IFN-γ) are enriched within SPOP mutant tumor and highly correlated with AFR Ancestry as well as several groups (A. M. Martin et al., 2015; Qian et al., 2018) showed that IFN-γ is likely driven by PD-L1 expression in the tumor microenvironment (TME), were interested in investigating the impact of PD-L1 on the AFR Ancestry tumor. Therefore, we categorized our samples based on PD-L1 expression, high PD-L1 (immunogenic), and low PD-L1 (non-immunogenic). Patients with AFR Ancestry have high PD-L1 (immunogenic subtype) compared to EUR Ancestry [Figure 6, A, Top]. Moreover, African populations have higher expression of CD8 T cells that co-express PD-1 within the PD-L1 immunogenic subtype compared to patients with low PD-L1 [Figure 6, A, Bottom]. When we took a look at the TCGA data, PD-L1 expression is highly correlated with immune cells, including CD8+ve T cells.

We used Spatial transcriptomic technology from Akoya Biosciences (multiplex staining at single cell level resolution) to validate our finding on AAM and EAM tissue sections. Our results showed that AAM tumors have higher immune cell infiltrations (CD8-Tcells) compared to EAM tumors **[Figure 7, A Top]**. Interestingly, when we performed segmentation and gating of the PD-L1 population (AAM vs. EAM), AAM tumors is associated with high expression of CD8- T cells and PD-1 within the PDL1 population compared to EAM counterpart **[Figure 7, A Bottom]**. The quantification of the immune cell populations reveals that PD-L1 (immunogenic) population is significantly higher in AAM compared to EAM **[Figure 7, B]** and within the immunogenic population CD8+ve and PD-1+ve populations are significantly higher in AAM than EAM.

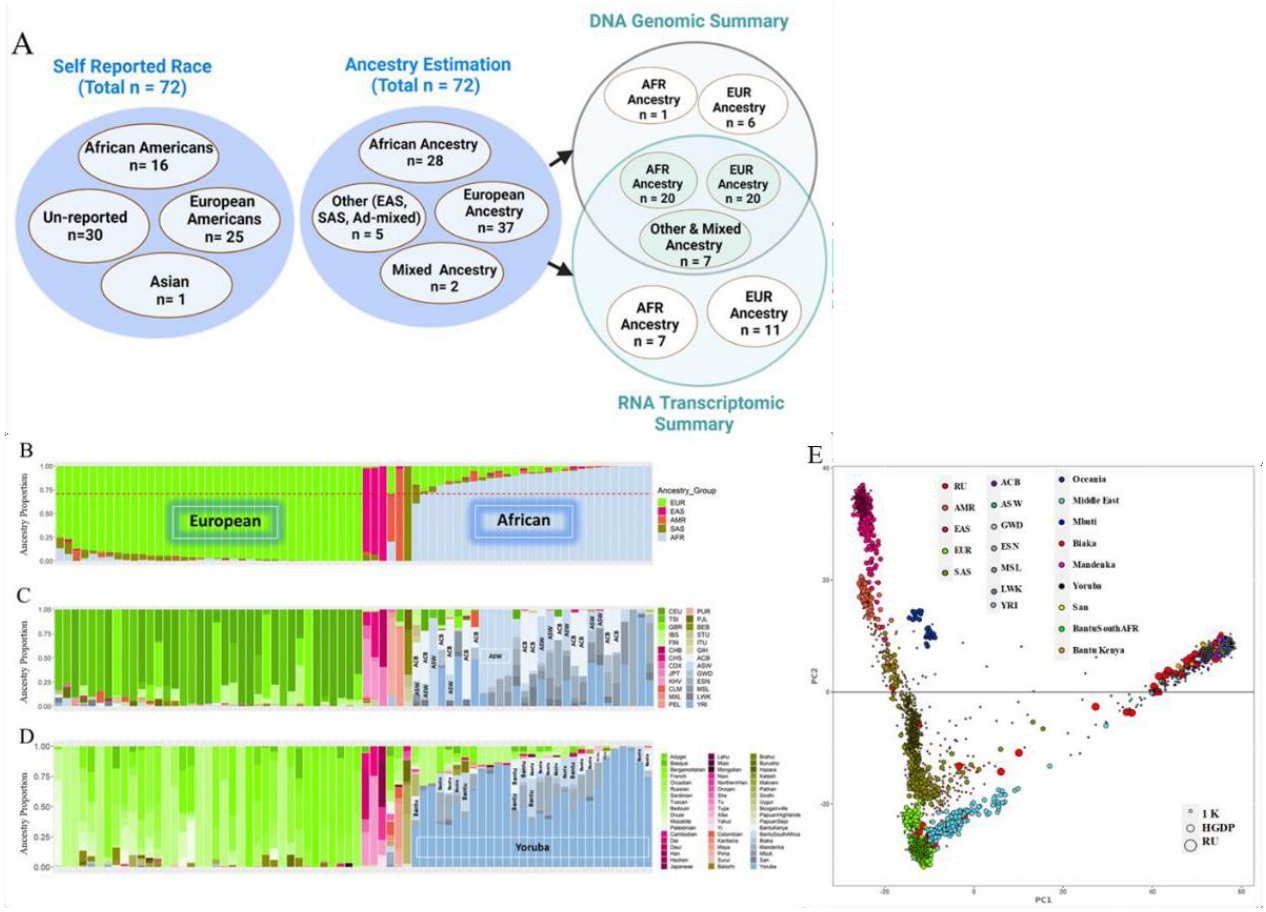


Figure 1: Patients' distribution and Genetic ancestry Estimation.

(A) Patient's SRR/ Ancestry distribution and Sequencing summary. The Global Ancestry (Super and Subpopulations) quantitative estimation of each individual ancestral composition is based on the 1000 genome populations reference using WES and RNA-Seq variants. For most of our cohortswho self-reported as AAM, their Superpopulations ancestry was assigned to AFR Ancestry (Ancestry proportion > 70%) **(B).** Particularly, AFR Ancestry patients' descent of African Caribbean subpopulations (ACB) and African of Southwest USA (ASW) based on 1000 genome **(C).** Moreover, we used HGDP populations reference to specify from where in Africa our African cohort descent, as shown in **(D)** Our African cohort descent from either the Bantu subpopulation in the Sub-Saharan area (western central Africa) and/or Yoruba (Nigeria) subpopulation **(E)** PCA distribution for African subpopulation from our cohort, 1 K and HGDP databases.

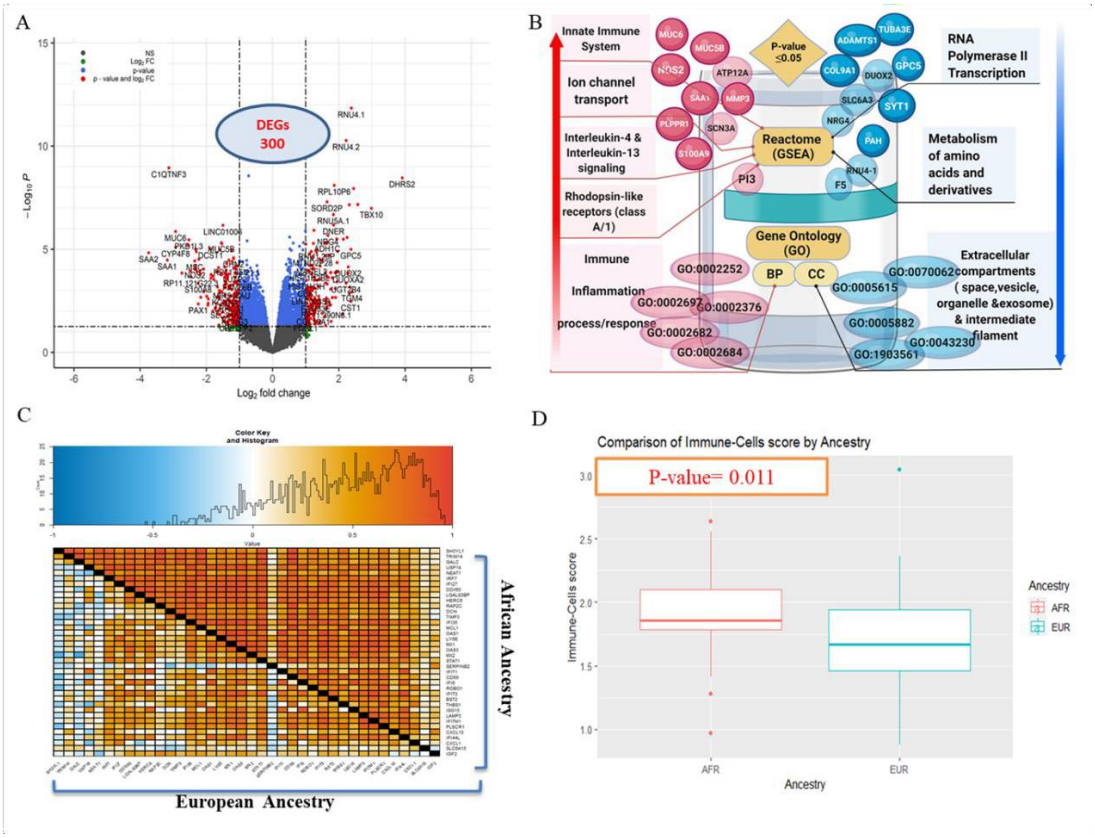


Figure 2: Ancestry-associated transcriptomic Signatures

Volcano Plot representation of log2 Fold Change (FC) and the P-value.), Based on the Ancestry model (AFR vs. EUR), 300 genes are differentially expressed in AFR vs. EUR patients

(A) GSEA classifications of the DEGs (FC>2, p<0.05) **(Red)** The innate immune system, Interleukin-4 &13 signaling, and immune-inflammation signaling is positively enriched in AFR Ancestry patients compared to the EUR counterpart **(Blue)** While gene sets such as RNA-Polymerase II and the metabolism of amino acids are negatively enriched in AFR Ancestry patients **(B)** Correlation plot showing IFN Signaling genes are highly correlated with AFR Ancestry compared to EUR Ancestry **(D)** CIBERSORTx deconvolutions analysis. Estimation of the abundances of immune cells (Immune score) in a mixed cell population, using gene expression based on Ancestry data

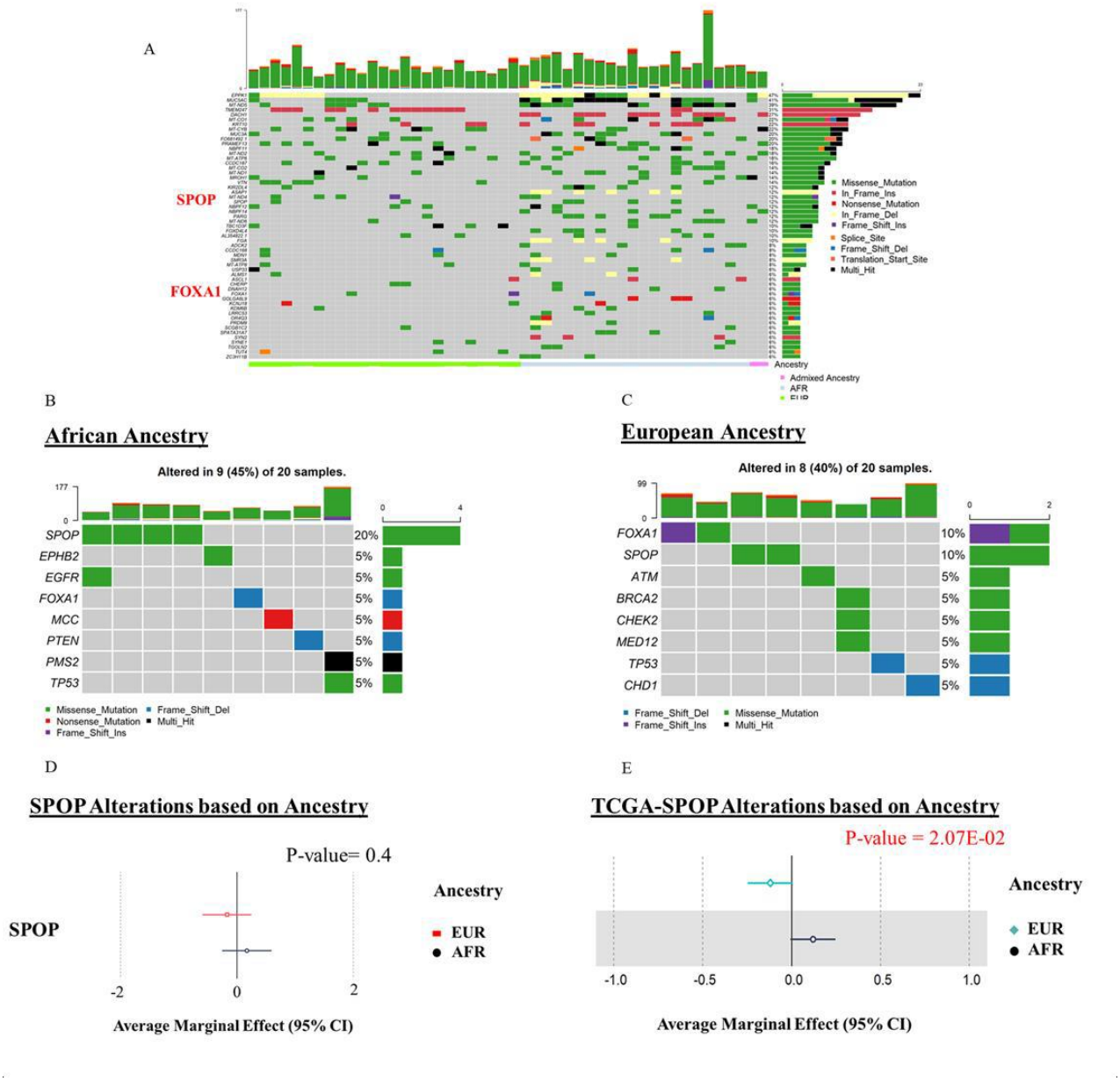


Figure 3: Somatic mutation analysis

(A) Oncoplot represents the expression of the Top 30 filtered mutation/Variants in AFR, EUR, and Admixed Ancestry patients (EAS &SAS and AMR ancestry was excluded), PCa Hot Spot (SPOP & FOXA1) are within the Top 30 somatic mutations. SPOP mutation on the top mutation (20%) in African Ancestry patients vs. 10% in European Ancestry counterparts as shown in **(B) and (C), respectively. (D) and (E)** Logistic Regression analysis shows that SPOP mutation is associated with AFR Ancestry patients, and this association is statistically significant in TCGA data

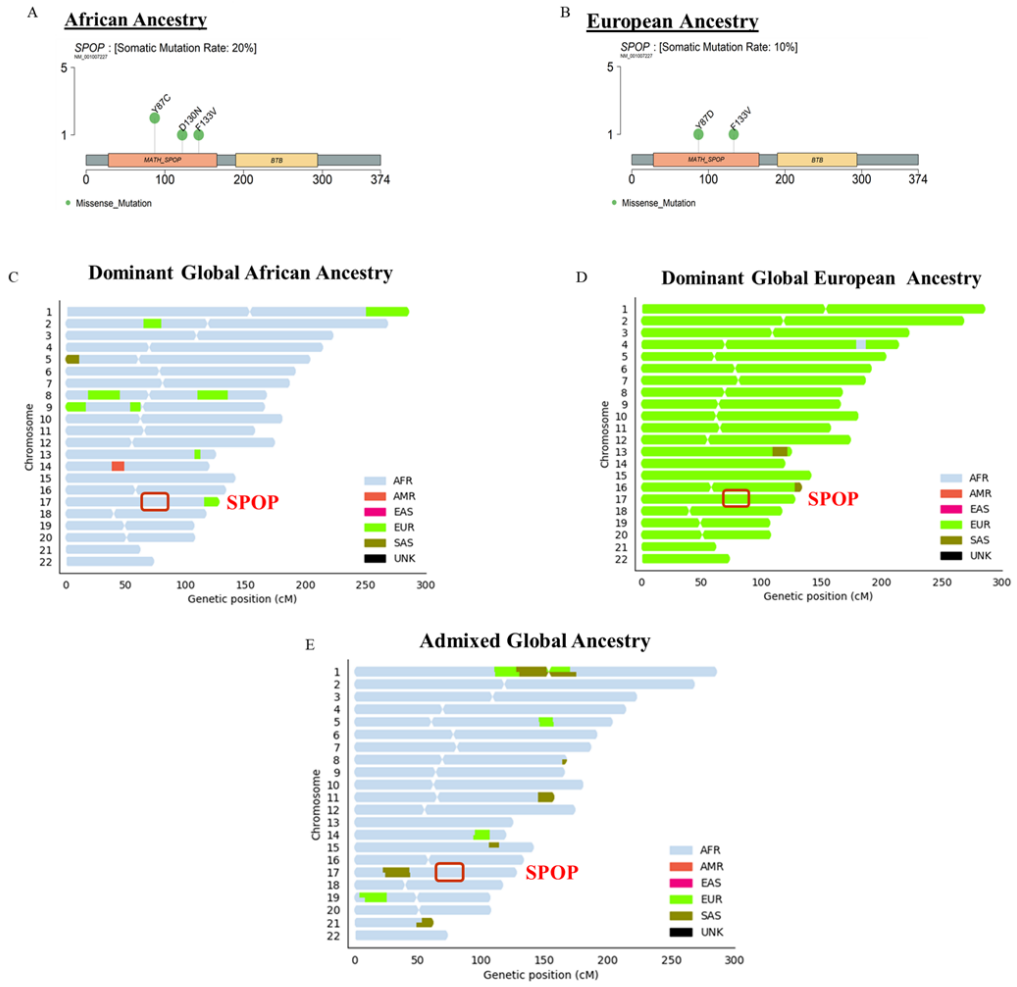


Figure 4: Lollipop plot of SPOP mutation residues and Local Ancestry

(A) and (B) the distribution of the vast majority of the mutation/variants is located within the SPOP gene (in the MATH domain). African and European Ancestry have several SPOP variants that unique to the Ancestry such as Y87C and D130N are link to AFR Ancestry, while Y87D are related to EUR Ancestry. F133V is associated with both AFR and EUR Ancestry in our cohort. To confirm and validate the association of SPOP residues with AFR/EUR ancestry, we ran Local ancestry to infer estimation of the regional ancestral origin of chromosomal segments in high AFR Ancestry patients as shown in **(C)** and EUR Ancestry (**D)** as well as Admixed ancestry patient **(E).**

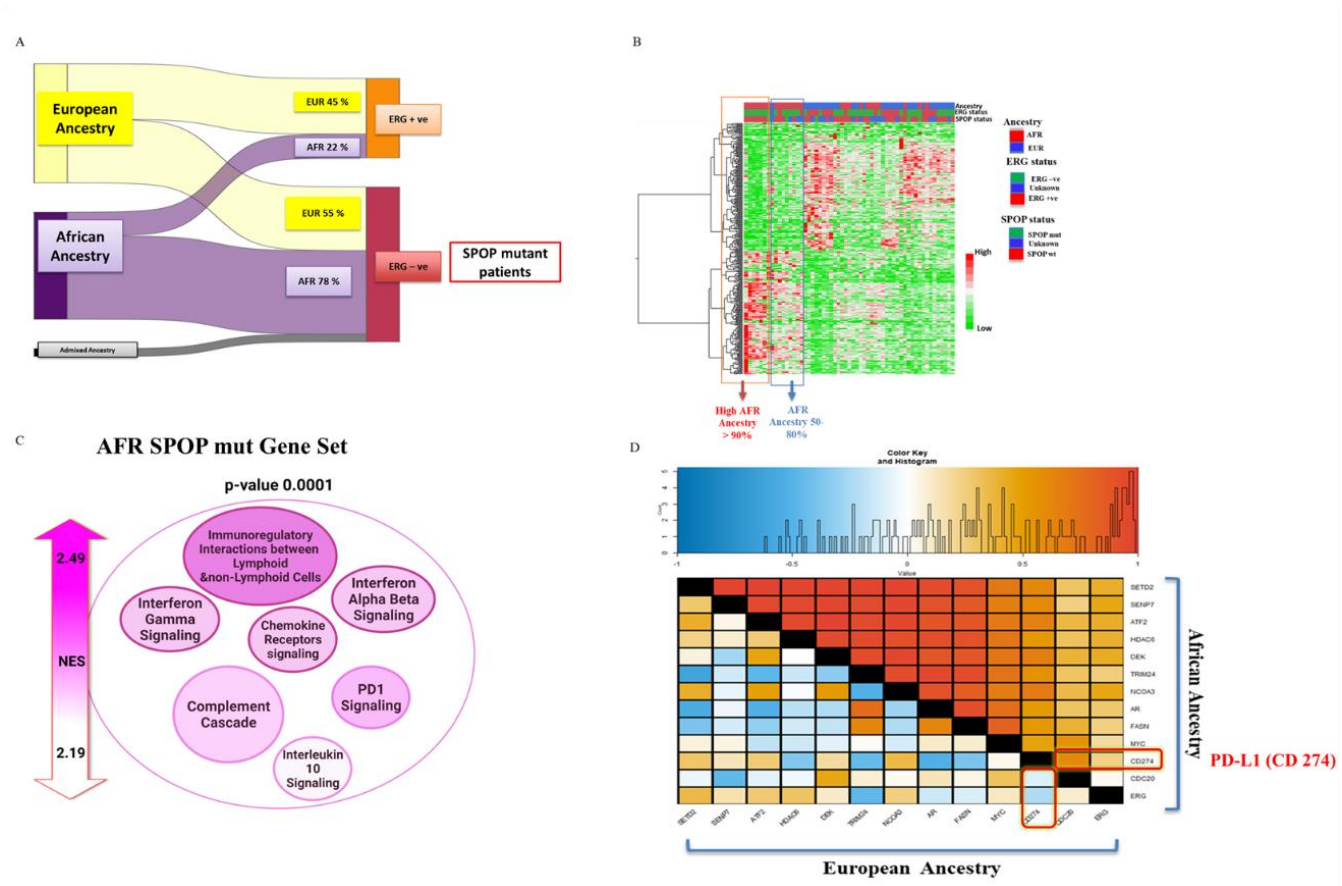
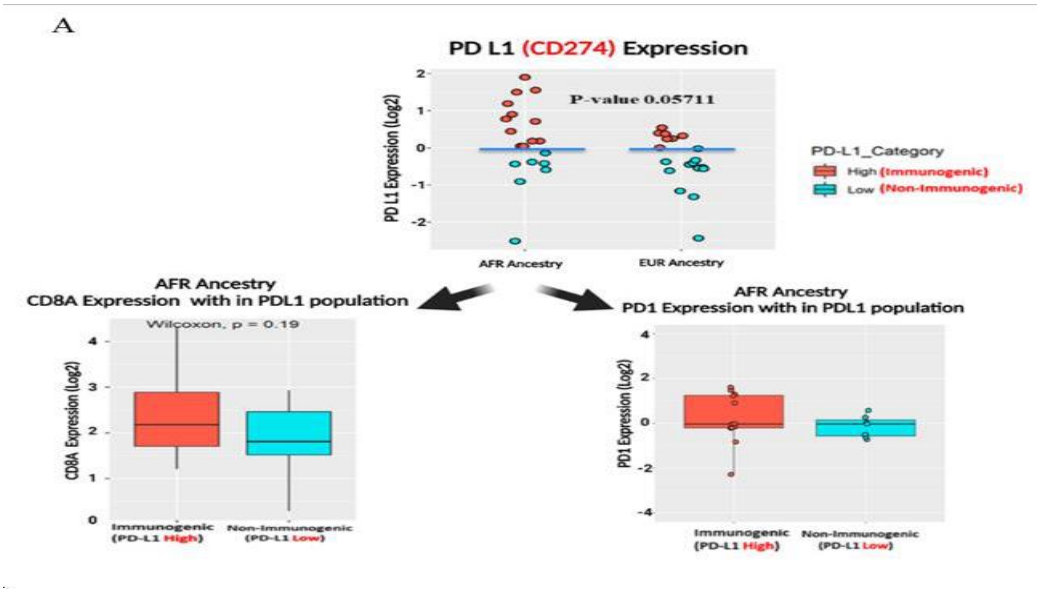


Figure 5: SPOP mutant transcriptomic signature and ERG fusion status analysis based on Ancestry

(A) The majority of the AFR Ancestry cohort are **ERG-Fusion Negative**, which represent 78% vs. 55 % of EUR Ancestry, and the SPOP mut patients are mutually exclusive with ERG Fusion status **(B)** transcriptomic signature changes with Ancestry spectrum, SPOP mutation, and ERG status **(C)** GeneSet-Enrichment Analysis showing the positively enriched geneSet in AFR ancestry patients with SPOP mutation compared to EUR patients with SPOP mut. **(D)** SPOP substrates are highly correlated with AFR ancestry, including PDL1 (CD274)



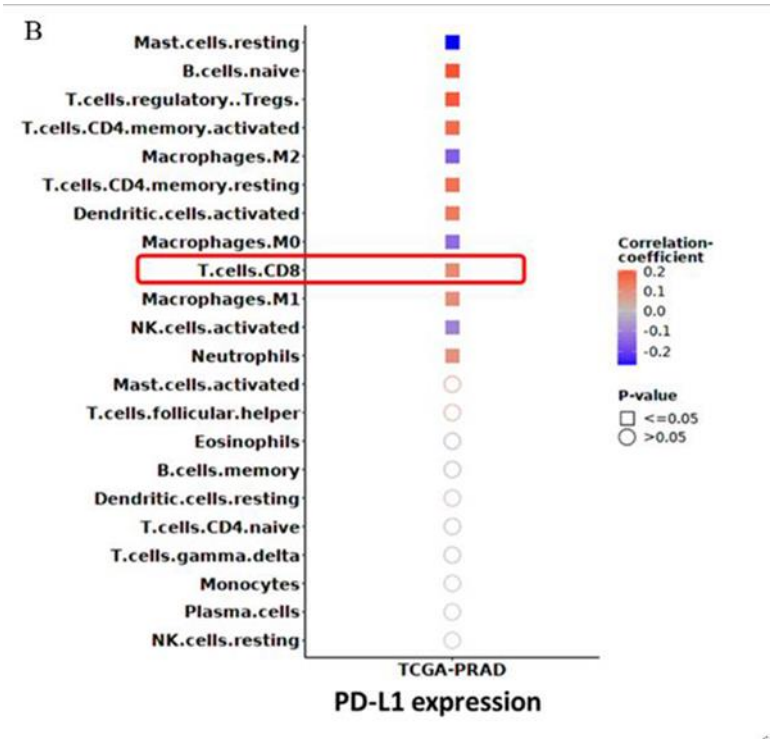


Figure 6: Prostate cancer Immunogenic (High PDL1 expression) and non-Immunogenic subtypes (Low PDL1 expression)

(**A, Top)** Higher PD-L1 expression in AFR Ancestry patients compared to EUR counterpart. Interestingly **(A, Bottom)**, when we stratified our AFR samples based on PD-L1 expression, patients with high PD-L1 (Immunogenic) have higher expression of CD8 T cells that co-express PD-1 compared to patients with low PD-L1 (Non-Immunogenic). **(B)** TCGA-PD-L1 expression is highly correlated with Immune Cells, including CD8+ve T Cell

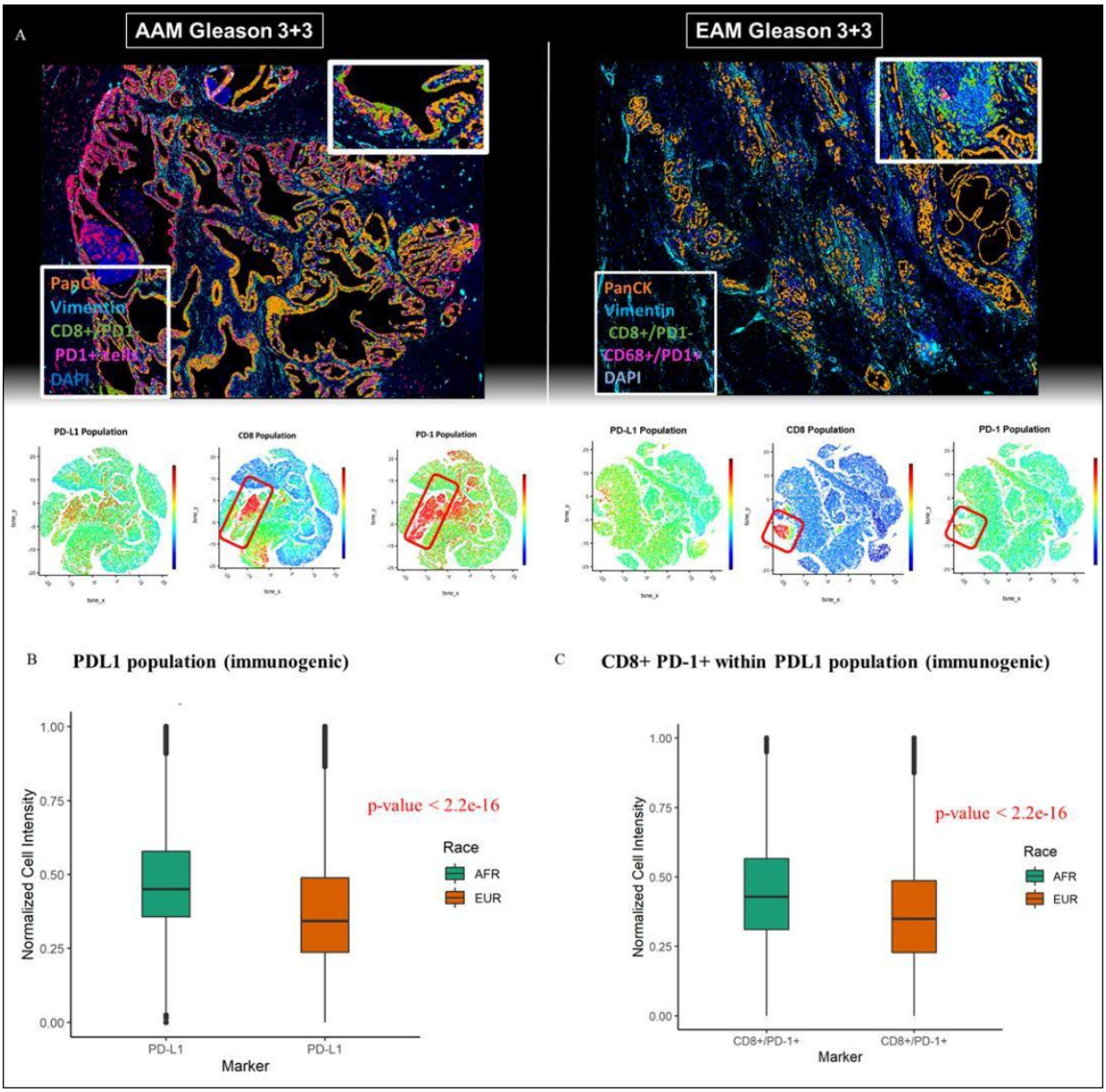


Figure 7: Spatial transcriptomic signatures revealing African tumors are Highly immunogenic

(A, Top) immune cells (CD8+ve) infiltration in AAM patients compared to EAM patients, even though those tumors were low grade. Interestingly **(A, Bottom),** segmentation and gating of the PD-L1 population (AAM vs. EAM), showed high expression of CD8-T cells and PD-1 within the PDL1 population **Quantification of the immune cell population (B)** PDL1 population (immunogenic) is significantly high in AAM vs. EAM. **(C)** Within the immunogenic population, CD8+ve and PD-1+ve populations are significantly high in AAM vs. EAM.

SPOTLIGHT Manuscript published.

As a result of our Ancestry specific DNA Damage Germline mutation data published in Cancer Research Communications and the presentation of Ancestry Specific SPOP at AACR meeting, we were asked to write a spotlight review for entitled **"Ancestry-defined Molecular Taxonomy of Prostate Cancer" that was published in Trends in Cancer**. Below is a summary of the text:

Prostate cancer (PCa) mortality rates are highest among men of African descent globally compared to their European counterparts. These disparities are a result of a complex interplay between genetics and non-genetic factors as well as PCa molecular profiling (Tan et al., 2016), which will lead to differences in disease risk and outcomes (Badal et al., 2020; Dasgupta et al., 2019). Vanessa Hayes and colleagues (Gong et al., 2022; Jaratlerdsiri et al., 2022) published joint papers in Nature and Genome Medicine that identified a new Ancestry-specific prostate cancer taxonomy in the largest African prostate cancer dataset explaining geo-ethnic differences in disease severity, particularly in Sub-Saharan Africa.

Prostate tumors are molecularly heterogeneous diseases with extensive intra and inter - tumoral complexity, especially among African descent patients. Unfortunately, the majority of the current data represents European ancestry populations which limits our knowledge of understanding the progression of advanced disease within the minority populations. To fill the gap of the under-representation of the African population in genomic studies, Vanessa Hayes group, generated the largest worldwide cancer genomics resource by sequencing treatment-naive prostate cancer tumors from 183 ancestrally (African versus European) and globally distinct patients. Furthermore, through generating a unified pipeline, and comprehensive analysis of the sequenced data, the Hayes group identified around 2 million somatic variants specific to African patients and described the impact of structural variations (SVs) interrogation on reducing the ethnic disparity.

This study consisted of prostate cancer patients from sub-Saharan Africa revealed novel subtypes and genomic alterations as well as therapeutic targets that could play a significant role in improving patient outcomes. Utilizing ancestry-specific cancer driver genes/hotspots particular to geographical regions/circumstances allows for the inclusion of endogenous contributions to disease progression and provides new insight into adapting personalized medicine across Africa. Interestingly, African Ancestry patients have a higher level of tumor mutational burden, genomic alterations, and damaging mutations. This led to the group reclassifying the existing molecular taxonomy for prostate cancer into global mutational subtypes (GMS) defined by ancestry [Figure 1]. The GMS module combines environmental factors/geographical location and PCa molecular profiling with the patients' clinical annotations to demonstrate novel Ancestry-specific taxonomy. Interestingly, GMS-A is ancestrally and geographically universal, whereas GMS-B and GMS-D are African-specific, with a new African–Asian GMS-E emerging, and GMS-C linked specifically to European–African module. Paralleled to previous taxonomy (Abeshouse et al., 2015), GMS modules complement known subtypes such as SPOP and FOXA1 mutations, which are associated with mutational signatures reported in the known catalog of somatic mutations in cancer (Alexandrov et al., 2020; Jaratlerdsiri et al., 2022). The implementation of the GMS module, which accounts for the contribution of regional ancestry to an individual patient's genome, could have a substantial impact to more robustly capture the heterogeneity of prostate tumors. Although it has been well recognized that prostate tumors are highly heterogeneous, these newly proposed GMS modules add a precision medicine approach to the molecular subtypes that will ultimately enrich the number of patients who benefit from targeted therapy with the promise of improving patient outcomes.

Diving deeper into the outcomes of the GMS-D (mutationally noisy) module, the authors provide an in-depth analysis of the contribution of the somatic SVs to the aggressive PCa in men of African ancestry. While it has been widely reported that non-European ancestry prostate tumors lack TMPRSS2-ERG fusions, the GMS-D module adds a pattern of novel ETS family members specific to African Ancestry patients. This novel GMS-D subtype is largely driven by translocations and rarely by deletion, inversion,

and duplication events, and categorized as the hyper-translocated subtype. Wide-ranging and informative analysis revealed that African-derived tumors had up to a 2.5-fold increase of duplication events (hyper-duplication SVs) which were significantly associated with CDK12 inactivation and MYC copy number gain. MYC expression alterations have widely been noted in castration-resistant prostate cancer (Qiu et al., 2022). However, this first report of MYC alterations in treatment-naïve primary prostate tumors, possibly indicating a biological contribution to the disease aggressiveness in African Ancestry men with PCa. Interestingly, the widely explored SPOP mutations, which was an outcome of the TCGA molecular taxonomy findings, are associated with a hyper-deletion subtype, and African patients were 1.3-fold more likely to present with SPOP-coding mutations. Since these hyper-SV alterations are observed within the primary tumor, it is likely an indicator of aggressive disease rather than a consequence of treatment response.

Collectively these two studies highlight how global inclusion in cancer genomics helps identify geo-ethnic restricted taxonomy and uncover PCa heterogeneity from a clinical standpoint as well as affecting an individual's predisposition to disease and response to therapy. Sub-Saharan African populations migrated across the continent over the past 5,000 years and admixed with resident populations, which plays a pivotal role in shaping the genomic landscape of Africa (Choudhury et al., 2020). The lack of sufficient genetic reference information from the African genome is a major barrier in investigating the benefits of precision oncology for both African Americans and native Africans (Marima et al., 2022). Furthermore, African genomes contain more genetic variation compared to non-African genomes, and since Africa is the source of the geographic expansions of ancestral populations into other regions of the world, there is a need for these types of high-depth studies within the African continent. Our lab recently reported ancestry specific genomic profiling of Nigerian men compared with prostate cancer to African American men (White et al., 2022) thus we are in agreement that ancestral and geographical attributes of patients should facilitate cancer population genomics as an added benefit to personalized genomics. Similar studies would increase the scientific opportunity to better understand the complex interplay of tumor biology and the impact of nature versus nurture on the human genome. Lastly, the application of a global mutational framework based on ancestral origin with more inclusion of diverse populations, has the potential to provide healthcare professionals with a guide to increase the chances of discovering drug targets for all patients and improve health outcomes.

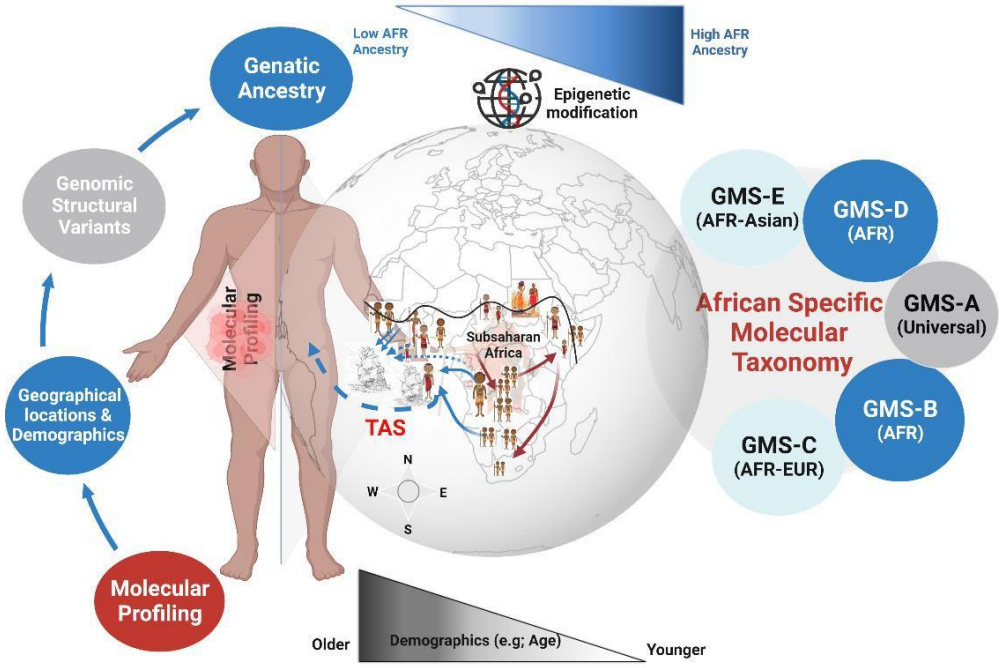


Figure 8: Prostate Cancer Genomic Alterations and Ancestral Subtypes.

A comprehensive, unified genomic analysis utilizing a large global database redefined prostate cancer based on an African-Specific Molecular Taxonomy. Within the Ancestry-specific classifications, both genetic and epigenetic (modifiable attributes) are identified and linked to aggressive subtypes of PCa. The classifications termed GMS are individual modules that define the global mutational subtypes based on Ancestry. High AFR Ancestry has the greatest impact on driving novel mutational signatures among individuals from divergent ancestral origins. Specifically, Sub-Saharan African migration contributes to the diverse ancestral spectrum in Africa as well as in the African Americans as a result of the transatlantic slave trade.
*Abbreviations: *PCa, Prostate Cancer; *AFR, African; *EUR, European; *GMS, Global Mutational Subtypes; *TAS, Trans-Atlantic Slave Trade*

IMPACT
Opportunities for training and professional development.

During this period, Dr. Isra Elhussin, who was added to this project during the last cycle was invited to platform presentations of this data at the AACR annual meeting as well as the AACR health disparities meeting. Both Jason White (First author on the Nigerian Whole Exome manuscript) and Isra Elhussin both successfully defended their dissertations and were awarded doctorate degrees as a result of this work.

Dissemination of results to communities of interest.

Isra Elhussin was selected as the 2022 AACR Next Gen Star as well as oral presentation at the AACR annual meeting in New Orleans, LA. Moreover, Isra's work was spotlighted in AACR Blog. (see link below)

https://www.aacr.org/blog/2022/09/12/a-researchers-multifaceted-approach-to-addressing-disparities-in-cancer/

Dr. Yates gave the following invited oral presentations:

2022 AACR Annual Meeting 2022 New Orleans, LA April 8-12, 2022

2022 AAMC Biomedical Research Leaders Conference May 4-5, 2022

2022 Johnson and Johnson, African Ancestry Leadership Council General Counsel Global Functions Chapter Feb 2, 2022

2022 Duke-NCCU "Evolutionary Medicine Summer Institute" May 17th, 2022

2022 National Academy of Sciences "Roundtable on Systemic Change in Undergraduate STEM Education" May 23, 2022

2022 University of Florida Cancer Center UFHCC Oncology Grand Rounds May 11, 2022
2022 CCR-DCEG Health Disparity Workshop taking place on May 25-26, 2022, in Bethesda MD

2022 City of Hope *First Annual Closing the Care Gap-C3E Health Justice Summit June 21,*

2022 Meharry Medical College Meharry Summer Undergraduate Cancer Program July 27, 2022

2022 Vitamin D Workshop Texas Sept 6-9, 2022

2022 Inaugural Health Equity in Precision Medicine Symposium, New York, NY

2022 Society for Basic Urological Research Plenary Session Orlando, Florida

AACR annual meeting, University of Florida (Grand Rounds) , City of Hope Health Disparities, Weill Cornell Health Disparities symposium Society for Basic Urological Research, Orlando Florida

Goals to accomplish during the next reporting period. A major focus during this second no-cost extension period will be analysis of sequencing data generated during the previous funding period. The first manuscript is now published, however we planning to integrate paired DNA/RNA data. Furthermore, we plan to analyze the single cells ATAC seq data to determine the chromatin changes proposed in AIM 3.

Publications

1. White J, Kaninjing E, Adeniji K, Jibrin P, Obafunwa JO, Ogo C, Faruk M, Popoola A, Fatiregun O, Olabode P, Karanam B, Elhussin I, Ambs S,Tang W, Davis M, Polak P, Campbell M, Francis D, Gibbs D, Brignole K, Rotimi S, Odedina F, Martin D, **Yates C**. Whole Exome Sequencing of Nigerian Prostate Tumors from the Prostate Cancer Transatlantic Consortium (CaPTC) Reveals DNA Repair Genes Associated with African Ancestry. Cancer Res Commun; 2(9) September 2022
2. Isra Elhussin and **Clayton Yates** "Ancestry-defined Molecular Taxonomy of Prostate Cancer*" Trends Cancer. 2022 Dec;8(12):973-975. doi: 10.1016/j.trecan.2022.10.008.*

4. Impact

The impact of our findings to-date are that we have a identified ancestry specific germline mutation, and gene mutations specifically in men of West African Ancestry. These findings will be significant to larger cancer communities including prostate cancers. Furthermore, this information could be useful to pharmaceutical companies that are interested in patient stratification.

5. Changes/Problems

Specific Aim 3, Major Task 1: The major challenge has encountered this cycle was the lack of quality DNA to perform large scale whole exome analysis for Nigerian samples. However, we were able to obtain quality RNA and thus we will continue with analysis if these data with subset for AA and Nigerian patients. Fortunately, we did obtain quality single cell sequencing data for ATAC-seq and thus this data will be analyzed during this period as well.

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	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 Amit Mitra at the Auburn University Genomics Core to perform the ATAC-seq which supports **Specific Aim 3**. We received the frozen Prostate Cancer samples from the Cooperative Tissue Network, and AA and EA were matched for age and Gleason grade. 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.