Serum proteomics links suppression of tumor immunity to ancestry and lethal prostate cancer

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Article

Keywords: Proteomics, inflammation, prostate cancer, ancestry, survival, disparity, immune signature, African, European

Posted Date: July 13th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-668276/v1>

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Version of Record: A version of this preprint was published at Nature Communications on April 1st, 2022.
See the published version at <https://doi.org/10.1038/s41467-022-29235-2>.

Serum proteomics links suppression of tumor immunity to ancestry and lethal prostate cancer (13 words, 92 characters)

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Running title: Immune-oncological markers and prostate cancer disparity

Key words: Proteomics, inflammation, prostate cancer, ancestry, survival, disparity, immune signature, African, European

Abbreviations: AA, African-American; EA, European-American; OR, odds ratio; CI, confidence interval; PSA, prostate-specific antigen.

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One sentence summary: A serum proteome-based immune function signature is upregulated in men of African ancestry and associates with lethal prostate cancer.

40 **Abstract (156 words):**

41 There is evidence that tumor immunobiology and immunotherapy response may differ between
42 African American and European American prostate cancer patients. Here, we determined if men of
43 African descent harbor a unique systemic immune-oncological signature and measured 82
44 circulating proteins in almost 3000 Ghanaian, African American, and European American men.
45 Protein signatures for suppression of tumor immunity and chemotaxis were significantly elevated in
46 men of West African ancestry. Importantly, the suppression of tumor immunity protein signature
47 associated with metastatic and lethal prostate cancer, pointing to clinical significance. Moreover,
48 two markers, pleiotrophin and TNFRSF9, predicted poor disease survival specifically among
49 African American men. These findings indicate that immune-oncology marker profiles differ
50 between men of African and European descent. These differences may contribute to the
51 disproportionate burden of lethal prostate cancer in men of African ancestry. The elevated
52 peripheral suppression of tumor immunity may have important implication for guidance of cancer
53 therapy which could particularly benefit African American patients.

INTRODUCTION

Men of African origin bear the highest prostate cancer burden in the U.S. and globally¹⁻³. They are at an increased risk of developing fatal prostate cancer in the U.S and England⁴ and present with more aggressive disease in the Caribbean and sub-Saharan Africa^{2,5}. The reasons for the observed global prostate cancer health disparities are unclear but may be related to an array of factors such as access to health care, lifestyle and environment, and ancestral and biological factors⁶⁻⁸.

Previously, we and others described that tumor immunobiology differs between African-American (AA) and European-American (EA) prostate cancer patients⁹⁻¹². A tumor-specific immune-inflammation gene expression signature was more prevalent in prostate tumors of AA than EA patients¹¹. The occurrence of this signature in prostate tumors was associated with decreased recurrence-free survival¹³. Furthermore, regular use of aspirin, an anti-inflammatory drug, may reduce the risk of aggressive prostate cancer, disease recurrence and the lethal disease in AA men^{14,15}. Combined, these findings suggest that inflammation and host immunity may contribute to prostate cancer progression but with notable differences between AA and EA men.

Ancestral factors can influence immune-related pathways¹⁶. Germline genetic variant prevalence and alternative splicing in immune-inflammation-related genes can show large differences amongst population groups¹⁷⁻¹⁹. Hence, the immune-inflammation gene expression signature identified in the tumors of AA prostate cancer patients could be due to either tumor biology and the associated microenvironment, ancestral factors, or systemic differences in immune-oncology marker expression. In the present study, we tested the hypothesis that a distinct systemic immune-inflammation signature exists in men of African ancestry that associates with prostate cancer. It is the novelty of our approach that we examined the serum proteome in a large cohort of

77 diverse men. Applying large-scale proteomics with Olink technology, we discovered the up-
78 regulation of circulating immune-oncological proteins that functionally relate to chemotaxis and
79 suppression of tumor immunity and their association with West African ancestry and lethal prostate
80 cancer. Our findings point to the clinical importance of a serum proteomic signature in prostate
81 cancer patients that may affect men of African ancestry more so than other men.

RESULTS

Large-scale evaluation of immune-oncological proteins in the NCI-Maryland and NCI-

Ghana prostate cancer studies.

To investigate if men of African descent are differentially affected by a systemic immune inflammation, we utilized two case-control studies with large representations of men of African ancestry: the NCI-Ghana and NCI-Maryland Prostate Cancer Case-Control Studies. Characteristics of the participants in the two studies have been previously described^{14,20}. We assayed 92 circulating immune-oncological proteins in a total of 3094 serum samples containing 1505 controls and 1432 cases along with 157 randomly selected blinded duplicates. To control for any batch effects, the serum samples were assayed in a random order along with the 5% blind duplicates for intensity normalization (see Methods). Ninety-five percent of the samples passed stringent quality control leaving 1482 controls (654 Ghanaian, 374 AA, and 454 EA) and 1308 cases (489 Ghanaian, 394 AA, and 425 EA) for our analysis (**table S1**). The average intra- and inter-plate CV calculated based on duplicates were very low at 1.7% and 2.6%, respectively. In addition, the proportion of variance explained by an inter-plate batch effect was rather minimal for each of the serum proteins even before intensity normalization (**fig. S1**). Out of the 92 serum proteins, 61 were detected in 100% of the samples tested and 78 were detected in > 50% of the samples (**fig. S2**). Because 10 out of the 92 serum proteins were detected in less than 20% of the samples (**fig. S2**), only the remaining 82 proteins were included in our analysis (**table S2**). Next, we assessed how the 82 serum markers correlate with one another in Ghanaian, AA, and EA men without prostate cancer using Pearson's pairwise correlation analysis (**fig. S3**). The top ten observed correlations for each population group is presented in **table S3**. Most of these relationships have not previously been described. Most notably, epidermal growth factor levels strongly correlated with CD40L [Ghanaian ($r=0.71$), AA ($r=0.83$), and EA ($r=0.80$) men], a marker

of activated T cells, whereas IL8 levels highly correlated with circulating caspase 8 in all three population groups [Ghanaian ($r=0.69$), AA ($r=0.82$), and EA ($r=0.80$)].

Clinical and socio-demographic characteristics are associated with immune-oncological proteins. Cytokine levels can be influenced by environmental exposures and disease. Therefore, we investigated the association between various socio-demographic and clinical characteristics (age, BMI, education, aspirin use, smoking, diabetes and PSA) with serum levels of immune-oncological proteins using a multivariable linear regression model (**Fig. 1**). We restricted this analysis to the control population in the NCI-Ghana and NCI-Maryland studies to exclude the potential confounding effect of prostate cancer in the analysis. Among the exposures, aspirin use and blood PSA levels showed only few relationships with the profile of the 82 immune-oncology markers. Other exposures and several demographics showed more robust relationships.

Aging is known to impact the immune system and is a risk factor for many diseases including cancer²¹. In our analysis, aging was most consistently associated with the level of the analytes across the three population groups, showing a significant correlation with almost half of these circulating immune-oncological proteins. For example, PGF, CXCL9, Gal9, Gal1, CX3CL1, TNFRSF12A, CCL23, MMP7, DCN, MMP12, CXCL13, CSF1, ADGRG1, CD4, and PTN positively associated with age in all three population groups. The top-ranked biological functions that associated with these age-related proteins were cell migration and positive regulation of cell adhesion (**fig. S4A**). Age was also positively associated with lymphocyte activation, represented by TNFRSF9, CRTAM, PDCD1, CD27, NCR1, TNFRSF4, KLRD1, CD83, IL12, and IL12RB1, but only in the NCI-Maryland EA and AA men (**fig. S4B**). On the other hand, hepatocyte growth factor (HGF) and vascular endothelial growth factor-A (VEGFA), two angiogenic cytokines, were positively associated with age exclusively in men of African ancestry (Ghanaian and AA men).

128 Lastly, VEGFR2, a tyrosine kinase receptor for VEGF, was negatively associated with age in EA
129 and AA men.

130 In contrast to the positive association of many of the immune-oncological proteins with
131 age, BMI tended to be negatively associated with these circulating immune-oncological analytes.
132 This finding may be surprising as obesity is generally thought to be associated with systemic
133 inflammation. CX3CL1 was negatively associated with BMI in all three population groups. The
134 soluble form of CX3CL1 stimulates recruitment of CX3CR1 expressing inflammatory immune
135 cells²². CAIX and LAMP3 were inversely associated with BMI exclusively in men of African
136 ancestry, suggesting that ancestral factors may influence the relationship between BMI and
137 expression of these markers. CAIX is a hypoxia regulated metalloenzyme that exists as both
138 membrane associated and soluble form²³ whose main cellular function is to catalyze the reversible
139 conversion of carbon dioxide to carbonic acid²⁴, thereby influencing local acidity, which is known
140 to affect immune function²⁵. LAMP3 is a member of lysosomal associated membrane glycoprotein
141 family that have a myriad of roles including lysosomal exocytosis and cholesterol homeostasis²⁶.
142 On the contrary, serum GAL1, a glycan binding protein that mediates the suppressive function of
143 T_{Reg} cells²⁷, showed the opposite trend and was positively associated with BMI in all three
144 population groups.

145 To explore how the social/behavioral environment may affect immune-oncological serum
146 protein levels, we investigated their relationship with educational attainment. For Ghanaian men,
147 27 of the 82 immuno-oncological markers were negatively associated with their education level
148 (**Fig. 1**). Yet only IL18 showed a significant inverse association with education for both Ghanaian
149 and AA men. Among EA men, 12 of the 82 immune-oncological proteins had significant inverse

relationships with the attained level of education (**Fig. 1**), with some of these markers showing a similar pattern among Ghanaian and EA men.

Previous studies have shown that tobacco smoking increases inflammation²⁸. Herein, we assessed the association between cigarette use (never, former, vs. current smoker) on the level of immune-oncological proteins in circulation. We found that current smoking was consistently associated with significantly increased level of analytes that regulate angiogenesis (ANGPT2), antigen presentation (CD83), and autophagy (LAMP3), in all three study populations (**Fig. 1**).

Innate immune system-driven inflammatory processes have been implicated in the pathogenesis of diabetes²⁹. In our analysis, among the cytokines that showed an association with self-reported diabetes, serum proteins belonging to tumor necrosis factor receptor super family (CD27 and TNFRSF12A), and a matrix metalloprotease enzyme (MMP7) were positively associated with diabetes in all three population groups (**Fig. 1**). Others, including PGF, CX3CL1, NCR1, TNFRSF4, and TNFRSF21 were positively associated with diabetes exclusively in men with African ancestry. Functional enrichment analysis revealed that diabetes-associated CX3CL1, TNFRSF4, and TNFRSF21 are all involved in negative regulation of cytokine secretion (**fig. S5**). CX3CL1 is known to regulate insulin secretion³⁰, is elevated in the serum of patients with type 2 diabetes³¹, and has been implicated in diabetic nephropathy³², validating the findings in our study.

C-reactive protein (CRP) is a commonly measured pro-inflammatory marker in the body and has been reported to be associated with worse prostate cancer prognosis^{33,34}. Because it was not part of our marker panel, we measured blood CRP in 156 plasma samples from population controls of the NCI-Maryland study. Smoking was the only socio-demographic variable that had a significant association with CRP (**table S4**), which is consistent with the literature. Furthermore, CRP showed significant positive associations with 24 of the 82 serum proteins (TNFRSF9, IL7,

PGF, IL6, Gal9, GZMH, CXCL1, TNFSF14, Gal1, PDL1, HGF, HO1, CD70, TNFRSF12A, CCL3, MMP7, ANGPT2, VEGFA, CCL20, KLRD1, CSF1, CD4, MCP3, and CXCL11).

The systemic immune-oncological cytokine profile in men of African ancestry is distinct from men of European ancestry. To investigate if ancestral population group differences may influence circulating levels of the immune-oncological markers, we performed an unsupervised clustering analysis examining how the levels of the 82 immune-oncological analytes would group men without prostate cancer from Ghana and the US. Notably, these analytes tended to cluster by population group, with levels in Ghanaian men being most distant from EA men while AA samples tended to cluster in between these two groups (**Fig. 2**), suggesting that the ancestral background may have a significant impact on this immune-oncological protein profile.

To further evaluate the influence of ancestry, we estimated West African ancestry in AA and EA population controls of the NCI-Maryland study and its relationship with the cytokine profile. West African ancestry was determined using 100 validated ancestry informative markers³⁵. The approach showed that, to some extent, the variance in the levels of several immune-oncological analytes is strongly influenced by the degree of West African ancestry of these individuals (**Fig. 3A**). The variance in 45 of the analytes were significantly ($P < 0.05$) influenced by degree of West African ancestry (**table S5**). The levels of 42 analytes were significantly accounted for by West African ancestry even after adjusting for age, BMI, aspirin use, education, income, diabetes, and smoking status (**table S6**). CXCL5, CXCL1, MCP2, MCP1, CXCL11, CCL23, PTN, TWEAK, NCR1, IL18 and CCL17 were the top-ranked proteins (**tables S5-S6**). Adjusting the significance threshold by Bonferroni ($P_B = 0.05/82 = 0.00061$), which is the most stringent criterion to adjust for multiple testing, the relationship of the top 28 proteins with West African ancestry remained significant. For instance, 41% and 50% of the variance in the serum

levels of CXCL1 and CXCL5, respectively, was accounted for by the degree of West African ancestry (**Fig. 3A** and **tables S5-S6**). When we compared the levels of these proteins across the 3 population groups, we observed a significant African ancestry-related trend (**Fig. 3B**), with 12 of the 82 circulating immune-oncological proteins (CXCL5, CXCL1, CXCL11, MCP2, CCL17, MCP4, CD70, MMP12, PDL2, MMP7, CCL19, and ANGPT2) being significantly elevated in both Ghanaian and AA men compared to EA men (**table S7**); twelve other markers (MCP1, IL12, CCL23, CD8A, NCR1, TNFRSF4, TNFSF14, TWEAK, IL7, HGF, HO1, TNFRSF21, and ANG1) were inversely related to West African ancestry (**table S8**).

Cytokines associated with suppression of tumor immunity and chemotaxis are upregulated in men of African ancestry. Levels of many of the 82 immune-oncology markers showed a marked association with ancestry. To better define the functional implications of these population group differences, we grouped the 82 proteins into six biological processes according to Olink guidelines (**table S9**): apoptosis/cell killing, autophagy/metabolism, chemotaxis/trafficking to tumor, suppression of tumor immunity (Th2 response, tolerogenic), promotion of tumor immunity (Th1 responses), or vasculature and tissue remodeling. To gain insight on how activation of these six processes/pathways may differ by population group, we compared process/pathway sum scores between Ghanaian, AA, and EA men without prostate cancer. Of these pathways, chemotaxis, promotion of tumor immunity, and suppression of tumor immunity were significantly different in their predicted activity between AA and EA men (**Fig. 4**). AA men had significantly higher scores for chemotaxis and suppression of tumor immunity when compared to EA men, indicating higher activity in AA men, but a lower score for promotion of tumor immunity. Ghanaian men had even higher scores for chemotaxis and suppression of tumor immunity than both AA and EA men (**Fig. 4C and E**), indicating a

possible association with West African ancestry. The latter was corroborated with our finding that the chemotaxis and suppression of tumor immunity scores positively correlated with the proportion of West African ancestry within the NCI-Maryland cohort (Spearman's $\rho=0.23$, $P < 0.001$, for chemotaxis score; Spearman's $\rho=0.15$, $P < 0.001$, for suppression of immunity score). Even though apoptosis and vasculature-associated cytokines were not significantly different between EA and AA men, we found both processes to be elevated in the Ghanaian men.

Suppression of tumor immunity is associated with reduced survival of prostate cancer

patients. Next, we examined the clinical implication of our findings and assessed the association of pathway activity with survival of prostate cancer cases or controls in the NCI-Maryland study. As of the end of 2018, out of the 819 cases, there have been 202 deaths in our case population, of whom 103 (51%) had a cancer diagnosis as the recorded primary cause of death, and 28% of all deaths ($n = 57$) were directly attributed to prostate cancer. On the other hand, 99 of the 828 population controls had died by the end of 2018. Median survival follow-up for cases and controls were 8.6 and 6.7 years, respectively. With these data, we built a multivariable Cox regression model with all biological processes/pathways and adjustment for other covariables (see Methods). Among the six defined pathways, only suppression of tumor immunity showed independent association with survival of cases (**Fig. 5**). Prostate cancer patients with an increased activity of this pathway had the highest risk of death from all causes (**Fig. 5A, table S10**). Moreover, prostate cancer patients with elevated suppression of tumor immunity at diagnosis had the highest risk of prostate cancer-specific mortality, albeit marginally significant ($P=0.057$) (**Fig. 5B, table S11**). Notably, suppression of tumor immunity was not associated with all-cause mortality of population controls (**table S12**), suggesting that the association with all-cause mortality among cases might be prostate cancer-related. Lastly, prostate cancer

patients with increased suppression of tumor immunity were also significantly more likely to die from any cancer (prostate cancer or secondary cancer) following the prostate cancer diagnosis (Fig. 5C, table S13), indicating a more general predisposition to cancer in patients with a high suppression of tumor immunity score.

Elevated suppression of tumor immunity is associated with metastatic prostate cancer. To further corroborate the significance of suppression of tumor immunity in the development of lethal prostate cancer, we assessed its association with prostate cancer aggressiveness per NCCN guidelines (see Methods). Information on TNM stage was only obtainable for the NCI-Maryland prostate cancer patients, hence only these cases were scored according to the NCCN guidelines. Patients with a high suppression of tumor immunity score were at substantially increased odds of being diagnosed with regional or distant metastasis (HR 3.79, 95% CI 1.59-9.04, > median vs. ≤ median) (Table 1), consistent with the disease survival data. The data showed a significant trend in the association of elevated suppression of tumor immunity with disease aggressiveness (P trend=0.004) (Table 1). Stratified analysis by race/ethnicity revealed that high suppression of tumor immunity was associated with metastatic prostate cancer more so among AA than EA men.

Blood levels of TNFRSF9 and pleiotrophin (PTN) predict lethal prostate cancer among AA men. To identify individual drivers of the relationship between immune-oncology markers and lethal prostate cancer, we applied a cross-validated, regularized Cox regression model using eNetXplorer (see Methods). Included in this model were the 82 immune-oncology markers and 6 covariates of clinical significance (age, education, BMI, smoking history, aspirin use, and diabetes). Lasso regression ($\alpha=1$) was selected based on overall performance (fig. S6). Utilizing this method, we could not identify a robust predictive signature of lethal prostate cancer

for EA patients. However, for AA patients, a signature primarily driven by TNFRSF9 and PTN (both positively associated with the risk of lethal disease) and regular aspirin use (negatively associated with risk) were the top predictors ($P < 0.05$) based on two selection criteria: the feature frequency (**Fig. 6A**) and the weight of the features' contribution to the prediction (**Fig. 6B**). These features combined predicted prostate cancer-specific mortality with an accuracy of 83.7% (SE=3.8%). Our finding that regular aspirin use was a predictor of improved survival among AA men is consistent with previously published data from this case-control study¹⁴ and the Southern Community Cohort Study¹⁵. The two proteins alone, TNFRSF9 and PTN, predicted prostate cancer-specific mortality with 78.2% (SE=4.2%) accuracy. AA prostate cancer patients with high levels (> median) of both TNFRSF9 and PTN in their blood at diagnosis had the worst prostate cancer-specific survival (**Fig. 6C**). By 10 years, 33% of cases with high levels of both TNFRSF9 and PTN died of prostate cancer compared to only 5% of cases with low levels of both or either of these proteins (**Fig. 6C**), highlighting the utility of these blood markers for risk stratification of AA prostate cancer patients.

DISCUSSION

In this study, we describe consistent differences in the expression of immune and chemotaxis-related markers in men from three population groups, with two of them – AA and Ghanaian men – having an ancestral relationship due to the trans-Atlantic slave trade. Most notably, expression of immune-oncology markers related to immune suppression were up-regulated in men of West African ancestry and were associated with lethal prostate cancer. While ancestry can explain some of the observations, other and yet unknown factors may contribute to these clinically significant differences in immune function and chemotaxis.

Infections endemic to certain regions have shaped the immune response in affected populations, leaving a lasting genetic and epigenetic footprint³⁶. As such, population differences in exposures to fatal pathogens have led to population heterogeneity in the immunome. It has been estimated that as many as 360 immune-related genes have been targets of positive selection and have functional variations between populations³⁷. Consistent with these observations, we now report population differences in circulating immune-oncological proteins among Ghanaian, AA, and EA men. We found that the serum proteome-defined immunome of Ghanaian men resembles the immunome of AA men more so than EA men. We identified CXCL5, CXCL1, MCP2, MCP1, and CXCL11 as the top immune-oncological proteins associated with West African ancestry. Four of these chemokines (CXCL5, CXCL1, MCP1, and CXCL11) are targets of Duffy Antigen Receptor for Chemokines (DARC) binding³⁸. DARC is a non-signaling receptor that binds to both CXC and CC family of chemokines and acts as a depot for chemokines on erythrocytes and as decoy receptor on endothelial cells³⁹. DARC expression modulates the susceptibility to clinical *Plasmodium vivax* malaria and loss of its expression on erythrocytes, which frequently occurs in sub-Saharan African populations due to germline

genetic variants, confers resistance against malarial infection⁴⁰. Its loss may also influence cancer susceptibility^{41,42}. Consequently, these individuals lack the ability to sequester the target chemokines, leading to elevated concentration of the chemokines in circulation⁴³. Accordingly, we found that CXCL5, CXCL1, and CXCL11 were 2-3-fold higher in sera of Ghanaian or AA men than EA men. Given the angiogenic properties of these chemokines⁴⁴, their role in cancer progression has been proposed⁴⁵.

As a key finding, we report that serum proteins driving chemotaxis and suppression of tumor immunity were elevated in men of African ancestry, suggesting persistent population differences in stimulation of leukocyte recruitment and T cell mediated immune response. Such differences may predispose men of African descent to a distinct tumor microenvironment. Although the direct impact of the peripheral immunome on the prostate tumor microenvironment requires further investigation, we and others have previously reported stark differences in the immune landscape of prostate tumors of AA men as compared to EA men⁹⁻¹³. For instance, programmed cell death ligand-1 (PD-L1), which suppresses T cell-mediated tumor immunity, was found to be overexpressed in AA prostate tumors⁴⁶. Recent work by Awasthi *et al.* reported that AA prostate tumors tend to be enriched for immune pathways that are associated with poor clinical outcomes⁴⁷. We show with our current work that elevated, peripheral suppression of tumor immunity associates with lethal prostate cancer. Hence, population differences in suppression of tumor immunity may contribute to the disproportionate burden of lethal prostate cancer among men of African ancestry. On the other hand, such differences may offer a therapeutic advantage for immunotherapeutic strategies that are tailored to target immune suppressive pathways. A recent study provided a first indication that differences in the response to cancer vaccines may lead to higher survival rates among AA men⁴⁸.

Differentiating men who have lethal forms of prostate cancer from those with a more slow-growing disease remains a major challenge in clinical oncology. Risk stratification strategies are particularly needed for AA prostate cancer patients who disproportionately bear the prostate cancer burden. This study identified TNFRSF9 and PTN as candidate predictive blood markers for prostate cancer mortality among AA patients. AA patients with high levels of both TNFRSF9 and PTN in their sera had the highest risk of dying from prostate cancer. The membrane form of TNFRSF9 possesses antitumor properties and agonistic anti-TNFRSF9 antibodies are currently in clinical trials^{49,50}. On the contrary, the soluble isoform of TNFRSF9 that we measured, generated by alternative splicing⁵¹, has been shown to antagonize antitumor immune response hence promote tumor survival most likely by acting as decoy receptor^{52,53}. Regulatory T cells described as Tregs are thought to be a major source of secreted TNFRSF9^{54,55}. Recently, TNFRSF9 mRNA level was shown to be a robust marker of tumor-infiltrating Tregs that suppress antitumor response⁵⁶. Moreover, high numbers of TNFRSF9-expressing Tregs were associated with poor survival outcomes across multiple human cancers⁵⁶, consistent with our findings that serum TNFRSF9 associates with lethal prostate cancer. Although pleiotrophin, the second protein marker associated with lethal prostate cancer in AA men, may not have the same immune function that soluble TNFRSF9 exhibits, it is a secreted cytokine with important roles in promoting angiogenesis and metastasis⁵⁷. Recently, pleiotrophin was described as a serum-based biomarker of pro-metastatic prostate cancer⁵⁸, consistent with our findings in this study.

To the best of our knowledge, this is the first study with a large representation of men of African descent who contributed to immune-oncological proteome profiling. With the advent of increasing immunotherapies in the drug development pipeline, such studies may inform research

on population differences in the immune landscape that need to be considered when designing therapies that exploit the immune response. Furthermore, our study may provide unique insights into variations in the manifestation and pathogenesis of different immune related diseases among different population groups.

In conclusion, it is a key finding of our study that suppression of tumor immunity was increased in Ghanaian and AA men, when compared to EA men, and associates with lethal prostate cancer. As such, these findings provide a novel insight into potential causes of the prostate cancer health disparity.

MATERIALS and METHODS

NCI-Maryland prostate cancer case-control study. This study and the eligibility criteria have been previously described^{14,59}. Race/ethnicity was assigned based on self-identification as either black or AA or as Caucasian or EA. The study was initiated to test the primary hypothesis that environmental exposures and ancestry-related factors contribute to the excessive prostate cancer burden among AA men. The study was approved by the NCI (protocol # 05-C-N021) and the University of Maryland (protocol #0298229) Institutional Review Boards and all participants signed an informed consent. Cases were recruited at the Baltimore Veterans Affairs Medical Center and the University of Maryland Medical Center. A total of 976 cases (489 AA and 487 EA men) were recruited into this study between 2005 and 2015. Controls were identified through the Maryland Department of Motor Vehicle Administration database and were frequency-matched to cases on age and race. A total of 1,034 population controls were recruited (486 AA and 548 EA men). At the time of enrollment, both cases and controls were administered a survey by a trained interviewer and a blood sample was collected. Serum samples were available for 846 cases (407 AA and 439 EA) and 846 controls (382 AA and 464 EA), therefore only these individuals were used for the study herein. Most of the 846 cases (85%) were recruited within a year of the disease diagnosis with a median of 5.1 months between disease diagnosis and blood collection.

NCI-Ghana prostate cancer case-control study. This case-control study has been previously described²⁰. The study was designed to study lifestyle, environmental, and genetic risk factors for prostate cancer in African men. The study was approved by institutional review boards at the University of Ghana (protocol #001/01-02) and at the National Cancer Institute (protocol #02CN240). Prior to study enrollment, all participants signed an informed consent. Prostate

cancer cases were recruited at Korle Bu Teaching Hospital in Accra, Ghana between 2008 and 2012. The cases were diagnosed using Digital Rectal Exam (DRE) and PSA tests, followed by biopsy confirmation. Immediately after diagnosis and before treatment, cases were consented and asked to submit blood specimen and questionnaire data. Controls were identified through probability sampling using the 2000 Ghana Population and Housing Census data to recruit approximately 1,000 men aged 50–74 years in the Greater Accra region between 2004 and 2006. These men were confirmed to not have prostate cancer by PSA testing and DRE. Serum samples were available for 586 prostate cancer cases and 659 population controls; hence, only these individuals were used for the study herein.

Serum sample processing. The participants in the two studies provided blood samples at time of recruitment. For the NCI-Maryland study, most blood samples were processed the same day, but always within 48 hours, after storage in a refrigerator. For the NCI-Ghana study, blood samples were processed within 6 hours. In this study, population controls provided fasting blood. Serum was prepared using standard procedures and aliquots were stored at -80°C . Serum samples were shipped from Ghana to the NCI in dry ice boxes.

Serum protein measurement. Serum levels of 92 immuno-oncology panel proteins were measured simultaneously using a proprietary multiplex Proximal Extension Assay (PEA) by Olink Proteomics (Boston). Olink utilizes a relative quantification unit, Normalized Protein eXpression (NPX), which is in a Log₂-format. Serum samples from NCI-MD study (846 cases and 846 controls) and NCI-Ghana study (586 cases and 659 controls) were completely randomized and were assayed in that order. In addition to the built-in internal controls, 5% blinded duplicates were randomly selected and were randomized along with the original set of samples. Protein levels were intensity normalized to adjust for batch effect. Because all our

samples were randomized across plates, a global adjustment was used to center the values for each assay around its median and across all plates. Ninety-five percent of the samples passed a stringent quality control (NCI-MD study: 819 cases and 828 controls; NCI-Ghana study: 489 cases and 654 controls) – with coefficients of variation (CV) among duplicates at < 10% for every marker. Out of the 92 proteins assayed, IL33, IL35, IL21, IL2, IFN β , IL13, IL1 α , CXCL12, IFN γ , and TNF were detected in less than 20% of the samples, hence the remaining 82 proteins were used for subsequent analysis (**table S2**).

Functional annotation and biological processes scores. Proteins were grouped into six biological processes based on their respective biological roles following the Olink guideline: apoptosis/cell killing, autophagy/metabolism, chemotaxis/trafficking to tumor, suppression of tumor immunity (Th2 response, tolerogenic), promotion of tumor immunity (Th1 responses), or vasculature and tissue remodeling. Apoptosis, autophagy, chemotaxis, suppression of tumor immunity, promotion of tumor immunity, or vasculature scores were calculated for each study participant as the mean z-score value for the proteins belonging to the respective biological process. For survival analysis, the biological process/pathway scores were evaluated as continuous variables. To evaluate the association of suppression of tumor immunity with aggressive prostate cancer, we grouped suppression of tumor immunity scores into low ($\leq$ median) and high ($>$ median) with cutoffs determined using the distribution of the score among population controls of the NCI-Maryland study.

Prostate Specific Antigen (PSA) measurement. For the cases in the NCI-Maryland cohort, PSA levels were obtained from medical record. For the controls of the NCI-Maryland study, total PSA was measured from stored serum aliquots using the human total PSA ELISA Kit (Abcam, ab188388). About 7% (n=56) of the controls in the NCI-Maryland cohort had PSA greater than

426 2.5 ng/ml, while only 3% (n=27) had blood PSA over 4 ng/ml. For the controls in the NCI-
427 Ghana study, close to 20% (n=132) had a PSA greater than 2.5 ng/ml, while about 11% (n=73)
428 had PSA over 4 ng/ml.

429 ***C-reactive protein (CRP) measurement.*** Plasma CRP was assayed using an ELISA assay (cat#
430 ab99995, Abcam, United States) according to the manufacturer's instructions. Two microliters of
431 plasma samples were added to 398 μ L of 1x Diluent D, followed by a second 1: 200 dilution
432 steps for each sample. One-hundred microliters of CRP standard (0 to 600 pg/mL) and the
433 diluted samples were loaded as duplicates into pre-coated 96-well plates. Samples were
434 incubated overnight at 4°C with gentle shaking, followed by incubations with the anti-human
435 CRP antibody and the horseradish peroxidase-streptavidin solution. CRP was quantified
436 measuring absorbance at 450 nm with a microplate reader.

437 ***West African ancestry estimation for participants in the NCI-Maryland case-control study.***

438 Genomic DNA was isolated from buffy coats (DNeasy Blood & Tissue Kit - Qiagen) or
439 mouthwash samples (standard phenol-chloroform technique). Isolated DNA was genotyped for
440 100 ancestry informative markers using the Sequenom MassARRAY iPLEX platform, as
441 previously described³⁵. Single nucleotide polymorphism genotype calls were generated using
442 Sequenom TYPER software. A genotype concordance rate of > 99% was observed for all
443 markers. Admixture estimates for each study participant were calculated using a model-based
444 clustering method as implemented in the program STRUCTURE v2.3. We applied
445 STRUCTURE v2.3 with an admixture model estimating K (number of sub populations) from 2
446 to 5 with 100 iterations and parental population genotypes from West Africans, Europeans, and
447 Native Americans, yielding three admixture estimations (West African, European, Native
448 American). For a subset (83%) of the cases and controls, additional West African ancestry

estimates were provided by the Cancer Genomics Research Laboratory/NCI-Leidos from a genome-wide association study using the Infinium HumanOmni5-Quad BeadChip array. West African ancestry estimates using the two approaches were very similar ($r=0.98$).

Association of clinical/socio-demographic characteristics with immune-oncological

proteins. The association of age, body mass index (BMI), education, aspirin use, smoking, diabetes, and PSA levels with the relative abundance of individual analytes was assessed by means of multivariable linear regression models implemented by the function `lm` in the base R package `stats` (version 3.6.1). These variables were chosen because they have either been linked to prostate cancer risk and survival or may influence the status of inflammation and host immunity. An analyte (as response variable) was considered significantly associated with clinical and socio-demographic covariables if the multivariable model yielded $P < 0.05$ on the F-statistic. If this condition was satisfied, the association between the target analyte and each individual covariable was characterized by the corresponding P value and coefficient.

Analysis of variance. Variance analysis for the levels of each of the 82 immune-oncological cytokines were simultaneously assessed as a function of genetic estimation of West African admixture among men without prostate cancer from the NCI-Maryland study. The analysis was implemented by the function `aov` in the base R package `stats` (version 3.6.1).

Heatmap plots. Heat map plots were generated using Broad Institute's web-based matrix visualization and analysis platform - Morpheus (<https://software.broadinstitute.org/morpheus>). To avoid spurious effects from outliers in heatmap plots, each protein's range of abundance values were set to saturate at the 1st and 99th percentiles. To account for widely different

abundance ranges for different proteins in the assay, each protein's measured abundances across the cohort were Z-score transformed.

Gene ontology (GO) enrichment analysis. GO terms with an enrichment in proteins of interest were identified using Over-Representation Analysis (ORA) as part of the web tool WebGestalt (WEB-based Gene SeT AnaLysis Toolkit). Enriched gene sets were further processed using affinity propagation (R package apcluster) to cluster gene sets according to functional similarity.

Survival analysis. Information on patient survival was only obtainable for the NCI-Maryland prostate cancer patients. Survival data was obtained from the National Death Index (NDI) database. We calculated survival for cases from date of diagnosis to either date of death or to the censor date of December 31, 2018. We built a multivariable Cox regression model with all biological processes scores and adjustment for other covariables to estimate adjusted hazard ratios (HR) and 95% confidence intervals (CI) for all-cause mortality, cancer-related mortality, and prostate cancer-specific mortality of cases. We adjusted for the following potential confounding factors: age at study entry (years), body-mass index (BMI, kg/m²), self-reported race (AA/EA), education (high school or less, some college, college, professional school), income (less than \$10k, \$10-30K, \$30-60K, \$60-90k, greater than \$90k), smoking history (never, former, current), diabetes (no/yes), aspirin use (no/yes), and treatment (0=none, 1=surgery, 2=radiotherapy, 3=hormone, 4=combination). Missing values for education (n=1), smoking history (n=5), and income (n=63) were imputed using the R package missForest, which implements nonparametric missing value imputation based on random forests. In the overall survival analysis of population controls, we calculated survival from the date of interview to either date of death or to the censor date of December 31st, 2018. We applied the Cox regression model to estimate adjusted HR and 95% CI and adjusted for all the confounding factors listed

above except for treatment. Missing values for education (n=1), smoking history (n=7), and income (n=67) were imputed using the R package missForest. The reported HRs indicate the change in risk of dying when the biological process z-score value increases by 1 while holding all the other biological processes' z-scores and covariates constant.

Classification of cases using National Comprehensive Cancer Network (NCCN) Risk Score.

Cases were assigned to risk groups based on the patients' TNM stage, Gleason score, Gleason pattern, and PSA level at diagnosis according to the 2019 NCCN guideline for prostate cancer⁶⁰. Information on TNM stage was only obtainable for the NCI-Maryland prostate cancer patients, hence only these cases were scored. Cases were categorized as localized, regional, and metastatic prostate cancer based on their clinical parameters at the time of diagnosis. Localized prostate cancer cases were further classified into low, intermediate, high, and very high risk based on the likelihood of their disease to progress to lethal prostate cancer per the 2019 NCCN guideline⁶⁰. Prostate cancer cases with lymph node involvement but no distant metastasis at diagnosis were classified as regional prostate cancer while those with distant metastasis at the time of diagnosis were classified as metastatic prostate cancer. For our analysis, we condensed these risk groups into 4 categories (low, intermediate, high/very high, and regional/metastatic).

Developing a predictive proteomic signature of lethal prostate cancer. The analysis was restricted to the cases from NCI-Maryland study for whom we had survival data. We stratified by race/ethnicity into AA cases (360 censored, 34 prostate cancer deaths) and EA cases (402 censored, 23 prostate cancer deaths). To identify a multi-analyte proteomic signature that is predictive of lethal prostate cancer, 88 features were evaluated [82 immune-oncological proteins along with six demographic/clinical variables (education, age, BMI (BMI, kg/m²), smoking history, diabetes, and aspirin use)]. Missing values for education (n=1) and smoking history

(n=5) were imputed using R package missForest. R package eNetXplorer (version 1.1.2)⁶¹ was implemented to build cross-validated, regularized Cox regression models with different elastic net mixture parameters from ridge (alpha=0) to lasso (alpha=1). Alpha was selected based on overall performance assessed as a function of the 5-fold cross-validated quality function (concordance) and the empirical *P* value generated from comparing the model against a statistical ensemble of null models created by random permutations of the response (i.e. survival time/status randomized across subjects in the cohort). These results comprise 10,000 Cox regression elastic net realizations arising from 200 randomly generated folds, each of them compared against 50 null model permutations. Features' performance as predictors was evaluated using two different, but complementary selection criteria: feature coefficients and feature frequencies. The feature frequency measure captures the significance of how often a feature is chosen in an in-bag model. When it is chosen, the feature coefficient measure captures the significance of the feature's weight in the in-bag model. See the publication by *Candia et al* for more details on this method⁶¹. Using only the significant protein features from both selection criteria, a multivariate Cox regression model was run. Risk stratification was used to generate Kaplan-Meier plots and log-rank tests of significance.

Statistical analysis. Data analyses were performed using Stata/SE 16.0 and R statistical software packages. All statistical tests were two-sided, and an association was considered statistically significant with $P < 0.05$.

Supplementary Materials:

Fig. S1. Variance explained by the inter-plate batch effect
Fig. S2. Detection frequencies of 92 immuno-oncological markers measured in 2937 serum samples
Fig. S3. Correlation matrix presenting Pearson pairwise correlations for each of the 82 serum protein pairs
Fig. S4. Gene ontology (GO) enrichment analysis
Fig. S5. Functional enrichment analysis of proteins positively associated with diabetes in men of African ancestry
Fig. S6. Performance of regularized cox regression models across alpha
Table S1. Characteristics of prostate cancer cases and population controls of the NCI-Maryland and NCI-Ghana Study
Table S2. List of 82 Immuno-oncological proteins detected in more than 20% of the serum samples
Table S3. Top 10 Pearson pairwise correlations in men without prostate cancer
Table S4. The association of blood CRP with clinical/sociodemographic variables estimated using multiple linear regression
Table S5. The fraction of variance in each of the serum proteins explained by degree of West African ancestry
Table S6. The fraction of variance in each of the serum proteins explained by degree of West African ancestry after adjusting for difference in age, bmi, aspirin use, education, diabetes status, smoking, and income
Table S7. Immune oncological markers that are significantly elevated in both Af and AA men compared to EA men
Table S8. Immune oncological markers that are significantly downregulated in both Af and AA men compared to EA men
Table S9. Serum proteins grouped by biological process
Table S10. Effect of biological processes scores on all-cause mortality of prostate cancer patients
Table S11. Effect of biological processes scores on prostate cancer-specific mortality
Table S12. Effect of biological processes scores on all-cause mortality of population controls
Table S13. Effect of biological processes scores on mortality from any cancer following a diagnosis with prostate cancer

Acknowledgments: We would like to thank personnel at the University of Maryland and the Baltimore Veterans Administration Hospital for their contributions with the recruitment of participants to the NCI-Maryland study. We would also like to thank Prof. Edward D. Yeboah as the original Ghana PI and Ms. Evelyn Tay as the original Study Manager for the NCI-Ghana study.

Funding:

DoD award W81XWH1810588 (to SA, CY)
U54 CA118623- CY (NCI) and U54-MD007585-26-CY (NIMHD) (to CY)
Intramural Research Program of the NIH, National Cancer Institute (NCI), Center for Cancer Research and Division of Cancer Epidemiology and Genetics (to SA, MBC)

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597 MBC, SA
598
599 **Conflicts of interest:** The authors declare that they have no competing interests.
600
601 **Data and materials availability:** All data are available in the main text or the supplementary
602 materials.

REFERENCES

- 1 Powell, I. J. Epidemiology and pathophysiology of prostate cancer in African-American men. *J Urol* **177**, 444-449 (2007).
- 2 Rebbeck, T. R. *et al.* Global patterns of prostate cancer incidence, aggressiveness, and mortality in men of african descent. *Prostate Cancer* **2013**, 560857 (2013).
- 3 Culp, M. B., Soerjomataram, I., Efstathiou, J. A., Bray, F. & Jemal, A. Recent Global Patterns in Prostate Cancer Incidence and Mortality Rates. *Eur Urol* **77**, 38-52, doi:10.1016/j.eururo.2019.08.005 (2020).
- 4 Butler, E. N., Kelly, S. P., Coupland, V. H., Rosenberg, P. S. & Cook, M. B. Fatal prostate cancer incidence trends in the United States and England by race, stage, and treatment. *Br J Cancer* **123**, 487-494, doi:10.1038/s41416-020-0859-x (2020).
- 5 Heyns, C. F., Fisher, M., Lecuona, A. & van der Merwe, A. Prostate cancer among different racial groups in the Western Cape: presenting features and management. *S Afr Med J* **101**, 267-270, doi:10.7196/samj.4420 (2011).
- 6 Wallace, T. A., Martin, D. N. & Ambs, S. Interactions among genes, tumor biology and the environment in cancer health disparities: examining the evidence on a national and global scale. *Carcinogenesis* **32**, 1107-1121 (2011).
- 7 Rebbeck, T. R. Prostate Cancer Disparities by Race and Ethnicity: From Nucleotide to Neighborhood. *Cold Spring Harb Perspect Med* **8**, doi:10.1101/cshperspect.a030387 (2018).
- 8 Lachance, J. *et al.* Genetic Hitchhiking and Population Bottlenecks Contribute to Prostate Cancer Disparities in Men of African Descent. *Cancer Res* **78**, 2432-2443, doi:10.1158/0008-5472.CAN-17-1550 (2018).
- 9 Hardiman, G. *et al.* Systems analysis of the prostate transcriptome in African-American men compared with European-American men. *Pharmacogenomics* **17**, 1129-1143, doi:10.2217/pgs-2016-0025 (2016).
- 10 Powell, I. J. *et al.* Genes associated with prostate cancer are differentially expressed in African American and European American men. *Cancer Epidemiol Biomarkers Prev* **22**, 891-897, doi:10.1158/1055-9965.EPI-12-1238 (2013).
- 11 Wallace, T. A. *et al.* Tumor immunobiological differences in prostate cancer between African-American and European-American men. *Cancer Res* **68**, 927-936, doi:10.1158/0008-5472.CAN-07-2608 (2008).
- 12 Yuan, J. *et al.* Integrative comparison of the genomic and transcriptomic landscape between prostate cancer patients of predominantly African or European genetic ancestry. *PLoS Genet* **16**, e1008641, doi:10.1371/journal.pgen.1008641 (2020).
- 13 Tang, W. *et al.* IFNL4-DeltaG Allele Is Associated with an Interferon Signature in Tumors and Survival of African-American Men with Prostate Cancer. *Clin Cancer Res* **24**, 5471-5481, doi:10.1158/1078-0432.CCR-18-1060 (2018).
- 14 Smith, C. J. *et al.* Aspirin Use Reduces the Risk of Aggressive Prostate Cancer and Disease Recurrence in African-American Men. *Cancer Epidemiol Biomarkers Prev* **26**, 845-853, doi:10.1158/1055-9965.EPI-16-1027 (2017).
- 15 Tang, W. *et al.* Aspirin Use and Prostate Cancer among African American Men in the Southern Community Cohort Study. *Cancer Epidemiol Biomarkers Prev*, doi:10.1158/1055-9965.EPI-19-0792 (2020).

- 16 Nedelec, Y. *et al.* Genetic Ancestry and Natural Selection Drive Population Differences in Immune Responses to Pathogens. *Cell* **167**, 657-669 e621, doi:10.1016/j.cell.2016.09.025 (2016).
- 17 Ness, R. B., Haggerty, C. L., Harger, G. & Ferrell, R. Differential distribution of allelic variants in cytokine genes among African Americans and White Americans. *Am J Epidemiol* **160**, 1033-1038, doi:10.1093/aje/kwh325 (2004).
- 18 Rotival, M., Quach, H. & Quintana-Murci, L. Defining the genetic and evolutionary architecture of alternative splicing in response to infection. *Nat Commun* **10**, 1671, doi:10.1038/s41467-019-09689-7 (2019).
- 19 Van Dyke, A. L., Cote, M. L., Wenzlaff, A. S., Land, S. & Schwartz, A. G. Cytokine SNPs: Comparison of allele frequencies by race and implications for future studies. *Cytokine* **46**, 236-244, doi:10.1016/j.cyto.2009.02.003 (2009).
- 20 Cook, M. B. *et al.* A genome-wide association study of prostate cancer in West African men. *Hum Genet* **133**, 509-521, doi:10.1007/s00439-013-1387-z (2014).
- 21 Nikolich-Zugich, J. The twilight of immunity: emerging concepts in aging of the immune system. *Nat Immunol* **19**, 10-19, doi:10.1038/s41590-017-0006-x (2018).
- 22 Ferretti, E., Pistoia, V. & Corcione, A. Role of fractalkine/CX3CL1 and its receptor in the pathogenesis of inflammatory and malignant diseases with emphasis on B cell malignancies. *Mediators Inflamm* **2014**, 480941, doi:10.1155/2014/480941 (2014).
- 23 Zavada, J., Zavadova, Z., Zat'ovicova, M., Hyrs, L. & Kawaciuk, I. Soluble form of carbonic anhydrase IX (CA IX) in the serum and urine of renal carcinoma patients. *Br J Cancer* **89**, 1067-1071, doi:10.1038/sj.bjc.6601264 (2003).
- 24 Supuran, C. T. Carbonic anhydrases: novel therapeutic applications for inhibitors and activators. *Nat Rev Drug Discov* **7**, 168-181, doi:10.1038/nrd2467 (2008).
- 25 Erra Diaz, F., Dantas, E. & Geffner, J. Unravelling the Interplay between Extracellular Acidosis and Immune Cells. *Mediators Inflamm* **2018**, 1218297, doi:10.1155/2018/1218297 (2018).
- 26 Alessandrini, F., Pezze, L. & Ciribilli, Y. LAMPs: Shedding light on cancer biology. *Semin Oncol* **44**, 239-253, doi:10.1053/j.seminoncol.2017.10.013 (2017).
- 27 Rabinovich, G. A. & Toscano, M. A. Turning 'sweet' on immunity: galectin-glycan interactions in immune tolerance and inflammation. *Nat Rev Immunol* **9**, 338-352, doi:10.1038/nri2536 (2009).
- 28 Arnson, Y., Shoenfeld, Y. & Amital, H. Effects of tobacco smoke on immunity, inflammation and autoimmunity. *J Autoimmun* **34**, J258-265, doi:10.1016/j.jaut.2009.12.003 (2010).
- 29 Wada, J. & Makino, H. Innate immunity in diabetes and diabetic nephropathy. *Nat Rev Nephrol* **12**, 13-26, doi:10.1038/nrneph.2015.175 (2016).
- 30 Lee, Y. S. *et al.* The fractalkine/CX3CR1 system regulates beta cell function and insulin secretion. *Cell* **153**, 413-425, doi:10.1016/j.cell.2013.03.001 (2013).
- 31 Shah, R. *et al.* Fractalkine is a novel human adipochemokine associated with type 2 diabetes. *Diabetes* **60**, 1512-1518, doi:10.2337/db10-0956 (2011).
- 32 Navarro-Gonzalez, J. F., Mora-Fernandez, C., Muros de Fuentes, M. & Garcia-Perez, J. Inflammatory molecules and pathways in the pathogenesis of diabetic nephropathy. *Nat Rev Nephrol* **7**, 327-340, doi:10.1038/nrneph.2011.51 (2011).

- 33 Liu, Z. Q. *et al.* Prognostic role of C-reactive protein in prostate cancer: a systematic review and meta-analysis. *Asian J Androl* **16**, 467-471, doi:10.4103/1008-682X.123686 (2014).
- 34 Saito, K. & Kihara, K. C-reactive protein as a biomarker for urological cancers. *Nat Rev Urol* **8**, 659-666, doi:10.1038/nrrol.2011.145 (2011).
- 35 Al-Alem, U. *et al.* Association of genetic ancestry with breast cancer in ethnically diverse women from Chicago. *PLoS One* **9**, e112916, doi:10.1371/journal.pone.0112916 (2014).
- 36 Rotimi, C. N. *et al.* The genomic landscape of African populations in health and disease. *Hum Mol Genet* **26**, R225-R236, doi:10.1093/hmg/ddx253 (2017).
- 37 Barreiro, L. B. & Quintana-Murci, L. From evolutionary genetics to human immunology: how selection shapes host defence genes. *Nat Rev Genet* **11**, 17-30, doi:10.1038/nrg2698 (2010).
- 38 Gardner, L., Patterson, A. M., Ashton, B. A., Stone, M. A. & Middleton, J. The human Duffy antigen binds selected inflammatory but not homeostatic chemokines. *Biochem Biophys Res Commun* **321**, 306-312, doi:10.1016/j.bbrc.2004.06.146 (2004).
- 39 Mantovani, A., Bonecchi, R. & Locati, M. Tuning inflammation and immunity by chemokine sequestration: decoys and more. *Nat Rev Immunol* **6**, 907-918, doi:10.1038/nri1964 (2006).
- 40 Miller, L. H., Mason, S. J., Clyde, D. F. & McGinniss, M. H. The resistance factor to Plasmodium vivax in blacks. The Duffy-blood-group genotype, FyFy. *N Engl J Med* **295**, 302-304, doi:10.1056/NEJM197608052950602 (1976).
- 41 Jenkins, B. D. *et al.* Atypical Chemokine Receptor 1 (DARC/ACKR1) in Breast Tumors Is Associated with Survival, Circulating Chemokines, Tumor-Infiltrating Immune Cells, and African Ancestry. *Cancer Epidemiol Biomarkers Prev* **28**, 690-700, doi:10.1158/1055-9965.EPI-18-0955 (2019).
- 42 Martini, R. *et al.* Investigation of Triple-Negative Breast Cancer Risk Alleles in An International African-Enriched Cohort. *scientific reports* **Preprint at: <https://www.researchsquare.com/article/rs-109841/v1>** (2021).
- 43 Yao, S. *et al.* Genetic ancestry and population differences in levels of inflammatory cytokines in women: Role for evolutionary selection and environmental factors. *PLoS Genet* **14**, e1007368, doi:10.1371/journal.pgen.1007368 (2018).
- 44 Strieter, R. M. *et al.* The functional role of the ELR motif in CXC chemokine-mediated angiogenesis. *J Biol Chem* **270**, 27348-27357, doi:10.1074/jbc.270.45.27348 (1995).
- 45 Bikfalvi, A. & Billottet, C. The CC and CXC chemokines: major regulators of tumor progression and the tumor microenvironment. *Am J Physiol Cell Physiol* **318**, C542-C554, doi:10.1152/ajpcell.00378.2019 (2020).
- 46 Calagua, C. *et al.* Expression of PD-L1 in Hormone-naïve and Treated Prostate Cancer Patients Receiving Neoadjuvant Abiraterone Acetate plus Prednisone and Leuprolide. *Clin Cancer Res* **23**, 6812-6822, doi:10.1158/1078-0432.CCR-17-0807 (2017).
- 47 Awasthi, S. *et al.* Comparative Genomics Reveals Distinct Immune-oncologic Pathways in African American Men with Prostate Cancer. *Clin Cancer Res* **27**, 320-329, doi:10.1158/1078-0432.CCR-20-2925 (2021).
- 48 Sartor, O. *et al.* Survival of African-American and Caucasian men after sipuleucel-T immunotherapy: outcomes from the PROCEED registry. *Prostate Cancer Prostatic Dis* **23**, 517-526, doi:10.1038/s41391-020-0213-7 (2020).

- 49 Segal, N. H. *et al.* Phase I Study of Single-Agent Utomilumab (PF-05082566), a 4-1BB/CD137 Agonist, in Patients with Advanced Cancer. *Clin Cancer Res* **24**, 1816-1823, doi:10.1158/1078-0432.CCR-17-1922 (2018).
- 50 Qi, X. *et al.* Optimization of 4-1BB antibody for cancer immunotherapy by balancing agonistic strength with FcγR affinity. *Nat Commun* **10**, 2141, doi:10.1038/s41467-019-10088-1 (2019).
- 51 Michel, J., Langstein, J., Hofstadter, F. & Schwarz, H. A soluble form of CD137 (ILA/4-1BB), a member of the TNF receptor family, is released by activated lymphocytes and is detectable in sera of patients with rheumatoid arthritis. *Eur J Immunol* **28**, 290-295, doi:10.1002/(SICI)1521-4141(199801)28:01<290::AID-IMMU290>3.0.CO;2-S (1998).
- 52 Michel, J. & Schwarz, H. Expression of soluble CD137 correlates with activation-induced cell death of lymphocytes. *Cytokine* **12**, 742-746, doi:10.1006/cyto.1999.0623 (2000).
- 53 Labiano, S. *et al.* Hypoxia-induced soluble CD137 in malignant cells blocks CD137L-costimulation as an immune escape mechanism. *Oncoimmunology* **5**, e1062967, doi:10.1080/2162402X.2015.1062967 (2016).
- 54 Itoh, A. *et al.* Soluble CD137 Ameliorates Acute Type 1 Diabetes by Inducing T Cell Anergy. *Front Immunol* **10**, 2566, doi:10.3389/fimmu.2019.02566 (2019).
- 55 Kachapati, K. *et al.* The B10 Idd9.3 locus mediates accumulation of functionally superior CD137(+) regulatory T cells in the nonobese diabetic type 1 diabetes model. *J Immunol* **189**, 5001-5015, doi:10.4049/jimmunol.1101013 (2012).
- 56 Freeman, Z. T. *et al.* A conserved intratumoral regulatory T cell signature identifies 4-1BB as a pan-cancer target. *J Clin Invest* **130**, 1405-1416, doi:10.1172/JCI128672 (2020).
- 57 Papadimitriou, E. *et al.* Pleiotrophin and its receptor protein tyrosine phosphatase beta/zeta as regulators of angiogenesis and cancer. *Biochim Biophys Acta* **1866**, 252-265, doi:10.1016/j.bbcan.2016.09.007 (2016).
- 58 Liu, S. *et al.* Discovery of PTN as a serum-based biomarker of pro-metastatic prostate cancer. *Br J Cancer* **124**, 896-900, doi:10.1038/s41416-020-01200-0 (2021).
- 59 Minas, T. Z. *et al.* IFNL4-DeltaG is associated with prostate cancer among men at increased risk of sexually transmitted infections. *Commun Biol* **1**, 191, doi:10.1038/s42003-018-0193-5 (2018).
- 60 Network, N. C. C. The NCCN Clinical Practice Guidelines in Oncology for Prostate Cancer, V4.2019. https://www.nccn.org/professionals/physician_gls/default.aspx#prostate (Accessed November 16, 2020).
- 61 Candia, J. & Tsang, J. S. eNetXplorer: an R package for the quantitative exploration of elastic net families for generalized linear models. *BMC Bioinformatics* **20**, 189, doi:10.1186/s12859-019-2778-5 (2019).

Fig. 1

Fig. 1. Association of socio-demographic and clinical characteristics with systemic immune-oncological proteins in Ghanaian (Af), AA, and EA men without prostate cancer. Association of the 82 immuno-oncological proteins with age, BMI, education, aspirin use, smoking, diabetes, and PSA was assessed in men without prostate cancer using a multivariable linear regression model. An analyte was considered significantly associated with clinical and socio-demographic covariables if the multivariable model yielded a $P < 0.05$ on the F-statistic. Analytes that did not have a significant association with any of the clinical/sociodemographic variables in at least one of the population groups are not presented in the heatmap. Blue represents negative association while red represents positive association. The significance level (P value-based) for each association is color-coded. TI = tumor immunity.

Fig. 2

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

Fig. 3

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

Fig. 4

Fig. 4. Population differences in the abundance of proteins driving (A) apoptosis, (B) autophagy, (C) chemotaxis, (D) promotion of tumor immunity, (E) suppression of tumor immunity, and (F) vasculature. Heatmaps showing levels of process/pathway-associated proteins in relationship to population group [Ghanaian (Af), AA, EA]. Shown to the right are the mean score differences for these processes/pathways among the three population groups. Profiles for Ghanaian (n=654), AA (n=374), and EA (n=454) men without prostate cancer. The process/pathway scores are derived from the average Z-scores of all the associated proteins. These scores are shown as violin plots. TI = tumor immunity.

Fig. 5. Suppression of the tumor immunity pathway associates with lethal prostate cancer. We assessed the association of the six pathways defined by the 82 immune-oncology markers with all-cause mortality, prostate cancer-specific mortality or mortality due to any cancer after a prostate cancer diagnosis. The pathway scores were evaluated as continuous predictor variables. Suppression of tumor immunity pathway was distinctively associated with all-cause mortality **(A)**, prostate cancer-specific mortality **(B)**, or a mortality due to any cancer after a prostate cancer diagnosis **(C)**. Multivariable cox regression analyses were used to assess if the pathways were independently associated with survival of prostate cancer patients in the NCI-Maryland study. We adjusted for the following potential confounding factors: age at study entry (years), body-mass index (BMI, kg/m²), self-reported race (AA/EA), education (high school or less, some college, college, professional school), income (less than \$10k, \$10-30K, \$30-60K, \$60-90k, greater than \$90k), smoking history (never, former, current), diabetes (no/yes), aspirin use (no/yes), and treatment (0=none, 1=surgery, 2=radiotherapy, 3=hormone, 4=combination). The hazard ratios (HR) indicate the change in risk of dying when the biological process z-score value increases by 1 while holding all the other biological processes' z-scores and covariates constant.

Fig. 6

Fig. 6. A signature of two serum markers is predictive of lethal prostate cancer in AA patients. Cross-validated, regularized Cox regression models with different elastic net mixture parameters from ridge ($\alpha=0$) to lasso ($\alpha=1$) were implemented to identify a predictive proteomic signature. **(A)** Heatmaps of feature frequencies across α . Features were ranked by P value for $\alpha=1$. **(B)** Heatmaps of feature coefficients across α . Features were ranked by P value for $\alpha=1$. **(C)** Kaplan-Meier plot comparing prostate cancer-specific mortality of AA cases with high levels (> median) of both TNFRSF9 and PTN (pleiotrophin) vs. low levels of either or both proteins. Log rank test was used to determine if there were statistically significant survival differences. Adjusted hazard ratio (HR) compares the risk of prostate cancer mortality for those with high levels of both TNFRSF9 and PTN vs. the remaining AA cases. HR estimates were adjusted for potential confounding factors: age, BMI, education, smoking history, diabetes status, aspirin use, treatment, and income. In **B** & **C**, P value significance was coded as <0.001 (***), <0.01 (**), <0.05 (*), and <0.1 (.).

Table 1. A high score for suppression of tumor immunity associates with National Comprehensive Cancer Network (NCCN) Risk Score for metastatic prostate cancer

NCCN Risk Score	Total OR (95% CI)*	AA OR (95% CI)*	EA OR (95% CI)*
Low	Ref	Ref	Ref
Intermediate	1.04 (0.68-1.59)	0.89 (0.46-1.70)	1.18 (0.65, 2.13)
High/Very High	1.47 (0.87-2.48)	1.33 (0.59-2.98)	1.72 (0.83, 3.54)
Regional/Metastatic	3.79 (1.59-9.04)	5.90 (1.43-24.34)	3.16 (0.95, 10.50)
P value for trend	0.004	0.019	0.040

Note: Bolded data indicate significant associations in the logistic regression analysis.

*Logistic regression adjusted for age at study entry, BMI (kg/m²), diabetes (no/yes), aspirin (no/yes), education (high school or less, some college, college, professional school), family history of prostate cancer (first-degree relatives, yes/no), self-reported race (not included in the stratified analysis), income (less than \$10k, \$10-30K, \$30-60K, \$60-90k, greater than \$90k), smoking history (never, former, current), treatment (0=none, 1=surgery, 2=radiation, 3=hormone, 4=combination)

High suppression of tumor immunity is defined by the median score in the NCI-Maryland control population (> median vs. ≤ median)

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