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PRINCIPAL INVESTIGATOR: Stephen J. Freedland, MD

CONTRACTING ORGANIZATION: Cedars-Sinai Medical Center

Los Angeles, CA 90048

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Stephen J. Freedland, MD, Adriana C. Vidal, PhD

E-Mail: Stephen.freedland@cshs.org

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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Cedars-Sinai Medical Center
8631 W 3rd St, Suite 430E
Los Angeles, Ca 90048

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14. ABSTRACT

Black men have a 67% higher prostate cancer (PC) risk than white men and over twice the mortality. While in general, black men have reduced access to care, within an equal access setting, we found they had higher grade disease at diagnosis and higher risk of cancer recurrence after surgery. These findings suggest underlying biological differences between black and white men. Recent data suggest differences in how the immune system and tumor interact may contribute to the more aggressive disease among black men. However, there are surprisingly limited data on inflammation and PC risk in black men despite compelling data suggesting inflammation varies by race. Nearly all the data linking inflammation and PC risk was derived from studies of largely white men. Our study will fill two major voids in the literature: 1) how inflammation links with PC and 2) how race links with inflammation. We will characterize and test which systemic and prostatic inflammatory markers are linked with PC risk and aggressiveness in black and white men, leading to the development of inflammatory-based biomarker panels to detect those most likely to have aggressive disease aiding risk stratification of whom to biopsy and treat; key PCRP focus areas.

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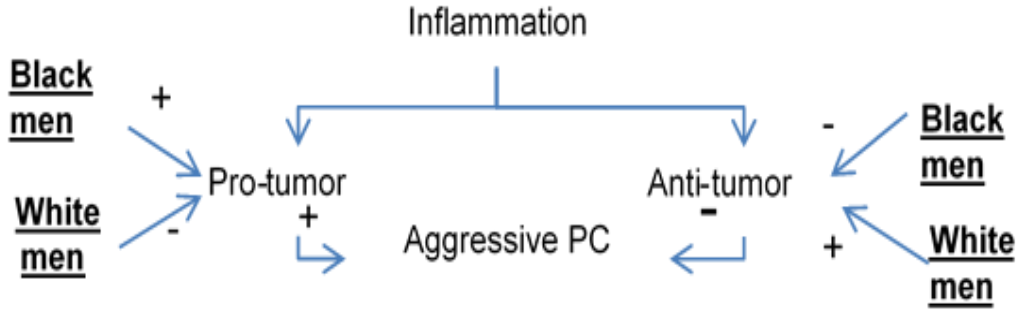
Introduction

This is a Health Disparity Prostate Cancer Research Award. The proposed studies will help understand race differences in the inflammatory milieu – both systemically and in the tumor microenvironment and how this contributes to PC race disparity. These data will also help identify inflammatory biomarkers that can be used clinically to identify which men have increased risk of PC, including aggressive PC. This, in turn, will allow clinicians to distinguish which patients need biopsy and which do not and among men with PC, to determine who needs aggressive therapy and who can be safely followed. Our underlying hypothesis is that dysregulated inflammation is more common in black men and that this contributes to PC race disparity. If confirmed, these data would suggest that targeting specific inflammatory markers or cell types, either through specific targeted therapy (e.g. anti-IL-1 antibodies) or lifestyle changes may reduce the excess burden of PC in black men, though testing this is beyond the scope of this study. Considerable progress has been achieved in year 1 of this proposal as explained below.

Body

We hypothesize that 1) inflammatory components can be pro- or anti-PC and 2) black men have more pro-PC and less anti-PC inflammation suggesting dysregulated inflammation in black men, which, in part, contributes to race disparity in PC risk and aggressiveness (Figure 1). The major goals of the project during this portion of the performance period were: **Aim 1: Examine the association between prostatic inflammatory markers and PC risk and aggressiveness,** and **Aim 2: Examine the association between serum inflammatory markers and PC risk and aggressiveness.** While **Aim 3: Assess the correlation between race and inflammatory markers,** will be conducted during the second year of this award.

Figure 1. Schematic showing overall study hypothesis



Key Research Accomplishments

Aim 1: Examine the association between prostatic inflammatory markers and PC risk and aggressiveness. We proposed to use multiplexed IHC to stain biopsy slides from 400 men (50% black), for neutrophils, B-cells (CD19), CD8+ T-cells, CD4+ T-cells, T-reg (CD4+ FoxP3+), M1/M2 macrophages (CD163,CD206), and MDSCs (CD14). An "exhausted" CD8 T-cell phenotype will be assessed by co-staining for CD8 and PD-1, LAG-3 and TIM-3. Expression of immune checkpoint ligands (PDL1, Class II MHC (for LAG-3) and GAL-9 (for TIM-3) will be assessed both on tumor cells and myeloid cells. A detailed list of inflammatory markers proposed to be stained are shown in Table 1.

Using machine learning, the tissue will be divided into stromal, benign glands, and PC glands. The amount of each cell type as a percent of all cells and of all inflammatory cells will be counted in each separate compartment (stroma vs. benign vs. PC glands).

Table 1. Immune Cells to be Studied in Aim 1

Cell type	Tissue Marker	Expected results: link with PC (pro or anti)
Myeloid lineage		
Neutrophils	CD66b+ CD11b+ CD63+	**anti**/pro
M1 macrophages	CD68	**anti**
M2 macrophages	CD163	pro
MDSCs	CD14+ CD15+	pro
Lymphoid lineage		
NK cell	CD56+ CD16+	**anti**
B-cells	CD19+ CD20+	pro
T-H cells	CD3+ CD4+	**anti/**pro
T-reg-cells	CD4+ CD25+ CTLA-4+ FoxP3+	*pro*
T-C cells	CD3+ CD8+	**anti**
Exhausted CD8+ T-cell	CD8, PD-1, LAG-3, TIM-3, *T-bet*	Pro
Other markers	PD-1, PDL-1, Ki67, granzyme	**anti**/pro

- **Key Accomplishments**:

1) Major activities included: meetings with Dr. Knudsen, at the Cedars-Sinai Biobank Core, to select prostate cancer cases tissue to be used to optimize the antibodies to stain the inflammatory markers; deciding which antibodies were good to use vs. those that failed; having regular meeting to discuss results; identifying prostate tissue and serum samples from patients at the Durham VA, to be transferred to Cedars-Sinai Medical Center.

2) Specific objectives included: optimizing the antibodies on prostate cancer tissue using the Cedars-Sinai Biobank specimens, *prior* to staining the Durham VA prostate

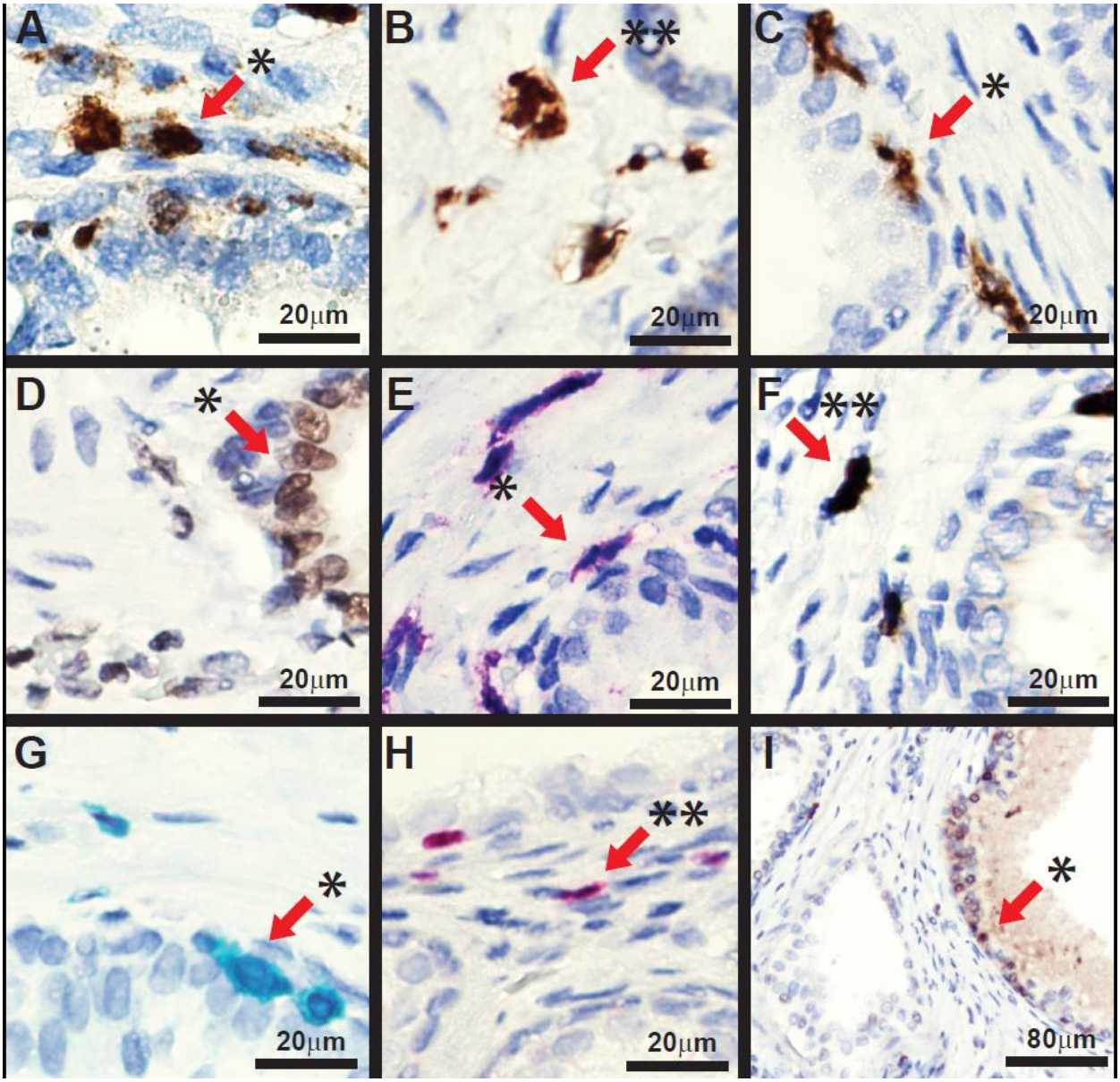
cancer cases as proposed in the study. Stained the first 20 biopsy slides from the Durham VA patients.

3) Key outcomes included: making significant progress in optimizing the staining of the inflammatory markers in prostate cancer cases from the Cedars-Sinai Biobank Core. **Table 2** summarizes the inflammatory markers that have been optimized so far. **Figure 2A** shows the images of the staining results. These data were presented as a poster at the Urology Care Foundation Honors Reception and Program during the AUA 2017 annual meeting in Boston, MA. (See Reportable Outcomes).

Table 2: Inflammatory markers that have been optimized

Antibody	**Prostate Staining Results**
CD68 (M1 macrophages)	1/6 RP cases were positive
CD163 (M2 macrophages)	6/6 RP cases were positive
LAG-3 (Exhausted T-cells)	0/6 RP cases were positive
CD11c (Dendritic cells)	6/6 RP cases were positive
TIM-3 (Exhausted T-cells)	5/6 RP cases were positive
CD3 (T-cells)	6/6 RP cases were positive
CD4 (T-Helper cells)	6/6 RP cases were positive
CD8 (Cytotoxic T-cells)	6/6 RP cases were positive
FoxP3 (T-regulatory cells)	3 cases positive, 1 case negative (2 cases failed stain)
CD20 (B-cells) pre-dilute	1 case positive
CD56 (NK cells)	1 case positive but not specific for NK cells
Elastase (Neutrophils)	1 case negative
Granzyme	1 case positive
PDL-1	5 cases negative
PD-1	5 cases positive

Figure 2A: Radical prostatectomy sections. A. CD68; B: CD163; C: CD11c; D: TIM-3; E: CD4; F: CD8; G: CD3; H: FoxP3; I: Granzyme. *: epithelial cell (glands); **: stroma.



4) Other achievements: Now that we have established which antibodies are appropriate to use for each inflammatory marker, the next step was to stain prostate cancer tissue from the patients at the Durham VA as proposed in this study. The methodology that we have been optimizing to achieve this, include:

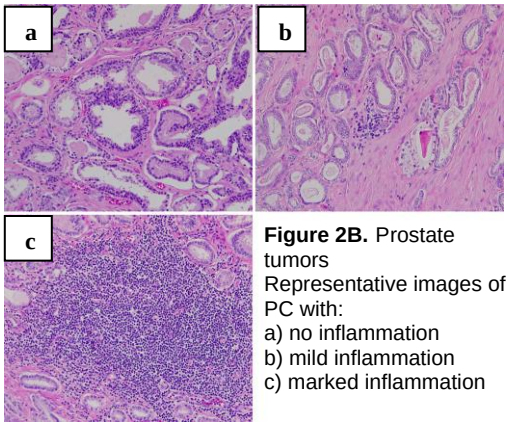


Figure 2B. Prostate tumors
Representative images of PC with:
a) no inflammation
b) mild inflammation
c) marked inflammation

Image analysis plan for inflammation quantification & characterization. Four cores, one more than the three cores often used, of the primary Gleason grade cancer will be harvested. Four-micron sections will be cut from the microarray and cancer will be assessed via CD3 and AMACR staining. H&E slides will be used to assess overall inflammatory infiltrate (**Figure 2B**).

To quantify the inflammatory infiltrate adjacent to the tumor, we will use computational quantification software developed at Cedars-Sinai Biobank Core. To characterize the peri-tumoral inflammatory infiltrate, we will assess the inflammatory infiltrate using both H&E and immunohistochemical methods. Briefly, following, deparaffinization in xylene and rehydration via ethanol, slides will be immersed in buffer and steamed for 30 min for antigen retrieval, immunostained with specific antibodies and immunoreaction products visualized with diaminobenzidine/H2O2. The mean number of stained cells in the representative cores for each patient will be calculated. Digital images of immune cell types present in the peri-tumoral inflammation: neutrophils, B-cells (CD19), CD8+ T-cells, CD4+ T-cells, Treg (CD4+ FoxP3+), M1/M2 macrophages (CD163,CD206), and MDSCs (CD14. An "exhausted" CD8 T-cell phenotype will be assessed by co-staining for CD8 and PD-1, LAG-3 and TIM-3. Expression of immune checkpoint ligands (PDL1, Class II MHC (for LAG-3) and GAL-9 (for TIM-3) will be assessed both on tumor cells and myeloid cells.) will be superimposed with the central image labeled with the tumor markers and two other images, one labeled for Granzyme-B (to measure T-cell activation) and another with Ki-67 (to measure proliferation).

Results:

a) T-cell panel. We have stained 104 biopsy tissue slides (50% African American men, 50% prostate cancer). The preliminary results are shown below. Briefly, we have found statistically significant differences in the amount of CD4 and CD8 cells, as well as in CD3 and FOXP3 T-cells between cancer

and no cancer and by race. We are now staining prostate biopsy tissue with the myeloid panel (macrophages) antibodies and we will have results soon. Once all these data are available we will match them with patients' demographics and Gleason score, for cancer aggressiveness.

CD8 T-cells in prostate biopsy tissues -preliminary analysis:

Green = p-value <0.0125
AA: African American Men
CA: Caucasian American Men

CD8 AA Epithelium	Cancer	Normal	Normal Adjacent
Cancer			
Normal	0.06134996		
Normal Adjacent	5.73594E-05	0.012552729	

CD8 CA Epithelium	Cancer	Normal	Normal Adjacent
Cancer			
Normal	3.53153E-09		
Normal Adjacent	0.000908009	0.007593163	

CD8 AA v CA Epithelium	AA Cancer	AA Normal	AA Normal Adjacent
CA Cancer	8.93983E-08	2.63921E-05	8.71821E-08
CA Normal	0.381854686	0.160803179	0.000188223
CA Normal Adjacent	0.055869334	0.005397399	5.09682E-06

CD8 Cancer v Normal Epithelium	Cancer	Normal	Normal Adjacent
Cancer			
Normal	0.001444177		
Normal Adjacent	0.00012342	0.279663044	

CD8 AA Stroma	Cancer	Normal	Normal Adjacent
Cancer			
Normal	0.199941791		

Normal Adjacent	0.062311932	0.018145702	

CD8 CA Stroma	Cancer	Normal	Normal Adjacent
Cancer			
Normal	0.624690599		
Normal Adjacent	0.038054441	0.05112835	

CD8 AA v CA Stroma	AA Cancer	AA Normal	AA Normal Adjacent
CA Cancer	0.535929596	0.561420174	0.034684969
CA Normal	0.225520877	0.898148893	0.019658808
CA Normal Adjacent	0.004727153	0.084023327	0.003877366

CD8 C v Normal Stroma	Cancer	Normal	Normal Adjacent
Cancer			
Normal	0.317724144		
Normal Adjacent	0.366434211	0.120608786	

CD4 T-cells in prostate biopsy tissues -preliminary analysis

CD4 AA Epithelium	Cancer	Normal	Normal Adjacent
Cancer			
Normal	0.07937665		
Normal Adjacent	0.000516951	0.033774245	

CD4 CA Epithelium	Cancer	Normal	Normal Adjacent
Cancer			
Normal	0.020025215		
Normal Adjacent	0.914325282	0.054994195	

CD4 AA v CA Epithelium	AA Cancer	AA Normal	AA Normal Adjacent
CA Cancer	0.019500939	0.003118212	3.23095E-05
CA Normal	0.797122845	0.053415809	0.000344181
CA Normal Adjacent	0.045111265	0.004512357	4.09327E-05

CD4 Cancer v Normal Epithelium	Cancer	Normal	Normal Adjacent
Cancer			
Normal	0.633467859		
Normal Adjacent	0.006172513	0.040460974	

CD4 AA Stroma	Cancer	Normal	Normal Adjacent
Cancer			
Normal	0.439421813		
Normal Adjacent	0.030707622	0.011521175	

CD4 CA Stroma	Cancer	Normal	Normal Adjacent
Cancer			
Normal	0.109089264		
Normal Adjacent	0.086038911	0.777353416	

CD4 AA v CA Stroma	AA Cancer	AA Normal	AA Normal Adjacent
White Cancer	0.703860358	0.664171415	0.018361461
White Normal	0.060734064	0.285568933	0.002771376
White Normal Adjacent	0.048751295	0.218856293	0.00224719

CD4 Cancer v Normal Stroma	Cancer	Normal	Normal Adjacent
Cancer			
Normal	0.028578608		
Normal Adjacent	0.434848244	0.024952243	

FOXP3 T-cells in prostate biopsy tissues -preliminary analysis

FOXP3 AA v CA Epithelium	AA Cancer	AA Normal	AA Normal Adjacent
CA Cancer	0.316115839	0.272753373	0.048630403
CA Normal	0.001022563	0.170705473	0.030482748
CA Normal Adjacent	0.105134531	0.229291046	0.040777037

FOXP3 AA v CA Stroma	AA Cancer	AA Normal	AA Normal Adjacent
CA Cancer	0.946264512	0.969793883	0.037211617
CA Normal	0.002313613	0.015457826	0.006814171
CA Normal Adjacent	0.418997389	0.423928535	0.020562258

CD3 T-cells in prostate biopsy tissues -preliminary analysis

CD3 AA Epithelium	Cancer	Normal	Normal Adjacent
Cancer			
Normal	0.227466134		
Normal Adjacent	0.007689746	0.098585598	

CD3 AA v CA Epithelium	AA Cancer	AA Normal	AA Normal Adjacent
CA Cancer	0.449429466	0.149416502	0.005000167
CA Normal	0.256417457	0.120198177	0.004041246
CA Normal Adjacent	0.729362303	0.192934263	0.006622093

CD3 AA v CA Stroma	AA Cancer	AA Normal	AA Normal Adjacent
CA Cancer	0.779808139	0.19476851	0.066577029
CA Normal	0.016757579	0.242431825	0.007102193
CA Normal Adjacent	0.159514856	0.688714519	0.014709959

Figures 3-8 show images of staining procedure and marker distributions in boxplots for CD4 and CD8.

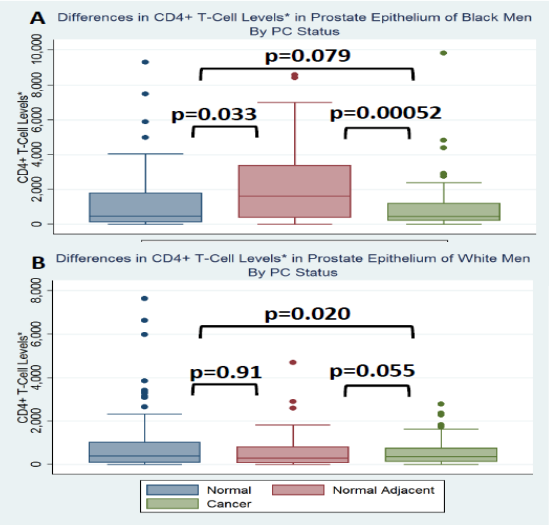


Figure 3. CD4+ T-cells/mm² differences in Epithelium of black (A) and white (B) men by PC status. Significant difference between Black Normal Adjacent and Cancer tissue (p=0.00052).

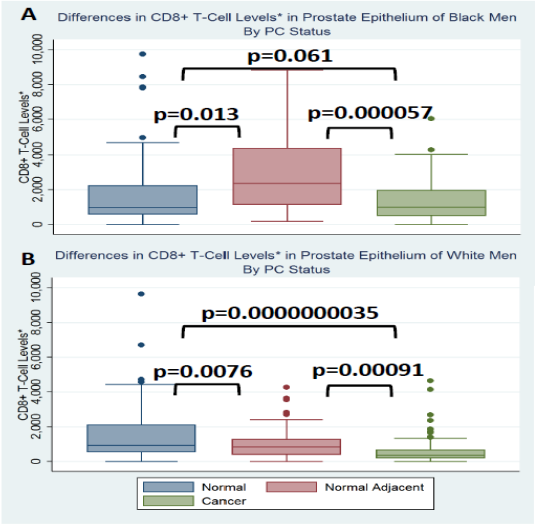


Figure 4. CD8+ T-cells/mm² differences in Epithelium of black (A) and white (B) men by PC status. Significant difference between Black Normal Adjacent and Cancer tissue (p=0.000057) and among all three White tissue types.

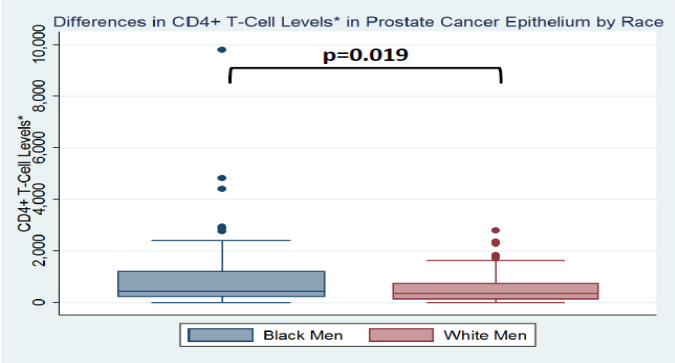


Figure 5. CD4+ T-cells/mm² differences in cancerous epithelial glands between black and white men. (p=0.019).

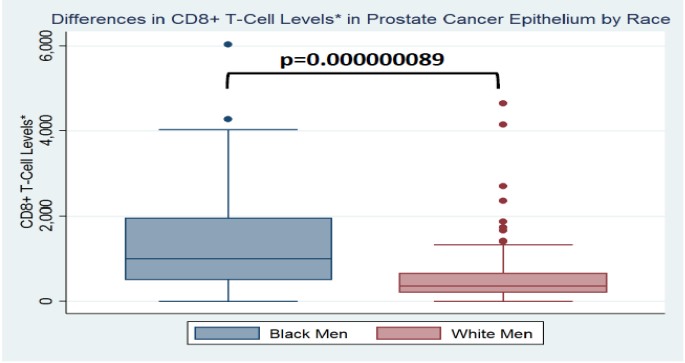


Figure 6. CD8+ T-cells/mm² differences in cancerous epithelial glands between black and white men. (p=0.000000089)

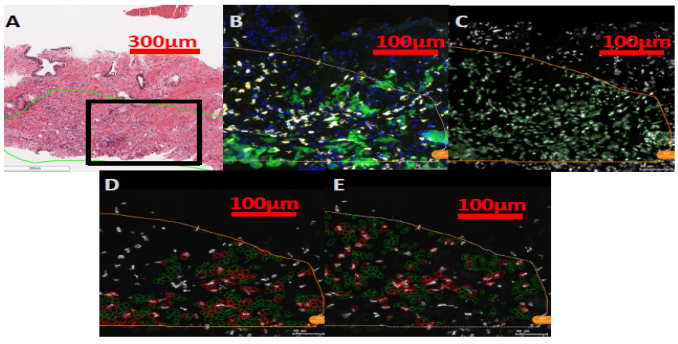


Figure 7. A: H&E staining of prostate biopsy with pathologist annotation of cancer. Insert viewed in following images. B: Composite Fluorescence Image of PC. C: DAPI filter with cells marked. D: Gold filter for CD8+ in epithelium with positive cells marked in red. E: Gold filter for CD8+ in stroma with positive cells marked in red.

Summary of Significant Differences in Prostate Epithelium	Black Normal	Black Normal Adjacent	Black Cancer
White Normal		CD3, CD4, CD8	FOXP3
White Normal Adjacent	CD4, CD8	CD3, CD4, CD8	
White Cancer	CD4, CD8	CD3, CD4, CD8	CD8

Summary of Significant Differences in Prostate Stroma	Black Normal	Black Normal Adjacent	Black Cancer
White Normal		FOXP3, CD3, CD4	FOXP3
White Normal Adjacent		CD4, CD8	CD8
White Cancer			

1 of 4 markers different | 2 of 4 markers different | 3 of 4 markers different

Figure 8. Summary tables of significant differences in T-cell markers between black and white prostate biopsies in epithelial glands and stroma. All given tissue markers were significant in testing with p≦0.0125.

b) Myeloid panel. Using 4-plex immunohistochemistry on prostate biopsy slides of AAM and EAM, with cancer patients and controls for each race, we quantified granulocytic (G) as well as monocytic (M) myeloid-derived suppressor cells (MDSCs), using the markers sown in **Table 3**.

Table 3: Cell types investigated and their associated markers for immunofluorescence identification

Cell Type	Markers
Monocytic MDSCs	$CD14^{+}HLA^{-}DR^{low/-}CD11b^{+}$
Granulocytic MDSCs	$CD14^{-}HLA\text{-}DR^{low/-}CD11b^{+}$
$CD14^{-}$ Myeloid Cells	$CD14^{-}HLA\text{-}DR^{+}CD11b^{+}$

Results: We have stained 49 biopsy tissue slides (51% African American men, 45% prostate cancer), as shown in **Figure 9**. The preliminary results are shown below. Overall, there were no significant differences in M-MDSCs among EAM and AAM or PC status (**Figure 10 a-b**). Prostate biopsies from AAM had statistically significant lower counts of G-MDSCs compared to those of EAM (p=0.014), although no significant differences were found by PC status (**Figure 11 a-c**). We will match these data with patients' demographics and Gleason score, for cancer aggressiveness.

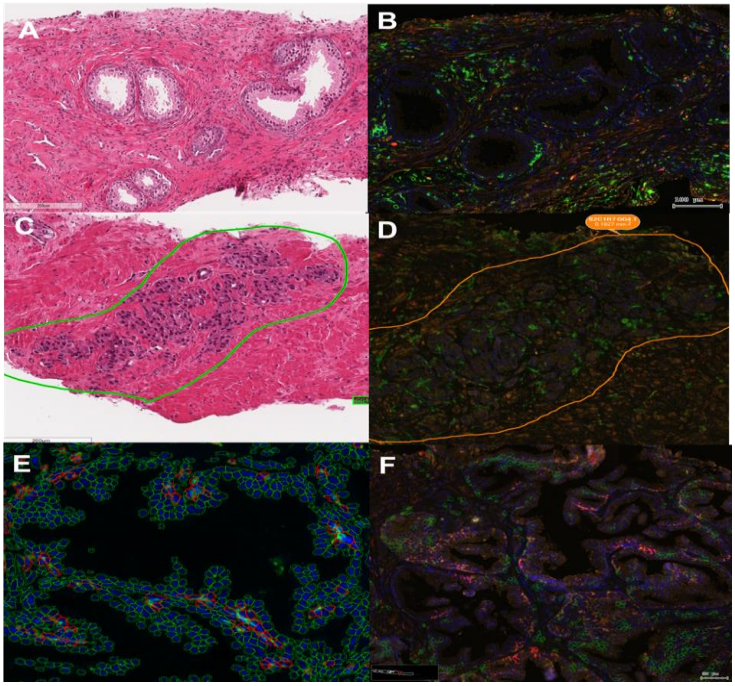


Figure 9. Scanned slides from prostate biopsies showing the following:
A.) H&E staining in healthy prostate tissue, B.) composite IF from the same biopsy sample as A. C.) H&E-stained biopsy from a cancerous region showing pathologist's annotation.
D.) A slice from the same biopsy as C showing composite IF.
E.) A representative region of positively identified cells, in this case M-MDSCs
F.) A representative sample showing a cell type that is not recorded in the literature

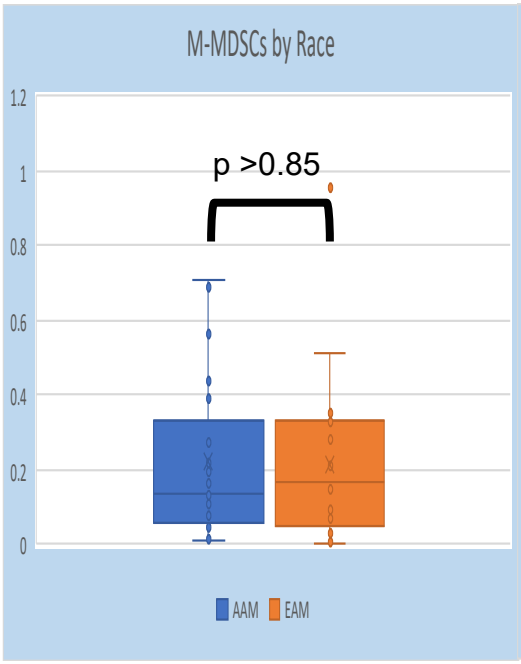


Figure 10a. M-MDSC in all men by Race. N=25 for AAM; N=24 for EAM

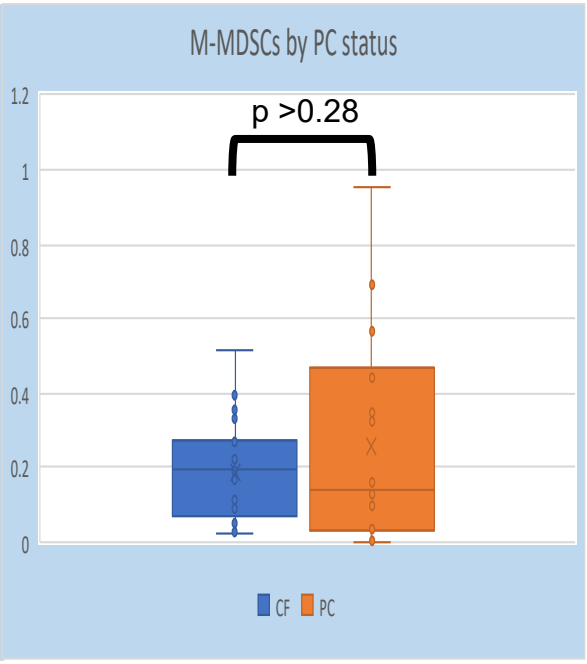


Figure 10b. M-MDSC in all men by PC status. N=27 for CF; N=22 for PC

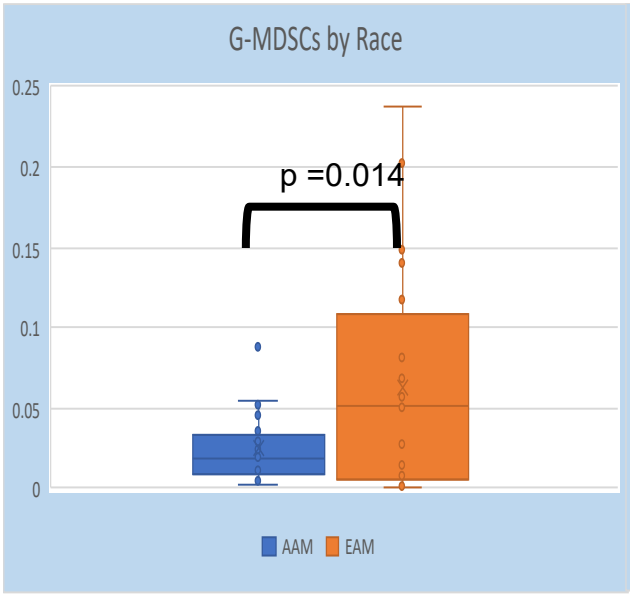


Figure 11a. G-MDSC in all men by Race. N=25 for

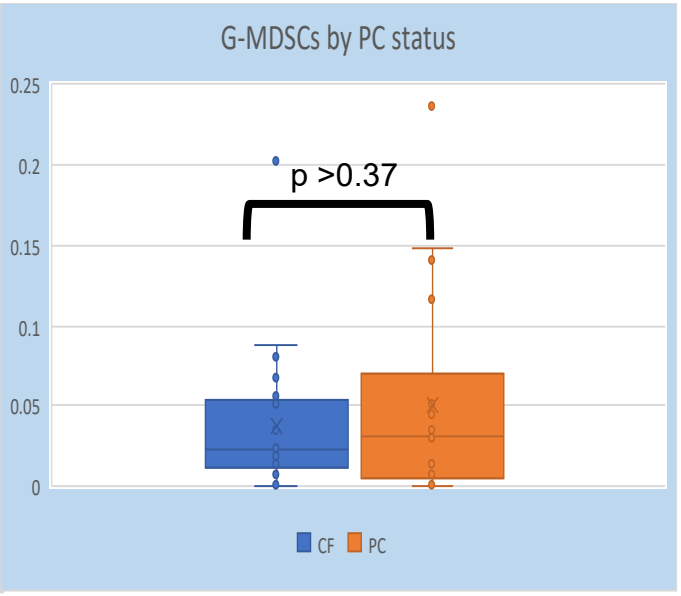


Figure 11b. G-MDSC in all men by PC status. N=27 for CF; N=22 for PC

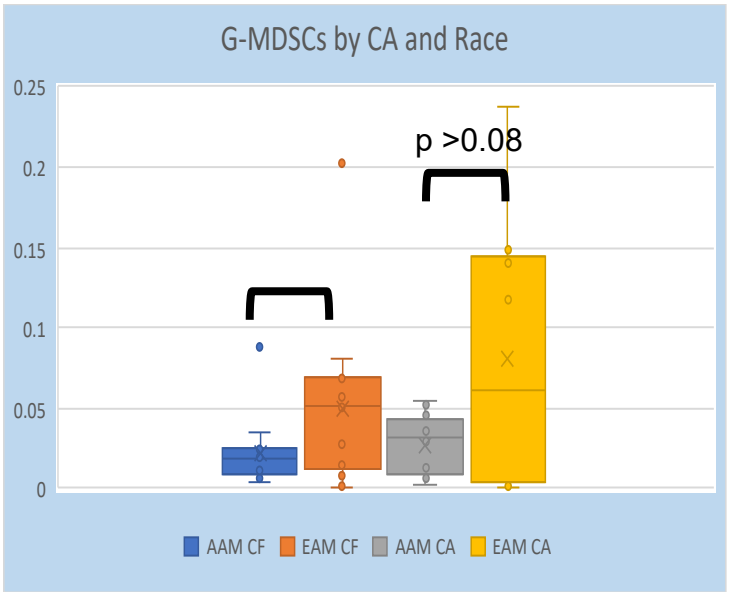


Figure 11c. G-MDSC in all men by PC status and Race. N= 13 for AAM CF; N=14 for EAM CF; N=12 for AAM PC; N=10 for EAM PC

c) Hyperion Imaging. Given that now we have access to new imaging technology at Cedars-Sinai, we are collaborating with Dr. Akil Merchant using Imaging Mass Cytomerty (IMC) or Hyperion technology. We have generated preliminary IMC data linked to this proposal's aims. To demonstrate the feasibility of performing IMC analysis on PC tissue microarrays (TMA) we labeled TMA slides from radical prostatectomy samples, with metal-conjugated antibodies and obtained imaging data on the Hyperion IMC system. We performed IMC on 8 cases of PC, including 3 cases with at least 3 replicate cores, for a total of 16 regions of interest (RO), to assess the feasibility of using this method to study changes in the tumor microenvironment. Using IMC, we could easily identify tumor and immune cells by visual inspection including cases with high and low immune infiltration and the presence of tertiary lymphoid structures (TLS), **Fig. 12**. A complete summary of the IMC work-low and analysis pipelines is presented in **Figure 13**. Thus, we have an established track record for successfully building multiplexed immunophenotyping panels for use on the IMC. We will adapt the panels we have developed for lymphoma and solid tumors for use in PC FFPE TMA. Our proposed panel will allow us to comprehensively profile the cells of the tumor microenvironment including immune subsets such as B, T, myeloid and dendritic cells phenotypes as well as non-immune cell types such as cancer associated fibroblasts, blood vessel, lymphatics, and other stromal cells (**Figure 14**).

Figure 12: TME heterogeneity revealed by IMC A. Large number of immune cells including a tertiary lymphoid structure (TLS) can be seen in this example. Immune cells are excluded from tumor glands. B. Dense sheets of tumor with high proliferative index (arrow), macrophages (M), and rare T cells (T)

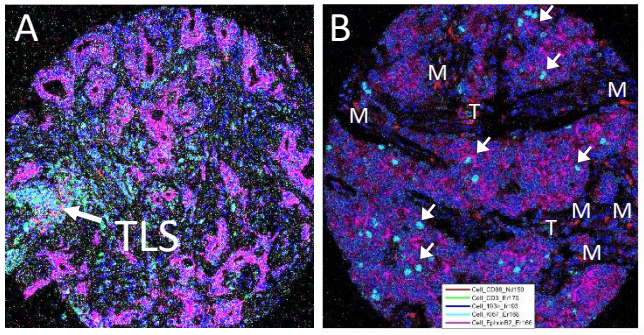


Figure 13: Imaging Mass Cytometry workflow. 1. Metal conjugated antibody panels are assembled. 2. Tissues are stained. 3. ROI selection/TMA generation guided H&E images. 4-5. Data generation. 6. Visual inspection, pixel classification and cell segmentation allow for single cell data extraction. 7. Single cell data can be further visualized by high dimensional methods like t-SNE, Phenograph, or spatial analysis markers (left to right)

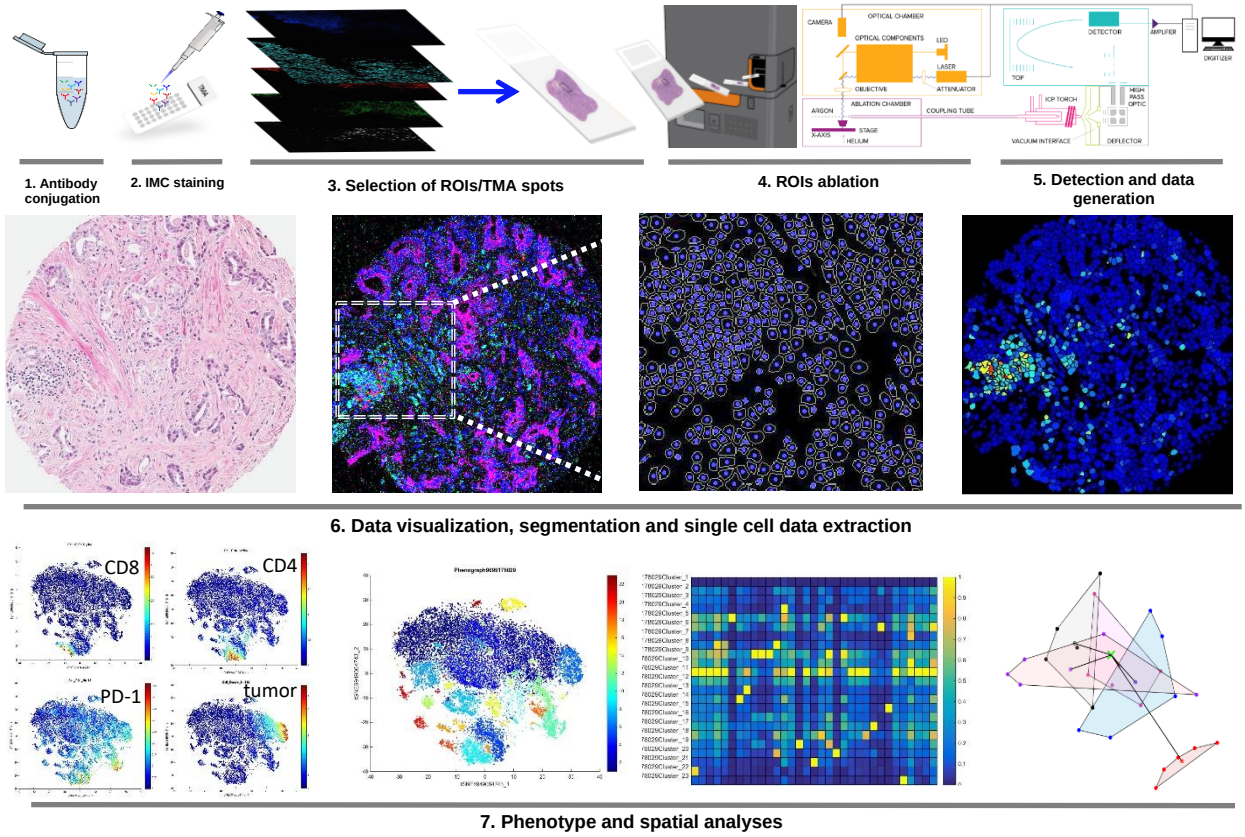
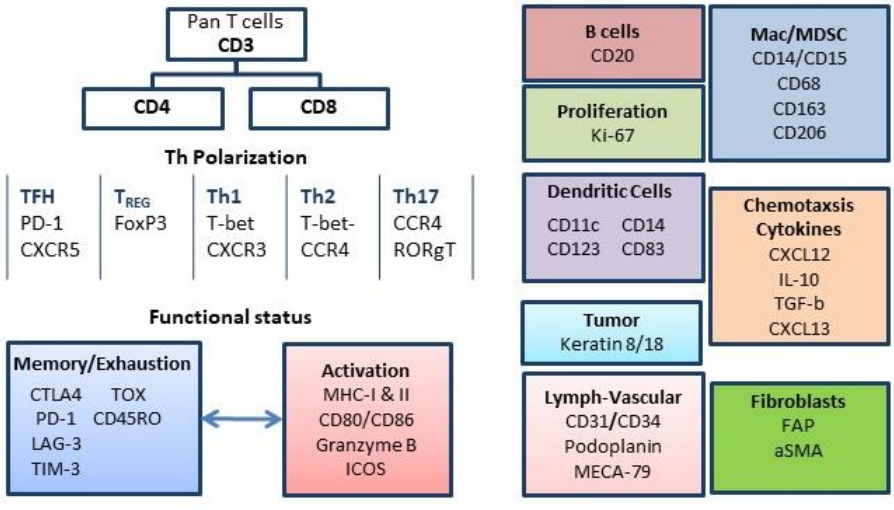


Figure 14: IMC panel. Markers are organized into modules based on function. Flexibility in IMC panel design allows modules to be swapped out as needed without revalidation.



Aim 2: Examine the association between serum inflammatory markers and PC risk and aggressiveness. We originally proposed to first analyze a 72-gene panel of inflammatory-related genes in peripheral blood RNA from 100 men (50 PC, 50 matched benign; 50/50 black/white). Subsequently, the expression of the top 10 cytokines linked with PC risk or PC aggressiveness in either black or white men will next be examined via multiplexed ELISA in serum from 400 men (200 PC, 200 matched benign; 50/50 black/white). Significant findings will be validated in a separate set of 400 men (200 PC, 200 matched benign; 50/50 black/white) from two independent cohorts. We expect some cytokines (IL-1, TNF-alpha, IL-6, IL-8, IL-4, IL-10, IL-12, IL-17, and IL-23) to be associated with higher PC risk/aggressiveness, while others (IL-12, IFN-gamma) will be associated with lower PC risk/aggressiveness. Although we have not performed the RNA analysis yet, we have run multiplex ELISA assays kits for 36 chemokines using the comprehensive kit shown in **Table 4.**

Table 4. V-PLEX Human Cytokine 36-Plex

Analyte	LLOQ - ULOQ, pg/mL
Eotaxin	12.3 – 1,120
Eotaxin-3	10.2 – 3,750
GM-CSF	1.90 – 750
IFN-γ	7.47 – 938
IL-1α	2.85 – 278
IL-1β	2.14 – 375
IL-2	0.890 – 938
IL-4	0.450 – 158
IL-5	6.28 – 562
IL-6	1.58 – 488
IL-7	1.37 – 563
IL-8	1.13 – 375
IL-8 (HA*)	713 – 43,400
IL-10	0.680 – 233
IL-12/IL-23p40	5.68 – 2,250
IL-12p70	1.22 – 315
IL-13	4.21 – 353
IL-15	1.40 – 525
IL-16	19.1 – 1,870
IL-17A	9.32 – 3,650
IL-21	1.65 – 650
IL-22	2.78 - 325
IL-23	4.55 - 3,250
IL-27	38.7 – 13,000
IL-31	4.22 – 650
IP-10	1.37 – 500
MCP-1	1.09 – 375
MCP-4	5.13 – 469
MDC	88.3 – 7,500
MIP-1α	13.8 – 743
MIP-1β	2.27 – 750
MIP-3α	0.588 - 325
TARC	3.32 – 1,120
TNF-α	0.690 – 248
TNF-β	1.15 – 458
VEGF-A	7.70 – 562

Instead of the above plan, we decided to perform RNA-seq on the blood of 84 men from the Durham VA (a technique that was cost prohibitive when the grant was originally submitted). These samples have been sent to Mt. Sinai (Dr. Taioli) and we have analyzed them. Moreover, we decided not to use these data as a filter for subsequent analyses as the costs for multiplexed cytokine analyses have come down that such a filtering step was no longer necessary.

- **Key Accomplishments:**

1) Major activities included: meetings with Drs. Shiao and You, at Cedars-Sinai Medical Center, to decide how to analyze RNA-seq and cytokines data, respectively.

2) Specific objectives included: complete analysis of the 84 Pax-gene RNA tubes and 400 cytokines serum samples from VA patients, PC cases and controls. See results below.

3) Key outcomes included: data on 400 serum samples has been analyzed by race and PC status for cytokines, not yet for RNA-seq of the 84 samples.

4) Major achievements: we have observed differences in RNA expression by two different genotypes; we have observed racial differences on cytokine profiles by PC status.

Results: a) Data analysis of RNA-seq expression

We analyzed differential RNA expression between samples with different genotypes i.e. with different single nucleotide polymorphisms (SNPs). The differentially expressed genes (DEGs) by SNPs data showed that expression is different according to which SNP is present. Two SNPs, CT vs. CC and GA vs. GG, were associated with RNA differential expression of genes,

Figure 15. Gene Set Enrichment Analysis (GSEA) (**Figures 16 and 17**) showed a commonality in high enrichment of autoimmune diseases such as lupus and asthma. This suggests that two SNPs are associated with immune reactivity in the samples analyzed.

Figure 15. Number of Common and Distinct DEGs between Two SNPs

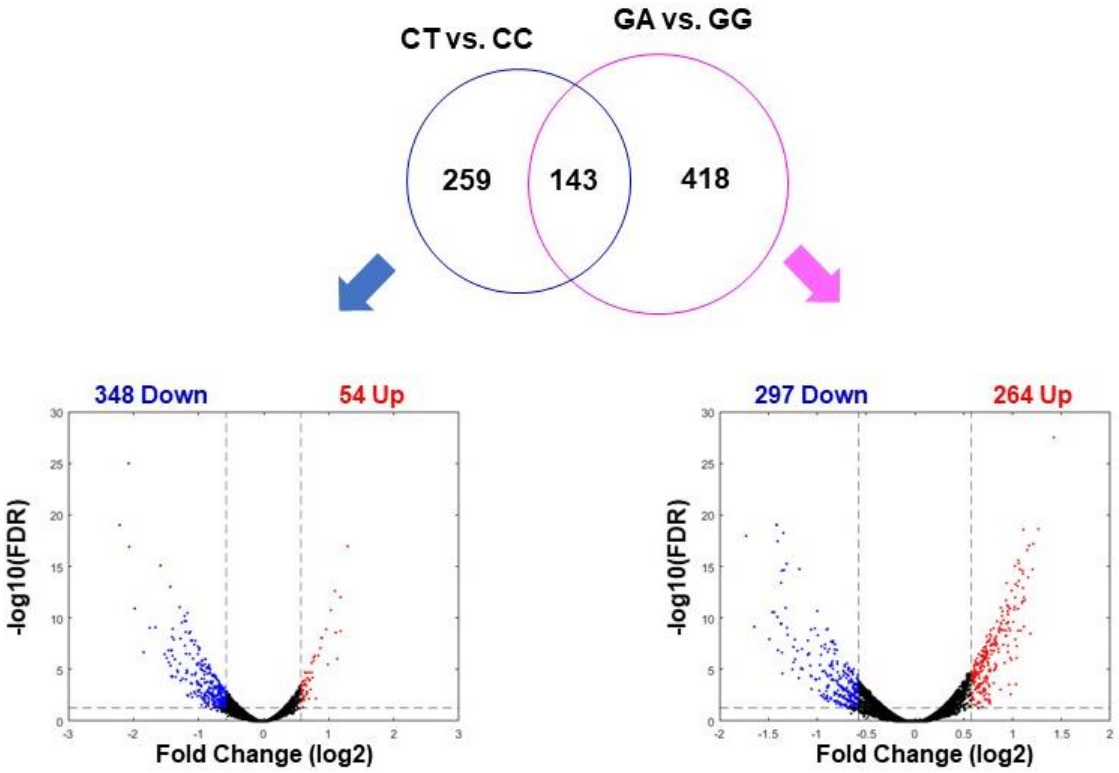


Figure 16. Top 10 Significantly Enriched Gene Sets in CT vs. CC

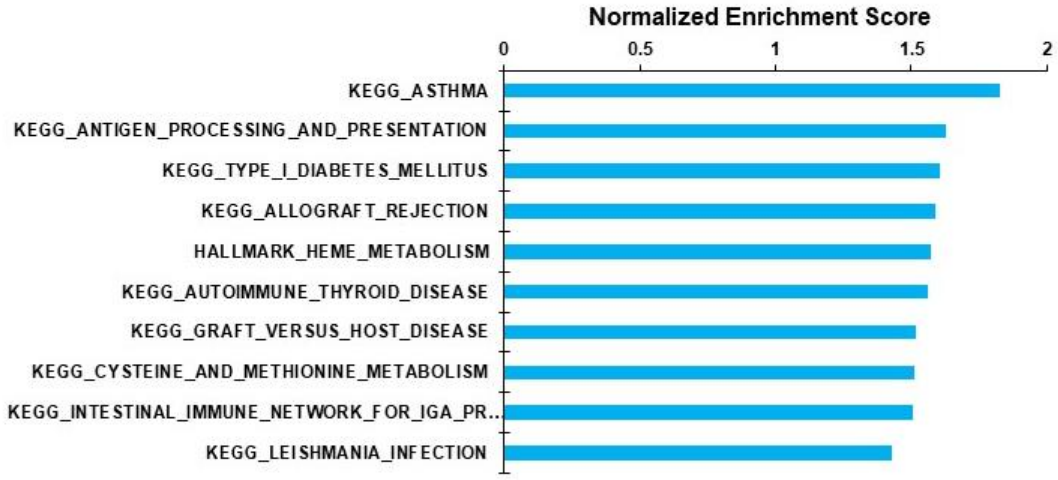
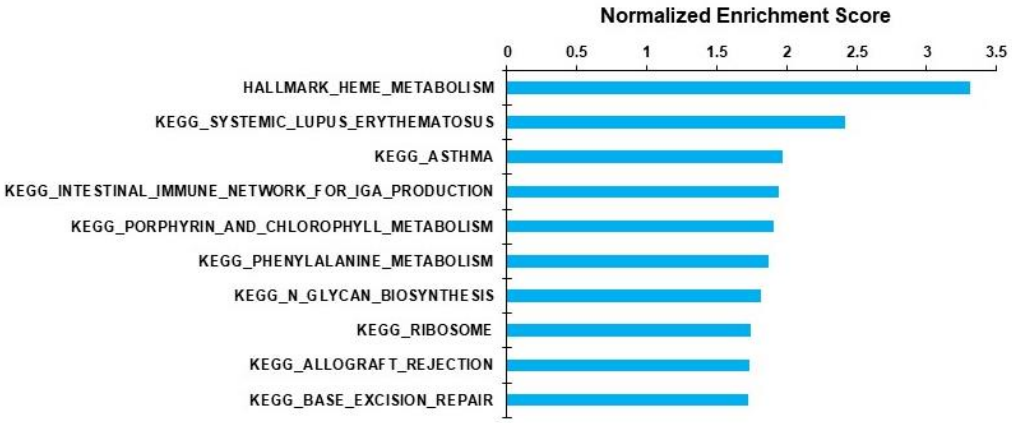


Figure 17. Top 10 Significantly Enriched Gene Sets in GA vs. GG



b) Data analysis of serum cytokines profile. We found that out of a panel of 37 cytokines, 8 (IL-12p70, IL-13, IL-1β, IL2, GM-CSF, IL-8(HA), IL-21 and IL-31) were differently expressed in black versus white men and associated with higher risk of PC, all p-interaction ≤0.041, **Table 5**.

When analyses were stratified by PC status, yes vs no, IL-12p70, IL-13, IL-1β, IL2, TNF-α, IL-15, IL-17A, IL-5, TNF-β, Eotaxin, MCP-1, MCP-4, MDC, IL-21, IL-22 and IL-27 were all detectable in statistically significantly higher levels in PC patients compared to controls all p≤0.020, **Table 6**.

When analyses were stratified by PC status for each race group, IL-12p70, IL-13, IL-1β, IL2, IL-4, GM-CSF, and IL-21 were all statistically significantly higher in PC cases vs. controls in black men (all p≤0.05), but not in white men, **Table 7**. In white men, the following cytokines were detected at higher levels in PC cases vs. controls: TNF-α, IL-12/IL-23p40, IL-15, IL-17A, IL-1α, IL-5, TNF-β, Eotaxin, IL-8(HA), IP-10, MDC, MIP-1α, MIP-1β, IL-17A (TH17), and IL-27, all p≤0.045), **Table 7**.

These data were presented at the 2019 AUA Annual Meeting in Chicago, IL.

Final analyses will be adjusted for PC grade, PSA, and other clinicopathological and demographic features that are abstracted via the computerized medical records.

Table 5: Unadjusted odds ratio (OR) and 95% confidence interval (CI) for the association between Chemokines and risk of Prostate cancer (PC), stratified by race and interaction between each Chemokine and race

	All men (346)			White Men (N=173)			Black Men (N=173)			p-interaction
	N	OR (95% CI)	p-value	N	OR (95% CI)	p-value	N	OR (95% CI)	p-value	
Pro-Inflammation Panel concentrations										
*IFN-γ	321	1.00 (0.99-1.01)	0.761	173	1.05 1.00-1.11)	0.067	148	1.00 (0.98-1.01)	0.511	0.443
*IL-10	312	1.77 (0.81-3.86)	0.150	171	1.89 (0.65-5.51)	0.243	141	1.66 (0.55-5.03)	0.367	0.539
**IL-12p70	228	2.29 91.44-3.64)	<0.001	125	1.85 (0.93-3.69)	0.079	103	2.81 (1.49-5.32)	0.002	**0.015**
**IL-13	130	1.63 (1.05-2.53)	0.028	62	0.56 (0.30-1.06)	0.073	68	4.88 (2.53-9.44)	<0.001	**<0.001**
**IL-1β	137	2.05 (1.32-3.17)	0.001	46	0.76 (0.39-1.51)	0.434	91	5.36 (2.78-10.33)	<0.001	**<0.001**
**IL-2	164	2.80 (1.81-4.33)	<0.001	83	1.16 (0.64-2.12)	0.621	81	7.54 (3.84-14.82)	<0.001	**<0.001**
**IL-4	142	1.15 (0.75-1.78)	0.521	81	0.78 (0.43-1.43)	0.424	61	1.83 (0.95-3.51)	0.071	0.102
*IL-6	339	1.00 (0.98-1.03)	0.691	173	0.96 (0.73-1.27)	0.763	166	1.00 (0.98-1.03)	0.674	0.664
*IL-8	340	1.00 (0.98-1.01)	0.463	173	1.00 (0.98-1.02)	0.807	167	0.99 (0.97-1.01)	0.229	0.408
*TNF-α	339	1.19 (1.02-1.39)	0.031	173	1.91 (1.31-2.77)	**<0.001**	166	1.00 (0.83-1.21)	0.976	0.135
Cytokines panel concentrations										
**GM-CSF	168	1.28 (0.84-1.95)	0.259	102	0.81 (0.44-1.49)	0.502	66	2.10 (1.12-3.94)	0.020	**0.041**
*IL-12/IL-23p40	346	1.00 (1.00-1.00)	0.913	173	1.01 (1.01-1.01)	**0.008**	173	1.00 (0.99-1.00)	0.335	0.093
*IL-15	345	1.18 (0.95-1.46)	0.145	173	2.91 (1.84-4.60)	**<0.001**	172	0.77 (0.58-1.02)	0.066	0.084
*IL-16	346	1.00 (1.00-1.00)	0.656	173	1.00 (1.00-1.00)	0.880	173	1.00 (1.00-1.00)	0.652	0.871
*IL-17A	345	1.10 (0.97-1.26)	0.146	168	1.37 (1.06-1.76)	**0.016**	156	1.02 (0.91-1.15)	0.723	0.478
**IL-1α	46	1.71 (0.91-3.21)	0.099	20	5.14 (1.63-16.19)	**0.005**	26	0.83 (0.36-1.93)	0.663	0.676
**IL-5	237	1.50 (0.94-2.37)	0.086	129	2.25 (1.09-4.62)	**0.028**	108	1.11 (0.60-2.06)	0.739	0.805
*IL-7	346	1.05 (1.01-1.09)	0.014	173	1.07 (1.01-1.13)	**0.032**	173	1.04 (0.98-1.10)	0.184	0.762
**TNF-β	216	4.46 (2.75-7.22)	<0.001	108	5.04 (2.52-10.08)	**<0.001**	108	3.95 (2.11-7.75)	<0.001	0.003
*VEGF	346	1.00 (1.00-1.00)	0.273	173	1.00 (1.00-1.01)	0.174	173	1.00 (1.00-1.00)	0.714	0.734
Chemokines panel concentrations										
*Eotaxin	343	1.00 (1.00-1.00)	0.005	173	1.00 (1.00-1.01)	**0.002**	170	1.00 (1.00-1.00)	0.383	0.238
*Eotaxin-3	312	0.99 (0.98-1.00)	0.222	170	0.99 (0.98-1.01)	0.242	142	1.00 (0.98-1.02)	0.767	0.287
**IL-8(HA)	29	0.34 (0.14-0.82)	0.016	6	6.13 (0.70-53.63)	0.101	23	0.09 (0.02-0.39)	0.001	**0.002**
*IP-10	346	1.00 (1.00-1.00)	0.194	173	1.00 (1.00-1.00)	0.065	173	1.00 (1.00-1.00)	0.544	0.816
*MCP-1	346	1.01 (1.00-1.01)	<0.001	173	1.01 (1.00-1.01)	**<0.001**	173	1.01 (1.00-1.01)	0.004	0.675
*MCP-4	346	1.01 (1.00-1.01)	<0.001	173	1.01 (1.00-1.01)	**0.030**	173	1.01 (1.00-1.01)	<0.001	0.513

***MDC**	346	1.00 (1.00-1.01)	0.059	173	1.00 (1.00-1.00)	**0.003**	173	1.00 (1.00-1.00)	0.945	0.321
*MIP-1α	297	1.00 (1.00-1.00)	0.401	165	1.00 (0.98-1.02)	0.851	132	1.00 (1.00-1.00)	0.398	0.399
***MIP-1β**	345	1.00 (1.00-1.01)	0.095	173	1.01 (1.00-1.01)	**0.014**	172	1.00 (1.00-1.00)	0.893	0.409
*TARC	346	1.00 (1.00-1.00)	0.133	173	1.00 (1.00-1.00)	0.463	173	1.00 (1.00-1.00)	0.181	0.697
***TH17 panel concentrations**										
****IL-**	115	1.67 (1.07-2.63)	0.025	46	2.24 (1.13-4.47)	**0.022**	69	1.35 (0.73-2.49)	0.336	0.405
****IL-21**	53	3.96 (2.06-7.61)	<0.001	22	1.81 (0.73-4.49)	0.200	31	8.47 (3.07-23.38)	<0.001	**<0.001**
*IL-22	316	1.06 (0.89-1.27)	0.522	163	0.92 (0.72-1.17)	0.500	153	1.37 (0.99-1.88)	0.054	0.180
***IL-23	0	-	-	0	-	-	0	-	-	-
*IL-27	346	1.00 (1.00-1.00)	0.214	173	1.00 (1.00-1.00)	0.785	173	1.00 (1.00-1.00)	0.118	0.636
****IL-31**	80	1.58 (0.96-2.61)	0.074	34	1.04 (0.49-2.21)	0.915	46	2.24 (1.13-4.47)	0.022	**0.035**
*MIP-3α	336	0.99 (0.98-1.07)	0.333	167	0.97 (0.93-1.02)	0.214	169	1.00 (1.00-1.00)	0.398	0.762

Undetectable value were replaced with a value of 0.

N=number of non-missing values of each Analyte; p^{int}=p-value for interaction between Analyte and race.

*Given that <25% of values are undetectable, variables were used as is (continuous).

**Given that ≥25% of values are undetectable, variables are dichotomized into “detectable vs. Not-detectable”

***All values were undetectable

Table 6. Comparison of the properties of Chemokines between (a) Race-White vs Black, (b) Cases and control						
	Race			**Treatment group**		
	Black (N=173)	White (N=173)	p value	Case (N=171)	Control (N=175)	p value
Pro-Inflammation Panel concentrations						
*IFN-γ			0.006[1]			0.740[1]
Median (Q1, Q3)	3.0 (1.4, 5.2)	3.5 (2.1, 5.7)		3.2 (1.7, 6.2)	3.1 (1.9, 5.2)	
*IL-10			0.003[1]			0.272[1]
Median (Q1, Q3)	0.2 (0.1, 0.3)	0.2 (0.1, 0.3)		0.2 (0.1, 0.3)	0.2 (0.1, 0.3)	
****IL-12p70,** *n (%)*			**0.013[2]**			**<0.001[2]**
Detectable	103 (59.5%)	125 (72.3%)		130 (76.0%)	98 (56.0%)	
Not-detectable	70 (40.5%)	48 (27.7%)		41 (24.0%)	77 (44.0%)	
****IL-13**, *n (%)*			0.505[2]			**0.009[2]**
Detectable	68 (39.3%)	62 (35.8%)		76 (44.4%)	54 (30.9%)	
Not-detectable	105 (60.7%)	111 (64.2%)		95 (55.6%)	121 (69.1%)	
****IL-1β**, *n (%)*			**<0.001[2]**			**0.001[2]**
Detectable	91 (52.6%)	46 (26.6%)		83 (48.5%)	54 (30.9%)	
Not-detectable	82 (47.4%)	127 (73.4%)		88 (51.5%)	121 (69.1%)	
****IL-2**			0.747[2]			**<0.001[2]**
Detectable	80 (46.2%)	83 (48.0%)		104 (60.8%)	59 (33.7%)	
Not-detectable	93 (53.8%)	90 (52.0%)		67 (39.2%)	116 (66.3%)	
**IL-4, *n (%)*			0.008[2]			0.541[2]
Detectable	53 (30.6%)	77 (44.5%)		67 (39.2%)	63 (36.0%)	
Not-detectable	120 (69.4%)	96 (55.5%)		104 (60.8%)	112 (64.0%)	
*IL-6			0.278[1]			0.656[1]
Median (Q1, Q3)	0.8 (0.4, 1.3)	0.8 (0.6, 1.2)		0.8 (0.5, 1.3)	0.8 (0.6, 1.3)	

*IL-8			0.001[1]			0.562[1]
Median (Q1, Q3)	9.1 (5.1, 15.1)	11.4 (7.6, 16.0)		10.8 (5.9, 16.1)	10.6 (6.8, 15.6)	
***TNF-α**			**<0.001[1]**			**0.001[1]**
Median (Q1, Q3)	2.3 (1.6, 3.0)	2.7 (2.3, 3.3)		2.8 (2.0, 3.4)	2.4 (1.8, 2.8)	
Cytokines panel concentrations						
**GM-CSF*, n (%)*			<0.001[2]			0.134[2]
Detectable	64 (37.0%)	102 (59.0%)		89 (52.0%)	77 (44.0%)	
Not-detectable	109 (63.0%)	71 (41.0%)		82 (48.0%)	98 (56.0%)	
*IL-12/IL-23p40			<0.001[1]			0.485[1]
Median (Q1, Q3)	72.7 (45.6, 111.9)	124.2 (94.7, 174.3)		110.3 (56.4, 160.7)	98.0 (66.7, 138.8)	
***IL-15**			0.165[1]			**<0.001[1]**
Median (Q1, Q3)	2.4 (1.5, 2.9)	2.3 (1.9, 2.9)		2.6 (1.9, 3.2)	2.1 (1.7, 2.6)	
*IL-16			0.003[1]			0.322[1]
Median (Q1, Q3)	215.5 (166.1, 264.0)	240.8 (183.5, 326.9)		231.6 (183.5, 288.6)	218.1 (166.1, 282.8)	
***IL-17A** (cytokines)			0.096[1]			**<0.001[1]**
Median (Q1, Q3)	1.1 (0.6, 1.9)	1.3 (0.8, 2.2)		1.5 (0.9, 2.3)	1.1 (0.6, 1.9)	
**IL-1α			0.263[2]			0.128[2]
Detectable	19 (11.0%)	26 (15.0%)		27 (15.8%)	18 (10.3%)	
Not-detectable	154 (89.0%)	147 (85.0%)		144 (84.2%)	157 (89.7%)	
****IL-5**			**0.015[2]**			**0.003[2]**
Detectable	129 (74.6%)	108 (62.4%)		130 (76.0%)	107 (61.1%)	
Not-detectable	44 (24.4%)	65 (37.6%)		41 (24.0%)	68 (38.9%)	
*IL-7			0.555[1]			0.174[1]
Median (Q1, Q3)	8.5 (5.6, 12.1)	8.7 (6.1, 11.6)		9.0 (6.1, 12.6)	8.5 (5.7, 11.2)	
****TNF-β**			1.000[1]			**<0.001[1]**
Median (Q1, Q3)	1.0 (0.0, 1.0)	1.0 (0.0, 1.0)		1.0 (1.0, 1.0)	0.0 (0.0, 1.0)	

*VEGF			0.660[1]			0.156[1]
Median (Q1, Q3)	95.1 (51.8, 176.0)	89.8 (55.1, 144.6)		93.1 (58.3, 179.5)	89.8 (50.4, 145.7)	
Chemokines panel concentrations						
***Eotaxin**			<0.001[1]			**<0.001[1]**
Median (Q1, Q3)	144.6 (89.3, 207.7)	273.9 (166.0, 362.1)		234.2 (137.3, 345.1)	160.9 (99.3, 273.9)	
*Eotaxin-3			<0.001[1]			0.479[1]
Median (Q1, Q3)	7.6 (2.6, 17.4)	13.1 ()		11.8 (4.7, 18.9)	10.3 (5.4, 21.8)	
****IL-8(HA),** *n (%)*			0.001[2]			**0.014[2]**
Detectable	23 (13.3%)	6 (3.5%)		8 (4.7%)	21 (12.0%)	
Not-detectable	150 (86.7%)	167 (96.5%)		163 (95.3%)	154 (88.0%)	
*IP-10			0.020[1]			0.369[1]
Median (Q1, Q3)	182.8 (46.2, 281.1)	195.7 (133.3, 323.1)		195.6 (102.2, 346.3)	188.2 (105.6, 275.0)	
***MCP-1**			<0.001[1]			**<0.001[1]**
Median (Q1, Q3)	165.1 (117.2, 210.6)	249.9 (181.2, 324.3)		231.0 (169.9, 306.0)	177.2 (127.5, 240.9)	
***MCP-4**			<0.001[1]			**<0.001[1]**
Median (Q1, Q3)	188.5 (135.1, 263.9)	139.9 (111.0, 205.7)		191.0 (135.6, 257.3)	149.7 ()104.3, 203.5	
***MDC**			0.138[1]			**0.012[1]**
Median (Q1, Q3)	1205.0 (318.0, 1673.4)	1202.9 (878.0, 1564.4)		1321.9 (746.4, 1788.5)	1146.4 (766.6, 1417.7)	
*MIP-1α			0.577[1]			0.204[1]
Median (Q1, Q3)	15.4 (1.7, 23.3)	13.5 (9.2, 18.6)		15.3 (8.5, 21.3)	12.3 (7.6, 19.4)	
*MIP-1β			0.233[1]			0.099[1]
Median (Q1, Q3)	93.0 (29.8, 145.7)	95.4 (64.7, 133.7)		102.2 (48.1, 149.5)	87.7 (55.1, 134.3)	
*TARC			0.242[1]			0.309[1]
Median (Q1, Q3)	399.3 (179.3, 603.8)	324.6 (166.4, 532.8)		351.0 (170.0, 610.6)	344.6 (171.1, 524.5)	
TH17 panel concentrations						
**IL-17A (TH17), *n (%)*			0.009[2]			0.102[2]
Not-detectable	104 (60.1%)	127 (73.4%)		107 (62.6%)	124 (70.9%)	

Detectable	69 (39.9%)	46 (26.6%)		64 (37.4%)	51 (29.1%)	
****IL-21, *n* *(%)***			0.179[2]			**<0.001[2]**
Detectable	31 (17.9%)	22 (12.7%)		41 (24.0%)	12 (6.9%)	
Not-detectable	142 (82.1%)	151 (87.3%)		130 (76.0%)	163 (93.1%)	
***IL-22**			0.238[1]			**0.006[1]**
Median (Q1, Q3)	0.7 (0.4, 1.2)	0.6 (0.3, 1.0)		0.7 (0.4, 1.3)	0.6 (0.3, 1.0)	
***IL-23			1.000[1]			1.000[1]
Median (Q1, Q3)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)		0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	
***IL-27**			<0.001[1]			**0.020[1]**
Median (Q1, Q3)	789.2 (481.1, 1228.9)	1091.7 (738.5, 1553.6)		1118.5 (610.5, 1539.9)	836.2 (588.9, 1208.3)	
**IL-31, *n (%)*			0.126[2]			0.164[2]
Detectable	46 (26.6%)	34 (19.7%)		45 (26.3%)	35 (20.0%)	
Not-detectable	127 (73.4%)	139 (80.3%)		126 (73.7%)	140 (80.0%)	
*MIP-3α			0.567[1]			0.091[1]
Median (Q1, Q3)	6.1 (4.0, 8.6)	5.6 (3.7, 8.8)		5.3 (3.5, 7.8)	6.4 (4.0, 9.0)	

*Given that <25% of values are undetectable, variables were used as is (continuous)
**Given that ≥25% of values are undetectable, variables are dichotomized into “detectable vs. Not-detectable”
***All values were undetectable
Q1=25th percentile; Q3=75th percentile

Table 7. Comparison of the properties of Chemokines between Cases and control within each race group (White and Black)

	Black Men (N=173)			**White Men (N=173)**		
	Cases (N=85)	**Control (N=88)**	p value	**Cases (N=86)**	**Cases (N=87)**	p value
Pro-Inflammation Panel concentrations						
*IFN-γ			0.964[1]			0.700[1]
Median (Q1, Q3)	3.0 (1.2, 5.9)	3.0 (1.5, 4.5)		3.5 (2.1, 6.2)	3.5 (2.2, 5.4)	
*IL-10			0.747[1]			0.085[1]
Median (Q1, Q3)	0.2 (0.0, 0.3)	0.2 (0.1, 0.3)		0.2 (0.1, 0.4)	0.2 (0.1, 0.3)	

****IL-12p70**, *n (%)*			**<0.001[2]**			0.099[2]
Detectable	63 (74.1%)	40 (45.5%)		67 (77.9%)	58 (66.7%)	
Not-detectable	22 (25.9%)	48 (54.5%)		19 (22.1%)	29 (33.3%)	
****IL-13**, *n (%)*			**<0.001[2]**			0.065[2]
Detectable	51 (60.0%)	17 (19.3%)		25 (29.1%)	37 (42.5%)	
Not-detectable	34 (40.0%)	71 (80.7%)		61 (70.9%)	50 (57.5%)	
****IL-1β**, *n (%)*			**<0.001[2]**			0.183[2]
Detectable	64 (75.3%)	27 (30.7%)		19 (22.1%)	27 (31.0%)	
Not-detectable	21 (24.7%)	61 (69.3%)		67 (77.9%)	60 (69.0%)	
****IL-2**			**<0.001[2]**			0.596[2]
Detectable	61 (71.8%)	19 (21.6%)		43 (50.0%)	40 (46.0%)	
Not-detectable	24 (28.2%)	69 (78.4%)		43 (50.0%)	47 (54.0%)	
****IL-4**, *n (%)*			**0.049[2]**			0.316[2]
Detectable	32 (37.6%)	21 (23.9%)		35 (40.7%)	42 (48.3%)	
Not-detectable	53 (62.4%)	67 (76.1%)		51 (59.3%)	45 (51.7%)	
*IL-6			0.411[1]			0.685[1]
Median (Q1, Q3)	0.8 (0.3, 1.3)	0.8 (0.6, 1.2)		0.8 (0.6, 1.2)	0.8 (0.6, 1.3)	
*IL-8			0.017[1]			0.075[1]
Median (Q1, Q3)	7.8 (1.1, 13.3)	10.5 (6.4, 16.5)		11.9 (8.8, 17.7)	10.9 (6.9, 15.4)	
*TNF-α			0.704[1]			**<0.001[1]**
Median (Q1, Q3)	2.3 (1.3, 3.1)	2.3 (1.8, 2.9)		3.1 (2.6, 3.7)	2.5 (2.1, 2.7)	
Cytokines panel concentrations						
****GM-CSF**, *n (%)*			**0.007[2]**			0.598[2]
Detectable	40 (47.1%)	24 (27.3%)		49 (57.0%)	53 (60.9%)	
Not-detectable	45 (52.9%)	64 (72.7%)		37 (43.0%)	34 (39.1%)	
***IL-12/IL-23p40**			0.093[1]			**0.008[1]**
Median (Q1, Q3)	70.5 (32.2, 111.9)	76.8 (55.0, 111.3)		143.1 (101.9, 203.2)	111.1 (90.2, 156.1)	
***IL-15**			0.958[1]			**<0.001[1]**
Median (Q1, Q3)	2.6 (0.7, 3.1)	2.3 (1.7, 2.7)		2.7 (2.3, 3.2)	2.0 (1.6, 2.5)	

*IL-16			0.754[1]			0.395[1]
Median (Q1, Q3)	216.8 (166.5, 266.7)	211.6 (165.5, 261.7)		247.1 (203.3, 315.6)	233.0 (167.4, 341.3)	
***IL-17A (cytokines)**			**0.010[1]**			**0.004[1]**
Median (Q1, Q3)	1.5 (0.7, 2.1)	1.0 (0.5, 1.8)		1.4 (0.9, 2.5)	1.1 (0.7, 2.0)	
****IL-1α**			0.742[2]			**0.007[2]**
Detectable	12 (14.1%)	14 (15.9%)		15 (17.4%)	4 (4.6%)	
Not-detectable	73 (85.9%)	74 (84.1%)		71 (82.6%)	83 (95.4%)	
****IL-5**			0.121[2]			**0.006[2]**
Detectable	58 (56.8%)	50 (56.8%)		72 (83.7%)	57 (65.5%)	
Not-detectable	27 (31.8%)	38 (43.2%)		14 (16.3%)	30 (34.5%)	
*IL-7			0.379[1]			0.303[1]
Median (Q1, Q3)	9.0 (6.1, 12.4)	8.3 (5.4, 11.5)		9.1 (6.3, 13.0)	8.6 (6.0, 11.1)	
****TNF-β**			<0.001[1]			**<0.001[1]**
Median (Q1, Q3)	1.0 (1.0, 1.0)	0.0 (0.0, 1.0)		1.0 (1.0, 1.0)	0.0 (0.0, 1.0)	
*VEGF			0.375[1]			0.289[1]
Median (Q1, Q3)	93.1 (60.4, 182.6)	96.5 (44.8, 164.5)		93.9 (58.3, 162.9)	86.3 (53.1, 134.2)	
Chemokines panel concentrations						
***Eotaxin**			0.108[1]			**<0.001[1]**
Median (Q1, Q3)	165.9 (84.3, 234.2)	128.0 (90.2, 181.5)		316.8 (232.1, 384.2)	210.8 (112.3, 290.3)	
*Eotaxin-3			0.280[1]			0.664[1]
Median (Q1, Q3)	8.0 (0.0, 18.0)	6.7 (3.3, 15.8)		13.2 (8.6, 19.4)	12.7 (7.4, 27.4)	
****IL-8(HA),** *n (%)*			**<0.001[2]**			**0.012[2]**
Detectable	2 (2.4%)	21 (23.9%)		6 (7.0%)	0 (0.0%)	
Not-detectable	83 (97.6%)	67 (76.1%)		80 (93.0%)	87 (100.0%)	
***IP-10**			0.903[1]			**0.045[1]**
Median (Q1, Q3)	171.3 (33.8, 363.7)	197.0 (126.8, 248.8)		207.6 (148.0, 328.5)	183.3 (95.7, 301.7)	
***MCP-1**			**0.001[1]**			**<0.001[1]**
Median (Q1, Q3)	178.6 (143.5, 232.0)	149.4 (99.2, 189.5)		279.4 (230.1, 370.3)	219.2 (170.7, 272.8)	
*MCP-4			**<0.001[1]**			**0.004[1]**

Median (Q1, Q3)	215.3 (166.2, 291.9)	170.0 (97.6, 214.1)		173.8 (119.8, 216.1)	129.0 (105.3, 187.5)	
***MDC**			0.453[1]			**<0.001[1]**
Median (Q1, Q3)	1228.0 (211.5, 1972.6)	1195.5 (823.7, 1462.7)		1334.6 (1073.7, 1644.3)	1025.8 (738.7, 1411.7)	
***MIP-1α**			0.945[1]			**0.014[1]**
Median (Q1, Q3)	16.8 (0.0, 27.0)	14.1 (7.9, 20.9)		15.0 (10.8, 19.2)	11.2 (7.5, 18.4)	
***MIP-1β**			0.721[1]			**0.005[1]**
Median (Q1, Q3)	96.1 (22.0, 164.6)	92.8 (63.2, 135.3)		105.2 (79.8, 134.9)	83.3 (53.3, 127.2)	
*TARC			0.139[1]			0.949[1]
Median (Q1, Q3)	436.4 (192.6, 629.3)	365.0 (167.4, 531.1)		312.3 (159.8, 577.9)	335.6 (176.2, 516.2)	
TH17 panel concentrations						
****IL-17A** (TH17)*, n (%)*			0.779[2]			**0.005[2]**
Detectable	33 (38.8%)	36 (40.9%)		31 (36.0%)	15 (17.2%)	
Not-detectable	52 (61.2%)	52 (59.1%)		55 (64.0%)	72 (82.8%)	
****IL-21***, n (%)*			**<0.001[2]**			0.064[2]
Detectable	26 (30.6%)	5 (5.7%)		15 (17.4%)	7 (8.0%)	
Not-detectable	59 (69.4%)	83 (94.3%)		71 (82.6%)	80 (92.0%)	
*IL-22			<0.001[1]			0.852[1]
Median (Q1, Q3)	1.0 (0.5, 1.5)	0.6 (0.3, 0.9)		0.6 (0.3, 0.9)	0.6 (0.3, 1.0)	
***IL-23			1.000[1]			1.000[1]
Median (Q1, Q3)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)		0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	
***IL-27**			0.985[1]			**0.007[1]**
Median (Q1, Q3)	861.6 (367.8, 1395.6)	775.1 (577.3, 1029.5)		1254.5 (882.1, 1608.7)	933.7 (600.9, 1477.1)	
****IL-31***, n (%)*			0.063[2]			0.970[2]
Detectable	28 (32.9%)	18 (20.5%)		17 (19.8%)	17 (19.5%)	
Not-detectable	57 (67.1%)	70 (79.5%)		69 (80.2%)	70 (80.5%)	
*MIP-3α			0.307[1]			0.181[1]
Median (Q1, Q3)	5.8 (4.0, 7.6)	6.4 (4.1, 9.0)		5.1 (3.0, 8.0)	6.4 (4.0, 8.9)	

*Given that <25% of values are undetectable, variables were used as is (continuous)

**Given that ≥25% of values are undetectable, variables were dichotomized into “detectable vs. Not-detectable”

***All values were undetectable

Q1=25th percentile; Q3=75th percentile

- **Key Accomplishments:**

5) Major activities included: meetings with Dr. Shiao, at Cedars-Sinai Medical Center, to decide which cytokines to be measured/analyzed; identifying serum samples from patients at the Durham VA, and transferred them to Cedars-Sinai Medical Center.

6) Specific objectives included: optimizing the cytokines kit (https://www.mesoscale.com/en/products/v-plex-human-cytokine-36-plex-kit-k15089d/) at Dr. Shiao's laboratory. Run the kits on 400 serum samples (200 biopsy positive/200 biopsy negative) from patients (50% black men) from the Durham VA.

7) Key outcomes included: making significant progress on successfully running the cytokines kits. **We are now validating these results in Dr. Fowke's cohort of black and white men undergoing biopsy.**

8) Other achievements: We are testing if the type and ratio of cytokine marker profiles is linked with PC risk and if these are modified by race. Data includes PC status, grade, PSA, and other clinicopathological and demographic features that are abstracted via the computerized medical records.

Aim 3: Assess the correlation between race and inflammatory markers. Race is a complex social construct. While black men have more aggressive PC, understanding the biological basis for this requires more than simply analyzing "black race". Thus, we proposed to test if prostatic and systemic inflammatory markers correlate with increased/decreased PC risk/aggressiveness in Aims 1 and 2 differ by race (we hypothesize black men will have more pro- and less anti-PC markers). We will then enter race in a multivariable model with factors such as SES, age, BMI, diet, exercise, comorbidities, PSA, and PC family history to assess which factors predict inflammatory marker expression and the degree to which race predicts these markers adjusting for other factors.

- **Key Accomplishments:**

1) Major activities included: Identified the adequate ancestry-informative SNPs markers chips to use (https://www.illumina.com/products/by-type/microarray-kits/infinium-multi-ethnic-global.html).

2) Specific objectives included: Prepared aliquots of de-identified DNA to be sent to Cedars-Sinai. Scheduled a time to run these chips at the Genomics Core at Cedars-Sinai Medical Center.

3) Key outcomes included: As mentioned above, we have aliquoted ~400 DNA samples matching the patients' biopsy tissue and serum used in Aims 1 and 2, and ran ancestry-informative SNPs markers (AIMs) chips.

4) Other achievements: We have data on SNPs AIMS for the 400 samples run. We are analyzing these data.

Reportable Outcomes

- Publications:

1. Racial differences in prostate inflammation: results from the REDUCE study.
Vidal AC, Chen Z, Howard LE, Moreira DM, Castro-Santamaria R, Andriole GL, Taioli E, Fowke JH, Knudsen B, Drake CG, Nickel JC, **Freedland SJ.**
Oncotarget. 2016 Jul 18;8(42):71393-71399. PMID: 29069714

2. Statin Use, Serum Lipids, and Prostate Inflammation in Men with a Negative Prostate Biopsy: Results from the REDUCE Trial.
Allott EH, Howard LE, **Vidal AC,** Moreira DM, Castro-Santamaria R, Andriole GL, **Freedland SJ.**
Cancer Prev Res (Phila). 2017 May 9. doi: 10.1158/1940-6207.CAPR-17-0019.

3. Neutrophil, lymphocyte and platelet counts, and risk of prostate cancer outcomes in white and black men: results from the SEARCH database.
Vidal AC, Howard LE, de Hoedt A, Cooperberg MR, Kane CJ, Aronson WJ, Terris MK, Amling CL, **Taioli E, Fowke JH,** Freedland SJ.
Cancer Causes Control. 2018 Jun;29(6):581-588. doi: 10.1007/s10552-018-1031-2. Epub 2018 Apr 17.PMID: 29663110

4. *Spatial Mapping of Myeloid Cells and Macrophages by Multiplexed Tissue Staining.*
Saylor J, Ma Z, Goodridge HS, Huang F, Cress AE, Pandol SJ, Shiao SL, **Vidal AC,** Wu L, Nickols NG, Gertych A, **Knudsen BS.**
Front Immunol. 2018 Dec 14;9:2925. doi: 10.3389/fimmu.2018.02925. eCollection 2018.

5. Geographic Differences in Baseline Prostate Inflammation and Relationship with Subsequent Prostate Cancer Risk: Results from the Multinational REDUCE Trial.
Allott EH, Markt SC, Howard LE, **Vidal AC**, Moreira DM, Castro-Santamaria R, Andriole GL, Mucci LA, **Freedland SJ.**
Cancer Epidemiol Biomarkers Prev. 2018 Jul;27(7):783-789. doi: 10.1158/1055-9965.EPI-18-0076.

6. Natural killer cell activity and prostate cancer risk in veteran men undergoing prostate biopsy.
Vidal AC, Howard LE, Wiggins E, De Hoedt AM, Shiao SL, Knott S, **Taioli E, Fowke JH, Freedland SJ.**
Cancer Epidemiol. 2019 Oct;62:101578. doi: 10.1016/j.canep.2019.101578.

7. Dietary inflammatory index (DII) and risk of prostate cancer in a case-control study among Black and White US Veteran men.
Vidal AC, Oyekunle T, Howard LE, Shivappa N, De Hoedt A, Figueiredo JC, **Taioli E, Fowke** JH, Lin PH, Hebert JR, **Freedland SJ.**
Prostate Cancer Prostatic Dis. 2019 Dec;22(4):580-587. doi: 10.1038/s41391-019-0143-4.

8. *Monocyte counts and prostate cancer outcomes in white and black men: Results from the SEARCH database.*
Yirga A, Oyekunle T, Howard LE, De Hoedt AM, Cooperberg MR, Kane CJ, Aronson WJ, Terris MK, Amling CL, **Taioli E, Fowke JH**, Klaanssen Z, **Freedland SJ, Vidal AC.**

Cancer Causes and Control, 2020, *in press.*

- Poster presentations:

1*. Racial Differences in Systemic and Prostatic Inflammation, by **Vidal et al**.*
Urology Care Foundation Honors Reception and Program, AUA 2017 Annual Conference, Boston, MA.
2. *Racial Differences in Systemic and Prostatic Inflammation, by **Vidal et al**.*
Urology Care Foundation Honors Reception and Program, AUA 2018 Annual Conference, San Francisco, CA.
3. *Serum cytokines and chemokines profile and risk of prostate cancer in a case-control study among black and white US Veteran men.*
Vidal AC, Oyekunle T, Howard LE, de Hoedt A, **Taioli E, Fowke JH, Drake CG, Freedland SJ.**
American Urological Association Annual Meeting AUA 2019, Chicago, May 3-6.
4. Black men have higher expression of inflammatory markers in prostate biopsies.
Bosomworth A, **Freedland SJ,** Ma Z, Fowke J, **Taioli E, Rogatko A, Knudsen B, and Vidal AC**.
American Urological Association Annual Meeting AUA 2019, Chicago, May 3-6.

Conclusions

We fulfilled our aims and made great progress on this project. However, challenges remained in regards to the timeline proposed to conduct and finish the experiments. For example, is worth mentioning that one of the Co-investigators in this study, Dr. Jay Fowke, has moved to a new Institution. Thus, we experienced delay in receiving the serum and tissue samples for the validation analyses proposed in the study. We will have that data soon.

Personnel receiving salary from award

Stephen J. Freedland, MD
Adriana C. Vidal, PhD
Beatrice Knudsen, MD, PhD
Andre Rogatko, PhD
Jay Fowke, PhD
Emanuela Tailoi, MD