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Brief Communications

# Analysis of Circulating Immune Biomarkers by Race in Men With Metastatic Castration-Resistant Prostate Cancer Treated With Sipuleucel-T

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## Abstract

Among racial subgroups, Black men have the highest prostate cancer–specific death rate, yet they also exhibit prolonged overall survival compared with White men when treated with standard therapies, including sipuleucel-T. Differential immune responses may play a role in these observations. We compared circulating immune markers from 54 men (18 Black and 36 White) with metastatic castrate-resistant prostate cancer who received sipuleucel-T and were enrolled on an immune monitoring registry. Markers included longitudinal serum cytokine concentrations, humoral responses, and cellular immunity from baseline until 52 weeks after sipuleucel-T administration. Black men had statistically significantly higher median concentrations of $T_H2$-type (interleukin [IL]-4, IL-10, and IL-13) and inflammatory cytokines (IL-2, IL-12, and IL-6) compared with prostate-specific antigen-matched White men both at baseline and 52 weeks after sipuleucel-T (2-sided $P < .05$). No differences by race were seen in either the antigen-specific T-cell response or the humoral responses to the immunizing antigen PA2024 and select secondary antigens.

BRIEF COMMUNICATIONS

Prostate cancer (PC) in Black men tends to exhibit more aggressive biology than in White men, with 2.2-times higher risk of PC-specific mortality (1). Paradoxically, mounting clinical data demonstrate that Black men with PC have improved survival with multiple therapies including chemotherapy, abiraterone acetate, Radium-223, and sipuleucel-T compared with White men (2–5). A 10-month median overall survival advantage (hazard ratio = 0.70, 95% confidence interval [CI] = 0.57 to 0.86; $P < .001$) was observed after sipuleucel-T treatment in PSA-matched Black men with metastatic castration-resistant PC compared with White men from the PROCEED (NCT01306890) registry (2).

Sipuleucel-T, approved for the treatment of men with asymptomatic or minimally symptomatic metastatic castration-resistant PC, is an autologous cellular immunotherapy that activates the immune system against the target antigen prostatic acid phosphatase (PAP), which is expressed on most PC cells (6). The results include increasing in antigen-presenting cell activity, increasing both cellular and humoral responses to PAP, stimulating cytotoxic T-lymphocytes, and generating a secondary immune response to additional tumor antigens (antigen spread) further augmenting T-cell activity (7–11). Differences in any of these mechanisms may contribute to reported differences in clinical outcomes.

Other immunologic studies observed racial differences, including the relative proportion of circulating immune cells, the allele frequencies responsible for upregulating pro-inflammatory cytokines, and B- and T-cell signaling (12–16). Black men with PC exhibit increased densities of immunosuppressive and stromal cell types and lower gene expression of key immune proteins (eg, major histocompatibility complex class I proteins) required to initiate an antitumor immune response compared with White men (17–19). Observed phenotypic differences in the tumor immune microenvironment may contribute to the more aggressive tumor biology seen in Black men with primary PC, but they do not explain the benefits observed

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**Table 1.** Baseline clinicopathologic characteristics of cohort

| Characteristics | Total (N = 54) | Black men (n = 18) | White men (n = 36) | P[a] |
|---|---|---|---|---|
| Mean age at enrollment (95% CI), y | 71.35 (68.88 to 73.82) | 67.78 (63.29 to 72.26) | 73.14 (70.21 to 76.07) | .10 |
| ECOG performance status, No. (%) | | | | |
| 0 (or missing) | 30 (55.6) | 10 (55.6) | 20 (55.6) | .86 |
| 1 | 21 (38.9) | 7 (38.9) | 14 (38.9) | |
| 2 | 2 (3.7) | 1 (5.6) | 1 (2.8) | |
| 3 | 1 (1.85) | 0 | 1 (2.8) | |
| Mean PSA at baseline (95% CI), ng/mL | 34.41 (21.94 to 46.89) | 33.43 (15.46 to 51.39) | 34.91 (17.89 to 51.92) | .71 |
| Gleason score at diagnosis, No. (%) | | | | |
| >8 | 27 (50) | 8 (44.4) | 19 (52.8) | .56 |
| ≤8 | 27 (50) | 10 (55.6) | 17 (47.2) | |
| Bone metastases, No. (%) | | | | |
| Low volume (≤5 metastases) | 37 (68.5) | 13 (72.2) | 24 (66.7) | .68 |
| High volume (>5 metastases) | 17 (31.5) | 5 (27.8) | 12 (33.3) | |
| Mean hemoglobin (95% CI), g/dL | 12.5 (12.0 to 12.9) | 11.8 (11.0 to 12.6) | 12.8 (12.2 to 13.4) | .09 |
| Additional systemic treatment during next 52 weeks, No. (%) | | | | |
| No | 27 (50) | 8 (44.4) | 19 (52.8) | .56 |
| Yes | 27 (50) | 10 (55.6) | 17 (47.2) | |

[a]P value of Wilcoxon signed-rank sum test (PSA), *t* test for all other continuous variables, and $\chi^2$ test for categorical variables. All P values are 2-sided. CI = confidence interval; ECOG = Eastern Cooperative Oncology Group; PSA = prostate-specific antigen.

with treatments including sipuleucel-T in Black men with advanced PC. A recent pathology-based study showed an increased inflammatory infiltrate in radical prostatectomy specimens from Black vs White men, which was associated with improved outcome (20).

Here, we describe studies examining the impact of race on immunologic responses to sipuleucel-T using peripheral blood samples from patients enrolled in the aforementioned PROCEED registry (2,21). Detailed methods and findings have been previously published (2,21); of note, race was self-reported.

A subset of PROCEED patients (n = 139) was concurrently enrolled in an immune monitoring trial (PRIME; NCT01727154) to identify potential biomarkers of immune response with provisions for collection of investigational blood samples at baseline, 6, 10, 14, 26, 39, and 52 weeks after the first infusion of sipuleucel-T. All Black men (n = 18) in PRIME who had an archived biospecimen were selected for this analysis and matched 1:2 to White patients (n = 36) based on group mean baseline PSA concentrations (33.43 vs 34.91 ng/mL, respectively; N = 54). Both PROCEED and PRIME protocols were reviewed and approved by institutional review board committees. Informed consent was obtained from all patients.

We analyzed longitudinal, peripheral immune biomarkers including serum cytokine concentrations using a 10-parameter electrochemiluminescence assay (Meso Scale Discovery, Rockville, MD) at baseline, 6, 26, and 52 weeks. T-cell proliferation, enzyme-linked immunosorbent assay, and enzyme-linked immuno spot assay ( ELISpot), to quantify cytokine-secreting T cells, were performed at all timepoints as described previously (22). Humoral (IgG) responses using protein microarray were determined using a Luminex XMAP assay system as previously detailed (8). We estimated overall survival in our total cohort (n = 54) using Kaplan-Meier methodology and a log-rank test. Descriptive statistics were used to summarize baseline clinical characteristics; group comparisons were performed using *t* test, Wilcoxon, and $\chi^2$ tests. We used nonparametric Wilcoxon signed-rank sum tests to compare observations by race per timepoint; Spearman rank correlation coefficient to compare continuous variables; aligned rank transformation for a nonparametric factorial analysis using analysis of variance procedure (23) to test the association of Gleason groups (high ≥ 8 vs low ≤ 7) with cytokine concentrations when controlling for repetitive sampling and longitudinal samples; and a gamma and exponential distribution model to test the association of PSA (continuous) with cytokine concentrations. All statistical tests were 2-sided, and a P value of less than .05 was considered statistically significant.

As shown in Table 1, Black and White men in PRIME cohort were similar in baseline demographic or clinical characteristics. Overall, mean PSA of the entire group was 34.41 ng/dL (95% CI = 21.94 to 46.89). Half had Gleason scores of 8 or greater. Most men (68.5%) had low volume disease (≤5 metastases). Consistent with previously published data, Black men had prolonged median survival time compared with White (median = not reached vs 32 months; log-rank $P = .07$). Interestingly, Black men had statistically significantly higher levels of $T_H2$-type (interleukin [IL]-4, IL-10, IL-13) and inflammatory (IL-2, IL-6, IL-12p40) cytokines at baseline (all $P < .05$). These differences were not apparent at treatment completion (week 6) or at the 26-week timepoint. However, 52 weeks after sipuleucel-T administration, elevated cytokine levels were again noted in Black men as compared with matched White men (Figure 1). When dichotomized by the median value, no cytokine was associated with increased survival by race in our small sample (data not shown). After controlling for all 10 cytokines, repeated measurements, and 4 timepoints, higher Gleason score was strongly associated ($P < .001$) with higher cytokine levels. We also tested race in this model, but it was not statistically significant. In the gamma and exponential model, somewhat counterintuitively, PSA was inversely associated with cytokine concentrations. In this second model, Black race was strongly associated with higher cytokine concentrations (estimate = 0.54, 95% CI = 0.12 to 0.95; $P = .01$) when adjusted for the same variables. There were no differences in humoral (B cell) responses to the immunizing antigen (PA2024), the target antigen (PAP), or selected secondary antigens by race at any timepoint. Counterintuitively, White men had statistically significantly increased T-cell responses to PA2024 compared with

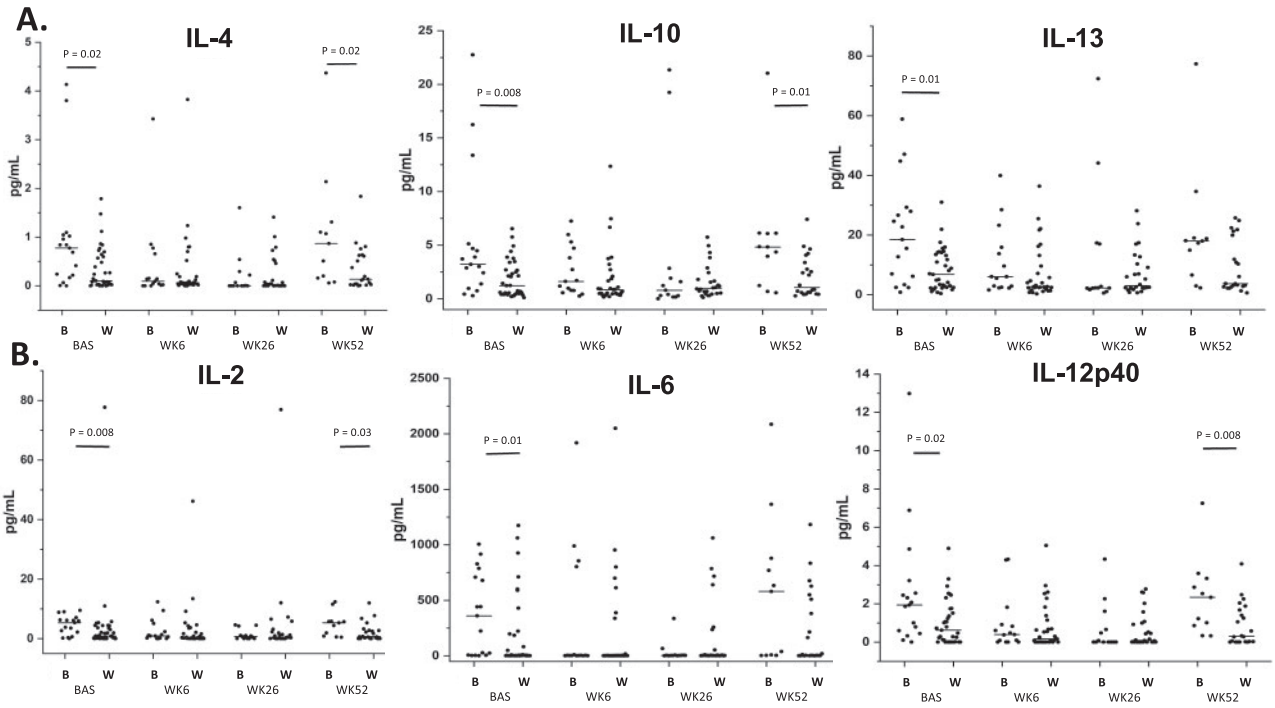


**Figure 1.** Differences in longitudinal measures of cytokines by race in men with metastatic castration-resistant prostate cancer over the course of sipuleucel-T treatment. **A)** $T_H2$-type and **B)** proinflammatory cytokine concentrations at baseline (BAS), 6 (WK6), 26 (WK26), and 52 (WK52) weeks in analyzed patients (n = 54). Results for Black (B) and White (W) men are presented. **Horizontal lines** represent the median values in each group. Wilcoxon signed-rank sum test was used to compare paired observations. **Horizontal bars** denote statistical significance between timepoints. All statistical tests were 2-sided. Individual observations represented by **dots**. IL = interleukin.

Black men as assessed by ELISpot at week 6 (59.67 vs 8.67 spot-forming cells; P = .04) and 39 weeks (18 vs 0; P = .03) (Supplementary Table 1, available online).

In summary, our data show higher baseline and posttreatment systemic inflammation in Black vs White men and are thus broadly consistent with prior studies (12–20). Treatment with sipuleucel-T transiently attenuated those differences, which reemerged later posttreatment. Unfortunately, our systemic data are unable to adequately explain the prolonged survival in Black men treated with sipuleucel-T as compared with White men. In particular, we hypothesized that increased $T_H2$-type cytokines would result in increased antibody titers; this was not the case. One might also suspect that increased systemic IL-2 and IL-12 levels would be reflected in increased T-cell responses; in fact, the opposite was noted, with White men showing increased ELISpot responses. One possible explanation for these data is that differences in the on-treatment tumor immune microenvironment are not adequately reflected in the systemic circulation. In addition, our studies were limited to a subset of circulating cytokines and biomarkers; therefore, it is also possible that a more extensive cytokine or proteomic analysis could reveal other racially disparate markers better associated with the improved outcome in Black men. Further studies, including careful tumor biopsies and incorporation of social determinants of health, will be required to more fully understand the genetic and environmental underpinnings of differential inflammation in Black vs White men, as well as their association with outcomes.

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**Author contributions:** JEH, NS, and CGD conceptualized the research. JEH, SP, HK, CGD performed the formal analysis. NS and CGD supervised the research. JEH and CGD wrote the manuscript. All authors reviewed, edited, and approved the final version of the manuscript.

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## Data Availability

Data for this paper were generated from two sources: experiments performed by Hawley et al. using samples provided by Dendreon Pharmaceuticals LLC and patient-level immune response testing performed by Dendreon. The data underlying this article may be shared on reasonable request after consideration of each request and pertinent patient privacy concerns. All requests should be sent to the corresponding author who will discuss the requests with the co-authors.

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