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IL-1β blockade enhances antiviral immunity and cytotoxic effector functions in ART suppressed PWHIV

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BACKGROUND

- Does therapeutic blockade of the pro-inflammatory cytokine IL-1β reverse immune dysfunction associated with chronic inflammation in PWHIV under ART suppression?

Persistent and chronic inflammation is a hallmark of immune dysfunction in PWHIV under ART suppression. Chronic inflammation also contributes to the development of co-morbidities experienced by PWHIV including cardiovascular disease (CVD). Therapeutic targeting of specific mechanisms of inflammation which drive immune dysfunction and co-morbidities in PWHIV represents an opportunity to improve outcomes. IL-1β is a highly potent inflammatory cytokine which is known to be induced during acute HIV infection and has been shown to be a critical driver of the development of CVD. Though IL-1b has been studied in great detail in the context of many pathogens, relatively little is known about how IL-1β contributes to chronic inflammation in HIV and how this directly regulates immune function or dysfunction. Existing FDA-approved drugs which block IL-1β and IL-1 signaling make this a promising mechanism for therapeutic targeting.

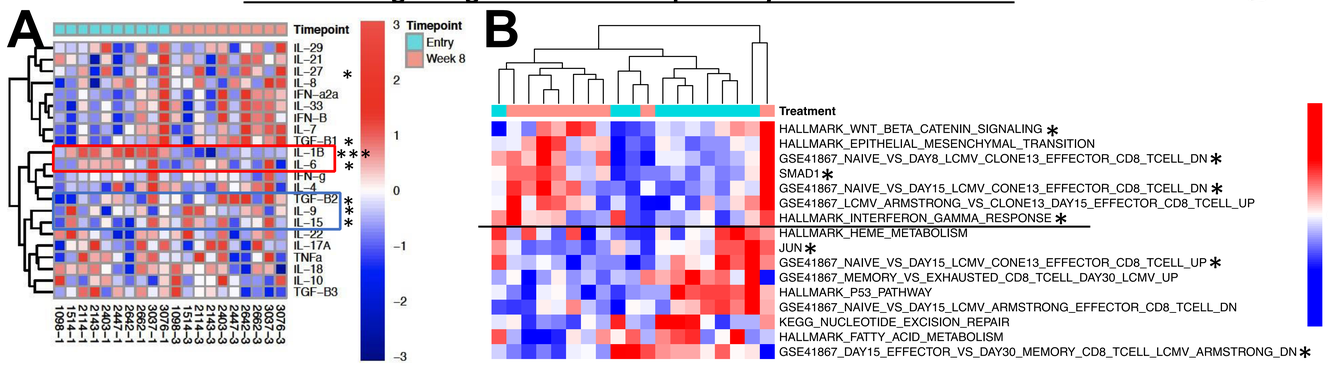
METHODS

- Accessed samples from the clinical trial (NCT002272946) studying the effect of Canakinumab, monoclonal blocking antibody that binds IL-1β, treatment on measures of safety, inflammation and cardiovascular risk factors
- Multi-plexed cytokine analysis was performed prior to Canakinumab treatment (entry) and at Weeks 4- and 8-post Canakinumab
- Performed RNA-sequencing on whole blood and sorted CD4+ T cells at entry and Week 8 post Canakinumab
- Single cell RNA-sequencing was performed on entry and Week 4 post-Canakinumab

Therapeutic blockade of the inflammatory cytokine IL-1β, using the monoclonal antibody Canakinumab, in PWHIV and CVD resulted in *reversal of pathways of chronic immune dysfunction* and *improved antiviral and cytotoxic effector immune responses*

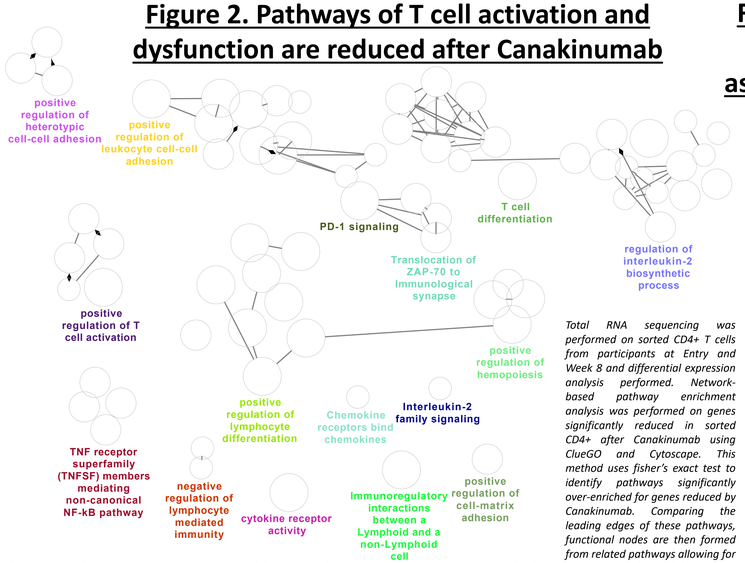
RESULTS

Figure 1. Reduction in circulating IL-1β levels results in increased Interferon signaling and reversal of pathways of CD8 exhaustion



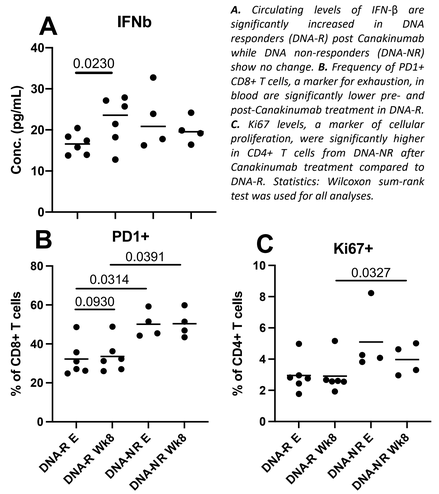
A. *Cytokine levels in the plasma of participants at Entry (pre-treatment) and Week 8 were quantified using the multiplexed U-Plex platform from MesoScale Discovery. Levels of IL-1ß and its target cytokine IL-6, both key cytokines driving chronic inflammation, were significantly reduced after Canakinumab. We also observed a significant increase in IL-15, cytokine that promote NK and CD8 effector function, and TGF-B2, a potent anti-inflammatory and immunosuppressive cytokine, highlighting a switch from a more inflammatory state to a more regulated environment that may promote effector function. IFN-α2a and IFN-ß levels are also elevated post-treatment, though not significantly. Stats: Wilcoxon sum rank test.* ***B.*** *Bulk RNA-sequencing was performed on whole blood samples (PAXGene tubes) from participants at Entry (Week 0) and Week 8. Differential gene expression analysis and pathway analysis by Gene Set Enrichment Analysis was performed. Key pathways highlighted by *. Among the top pathways significantly by Canakinumab were: Interferon Gamma signaling (genes map to antiviral function and MHC Class I antigen presentation), pathways associated with CD8 exhaustion, and SMAD signaling (TGF-β/BMP responses). Among pathways increased by Canakinumab were Jun signaling, a key transcription factor involved in inflammation, and pathways of Naïve and Memory CD8 T cells. Stats: All pathways pass adjusted p value cut-off of 0.05*

Figure 2. Pathways of T cell activation and dysfunction are reduced after Canakinumab



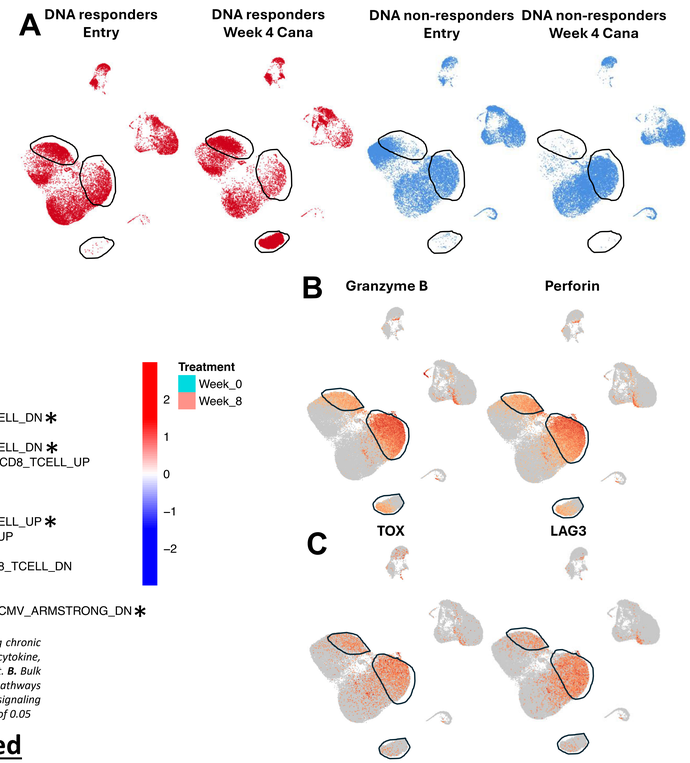
Total RNA sequencing was performed on sorted CD4+ T cells from participants at Entry and Week 8 and differential expression analysis performed. Network-based pathway enrichment analysis was performed on genes significantly reduced in sorted CD4+ after Canakinumab using ClueGO and Cytoscape. This method uses fisher's exact test to identify pathways significantly over-enriched for genes reduced by Canakinumab. Comparing the leading edges of these pathways, functional nodes are then formed from related pathways allowing for network visualization of overall changes in cellular status and function. Key functional nodes of T cell activation, T cell differentiation, PD-1 signaling, and response to cytokines including IL-2 were reduced in CD4+ T cells after Canakinumab highlighting significant reprogramming of CD4+ T cells to be less activated and dysfunctional. Pathways of inflammation, cell cycling, and glycolysis were also significantly reduced in CD4+ T cells after Canakinumab (not depicted). All pathways pass adjusted p value cutoff of 0.05

Figure 3. Heightened IFN and reduced CD8 exhaustion and CD4 cycling are associated with reduced HIV DNA levels



A. *Circulating levels of IFN-β are significantly increased in DNA responders (DNA-R) post Canakinumab while DNA non-responders (DNA-NR) show no change.* ***B.*** *Frequency of PD1+ CD8+ T cells, a marker for exhaustion, in blood are significantly lower pre- and post-Canakinumab treatment in DNA-R.* ***C.*** *Ki67 levels, a marker of cellular proliferation, were significantly higher in CD4+ T cells from DNA-NR after Canakinumab treatment compared to DNA-R. Statistics: Wilcoxon sum-rank test was used for all analyses.*

Figure 4. scRNA-seq confirms reduced CD8 exhaustion in participants with reduced HIV DNA levels



A. *Single cell sequencing was performed on PBMCs from DNA-R and DNA-NR (n=3 each) at Entry and Week 4 post Canakinumab and analyzed using 10x Genomics pipelines. Clustering analysis revealed 3 clusters of CD8+ T cells and NK cells differentially regulated between DNA-R and DNA-NR. The top and bottom outlined clusters are features of DNA-R pre- and post-Canakinumab. The outlined middle cluster is a feature of DNA-NR pre- and post-Canakinumab.* ***B.*** *Overlay of expression of Granzyme B and Perforin reveals these all 3 clusters contain cells expressing high levels of these cytotoxic effector proteins, indicating these are cytotoxic cells.* ***C.*** *Critically, the cluster of cytotoxic cells that is a feature of DNA-NR (middle outline) had increased expression of the exhaustion markers TOX and LAG3, suggesting that cytotoxic cells from DNA-NR are more exhausted pre- and post-Canakinumab. These data indicate there may be a point at which exhaustion can no longer be reversed just by blocking IL-1ß.*

CONCLUSIONS

- Our findings support the hypothesis that blockade of IL-1β reduces immune dysfunction in 3 key areas:
  1. Decreased inflammation (Fig. 1A;Fig. 2) and immune activation/cycling (Fig. 2, Fig. 3C)
  2. Increased Interferon cytokine levels (Fig. 1, Fig. 3B) and Interferon signaling (Fig. 1B)
  3. Reduced CD8 T cell exhaustion (Fig. 1B, Fig. 3B, Fig. 4)
- This "triple threat" has the potential to dramatically improve the dysregulated immune environment of PWHIV, setting the stage for more effective cure strategies and reduced incidence of co-morbidities

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