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Inflammatory Monocytes Increase During HIV-1 Treatment Interruption Before Detectable Viremia

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BACKGROUND

There are currently no reliable non-viral biomarkers of HIV rebound in people living with HIV (PLWH) undergoing analytical treatment interruption (ATI). Such a biomarker may illuminate early mechanisms underlying immune control of viral recrudescence, improve the safety of ATIs, and inform individuals of potential controller status (1). **We hypothesized that low levels of virus replication in tissues may create measurable changes in circulating cells or proteins prior to detectable viremia.** Previous studies demonstrated changes in innate immune responses and circulating plasma metabolites that precede HIV rebound (2,3). Here, we computationally analyze changes in the immune landscape that may indicate early responses to viral rebound in individuals with HIV-1 during ATI (Table 1) (4,5,6). We compared three distinct timepoints: on-ART (before ATI), pre-rebound (during ATI, before detectable viremia), and post-rebound (detectable viremia) to assess changes that indicate imminent rebound (Figure 1). A subset of participant samples were used for single cell RNAseq (scRNAseq) (n=10) and for Olink plasma protein analysis (n=23).

Table 1. Cohort demographics

Participants	24
Median Age (median, range)	40 (27-65)
Male (% sex)	87.5
Female (% sex)	12.5
Black Non-Hispanic (%)	4.2
Asian, Pacific Islander (%)	4.2
White Non-Hispanic (%)	75.0
Hispanic, Regardless of Race) (%)	16.6

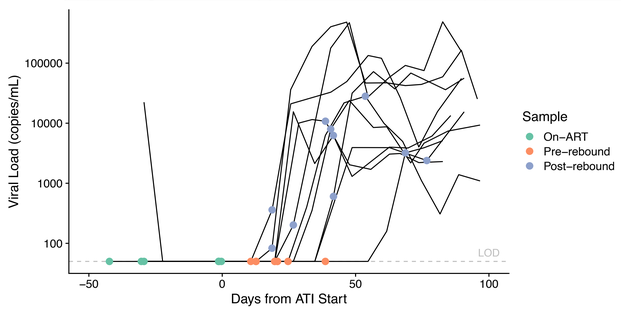

Figure 1. Participant viral load curves with a limit of detection of 50 copies/mL for the group of samples analyzed by scRNAseq. Colored circles indicate sample collection and assigned groups; green: on-ART, orange: pre-rebound, and blue: post rebound.

METHODS

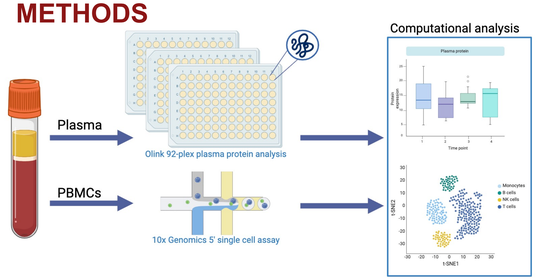

Figure 2. Experimental workflow. PBMCs from 10 participants were isolated and processed with 10x Genomics 5' scRNAseq. Data was analyzed using Cell Ranger, Seurat, and DeSeq2. Plasma from 23 participants was analyzed by Olink using the Immuno-oncology, Biological Processes and Inflammation panels.

RESULTS

Single cell RNA sequencing data was computationally processed, and cells were clustered by predicted cell type based on PMBC gene expression reference data (Figure 3).
Cell type frequency between timepoints was determined.
We observed an **increase in monocyte subsets prior to detectable viral rebound.** The proportion of predicted $CD14_{dim}CD16+$ monocytes increased by 1.9% (p=0.008) and predicted CD14+CD16- monocytes by 3.6% (p=0.054) (Figure 4).

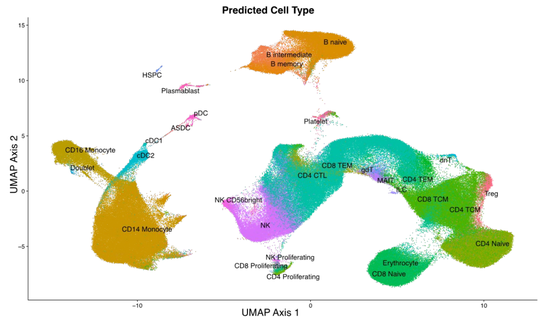

Figure 3. A UMAP projection of single cell RNAseq data using the normalized mRNA expression of the top 3000 genes in the data set showing cell clusters within PBMC samples. Analysis of single cell data was done using the Seurat R package.

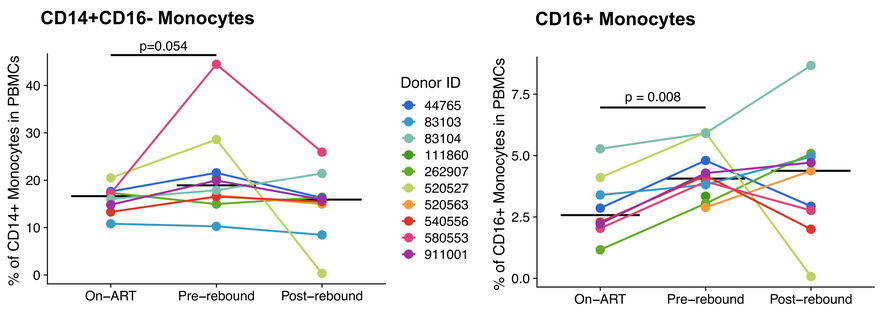

Figure 4. Percentage of total monocytes in PBMCs of ATI participants compared between on ART, pre-rebound (during ATI), and post-rebound (detectable viremia). P-values show the results of a paired Wilcoxon test between time points.

Pre-rebound monocytes have distinct gene expression compared to on ART time points (Figure 5).
Pathway analysis indicates an increase in activation, enrichment for IFNγ, IFNα, and inflammatory response pathways within monocyte subsets (Figure 6).

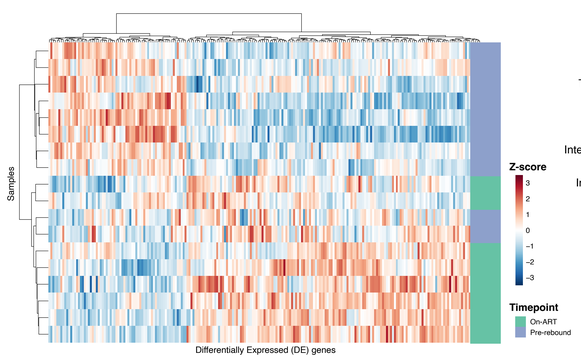

Figure 5. A heatmap showing the differentially expressed genes between CD16+ monocytes from samples on ART and Pre-rebound timepoints. Differentially expressed genes were found using DeSeq2 with normalized gene expression.

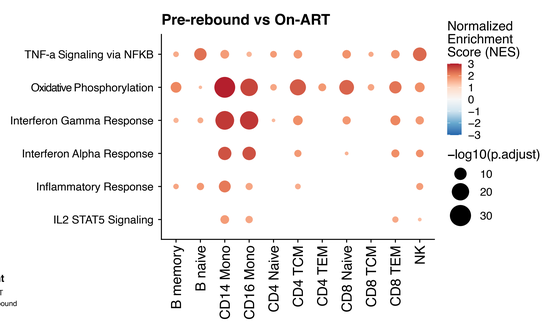

Figure 6. GSEA Hallmark pathway analysis of differentially expressed genes in scRNAseq data on ART vs. Pre-rebound showing the normalized enrichment score for significantly enriched pathways. The size of the circles shows the statistical significance of the log fold association with the cell type and listed pathway.

There was an increase in inflammatory plasma proteins post-rebound during detectable viremia likely due to immune activation (PD-L2, TRAIL), cytokine signaling (TNF, LAG3, GZM proteins), and NFkB signaling (CXCL9, CXCL10, IL12) determined by Olink plasma protein analysis (Figure 7).

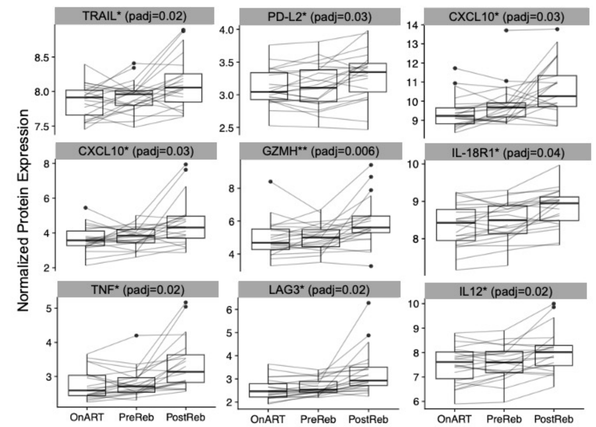

Figure 7. Olink plasma soluble, circulating protein measurements between time points using normalized protein expression values show inflammatory proteins increase post-rebound during detectable viremia.

CONCLUSIONS

- **Increasing monocytes in circulation may signal an initial innate immune response to low levels of replicating virus.**
- Total monocyte count and composition may serve as a potential non-viral biomarker for rebound in people living with HIV-1 who undergo ATI.
- After ATI, a subset of circulating monocytes well suited to encounter virus activate and shift to a more inflammatory phenotype as a possible response to low level viral replication.
- This change is measurable across donors who have significant viral load heterogeneity, as shown in Figure 1.
- This may have implications for measurements during future ATI studies.

Acknowledgments

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