MULTIOMIC BIOMARKERS ACROSS ART INITIATION TIMEPOINTS REVEAL INTERFEROME ENRICHMENT

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BACKGROUND

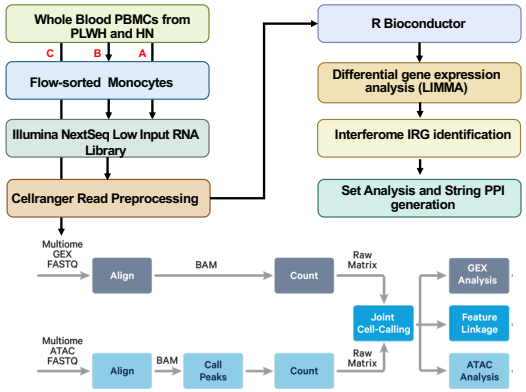
People with HIV (PLWH) have increased risk of comorbidities even with antiretroviral therapy (ART). Based on our prior studies, we hypothesized that persistent immune activation in PLWH, particularly in the monocyte compartment, undermines immunoregulation in some PLWH. We analyzed peripheral blood gene expression profiles of monocytes and lymphocytes from PLWH before and after ART to assess regulation of the interferome in chronic immune dysregulation after ART.

METHODS

Our first RNASeq analysis was performed on select cryopreserved PBMCs from African PLWH enrolled in three HIV-prevention trials prior to ART initiation (preART; CD4 >200 cells/mL, n=17) and <1 yr post-ART initiation (postART, n=17); and matched HIV-negative individuals (HN, n=12), as well as flow-sorted immune cell subsets to determine gene expression. Samples were sub-grouped to early post-ART (sampled ≤60 d after ART initiation), and late post-ART (>100 d after ART initiation) to identify longitudinal changes. Table below contains full cohort demographics.

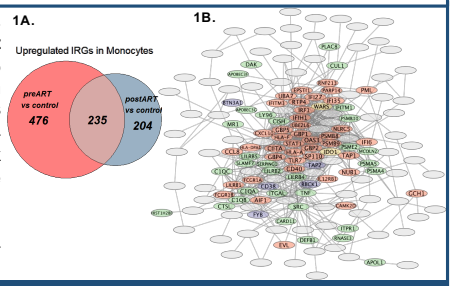
	Sample Size	Mean age (years)	Biological Sex	Country
Control	23	36	61% Female	Kenya: 39% South Africa: 13% Uganda 48%
On ART	25	33	72% Female	Kenya: 44% South Africa: 12% Uganda 44%

RNASeq was performed (Illumina NextSeq 550 platform) on (A) total PBMC and (B) flow cytometry-sorted cell subsets. Linear contrasts were performed to identify differentially expressed genes (DEG, p≤0.05, n≥3) between pre-ART, post-ART and HN. We employed 10X Genomics single cell multiomic analysis (C) to delve deeper into potential epigenetic mechanisms driving differential gene expression in PLWH at pre- vs post-ART timepoints.

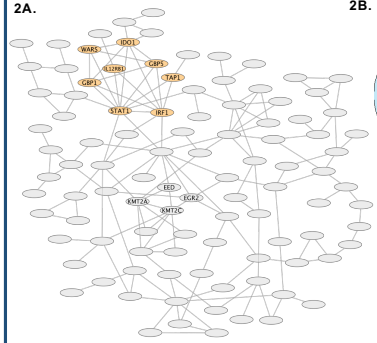


RESULTS

Figure 1. Gene interaction network of IRGs upregulated in monocytes from PLWH at pre- and post-ART timepoints relative to HIV-negative individuals. 1A Venn diagram indicating numbers of upregulated IRGs identified in contrasts of pre-ART and post-ART groups vs. HN. **1B** Interactive network IRGs coded in red represent members in the interferon response pathway, those in green innate immunity, those in blue antigen presentation, and those in orange tryptophan-related interferon response.



Core interferon regulatory gene networks potentially related to ongoing **inflammation** and **epigenetic changes** *remain upregulated* post-ART administration in PLWH.



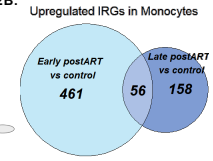
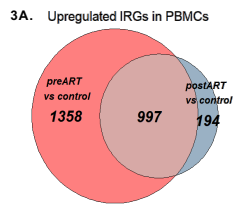
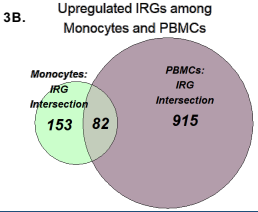
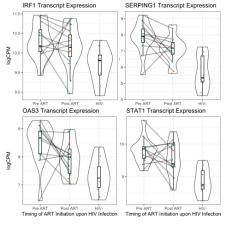


Figure 2. IRGs upregulated in PLWH at later time points after ART administration are related to interferon regulation and epigenetic remodeling. Interaction network of differentially expressed genes that remain enriched in monocytes in PLWH after 100 days of ART administration reveals gene modules related to inflammation and alteration of gene expression. All genes in the interferon regulation module interact with STAT1, a transcription factor stimulated by type I, II, and III interferons. Another gene module two degrees away contains genes such as EED and KMT2A, which drive changes in the modification of histones.

Figure 3. Gene interaction network of IRGs upregulated in whole blood PBMCs from PLWH before and after ART administration in contrast to HIV-negative individuals. 3A shows that only a small fraction of IRGs identified in the post ART versus HN contrast in PBMCs are exclusively upregulated in post ART PLWH. **3B** reveals that more than half of IRGs present in the monocyte intersection of pre- and post-ART are present in the PBMC intersection, indicating the monocyte expression signal is discernable in the PBMC contrast. **3C** profiles the expression of selected IRGs in monocytes across pre-ART, post-ART, and HN.



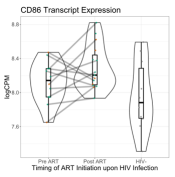




RESULTS

Figure 4. Multiome analysis reveals epigenetic changes in IRGs differentially expressed between ART administration groups.

A single-cell RNA sequencing approach combining gene expression and chromatin accessibility (ATACSeq) provides both epigenetic and gene expression data for each cell assayed at pre- and post-ART time points. **4A**. CD86 contains an internal genetic element that is differentially accessible between pre- and post-ART treatment conditions. **4B**. CD86 transcript read counts from monocyte bulk RNAseq were validated for increased expression post-ART in PLWH.



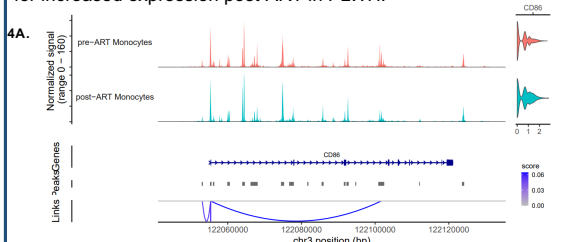
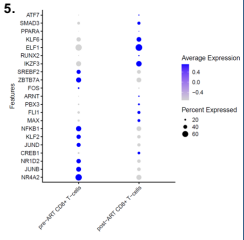


Figure 5. Enrichment of chromatin accessibility reveals transcription factors likely influencing enriched pathways in CD8⁺ calls from PLWH. Chromatin regions differentially altered for genes in enriched pathways between treatment conditions reveal a set of transcription factors which are also IRGs (except IKZF3) likely to drive these phenotypic differences.



CONCLUSIONS

This multiomic approach has revealed candidate biomarkers and potential therapeutic targets for reducing ongoing inflammation in PLWH, and increases our understanding of the role this ongoing response plays in immune dysfunction observed in PLWH on ART at the single cell level. Validation of these signatures in an independent cohort is needed to pursue these potential translational targets.

ACKNOWLEDGEMENTS

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