Refractoriness to SIV re-infection is induced by anti-IL-10/PD-1 therapy in rhesus macaques

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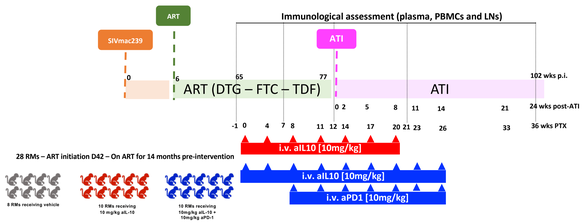
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Introduction

The persistence of the HIV reservoir under long term cART (combined Antiretroviral Therapy) is supported by virus rebound upon ATI (Analytical Treatment Interruption). Among the factors contributing to HIV reservoir persistence are the heightened levels of circulating IL-10 and PD1 expression. IL-10 contributes to the lack of effector function and antigen-presentation, additionally it promotes HIV reservoir survival. PD-1 plays a major role in inducing T cell exhaustion and HIV latency. *We hypothesized that the dual blockade of IL10 and PD1 could synergize and lead to SIV-functional cure by triggering intrinsic antiviral immunity in monocytes and CD4 T cells resulting in lack of viral re-seeding post-ATI (Analytical Treatment Interruption) and by boosting innate and adaptive cellular effector function*

Study Design



Methods

Twenty-eight rhesus macaques (RMs) were infected intravenously with SIVmac239. ART (TDF/FTC/DTG) was initiated at day 42 post-infection and maintained for 14-months prior to immune-therapeutic interventions. RMs received de-immunized aIL-10 (n=10), aIL-10/aPD-1 (n=10), or vehicle (n=8), with infusions every 3-weeks. ATI was performed at 12-weeks after the first dose of therapy has started. Infusions continued for 14-weeks after ATI. RMs were followed longitudinally, importantly during ATI and after discontinuation of the immune-therapy protocol. Flow cytometry longitudinally, gene expression and chromatin accessibility was measured in LNs and PBMC by bulk RNA-seq and scMultiome (GEX + ATAC) (pre-ATI).

Results

Durable viral load control post-ATI (<1000 cps/mL in 9/10 animals) was observed in the combo treated RMs up to 6 months post-ATI (**Fig.1**). Post-ATI stable IPDA and 2LTR readouts was also observed in combo-treated animals as compared to the control group (**Fig.2**). Gene expression analysis revealed significantly elevated restriction factor machinery in the lymph nodes (LN) cells from the combo treated group pre-ATI (APOBECs, SAMDH, ISGs) that was persistent till the end of the follow up (6 months post-ATI) (**Fig.3**). Monocytes, CD8 and CD4 T cells presented significantly higher protein-expression of pIRF3 and pIRF7 (**Fig.4**). Use of multiome platform that allows simultaneous single cell assessment of transcription and epigenetic profiles showed that the enhanced expression of antiviral ISGs was concomitant with the increased chromatin accessibility of the IRF3, IRF7 and Stat1 transcription factors, known to confer refractoriness to viral infections by inducing Interferon antiviral genes (**Fig.5-6**). This poised antiviral environment was associated with viral rebound control. The combo therapy triggers an epigenetic reprogramming of innate and adaptive immune cells highlighted by poised intrinsic antiviral machinery and poised effector molecules of T cell function that together synergize to block the *de novo* CD4 T cell infection post-ATI and viral dissemination.

Dual Treatment leads to viral rebound control post-ATI and lack of virus re-seeding as shown by stable IPDA and 2LTR circles

FIG 1

IL10 and PD1 dual blockade leads to SIV viremia rebound control for up to 6 months post-ATI

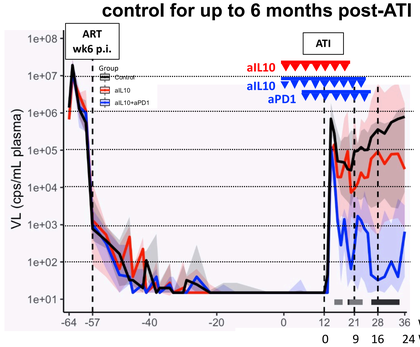
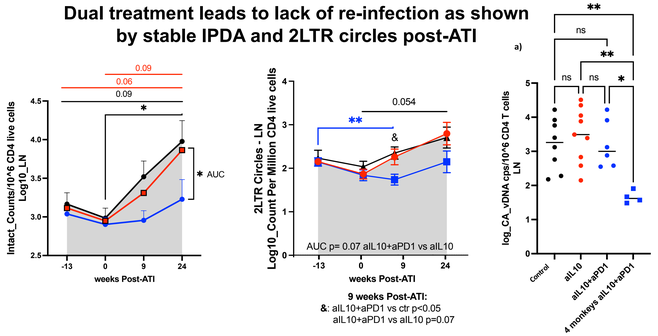


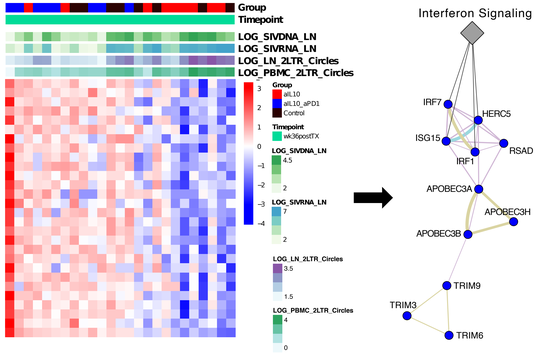
FIG 2

Dual treatment leads to lack of re-infection as shown by stable IPDA and 2LTR circles post-ATI



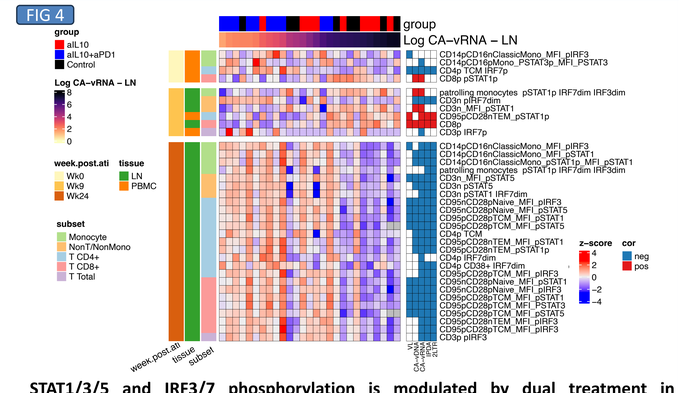
Dual therapy leads to significant reservoir decay in 40% of the treated animals

FIG 3



Anti-viral ISGs are negatively correlated to outcomes at week 24 post-ATI

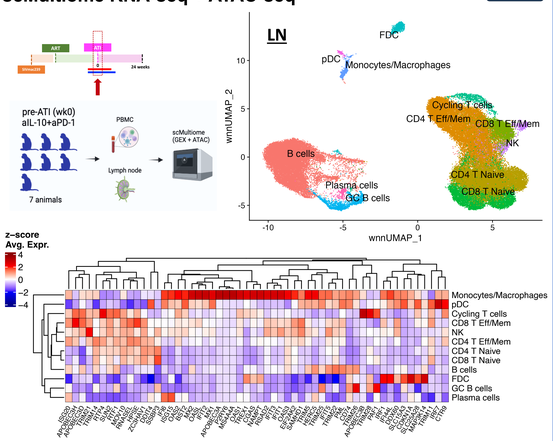
FIG 4



STAT1/3/5 and IRF3/7 phosphorylation is modulated by dual treatment in monocytes and T cells and negative correlated to viral readouts at week 24 post-ATI

scMultiome RNA-seq + ATAC-seq

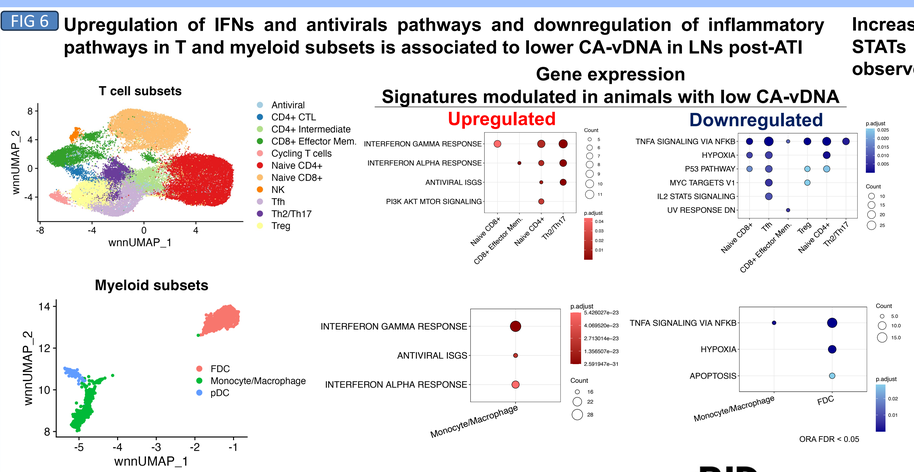
FIG 5



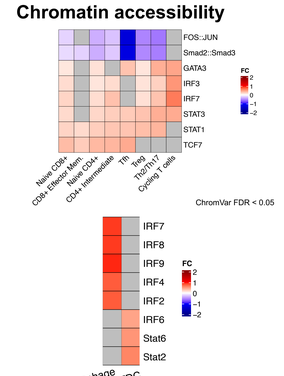
Anti-viral ISGs and HIV RFs are expressed across different immune subsets pre-ATI in lymph nodes from dual treated animals

FIG 6

Upregulation of IFNs and antivirals pathways and downregulation of inflammatory pathways in T and myeloid subsets is associated to lower CA-vDNA in LNs post-ATI



Increased motif accessibility of IRFs and STATs and decreased FOS/JUN is observed in animals with low SIV-DNA



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RID HIV

Concluding Remarks

- RMs treated with aPD1+aIL10 controlled viral rebound post-ATI and had lower SIV-DNA in LNs
- These RMs induced anti-viral ISGs/RFs at week 12 and CD4 stem-like signatures at week 36
- Lymphoid and myeloid subsets of combo treated animals express antiviral ISGs and HIV restriction factors pre-ATI even in the absence of detectable viremia.
- Lower viral reservoir 24 weeks post-ATI is associated to increased expression of IFN and decreased inflammatory pathways pre-ATI in lymph nodes
- This condition is associated to increased chromatin accessibility of IRFs and STATs binding sites and decreased FOS/JUN.

