IL-10/PD-1 Blockade in ART-treated SIV Infected RMs leads to Enhanced BA Signaling and Type I IFN Production

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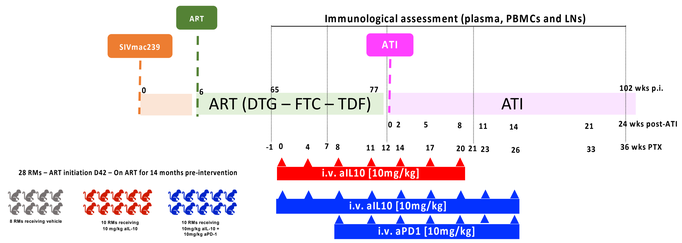
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Introduction

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 blockade of IL-10 and PD-1 could act synergistically to improve immune responses and promote viral load control upon ATI in the treated macaques.*

Study Design



Methods

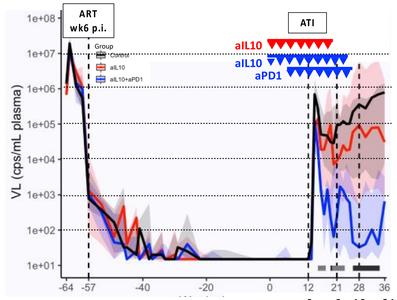
28 rhesus macaques (RMs) infected with SIVmac239 started antiretroviral therapy (ART) 42 days post-infection, maintained for 14-months. Rhesusized aIL-10 (n=10), aIL-10/aPD-1 (Combo therapy - n=10), or vehicle (n=8), infused every 3-weeks was administered starting 12 weeks pre-ATI and stopped 14-weeks post-ATI (week 26). Animals were followed longitudinally for biospecimen collections. Here, we utilized transcriptomic and metabolomic data from PBMCs and LNs to decipher the mechanisms of VL control observed in aPD1+aIL10 treated RMs.

Results

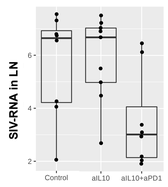
Week 12 (pre-ATI) PBMC and LN transcriptomic profiling of aPD1+aIL10 treated RMs revealed a significant enrichment of Bile Acid (BA) metabolism and synthesis characterized by elevated FXR (NFKB inhibitor) levels and enhanced BA and BA salt synthesis. We observed that targets of BA nuclear receptors FXR and VDR were enriched in combo-treated RMs. Concordantly, elevated metabolites (tauro-litho-cholate sulfate, litho-cholate sulfate) mapping to primary and secondary BA metabolism were elevated in dual-treated RMs. Additionally, enhanced levels of Type I IFN production characterized by innate anti-viral restriction factors RFs (IRF1, IRF7, APOBECs, TRIMs) were elevated in aPD1+aIL10 treated RMs. Notably, we observed a positive correlation between BA synthesis and Type I IFN RFs. Importantly, both mechanisms were negatively associated with the magnitudes of SIV RNA/DNA and 2LTR circles measured in LN. A multi-variate regression model highlighted the interplay between the innate interferon and BA signatures and their negative association with the magnitude of VL. The combination of both transcriptomic machineries was associated with better viral load control (p=0.0031). Altogether, these data demonstrate the potential role of intact BA synthesis/metabolism in regulating the innate anti-viral response as early as week 12 (pre-ATI) which can be critical to VL control.

Impact of Dual Treatment on SIV viremia

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

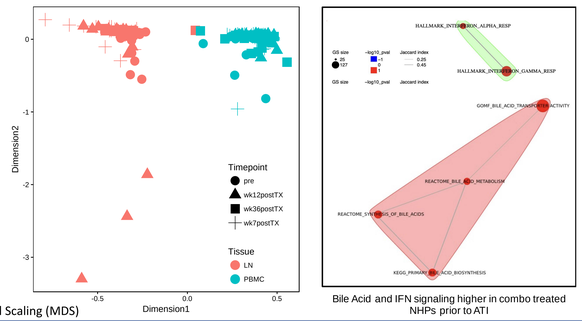


Week 36 (24 weeks post-ATI)



Lower SIV-RNA in LN post-ATI in NHPs receiving combo therapy

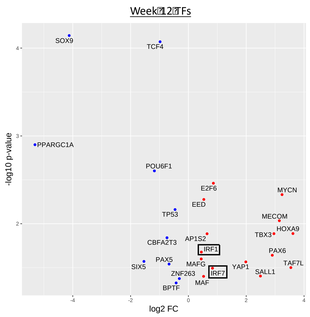
RNAseq analysis reveals IFN signaling and BA synthesis as 2 modules induced in PBMCs and LNMCs of combo-treated RMs



Method: Multi-dimensional Scaling (MDS)

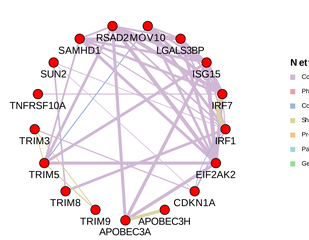
Bile Acid and IFN signaling higher in combo treated NHPs prior to ATI

RNAseq analysis reveals lack of viral re-seeding is associated with early induction of restriction factors, Bile Acid Signaling and antiviral pathways in LNs

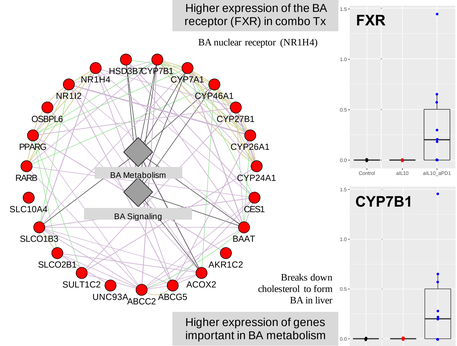


NAME	NES	NOM_pval	FDR_qval
CHEA_IRF1_Targets	1.57	0	0.0381
TRANSFAC_IRF7_Targets	1.41	0.0067	0.0523

Method: GSEA
Database: Hallmark, CHEA, TRANSFAC



Higher expression of the BA receptor (FXR) in combo Tx



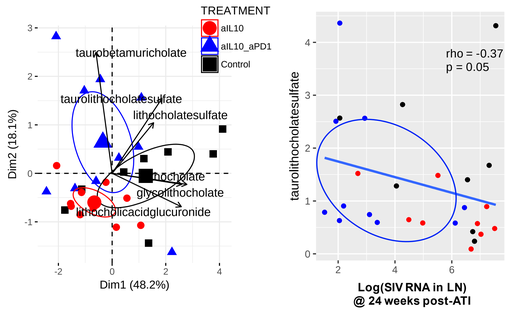
Breaks down cholesterol to form BA in liver

Higher expression of genes important in BA metabolism

IRFs including IRF1 and IRF7 among TFs induced at week 12 pre ATI

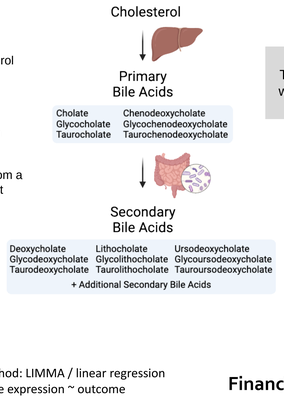
Pre-ATI: Plasma metabolome of Combo treated NHPs enriched in IFN inducing secondary BAs

- Plasma from combo treated NHPs is enriched in LCA derivates
- Note: LCA was observed to promote ISGs in vitro
- Sulfated TLCA prior to ATI associated with viral control 24 weeks post-ATI
- Data suggest, a role of ISG inducing BAs in shaping long-lasting anti-viral responses

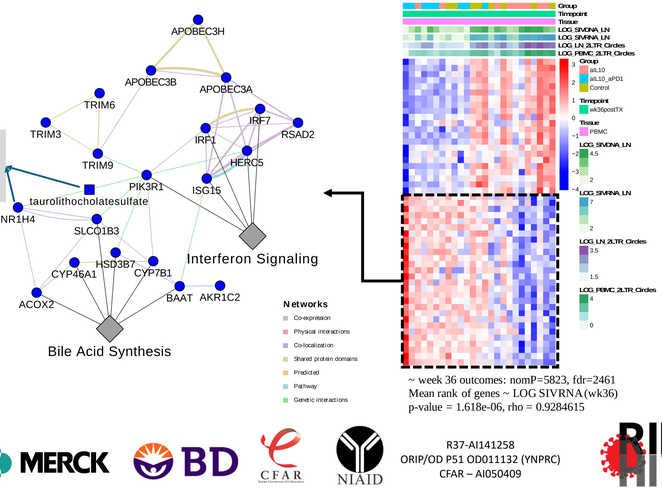


Higher expression of BA-IFN network pre-ATI… associates with control of SIV outcomes during ATI

- Bile acids (BAs) are products of cholesterol degradation in the liver.
- Are considered primary or secondary.
  - Primary: produced in the liver.
  - Secondary: primary bile acids which have been modified in the gut.
- Complex signaling cascades resulting from a diversity in BAs, receptor, and cell subset diversity.



Note: Plasma sulfated TLCA and FXR (NR1H4) were also components of this network

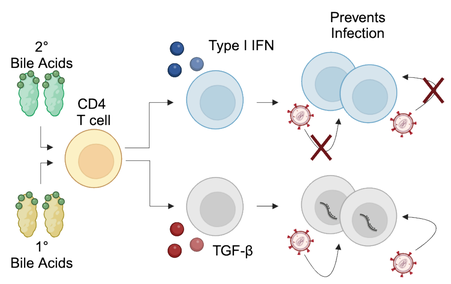


~ week 36 outcomes: nomP=5823, fdr=2461
Mean rank of genes ~ LOG SIVRNA (wk36)
p-value = 1.618e-06, rho = 0.9284615

Method: LIMMA / linear regression
Gene expression ~ outcome

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Working Model



- Hypothesis: Higher proportion of secondary BAs that promote type I IFN over TGF-b secretion can enhance anti-viral response and restrict HIV persistence.