0352: IMPACT OF RAPAMYCIN ON SIV PERSISTENCE IN RHESUS MACAQUES ON ANTIRETROVIRAL THERAPY

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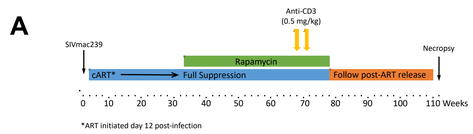
Background

Rapamycin is an immunosuppressive macrolide antibiotic that inhibits the mammalian target of rapamycin (mTOR), a regulatory kinase that controls glucose metabolism and cell growth. Inhibition of mTOR has been linked with several immune regulatory functions that may restrict HIV persistence, including reducing CCR5 expression[1-2], reducing cytokine driven expression of negative receptors (i.e. PD-1)[3] and limiting T cell homeostatic proliferation[4]. These immune modulatory effects of rapamycin may be linked to lower levels of HIV DNA in PBMC observed in HIV-infected kidney-transplant recipients who were treated with rapamycin for immune suppression[5]. Interestingly, rapamycin has also been shown to have immune stimulatory effects, by enhancing the quantity and quality of anti-viral CD8+ T cells responses[6]. As such, rapamycin has the potential to reduce inflammation and preserve viral-specific adaptive immune function. Indeed, rapamycin was recently shown to block cytokine production and T cell proliferation induced by pan-T cell activation without blocking latent HIV transcription or the killing of latently-infected cells[7]. Here we used SIV-infected rhesus macaques (RM) on combination antiretroviral therapy (cART) to evaluate the impact of long-term treatment of rapamycin on CD4+ T cell homeostasis, cell-associated viral loads and post-cART virus rebound. We also assessed whether pan-T cell activation, using a non-depleting anti-CD3LALA monoclonal antibody (mAb), in the presence of rapamycin can be used to disrupt SIV latency *in vivo*.

References

1. *Gilliam et al., AIDS. 2007. 21:2108-2110.*
2. *Heredia et al., PNAS 2003. 100:10411-10416*
3. *Taylor et al., AIDS. 2018. 32(18):2847-2851.*
4. *Ponticelli C. Expert Rev Clin. Immunol. 2014. 10:295-305*
5. *Stock et al., Am. J. of Transplant. 2014. 14:1136-1141*
6. *Araki et al., mTOR regulates memory CD8 T cell differentiation. Nature. 2009. 2;460(7251):108-112.*
7. *Martin et al., JCI. 2017. 127(2):651-656.*

Experimental protocol



- *14 rhesus macaques (RM) were intravenously (IV) inoculated with SIVmac239 and placed on cART (TDF/FTC/DTG) 12 days later.*
- *At 33 weeks post-infection (pi), RM received twice-daily intramuscular (IM) injections of rapamycin at 0.02mg/kg (n=7) or vehicle control (n=7) for 312 days.*
- *At 68 and 71 weeks post-infection, rapamycin-treated RM received IV infusions of a non-depleting anti-CD3 LALA monoclonal antibody (mAb) at 0.5mg/kg.*
- *PBMC, lymph node (LN), bone marrow (BM) and small intestinal biopsies were obtained at week 40, 46, 57, 66, 76 and 80 post-infection to determine changes in cell-associated SIV DNA.*
- *At 77 weeks post-infection, cART was stopped to determine impact of rapamycin on virus rebound dynamics.*

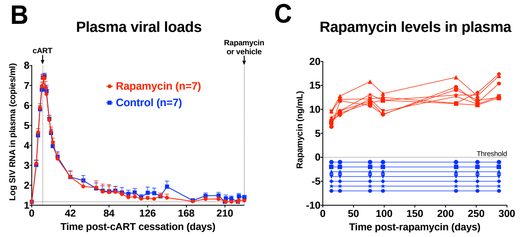


Figure 1: (**A**) Schematic representation of the animal protocol. (**B**) Plasma viral load profiles of RM after IV inoculation with SIVmac239 and cART administration 12 days post-infection. At 33 weeks post-infection, 7 RM received IM injections of rapamycin or vehicle control for 312 days. (**C**) Rapamycin drug levels achieved in plasma.

Rapamycin significantly disrupts T cell homeostasis

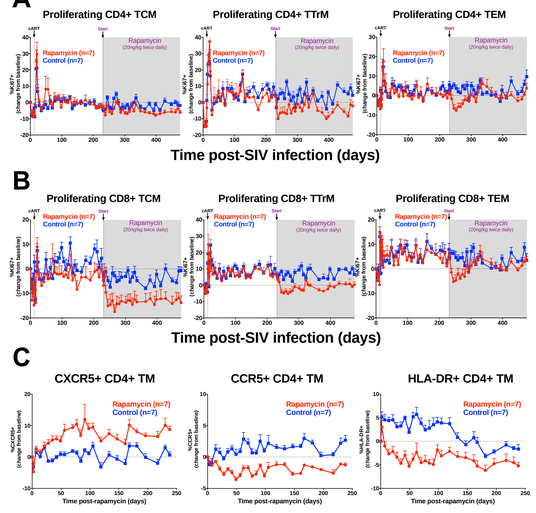


Figure 2: (**A**) Showing mean (+SEM) change in Ki-67 expression on CD4+ and (**B**) CD8+ memory T cell subsets in rapamycin-treated RM vs. controls. (**C**) Showing mean (+SEM) change in CXCR5, CCR5 and HLA-DR on CD4+ memory T cells in blood.

Rapamycin disrupts T cell activation and metabolic pathways

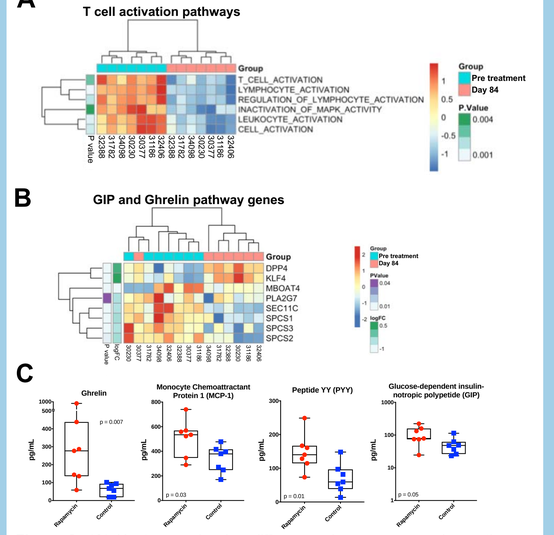


Figure 3: (**A**) Heatmaps showing differences in gene expression pathways associated with T cell activation and (**B**) genes enriched in GIP and Ghrelin pathways before and 12 weeks post-rapamycin treatment. (**C**) Comparison of ghrelin, MCP-1, PYY and GIP levels in plasma after 238 days of rapamycin.

Rapamycin has a significant effect on circulating miRNAs

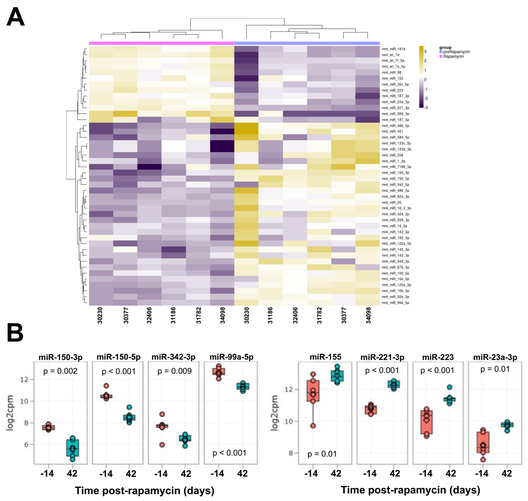


Figure 4: (**A**) Showing differentially expressed miRNAs isolated from the plasma of RM after 6 weeks of rapamycin treatment. (**B**) Individual miRNAs that were significantly down regulated (left panel) or upregulated (right panel) between day -14 and day 42 post-rapamycin treatment.

Anti-CD3LALA mAb in the presence of rapamycin can induce SIV production

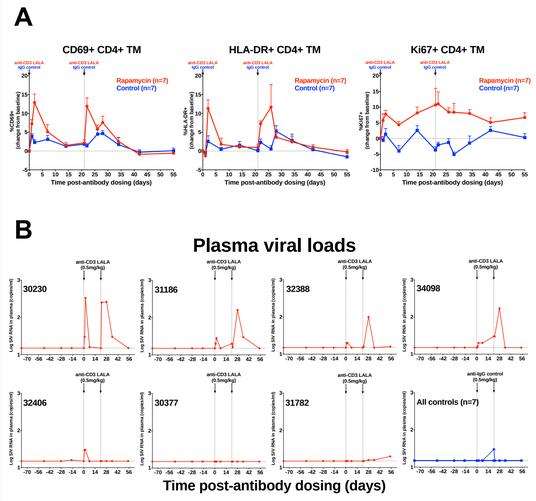


Figure 5: (**A**) Showing mean (+SEM) change in CD69, HLA-DR and Ki-67 expression on CD4+ memory T cells in response to anti-CD3LALA mAb dosing in rapamycin treated RM. Note that control RM received an IgG control antibody. (**B**) Showing 4 of 7 RM with >1 log increases in plasma viral loads above threshold (15 SIV RNA copies/ml) in response to anti-CD3LALA mAb.

Rapamycin had no effect on SIV DNA levels or virus rebound kinetics

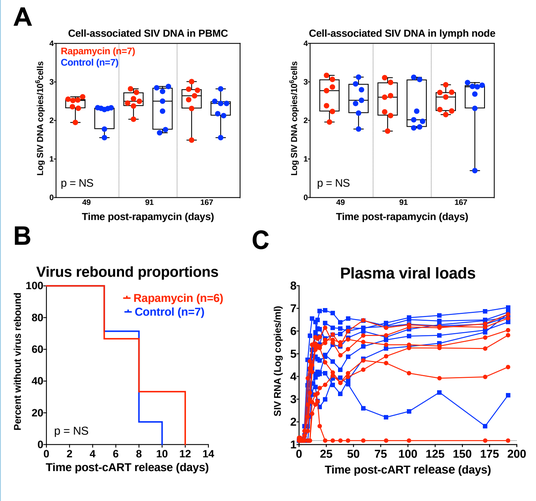


Figure 6: (**A**) Comparison of SIV DNA in PBMC and lymph nodes at 49, 91 and 167 days post-rapamycin. (**B**) Kaplan-Meier curve of time to SIV rebound. Note 1 RM in the rapamycin group was taken to necropsy prior to cART-release. (**C**) Individual post-cART plasma viral load profiles of rapamycin treated RM (n=6) and controls (n=7).

Summary

- ❖ Chronic rapamycin dosing to SIV-infected RM on cART resulted in severe but often transient decreases in proliferating CD4+ and CD8+ memory T cell subsets in blood.
- ❖ Rapamycin dosing led to profound changes in CXCR5, CCR5 and HLA-DR expression on CD4+ memory T cells. Rapamycin also induced a significant decrease in gene expression pathways associated with T cell activation and circulating miRNAs.
- ❖ Despite observed perturbations in T cell homeostasis and immune activation, cell-associated SIV DNA in blood and peripheral lymph nodes were not significantly different between treatment groups. In addition, rapamycin did not alter the kinetics of virus recrudescence after cART release.
- ❖ Interestingly, 4 of 7 rapamycin-treated RM showed increases in plasma viremia (>1 log) in response to anti-CD3LALA mAb, suggesting pan-T cell activation in the presence of rapamycin can disrupt SIV latency.
- ❖ Although these data suggests that rapamycin may not significantly affect the stability of the SIV reservoir, it does suggest that rapamycin may be used in conjunction with potent T cell activators to induce viral gene expression while also limiting potential global immune activation and T cell proliferation.

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