Latency Reversing Agents activate latent reservoirs in the brain of SIV-infected macaques

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Background: Our group has been testing the PKC activator ingenol-3-hexanoate (Ing-B) as a potential candidate for a "Kick and Kill" HIV eradication strategy. Preliminary data show that Ing-B treatment caused a temporary but significant increase in plasma viral load (VL) of two virally suppressed SIVmac251-infected cART-treated rhesus macaques. Here we report results from Ing-B treatment in our consistent and accelerated SIV macaque model for HIV/AIDS and HAND.

Methods: Three pigtailed macaques were dual inoculated with SIVDeltaB670 and SIV/17E-Fr, and treated at 12 days p.i. with CNS-penetrant cART (TNF, DRV, RTN, L-870812). After 500 days of viral suppression (< 100 copies/mL), one animal was kept as control while two macaques received daily oral doses of Ing-B (0.2 mg/kg BID) for 40 days. After a 2-week washout, the same animals received a 10-day treatment of Ing-B (0.2 mg/kg BID) in combination with vorinostat (4 daily IV doses of 6 mg/kg in 10 days). Animals were kept on cART until necropsy. SIV RNA was quantitated in plasma and CSF by qPCR and confirmed by ddPCR. SIV DNA was assessed in tissues by qPCR. In situ hybridization (ISH) for SIV RNA was performed in samples from brain, mesenteric lymph node, spleen, and lung. Resting CD4+ T cells were collected before and after latency reversing agents (LRA) treatment for quantitative viral outgrowth assay. Blood cells were analyzed longitudinally by cytometry. Immuneactivation markers in the CNS were assessed by ELISA.

Results: LRA induction caused a significant increase of plasma and CSF VL in one of the LRA-treated macaques (Figure 1). CSF viral load was 10x higher than in plasma, and the animal had to be euthanized due to encephalitis-related symptoms. SIV RNA could be detected by ISH in occipital cortex (Figure 2), despite undetectable levels of SIV DNA measured by qPCR (Figure 3). No change was observed in the other LRA-treated macaque. However, the number of SIV-infected resting CD4+ T cells was reduced after LRA-induction in both treated animals when compared to control (Figure 4). Changes in activation markers in the CNS were observed mostly in the animal with increased viral load (Figure 5). Increase in CD69+ T cells, together with a decrease in CD62L+ CD8 T cells, was observed in both LRA-treated macaques (Figure 6).

Conclusions: Treatment with LRAs led to a decrease in latent reservoirs in SIV-infected cART-treated macaques. In one animal, treatment activated viral genomes in occipital cortex leading to CNS disease, indicating that the brain harbors latent virus and should be seriously considered when novel "Kick and Kill" strategies are designed for HIV eradication.

Figure 1. Plasma (A) and CSF (B) viral load of three SIV-infected pigtailed macaques treated with cART starting at 12 days p.i. Each line represents one animal. Macaque Pt0 (red) was kept as control while macaques Pt1 (blue) and Pt2 (green) were treated with Ing-B for 40 days and then Ing-B plus vorinostat for 15 days (inserts).

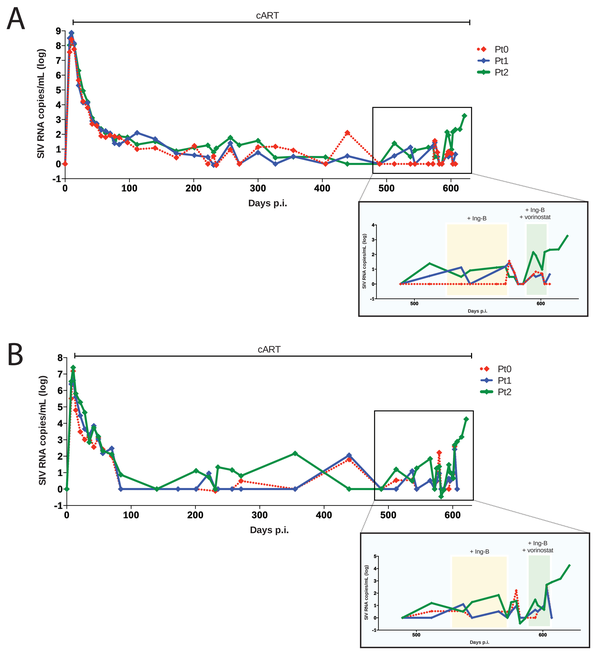


Figure 2. SIV RNA in occipital cortex of macaque Pt2 assessed by *in situ* hybridization (20X magnification). Yellow arrows show positive foci. No positive results were observed in mesenteric lymph nodes, spleen, or lung (not shown).

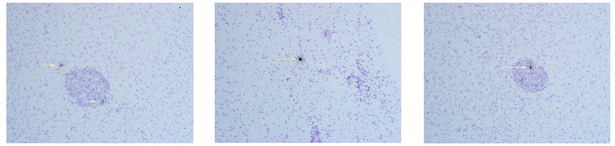


Figure 3. Levels of SIV DNA (qPCR) and RNA (ddPCR) in postmortem tissues of virally suppressed macaques treated (Pt1 and Pt2) or untreated (Pt0 - red column) with LRAs.

	SIV DNA copies per 10^6 cells			SIV RNA copies per µg total RNA		
TISSUES	**Pt0**	**Pt1**	**Pt2**	**Pt0**	**Pt1**	**Pt2**
Occipital cortex	NA	NA	NA	3	0	1700
Basal Ganglia	18	0	0	3	2	3
Parietal Cortex	5	0	7	4	0	19
Lung	5	19	9	5	12	4
Spleen	22	68	21	18	120	124
Liver	0	8	0	15	359	3
Kidney	0	0	4	84	5	16
Axillary LN	21	32	29	3	7	10
Bronchial LN	11	59	0	4	10	7
Colonic LN	57	52	59	15	17	50
Retropharyngeal LN	57	29	0	6	3	NA
Submandibular LN	19	65	12	7	5	14

Figure 4. Infected resting CD4+ T cells were measured by quantitative viral outgrowth assay. Cells were isolated form PBMCs (at 188 and 617 days p.i.) or spleen biopsies (at 231 days p.i.). Each color represents a macaque as described in Figure 1.

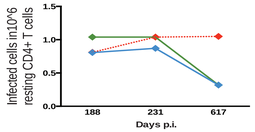


Figure 5. Changes in activation markers in the CSF, measured by ELISA. Each color represents a macaque as described in Figure 1. Time of treatment with Ing-B is marked in yellow, and with Ing-B and vorinostat, in green.

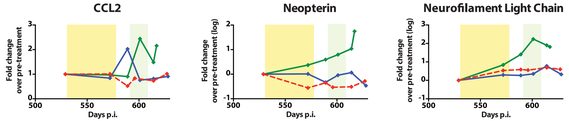
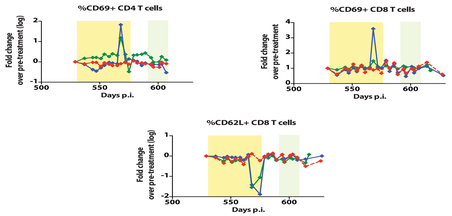


Figure 6. Changes in CD4 and CD8 T cell phenotypes during induction with LRAs. Each color represents a macaque as described in Figure 1. Time of treatment with Ing-B is marked in yellow, and with Ing-B and vorinostat, in green.



Acknowledgments

We would like to thank Amazônia Fitomedicamentos (Brazil) and Merck for generously providing, respectively, Ing-B (patent pending) and vorinostat for this study. Many thanks to the Retrovirus Laboratory at Johns Hopkins for the dedication during drug treatment, sample collection, and data preparation. Thanks to Dr. Ashley Haase at the University of Minnesota for helping with the ISH analysis. cART drugs were kindly provided by Gilead, Janssen, Abbvie, and Merck.

Funding Sources

NIH grants P40 OD013117 and U10 AI096113; Johns Hopkins CFAR

HIV CARE Collaboratory of AIDS Researchers for Eradication

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