Effect of combination latency reversing agents and bNAb in SHIV-infected rhesus macaques on antiretroviral therapy

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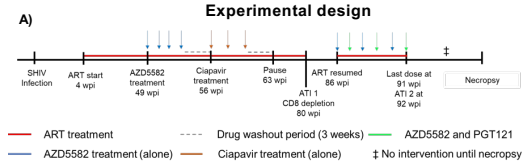
BACKGROUND

The combination of latency reversing agents (LRA), together with antiretroviral therapy (ART), known as the "Shock and Kill" strategy, is not able to clear established HIV reservoirs. These reservoirs include cells from the myeloid and lymphoid lineage that express the CD4 receptor, and are located throughout the body[1]. This study aims to assess the impact of an LRA with a broadly neutralizing antibody (bNAb) on tissue reservoirs in animals on long-term ART and on the prevention of viral rebound upon ART interruption (ATI).

METHODS

Eight Chinese rhesus macaques were infected with the barcoded virus SHIVAD8E0M[2] and underwent ART starting at 4 weeks post-infection (wpi). These ART-suppressed animals were divided into 2 groups at 49 wpi. The active arm was dosed with AZD5582, then Ciapavir prior to a 6-week ATI (ATI-1) at 80 wpi with a dose of anti-CD8 antibodies to facilitate viral rebound. At 86 wpi, ART was re-initiated with a combination of AZD5582 and PGT121. The control group received daily ART only. At 92 wpi, animals from both groups entered a 2nd ATI (ATI-2) for up to 9 weeks before necropsy (**Figure 1A**). Viral nested PCR, Q-RT-PCR, intact proviral DNA assay (IPDA), immunofluorescence analysis, and RNAscope were used to evaluate the reservoir size and the potential rebounding sources from different cell subsets along different tissues. ELISA test was used to determine levels of PGT121 concentration in blood.

RESULTS



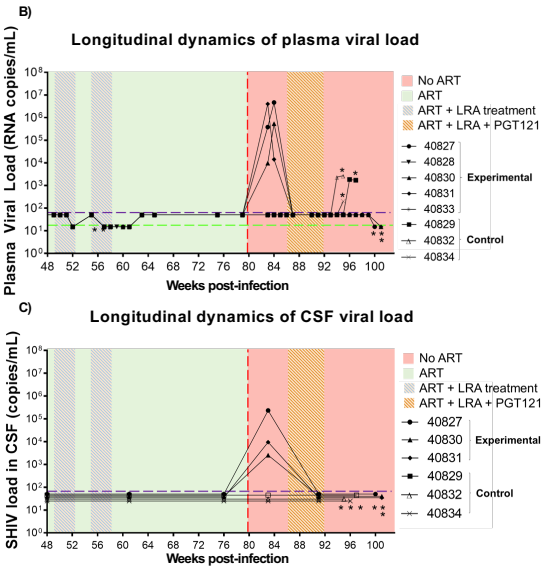
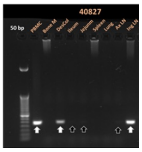


Figure 1: A) Experimental design **B)** Dynamics of plasma viral load. **C)** Dynamics of CSF viral load. Vertical dashed red line represent anti-CD8 antibody infusion. Purple and green horizontal dashed lines represent the limit of detection for the quantification of SHIV of 81 copies/mL and 22 copies/mL respectively. * marks the necropsy of the animal.

PVL maintained < 81 copies/ml while on ART. During the ATI-1 period, experimental animals experienced a rebound that was detected both in PVL and CSF (**Figure 1B and C**). Viral rebound was not detected in experimental animals during ATI-2 neither in plasma or CSF, while control animals experienced a rebound detected in plasma. Further SHIV detection analysis in tissues at different timepoints and necropsy revealed comparable viral detection by different methods (**Figure 2**). Single LRA-treatment with AZD5582 first, followed by Ciapavir while under ART seemed to induce a significant decrease in the intact proviral reservoir found in axillary lymph nodes compared to the control group which only received ART. In addition, the control group showed a mean of 32 intact provirus/10^6 cells in inguinal lymph nodes at necropsy, but none was detected in the experimental animals. Moreover, SHIV was detected in multiple regions of the brain, being readily detectable in basal ganglia, hippocampus and hypothalamus (**Figure 3A**). Further viral quantification revealed low concentrations in most regions, while still detectable for some animals in temporal lobe, hypothalamus and thalamus (**Figure 3B**). In addition, some intact proviral levels ranging between 7.5 and 30.4 intact provirus/10^6 cells were detected in temporal and parietal lobe, hippocampus, and hypothalamus in some of the animals from both groups (**Figure 3C**).

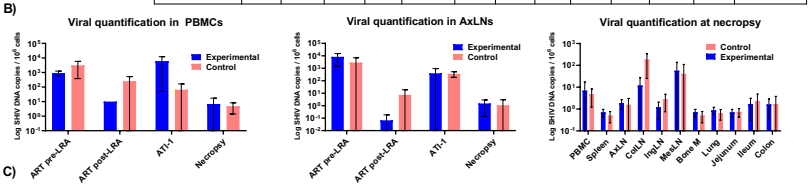
Table 1. SHIV detection in multiple tissues

A)



		PBMC	BoneM	Colon	Ileum	Jejunum	Spleen	Lung	AxLN	CoILN	IngLN	MesLN	Brain	Positive %	
Control	40829	+	+	+	n/a	+	+	-	-	+	-	+	+	75%	68%
	40832	-	-	+	+	+	+	-	+	+	+	+	-	63%	
	40834	+	-	+	+	+	+	-	-	+	+	+	+	75%	
LRA treated	40827	+	-	+	+	+	-	-	-	n/a	+	+	+	64%	65%
	40830	-	+	+	+	-	-	+	+	-	+	-	+	58%	
	40831	-	-	-	+	+	+	+	+	+	n/a	+	+	73%	
Positive %		50%	33%	83%	100%	83%	66%	33%	50%	80%	80%	83%	83%		

B)



C)

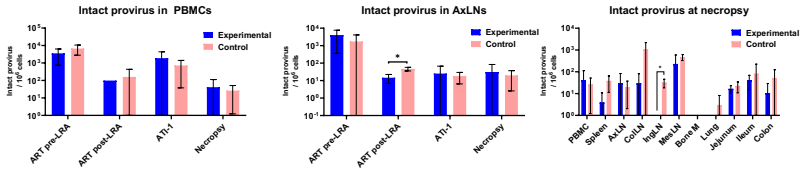


Figure 2: A) Viral detection in tissues. **B)** Cell-associated viral DNA load in tissues. **C)** Level of intact provirus in tissues. * $p < 0.05$.

Table 2. Comparative SHIV detection in different brain regions

A)

		Frontal lobe	Temporal lobe	Parietal lobe	Basal ganglia	Brain stem	Hippo campus	Hypo thalamus	Thalamus	Positive %	
Control	40829	+	+	+	+	+	+	+	-	88%	84%
	40832	+	-	+	+	+	+	+	-	75%	
	40834	+	+	+	+	+	+	+	-	88%	
LRA Treated	40827	+	+	+	+	-	+	+	+	88%	80%
	40830	-	+	+	+	+	+	+	+	88%	
	40831	+	-	-	+	+	+	+	-	63%	
Positive %		83%	67%	83%	100%	83%	100%	100%	33%		

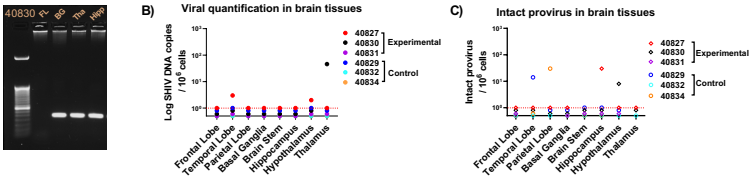


Figure 3: A) Viral detection in brain tissues. **B)** Viral quantification in brain tissues. **C)** Intact provirus in brain tissues. Red dashed lines represent the limit of detection of the assay of 1 intact provirus per 10^6 cells.

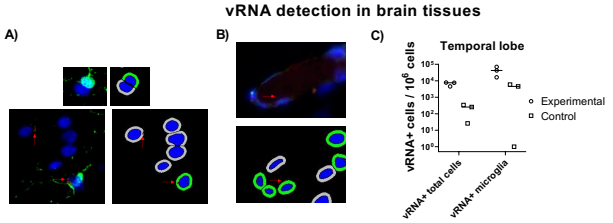


Figure 4: vRNA transcription quantification A) HALO analysis of microglia (Iba-1). Left panels are representative IF images. Right panels are representative viral and cellular detection. **B)** HALO analysis of pericytes (PDGFR-β). Top panel is a representative IF image. Bottom panel is a representative viral and cellular detection. **C)** vRNA quantification in temporal lobe in microglia and total cells.

Viral RNA (vRNA) transcription was detected in microglia and pericytes from the temporal lobe (**Figure 4A and B**). The analysis of the microglia and the total cell population suggested higher levels of viral transcription in the experimental group in this lobe.

PGT121 concentration levels may have prevented a systemic viral rebound in the experimental group at necropsy (**Figure 5A**). In addition, ADA analysis suggested that PGT121 treatment did not induce an immune response in the experimental group (**Figure 5B**).

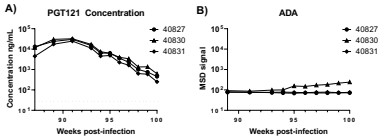


Figure 5 A) PGT121 concentration in blood. **B)** Anti-drug antibodies (ADA) analysis.

CONCLUSIONS

The sustained viral suppression observed in the experimental group suggests that treatment with activators of the non-canonical NF-kB pathway, such as AZD5582, together with ART and PGT121 may decrease LN reservoirs and provide sustained viral control. Further testing in people living with HIV may provide additional evidence of the effectiveness of this strategy upon the need of an ATI. These data also suggest that intact viruses in certain brain regions may serve as HIV reservoirs that could be challenging for eradication, specially, if they are found in different cell types, including microglia or other cell types. Future HIV cure strategies may need to include interventions that specifically target these resilient and varied reservoirs.

REFERENCES

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