Impact of CD4 binding site bNAbs on barcoded TF-SHIV-D rebound in macaques at ATI

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INTRODUCTION

Passive administration of HIV-1 broadly neutralizing antibodies (bNAbs) is a promising strategy to achieve durable viral suppression in the absence of ART. In human clinical trials, bNAb monotherapy has been shown to modestly delay viral rebound and combination therapy has been demonstrated to more durably suppress viral rebound following analytical treatment interruption (ATI)[1-3].

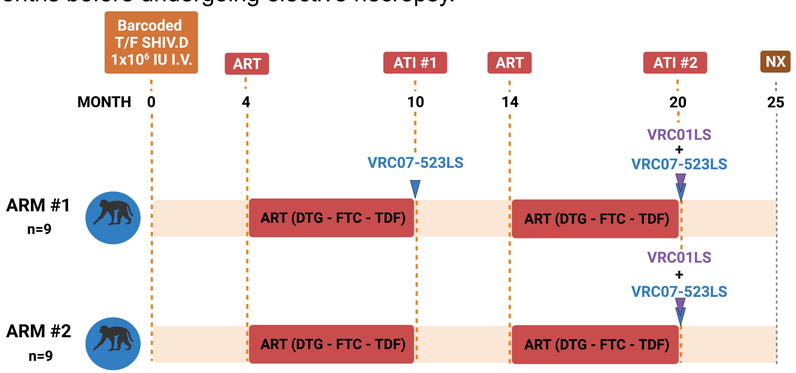
The Simian-Human Immunodeficiency Virus (SHIV) rhesus macaque (RM) model of HIV-1 infection has been used extensively to evaluate bNAbs for HIV-1 prevention, treatment, and functional cure. However, the majority of existing SHIVs are derived from HIV-1 variants isolated from chronic stages of infection and do not accurately recapitulate key properties of transmitted/founder (T/F) viruses[4-5]. Here, we evaluated a novel barcoded T/F SHIV.D.191859 (referred to as barcoded T/F SHIV.D), as a pre-clinical model for bNAb immunotherapy at ATI using CD4 binding site (CD4bs)-directed bNAbs VRC07-523LS and VRC01LS[6-7].

AIMS

- ❖Validate barcoded T/F SHIV.D for robust infection, elicitation of autologous neutralizing antibodies, persistence on ART, and viral rebound following ATI
- ❖Evaluate barcoded T/F SHIV.D as a model for bNAb immunotherapy at ATI
- ❖Characterize the mechanisms of viral escape from bNAbs

RESEARCH DESIGN

18 Mamu-A*01$^-$, B*08$^-$, B*17$^-$ RMs were infected intravenously with 1x10^6 infectious units barcoded T/F SHIV.D and initiated daily combination ART at 4 months post-infection. After 6 months of ART, all RMs underwent analytic treatment interruption (ATI #1), during which 9 RMs received a single, 30mg/kg i.v. dose of VRC07-523LS while the other 9 RMs remained untreated. ART was re-initiated 4 months after ATI #1, maintained for 6 months, and then interrupted for a second time. At ATI #2, all 18 RMs were treated with 30mg/kg each of VRC07-523LS and VRC01LS and followed for 5 months before undergoing elective necropsy.

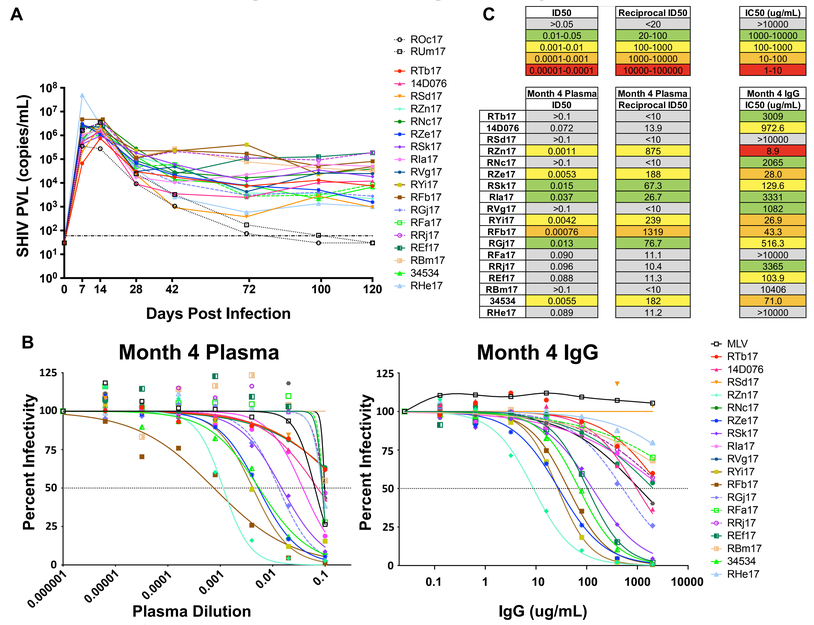


METHODS

Blood was collected frequently during acute infection and following both ATIs to determine viral kinetics. Autologous neutralizing antibody titers of plasma and purified IgG extracted from plasma at 4 months post infection were evaluated by TZM-bl assay. To characterize rebounding viral populations at ATI #1, high-throughput Illumina based sequencing on the barcode region was performed in parallel with 3' SGS of half genomes.

RESULTS

Barcoded T/F SHIV.D established robust infection and elicited autologous neutralizing antibody responses in RMs



C

	ID50	Reciprocal ID50	IC50 (ug/mL)
	>0.05	<20	>10000
	0.01-0.05	20-100	1000-10000
	0.001-0.01	100-1000	100-1000
	0.0001-0.001	1000-10000	10-100
	0.00001-0.0001	10000-100000	1-10

	Month 4 Plasma ID50	Month 4 Plasma Reciprocal ID50	Month 4 IgG IC50 (ug/mL)
RTb17	>0.1	<10	3009
14D076	0.072	13.9	972.6
RSd17	>0.1	<10	>10000
RZn17	0.0011	875	8.9
RNc17	>0.1	<10	2065
RZe17	0.0053	188	28.0
RSk17	0.015	67.3	129.6
RIa17	0.037	26.7	3331
RVg17	>0.1	<10	1082
RYI17	0.0042	239	26.9
RFb17	0.00076	1319	43.3
RGj17	0.013	76.7	516.3
RFa17	0.090	11.1	>10000
RRj17	0.096	10.4	3365
REf17	0.088	11.3	103.9
RBm17	>0.1	<10	10406
34534	0.0055	182	71.0
RHe17	0.089	11.2	>10000

Figure 1. Barcoded T/F SHIV.D-infected RMs demonstrated high peak viral loads and robust set point viral loads in the majority of animals and autologous neutralizing antibodies in approximately half of the animals at 4 months post infection. Barcoded T/F SHIV.D RNA levels in plasma of RMs during acute and chronic infection expressed as copies/mL (LOD, 60 copies/mL). All RMs established high peak viral loads (average 4.61E+06 copies/mL) and the majority of animals reached stable set-point viral loads, with the exception of ROc17 and RUm17 that spontaneously controlled by day 100 post-infection and were removed from the study. The remaining 18 animals continued to study completion (**A**). Neutralization curves of plasma and purified IgG (**B**), 50% inhibitory dilution (ID_{50}), reciprocal ID_{50} of plasma, and 50% inhibitory concentration (IC_{50}) of purified IgG (**C**) from RMs at 4 months post infection against barcoded T/F SHIV.D. Autologous neutralizing antibody responses were elicited against the inoculating virus in approximately half of RMs by 4 months post-infection, with 8 of 18 RMs having reciprocal plasma ID50 titers >20, and 5 of 18 with reciprocal ID50 titers > 100.

Modest delay in time to viral rebound in VRC07-523LS-treated vs. control RMs following ATI #1

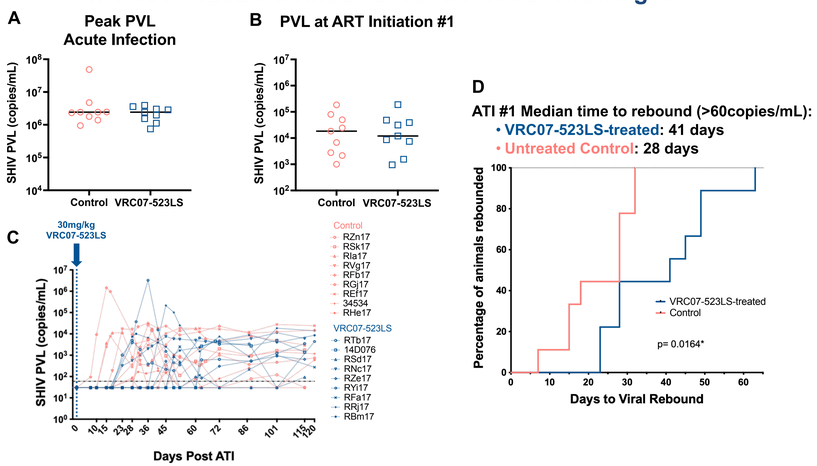


Figure 2. All 18 RMs rebounded following ATI #1, validating barcoded T/F SHIV.D for persistence on ART; RMs treated with a single 30mg/kg i.v. dose of VRC07-523LS rebounded later than untreated RMs following ATI #1. RMs were stratified into control and VRC07-523LS treatment groups for ATI #1 by plasma viral load at peak infection (**A**) and ART-initiation (**B**). Barcoded SHIV.D RNA levels in plasma of RMs were frequently measured following ATI #1 to determine time to viral rebound (LOD, 60 copies/mL) (**C**). All 18 RMs that underwent ATI #1 rebounded by 63 days post ATI (rebound defined as one pVL >60 copies). Kaplan-Meier survival curve analysis revealed that there was a statistically significant (p=0.0164, Log-rank test) delay in time to viral rebound in VRC07-523LS-treated animals as compared to untreated controls (median time to viral rebound of 41 days vs. 28 days respectively) (**D**).

Untreated controls and VRC07-523LS-treated RMs at ATI #1 possessed similar numbers of rebound lineages

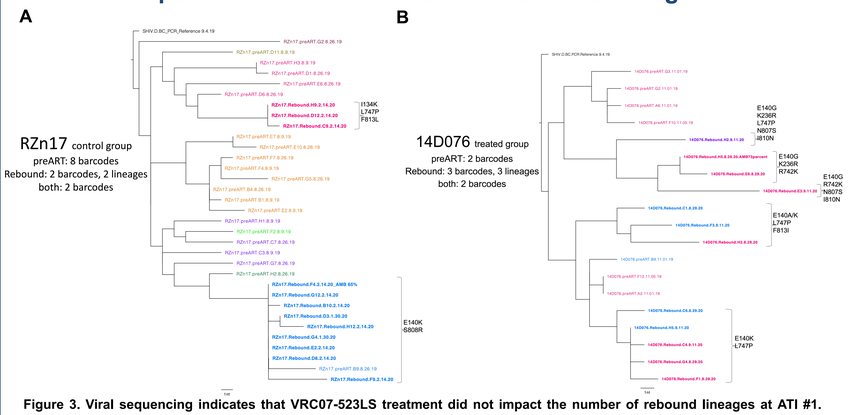


Figure 3. Viral sequencing indicates that VRC07-523LS treatment did not impact the number of rebound lineages at ATI #1. Representative phylogenetic trees of pre-ART and ATI #1 SHIV.D barcodes in untreated control animal RZn17 (**A**) and VRC07-523LS-treated animal 14D076 (**B**). Rebound lineage number was not statistically different between untreated controls and VRC07-523LS-treated animals at ATI #1 (median 3 for untreated (range 1-7), median 2 for VRC07-523LS-treated (range 1-4)). The T/F SHIV.D sequence is shown in black and different colors indicate different barcodes. Genetic distance is indicated by scale.

Effects of combination CD4bs bNAb therapy on ATI #2 time to viral rebound in RMs that were previously treated or untreated at ATI #1

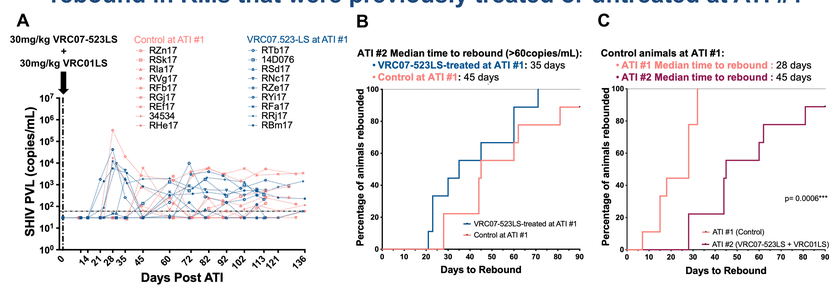


Figure 4. 17/18 RMs rebounded after ATI #2 (A), further validating barcoded TF-SHIV-D for persistence on ART and rebound after ATI. Barcoded SHIV.D RNA levels in plasma of RMs were frequently measured following ATI #2 to determine time to viral rebound (LOD, 60 copies/mL) (**A**). Survival analysis showed a trend towards faster time to rebound following ATI #2 in previously treated vs. untreated RMs (median 35 vs. 45 days) (**B**), potentially due to archived VRC07-523LS resistance. Comparing the survival curves of ATI #1 vs. #2, the 9 RMs that were untreated at ATI #1 experienced a more delayed time to viral rebound at ATI #2 with combination CD4bs bNAb treatment (p=0.0006, Log-rank test) (**C**).

CONCLUSIONS

- ❖ Validated barcoded T/F SHIV.D for robust infection, persistence on ART, and viral rebound at two sequential ATIs
- ❖ At 4 months post-infection, autologous neutralizing antibodies were elicited by approximately half of T/F SHIV.D-infected RMs
- ❖ Effects of CD4bs bNAb monotherapy on barcoded T/F SHIV.D rebound recapitulated results of human clinical trials, with modest delays in time to viral rebound

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