Macrophage-tropic TF SHIV Model of Systemic/CNS Pathogenesis and Persistence on ART

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Suvadip Mallick [1], Ryan Krause[1], Alexander G Mcfarland[1], Hannah Schrader[1], Richard W Camp[1], Gregory D. Whitehill[1], Francesco Elia Marino[1], Rachel Podgorski[2], Emily Lewis[1], Himanshu Garg[1], George M. Shaw[1], Tricia H. Burdo[2,3], Katharine J. Bar[1].

[1]Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA. [2]Lewis Katz School of Medicine, Temple University, Philadelphia, PA, USA.

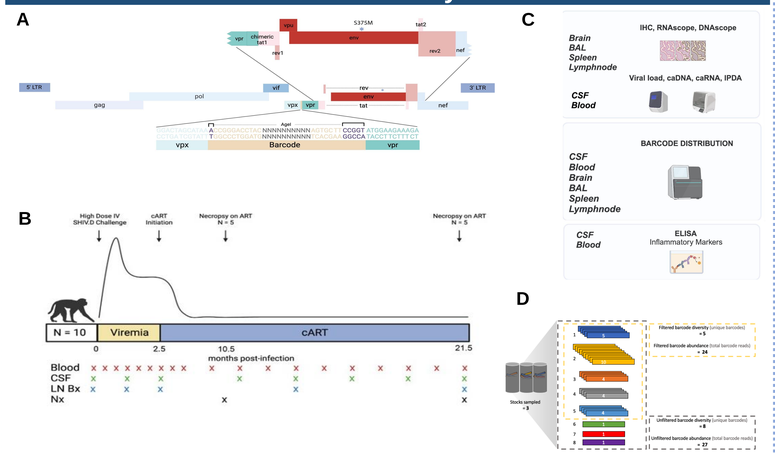
[3]Rutgers Institute for Translational Medicine and Science, New Brunswick, NJ, USA

Background

- Human studies have shown that brain macrophages harbor HIV DNA and are persistently activated and dysfunctional in ART-suppressed PLWH. Definitive proof of long-lived CNS cells latently infected with replication competent virus, however, is challenging to obtain in humans.[1]
- Biologically relevant and predictive animal models are needed to elucidate the mechanisms of systemic and CNS persistence.
- Clade D HIV-1 is known to infect both CD4 T cells and Macrophages, thus we have developed a barcoded SHIV encoding a clade D TF HIV-1 Env.

Here, we characterize this physiologically relevant TF SHIV.D-NHP model of HIV CNS and systemic pathogenesis and persistence.

Methods and Analysis Plan



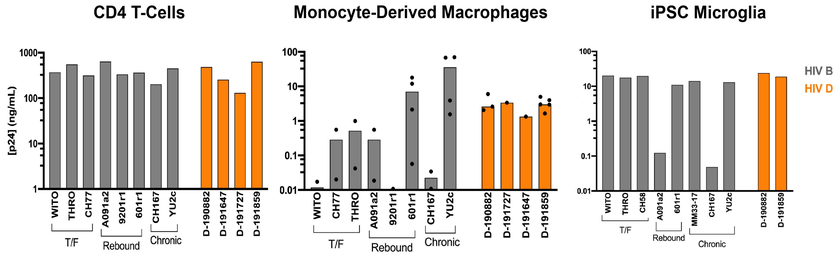
A) **SHIV schema:** Gene organization of barcoded SHIV.D.191859.375M.dCT (SHIV D). HIV-1 Env D.191859 (red) is incorporated into SIVmac766 and substitutes S375M to enhance CD4 binding and SHIV replication in Rhesus macaques.[2] A 34-base cassette with 10 random bases was inserted between Vpx and Vpr allowing for each virion or infected cells to contain unique genetic tags without sacrificing viral fitness.[3]

B) **NHP Study Schema:** 10 animals were infected with TF SHIV D. cART started 10 weeks post infection (WPI). Longitudinal samples were collected through out pre-viremic, viremic and on treatment time points. 5 animals were necropsied after 9 months (short term ART) and 5 remain to be necropsied after 20 months (long term ART).

C) **Experimental Plan:** We will assess *i)* viral kinetics in blood, systemic tissue, and brain; within both CD4 T cells and macrophages, ii) Reservoir quantification across tissues and cell type, iii) Barcode proportion and numbers across tissues and cells, and protein analytes in tissues and plasma.

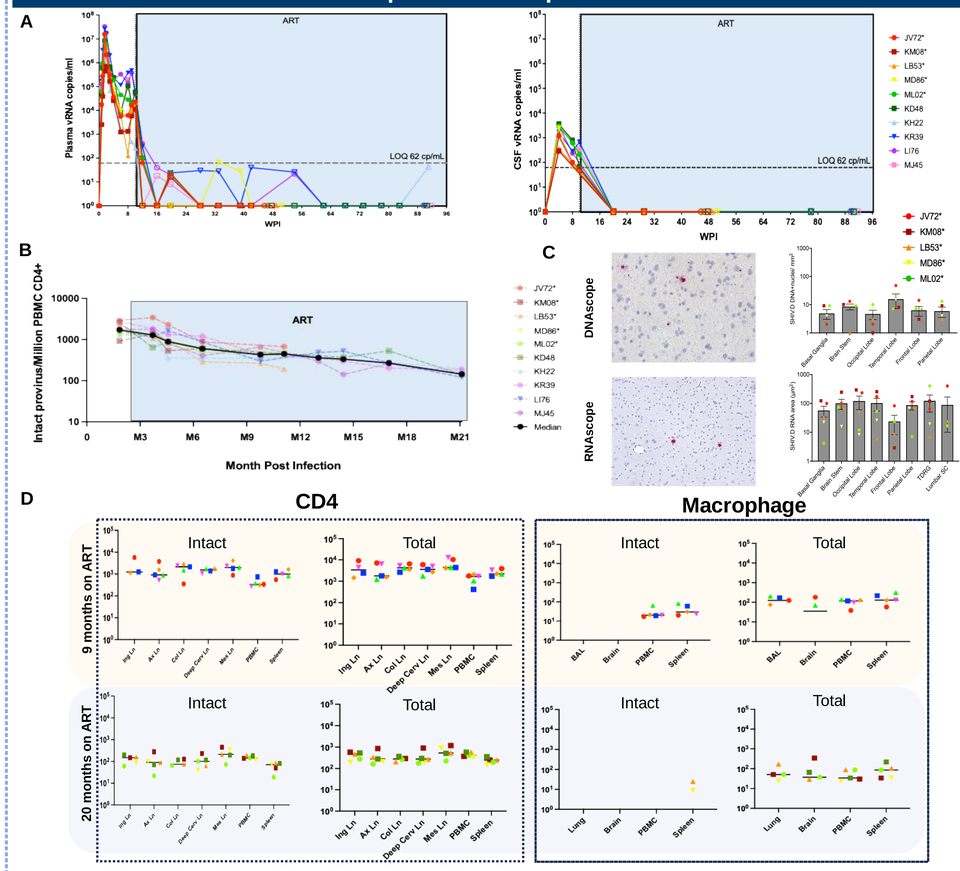
D) **Barcode analysis schema.**

Clade D TF viruses infect CD4 and Macrophage lineage cells *in vitro*



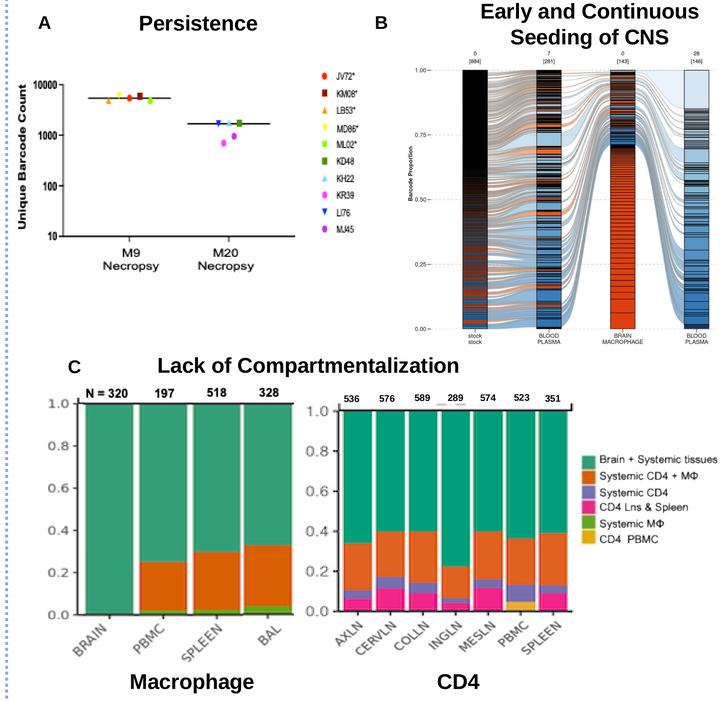
Primary Clade D HIV-1 isolates replicate well in CD4 T cells, Monocyte-derived Macrophages and iPSC-derived Microglia.

TF SHIV D replication and persistence *in vivo*



(**A**) Plasma (left) and CSF (right) viral load (VL) kinetics study for 10 animals. Peak VL median was $2.5x10^6$ copies/mL, and setpoint median was $2x10^4$ copies/ mL. Viral suppression was achieved rapidly and durably after ART initiation at 10 WPI. In the CSF, VL was ~2 logs lower than plasma and became undetectable after ART initiation. **(B)** Intact Proviral DNA Assay (IPDA) by ddPCR to detect the persistence of intact proviral DNA and decline over time in PBMC derived CD4+ T cells. **(C)** Representative DNAscope/RNAscope images (left) showed persistence of proviral DNA (top) and vRNA (bottom) in CNS at 9 months on ART. Average of vDNA+ nuclei/mm^2 or RNA+ area (μm^2), respectively, from 10 non-overlapping 20X images/brain section of each RM (20Xframe area=393,880 μm^2).[4] **(D)** ddPCR analysis was used to assess the intact and total proviral DNA persistence in CD4+ T cells and Myeloid (CD11b+ and CD206+ for BAL) cells at necropsies at 9 months on ART (n=5;*Starred) and 20 months on ART (n=5).

SHIV.D barcode distribution in reservoir



A) Total unique barcodes detected in animals (across all tissues) at necropsy are a rough measure of SHIV-infected cell diversity and persistence. Between month 9 and month 20 necropsy timepoints, we see a decline in counts, but continued persistence. **B**) The total number and overlap of unique barcodes from stock, day 7 plasma virus, day 28 plasma virus, and brain macrophage tissues at necropsy are shown in an alluvial plot from the representative animal, LI76. We see barcodes replicating at day 7 and not at day 28 can be found in brain tissues at necropsy, indicating early seeding of the brain. Similarly, barcodes found only at day 28 are also recovered from brain tissue at necropsy. **C**) Overlap plot of one representative animal (ML02) comparing barcode abundance in brain macrophages to macrophage and CD4+ T cells in the systemic reservoirs showed no evidence of compartmentalization.

Summary

- TF SHIV.D infection of NHP reproduces key features of HIV-1 persistence on ART. CNS and systemic reservoirs decrease in size but remain detectable over long-term ART.
  - TF SHIV D persists in both CD4+ cells and macrophages, with ~1-2 logs higher frequency in CD4+ cells.
  - Barcode analyses demonstrate (i) early and continuous seeding of the brain with viruses replicating in plasma pre-ART, (ii) no evidence for this dual tropic virus compartmentalization between systemic and CNS, or CD4+ T cells and macrophages with only 10 weeks of viremia pre-ART.
- Recapitulation of appropriate persistence in systemic and CNS reservoirs enable ongoing study to model the viral kinetics and decay on prolonged ART suppression and cure interventions.

References

1. Gonzalez-Perez *et al*., *J Virol,* 2017; 91(20).
2. Li H *et al*., *J Virol,* 2021; 95(11).
3. Fennessey CM *et al*., *PLoS Pathog,* 2017; 713(5).
4. Podgorski RM *et al*., *Retrovirology,* 2023; 20(1): 13.

Method images prepared with BioRender software.

Supported by NIMH & NIAID: R01-MH128155; R01-AI-162646; R01-AI-179666; P30-AI-045008; UM1-AI1-64570