Dynamics of HIV Reservoirs in the brain during intermittent Antiretroviral Therapy.

Author list
Sara Gianella[1], Guochun Jiang[2,3*], Yuyang Tang[2,3], Magali Porrachia[1], Gemma Caballero[1], Caroline Ignacio[1], Davey M Smith[1], Antoine Chaillon[1]

Affiliations
[1]University of California, San Diego (UCSD),
[2]University of North Carolina (UNC) HIV Cure Center,
[3]Division of Infectious Diseases, Department of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA.

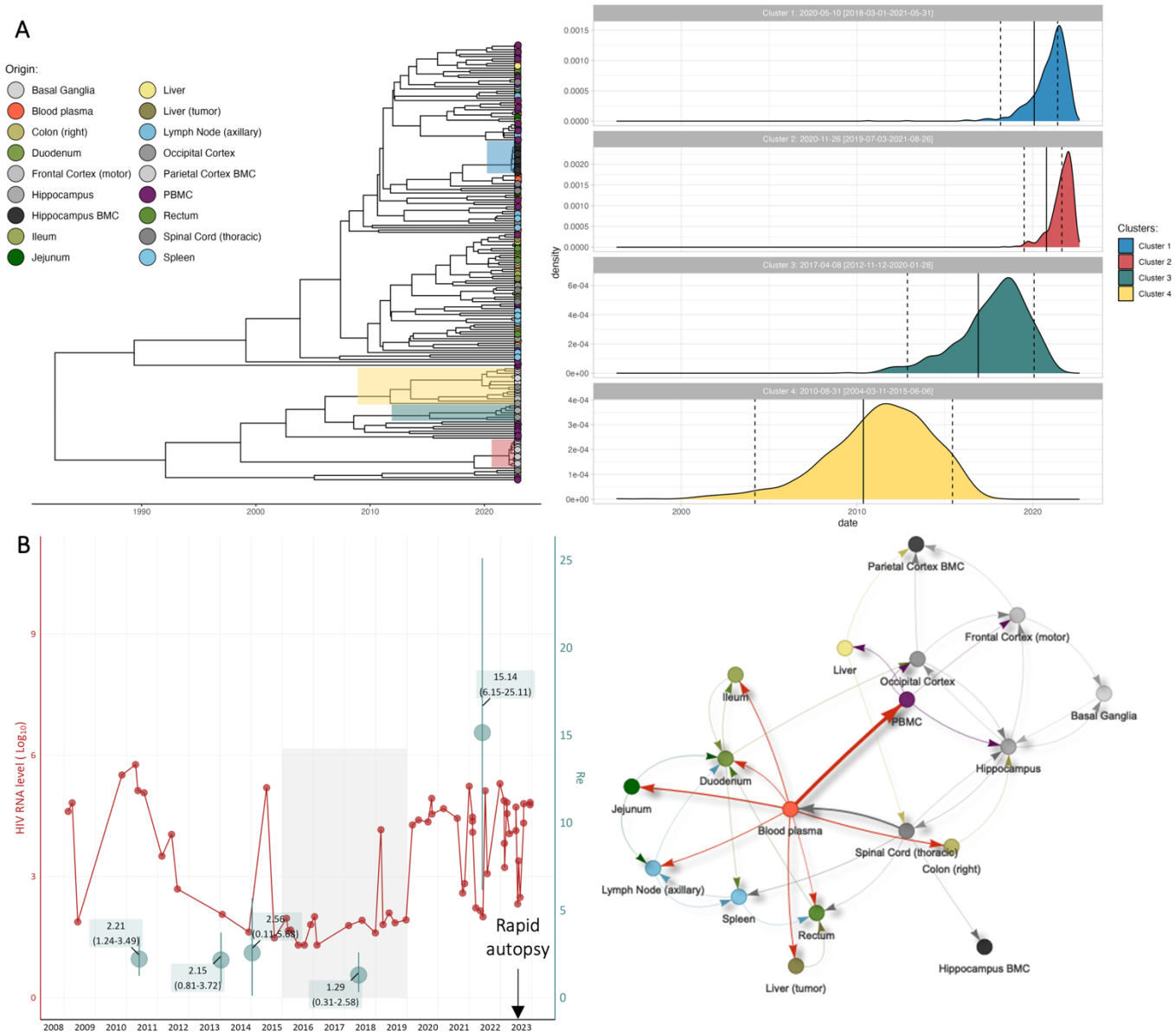
Fundings
This work is supported by the James B. Pendleton Charitable Trust and by the San Diego Center for AIDS Research (SD CFAR), an NIH-funded program (P30 AI036214), which is supported by the following NIH Institutes and Centers: NIAID, NCI, NHLBI, NIA, NICHD, NIDA, NIDCR, NIDDK, NIGMS, NIMH, NIMHD, FIC, and OAR , and by the NIH NIDA (R01DA055491).

ABSTRACT
Background. Determining the dynamics of rebounding HIV after antiretroviral therapy (ART) interruption and identifying the source of viral re-seeding in deep tissues is critical. We applied Bayesian phylogenetic and phylogeographic models to investigate the establishment and dynamics of HIV across brain regions and peripheral compartments in one person with HIV and with intermittent ART intake.

Method. One 62 years old male participant with hepatocarcinoma was followed as part of the Last Gift rapid autopsy program for 10 months prior to death. Single genome full *env* sequencing of HIV was performed in serial pre-mortem blood plasma (RNA) and peripheral blood mononuclear cells (DNA) samples and in fresh peripheral tissues collected during rapid autopsy (DNA) and Brain Myeloid Cells (BMCs) isolated from fresh parietal cortex and hippocampus (outgrowth RNA). Using 189 proviral sequences across 18 sites, Bayesian Discrete Trait Analysis (DTA) was performed to infer viral dynamics within the brain and across the Blood-Brain barrier (BBB). A Birth-Death Skyline (BDSKY) model was applied to estimate changes in the viral reproductive number (R_e), where $R_e>1$ indicates growing population size.

Results. Phylogenetic analyses of all CCR5-tropic variants revealed 2 divergent HIV populations forming 4 clusters across different brain regions (**Fig1A)**. Notably, 2 highly divergent clusters 1 and 2 (mean pairwise distance [PWD] = 0.043) in BMCs from parietal cortex (n=13 sequences) and hippocampus (n=14) arising from 2 distinct populations were established in early 2020 (times to most recent common ancestor [tMRCAs] in January 2020), 2 years prior to death. These clusters remained homogeneous with limited viral diversity (mean PWD 0.0013 and 0.0001). Two additional clusters from different brain regions were identified with estimated tMRCAs in 2017 and 2010. DTA model revealed viral migrations within the brain and across the BBB. BDSKY model showed an increased R_e (15.1 [95%CI: 6.1-25.1]) after 2018 as compared to the 2015-2018 period of viral suppression (Re 1.3 [95%CI: 0.3-2.6]), (**Fig1B**).

Conclusions. We identify distinct HIV clusters within the brain, originating from different viral strains with varying R_e during periods of viral replication. These findings highlight how the resurgence of the virus plays a role in establishing and replenishing deep tissue reservoirs which can persist for decades and emphasize that HIV reservoirs within the brain are heterogenous across brain regions.



A. Molecular clock tree reconstructed from 189 HIV single genome full length env sequences across 18 sites (blood plasma, peripheral mononuclear cells [PBMC], tissues or Brain myeloid cells [BMC]). Brain clusters are highlighted, and tips are colored by sampling source (left). Times to most recent common ancestor [tMRCA] posterior densities of the four CNS clusters are shown on the right panel (right).

B. HIV RNA levels (left y axis, in red) and estimates of the effective reproductive number (R_e). Circles and vertical bars represent the mean and 95% confidence interval (CI) of the Re obtained from the Bayesian skyline model. Grey area represents the estimated period of viral suppression (left). Bayesian discrete trait analysis (DTA) of 189 full length *env* sequences sampled across 18 locations. Nodes are colored according to the sampling location with brain tissues and cells depicted in shades of grey. Edges are colored according to the tissue sources. Only migration events with Bayes Factors ≥ 3 are considered (right).