PRESENCE OF INTACT HIV DNA VARIANTS IN THE BRAIN AND LYMPHOID TISSUES DURING ART

Michelli F. Oliveira[1], Ben Murrell[1], Andrej Vitomirov[1], Thomas Vollbrecht[1,2], Venkatesh Kumar[1], Nikesh Kumar[1], Ben Gouaux[1], David Moore[1], Alan Jarmusch[1], Fernando Vargas[1], Pieter Dorrestein[1], Ronald Ellis[1], Davey Smith[1,2], Sara Gianella[1].
[1]University of California San Diego, La Jolla, CA, USA
[2]Veterans Affairs San Diego Healthcare System, San Diego, CA, USA

Michelli Faria de Oliveira, Ph.D.
University of California San Diego
9500 Gilman Drive MC 0679
La Jolla, CA 92093-0679, USA
Tel: (858) 552-5955
E-mail: mfariadeoliveira@ucsd.edu

CROI Conference on Retroviruses and Opportunistic Infections

Abstract #0452

CFAR CENTER FOR AIDS RESEARCH *University of California San Diego*

BACKGROUND

- HIV persists in cellular reservoirs in tissues, despite long-term antiretroviral therapy (ART).
- During the course of infection, HIV-1 may also adapt to anatomic tissues, leading to compartmentalized reservoirs.

OBJECTIVE

Evaluation of the genetic features of the HIV DNA reservoirs in brain and lymphoid tissues during suppressive ART.

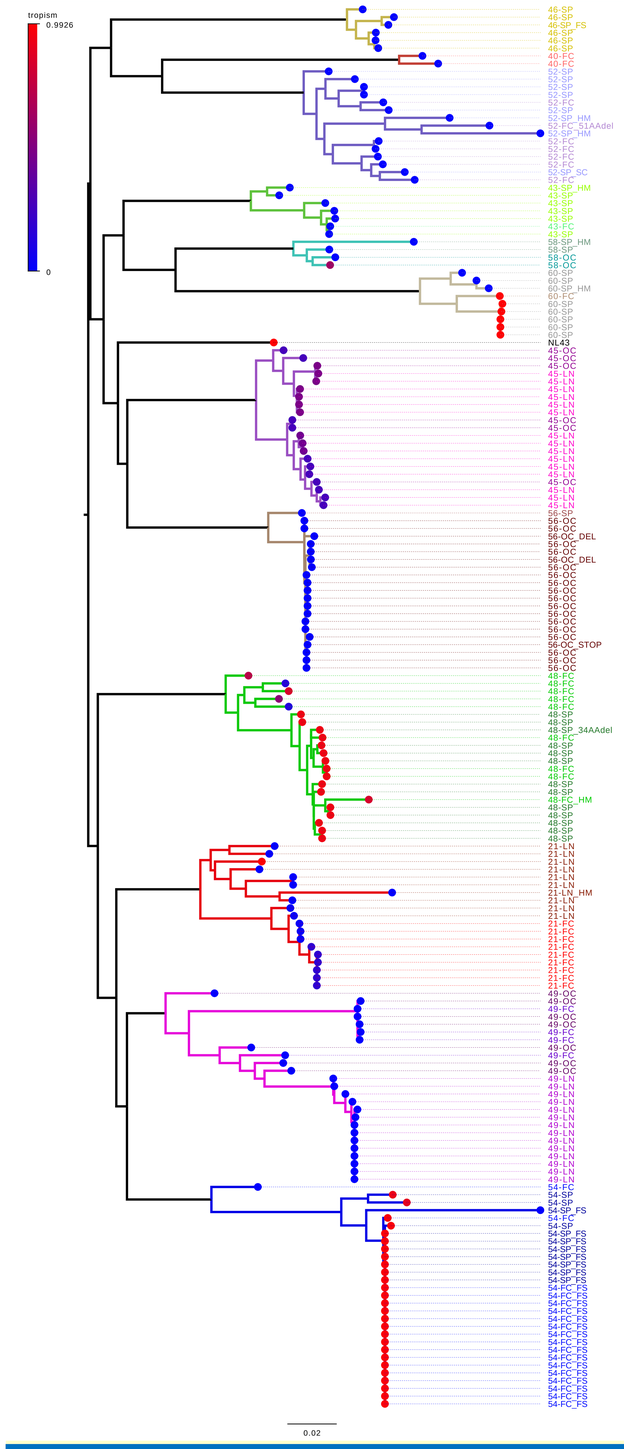
MATERIAL AND METHODS

- We evaluated post-mortem brain (frontal [FC] or occipital cortex [OC]) and peripheral lymphoid tissues (lymph node [LN] or spleen [SP]) from 12 HIV+ decedents from the National NeuroAIDS Tissue Consortium (NNTC).
- All donors passed away between 2001-2014, with virologic suppression on ART (<50 or 400 copies/ml, assay-dependent), and without evidence of central nervous system opportunistic disease.
- Total DNA was extracted from each tissue sample and levels of HIV DNA (*gag*) were quantified by droplet-digital PCR (normalized by number of total cells [RPP30 gene]).
- The genotypic composition of the HIV DNA populations was evaluated by high-throughput single genome amplification using the PacBio platform to sequence full-length HIV *envelope* (FL HIV-*env*)
- Tropism of the FL HIV-*env* sequences was predicted *in silico* (geno2pheno).
- Presence of ART (tenofovir and lamivudine) in brain tissue (frontal cortex) was evaluated by mass spectrometry.

Acknowledgments

This work was supported by the Department of Veterans Affairs and grants from the National Institutes of Health: AI100665, AI131385, AI007384, AI106039, AI106039, MH100928, AI036214, CFAR Translational Virology Core. the James B. Pendleton Charitable Trust, CNPq-Brazil, HNRP developmental grant PST-HN39, tissues were provided through a request (R442) from the National NeuroAIDS Tissue Consortium.

Most FL HIV-*env* sequences were intact, with variable predicted tropisms and often compartmentalized



- Presence of ART was confirmed in 6 out of 8 donors' post-mortem brain (frontal cortex) tissues.
- 180 individual FL HIV-*env* sequences were obtained across donors: FC, n= 7 donors; OCC, n=3; LN, n=3; SP, n=8 (**Figure**).
- For 10 donors, FL HIV-*env* sequences were obtained from paired brain and lymphoid tissues.
- *In silico* tropism prediction varied between samples.
- HIV compartmentalization patterns differ between donors, with four donors (#21, 48, 49, 54) showing evidence of HIV DNA compartmentalization (Fst test, $p<0.05$).
- 143 FL HIV-*env* sequences were genetically intact, while 37 sequences appeared non-functional.
- In one donor, we found 23 clonal sequences with a frameshift mutation that was present in both, brain and spleen, suggesting migration of cells with clonal provirus between tissue compartments.

Table. Summary Characteristics of Study Participants

Sex	Female n=3; Male n= 9
Age (years)*	52 (40 – 66)
EDI*	13.5 (6 – 19)
Last visit prior death (months)*	3 (1 -4)
Levels of HIV DNA (copies/10^6 total tissues cells)*:	
Frontal Cortex	12 (3 – 1110)
Occipital Cortex	9 (3 – 284)
Lymphoid Tissue	99 (9.3 – 1388)

*median (range)
EDI= Estimated Duration of HIV infection

Figure. Maximum likelihood phylogeny tree showing FL HIV-*env* sequences obtained from post-mortem brain and lymphoid tissues from 12 PLWH donors enrolled as part of the NNTC cohort. Lineage colors represent different participants and shades of colors represent different body tissues. Non-intact *env* sequences contained major deletions (DEL), hyper-mutations (HM), stop-codon mutations (STOP) or frameshift (FS) mutations. Sequence nodes are colored by Geno2Pheno predicted co-receptor tropism, showing CCR5 (blue) and CXCR4 (red). FC=frontal cortex, OC=Occipital cortex, LN=Lymph Node, SP=Spleen

CONCLUSIONS

- Based on FL HIV-*env* sequencing, most HIV DNA populations in brain and lymphoid tissues appeared to have intact *env* genes, with different predicted viral tropisms and were often compartmentalized.
- Understanding the composition of the HIV reservoirs in anatomic compartments is crucial for HIV cure strategies.