ANALYSIS OF COMPARTMENTALIZATION OF HIV-1 IN BONE MARROW

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Thuy Nguyen[1], Jenna Honeycutt[1], Christopher Nixon[1], Zakharova Oksana[2], Christopher Evans[2], JoAnn Kuruc[2], Ben Murrell[3], Cynthia Gay[2], Douglas Richman[4,5], J. Victor Garcia[1]

[1] Division of Infectious Diseases, International Center for the Advancement of Translational Science, Center for AIDS Research, University of North Carolina, School of Medicine, Chapel Hill, NC, USA.[2] Department of Medicine, University of North Carolina, Chapel Hill, North Carolina, USA.
[3] Department of Microbiology, Tumor and Cell Biology, Karolinska Institute, Stockholm, Sweden. [4] University of California San Diego, La Jolla, California, United States of America. [5] Veterans Affairs San Diego Healthcare System, San Diego, California, United States of America.

BACKGROUND

- A human immunodeficiency virus (HIV) reservoir is established and compartmentalized in multiple tissues and anatomic sites early after infection due to tissue-specific genetic differentiation and restricted patterns of cell migration. [1]
- Independent replication of HIV in different tissues may contribute to various patterns of virologic and immunologic evolution as well as disease pathogenesis. [2,3]
- HIV infection frequently results in hematological disorders observed in the late stages of disease; [4,5] however, little is known about the HIV viruses in the bone marrow (BM). We evaluated the compartmentalization of HIV in BM from participants not on antiretroviral therapy (ART).

METHODS

- HIV-RNA was extracted from BM and peripheral blood (PB) plasma and cDNA was generated using SuperScript III system. Total HIV-1 cDNA was used to amplify full-length HIV-1 *env by* PCR. Pacific Biosciences' Single Molecule, Real-Time libraries were prepared from purified HIV-1 *env* amplicon using the PacBio P6/C4 chemistry. [6]
- High-quality consensus sequences (Full-Length Envelope Analyzer software) [6] were used to build phylogenetic trees (FastTree v.2.1.11) and assessed for compartmentalization by
  - ➢Distance-based tests: a) genetic diversity with the average pairwise distance (APD) (MEGA7), b) divergence using a test for panmixia, c) Wright's measure of population subdivision (Fst), d) nearest-neighbor statistics (Snn)
  - ➢ Tree-based tests: a) Simmonds Association Index, b) Slatkin-Maddison, c) Correlation coefficients (HYPHY v.2.3.14).
- Viral tropism was predicted at 2.5% of false-positive rate (https://coreceptor.geno2pheno.org/).
- Compartmentalization was established when results from phylogeny and compartmentalization analyses were concordant.

Viral compartmentalization and the presence of CXCR4-tropic virus were detected in the bone marrow, which may suggest pathogenic consequences of HIV-1 infection in this compartment.

RESULTS

1) Patients' characteristics and virologic analyses

Table 1: Patients characteristics

Participant	Age	Sex	Current and historic ART	CD4 count in PB (cells/mm3)	CD4/CD8 ratio in PB	CD4/CD8 ratio in BM
1	34	M	- NAÏVE	352	0.525	0.314
3	43	M	- Self discontinued TDF/FTC, DRV/c before sample collection date - On continuous ART from 2009-2016: TDF/FTC/ATV/r and TDF/FTC/DRV/c	409	0.427	0.243
2	26	M	- Self discontinued TDF/FTC/EVG before sample collection date - Lack of ART adherence from 2015-2017 due to treatment side effects and financial access to ART	304	0.592	0.438

2) Phylogenetic analyses

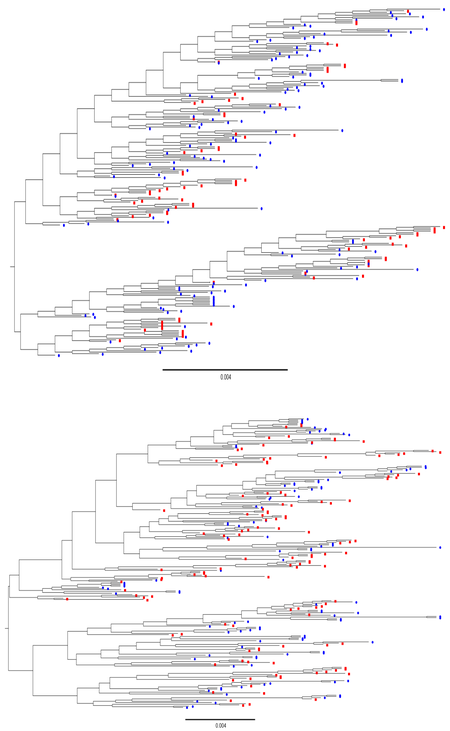


Fig.2: The phylogenetic trees constructed from full *Env* sequences from BM and PB plasma of participant 1 (above) and participant 2 (below). No compartmentalization was observed.

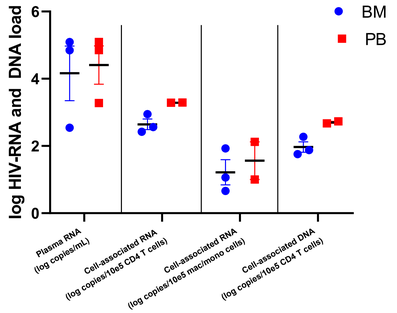


Fig.1: Plasma RNA, Cell-associated RNA and DNA in BM and PB samples. No significant difference in RNA and DNA levels between compartments

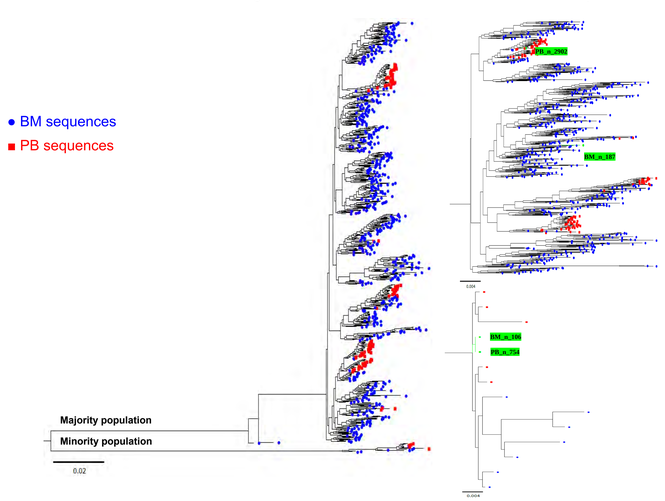


Fig.3: The phylogenetic tree (left) constructed from full *Env* sequences from BM and PB plasma of participant 3. The participant harbored two distinct viral populations. Separate phylogenetic trees were constructed from sequences of the majority (upper right) and minority populations (lower right). Phylogenetic trees reveal a clustering of BM and PB sequences into separate lineages, which is consistent with results from compartmentalization tests (table 2). In the majority viral population, the predominant consensus sequences in BM and PB are shown in green, BM_n_187 and PB_n_2902 respectively, in which n represents the number of individual sequences assembled in each consensus. Similarly, in the minority population, the predominant consensus sequences in each compartment are shown, BM_n_106 and PB_n_754. These two consensus sequences are identical, but the phylogenetic tree shows a compartmentalized and more diversified viral population in BM than in PB (average pairwise distance, APD = 1.38% in BM vs 0.94% in PB, p-value = 0.0118), suggesting a phenomenon in which HIV concomitantly migrates between BM and PB and distinctly replicates in BM. CXCR4-tropic virus was present at 3.04% in BM and 7.27% in PB from this participant.

4) Compartmentalization analyses

Table 2: Compartmentalization tests performed in BM and PB sequences of participant 3

Methods	Compartmentalization tests	PB sequences	BM sequences	Statistical significance
Distance-based (non-parametric)	Average pairwise distance (% substitutions/site)	2.65	1.78	P = 0.0005
	Panmixia	NA		$P < 10^{-7}$
	Root-to-tip distance	0.00009		P < 0.0001
	Wright's measure of population subdivision, Fst	0.099		P = 0
	Nearest neighbor statistic (Snn)	0.981		P = 0
Tree-based (analogous to a parametric analysis)	Simmonds Association Index and bootstrap significance	0.0313		1000/1000
	Modified Slatkin-Maddison	9 migration events		P <0.000
	Correlation coefficient path lengths (r)	0.1729		P = 0.0009
	Correlation coefficient branch count (r_b)	0.3199		P = 0.0009

CONCLUSIONS

- HIV-1 compartmentalization has been previously observed in the central nervous system and shown to be correlated with neurocognitive impairment. [2,7,8]
- No statistically significant difference between compartments with respect to HIV-RNA, HIV-DNA levels, cytokine/chemokine levels, percentage of T cell activation, and T cell phenotypes (data not shown) was observed.
- However, the presence of compartmentalized virus in the bone marrow might have pathogenic consequences and should be further studied for implication in the cure of HIV.

ADDITIONAL KEY INFORMATION

References:

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Author contact: thuy_nguyen@med.unc.edu