ATTACK OF THE CLONES: CLONAL EXPANSION IN THE HIV RESERVOIR
P-E08

HIV DYNAMICS AND REPOPULATION OF RESERVOIRS IN THE HUMAN BODY

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Background

- Upon transmission, HIV-1 disseminates to the lymph nodes within days, and to the bloodstream and throughout the human body within 1-2 weeks.
- The persistence of replication competent HIV in these anatomical sites, even in the setting of longstanding, potent antiretroviral therapy (ART) that suppresses HIV replication, is the main barrier to curing HIV.
- Understanding HIV dynamics across the body is important for cure efforts.
- This goal has been hampered by technical difficulties and the challenge to obtain fresh tissues.

Objective

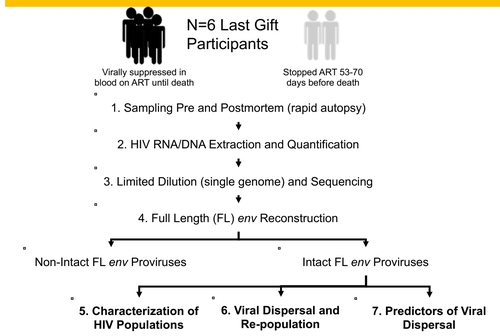
To characterize HIV reservoirs persistence and dynamics across the human body and evaluate factors associated with dispersal.

Methods

- **Study Design and Cohort**. See Figure 1.
- **HIV DNA Quantification and Sequencing**. HIV DNA extracted from PBMC and tissues was quantified by droplet digital PCR (ddPCR). HIV full length (FL) env single genome amplification (SGA) and sequencing was performed on DNA extracted from ante-mortem PBMCs and post-mortem tissues.
- **Sequence analyses** .
- *Defective or hypermutant sequences*. FL env containing large deletion, stop codons or APOBEC-induced hypermutations were considered defective.
- *Clonality*. FL *env* sequences were defined as identical (100%) or nearly identical (99% identity).
- *Viral migration*. BEAST software[1] was used for all analyses. Discrete trait analyses were performed using the asymmetric diffusion model[2,3] and an averaging procedure model (Bayesian stochastic search variable selection)[2]. Bayes factor (BF) support for all possible types of location exchanges were calculated with SpreaD3[4]. Only BFs that remain significant after accounting for the sampling heterogeneity (i.e. adjusted BF [BF_{adj}]) were taken into account to further reduce the false positive rate (see [5] for details).
- *Predictors of within-host spread*. The generalized linear model (GLM) extension of the discrete trait model, implemented in BEAST, was used to investigate potential explanatory variables (predictors) of viral dispersal.

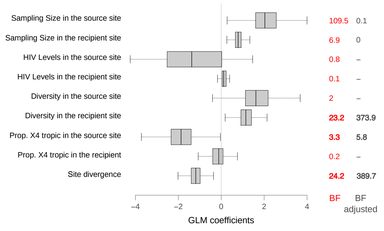
Results

Fig. 1. Study Design



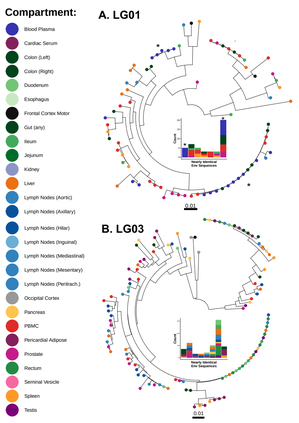
Six participants enrolled in the Last Gift cohort (n=2 who stopped ART and n=4 who remained virally suppressed on ART until death) were included in this study. Pre-mortem blood plasma (n=2 participants) and peripheral blood mononuclear cell (PBMC) samples (n=6 participants) were collected before death and tissues were collected during a rapid autopsy procedure. Intact full length *env* sequences from all samples were used to characterize the HIV populations within each compartment, to assess dispersal across tissues using Bayesian phylodynamic models and to evaluate factors associated with dispersal.

Fig. 4. Viral dynamics are associated with low HIV divergence between sites and high HIV diversity



Box plots of GLM coefficients for all tested predictors in one participant. Bayes Factors (BFs) of each predictor in the model is indicated in red on the right. The original BF support are presented along with the BF_{adj} (in dark grey) (see [5] for details). BF_{adj}≥3 are in bold.

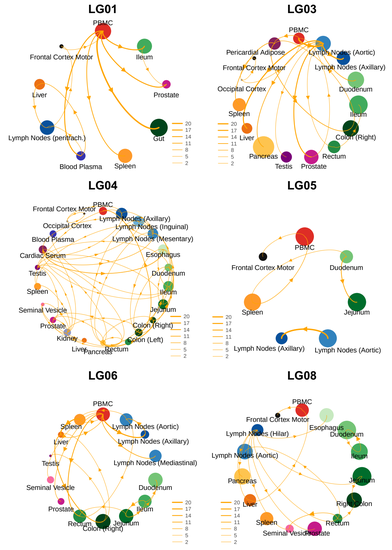
Fig. 2. Large identical HIV *env* populations within and across tissues



Maximum Likelihood (ML) Phylogenies and Clonal Populations (FL env) for 2 Participants Who Stopped ART **(A)** or Remained Virally Suppressed on ART **(B)**.
For LG01 (who stopped ART) the rebounding HIV RNA population was 100% intact (17/17) and predominantly clonal (13/17). Phylogenetic analyses also revealed a large nearly identical FL *env* population of 20 X4 tropic proviruses in post-mortem gut (n=5), PBMC (n=4) prostate (n=3) and blood plasma (n=8)

- All 6 participants initiated ART during chronic infection and were virally suppressed for a mean 7.5 years before enrolling into the Last Gift study (range: 3-14 years). Two participants, LG01 and LG04, voluntarily interrupted ART 53 and 70 days prior to death.

Fig.3. Multiple sites acted as hubs for dissemination of HIV



Lineage Dispersal Events between Compartments. Circle size is proportional to HIV DNA level (or RNA for plasma) in each compartment. The thickness of the arrows corresponds to the average number of inferred migration events between compartments. Only transition events between locations for which the adjusted BF ≥ 3 (at least positive evidence) are shown.
Among participants who remained virally suppressed (LG03, LG05, LG06 and LG08), the majority of transition events originated from lymph nodes and gut tissues.
For LG01 (who stopped therapy), there was strong evidence of viral dispersal from PBMC (BFs≥20) toward gut sites, prostate, spleen, and blood plasma (adjusted BF_{adj} of 449.7, 62, 27 and 22.7 respectively). There was also evidence (BFs≥10) for transition events from blood plasma toward lymph nodes (adjusted BF_{adj} = 16.6) and liver (adjusted BF_{adj} = 10.3) and from liver toward lymph nodes (BF_{adj} = 11.8).
For LG04 (who stopped therapy), 9 sources of viral migration were strongly-supported (BF_{adj}≥20), including migration from blood plasma toward gut tissues and intense viral exchanges between the sampled gut sites. We also found evidence of bidirectional transition events within the central nervous system.

- *Reservoir size*. HIV DNA levels was lowest in CNS samples varying from 0-4.6 copies/10^6 cells in the occipital lobe to 1.8-33.6 copies/10^6 cells in the frontal lobe. There was no discernible difference in tissue between participants who did and did not interrupt ART (p>0.05).
- *Diversity and Clonality*. 676 single genome FL *env* sequences were recovered from 28 sites. Analyses of the 605 intact sequences showed no significant difference in viral diversity across compartments or participants. Large populations of nearly identical sequences were found across up to 10 different compartments (Figure 2).

Conclusions

- This study leveraged the unique Last Gift cohort of participants to reconstruct the spatial dynamics of HIV across the body.
- Multiple body compartments act as hubs for dissemination of HIV, and that blood is the conduit for dispersal.
- For participants stopping their therapy, rebounding virus was likely derived from circulating reservoirs, and it quickly populated deep tissue reservoirs throughout the body via the blood.
- These finding may explain how bone marrow transplants that make circulating CD4 T cells impervious to HIV can induce ART-free remission.

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