Clonal Expansion and Driving Forces of HIV-1 Persistence in Anatomic Compartments

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Background:

- Upon transmission, HIV disseminates widely to lymphoid and nonlymphoid anatomic compartments[1]. Most infected cells are rapidly eliminated; a subset of infected cells persist and undergo clonal expansion[2]. Introduction of antiretroviral therapy (ART) blocks new infection, but does not eliminate infected cells that persist and undergo clonal expansion
- Proviral populations that persist during ART undergo dynamic changes in composition, with relative enrichment of defective proviruses. Although defective proviruses cannot produce infectious HIV, they can contribute to ongoing immune activation, which is associated with morbidity and mortality during ART[3].
- Mechanisms responsible for changes in proviral landscape are not well understood, particularly in anatomic compartments[2,4]. The roles of immune factors and comorbid illnesses (such as neoplastic diseases) driving the proviral landscape are not well understood. HIV infected cells, including clones with replication-competent proviruses, have been reported and enriched in neoplasms that occur during ART, suggesting immune cells infected with HIV traffic to neoplastic lesions, including lesions that arose after ART is initiated.
- Distinct immune environments in tissues established through heterogenous cell types and therefore different host immune responses may contribute to differential mechanisms of persistence of HIV in tissues compared to blood.
- In this study, **we hypothesize that the composition of HIV populations are distinct across anatomic compartments, including neoplastic tissue.** To investigate the distribution of HIV proviruses we quantified the levels of proviruses, investigated phylogenetic relationships, and identified integration sites.

Methods:

1. Study participants and samples

- Persons living with HIV (PLWH) and co-morbid neoplasms enrolled in HIV protocols at NIH Clinical Center with suppressed HIV RNA levels during long term ART consented to research autopsy and underwent post-mortem examination after expiration (12 hours – 7 days).

2. Quantitative measurement of HIV proviruses

- Genomic DNA was extracted from tissues using the HMW Mag Extraction Kit (Qiagen) to reduce DNA shearing[4].
- HIV DNA levels were measured by single copy PCR and multiplexed HIV-LTR/*gag* digital droplet PCR assays[5].
- IPDA assays were performed as described[6].

3. Qualitative measurement of HIV proviruses

- We determined the end-point dilution for single copy of HIV via limiting dilutions and performed **single genome sequencing** (SGS) of HIV *gag* (1.1kb, positions 802-2340 on HXB2).
- Hypermutants were identified and removed from phylogenetic analyses

4. Integration site analyses

- Proviral integration sites (IS) were obtained as previously described
- To assess diversity and clonal expansion rates and patterns, alpha (Simpson) and beta diversity (Bray-Curtis) indices were calculated to quantify intra- and inter-tissue clonal diversity.

5. Population genetics analyses

- Populations with identical SGS were analyzed with and without collapsing identical sequences.
- The average pairwise distance (APD) of unique and non-hypermutated sequences was measured to evaluate the **diversity** of populations.
- Neighbor joining phylogenetic trees were constructed for each PLWH using FastTree and visualized in FigTree[7,8] to investigate the **proviral structures across tissues**.

Table 1. Patient characteristics

PLWH	Comorbid conditions	Sex	Age (years)	Race and Ethnicity	ART duration (years)	Nadir CD4 (cells/ul)	Last CD4 (cells/ul)	HIV Viral Load (copies/mL)
HAMB-1	KSHV*-Associated Primary Effusion Lymphoma	M	61.4	White/Non-Hispanic	22	195	259	<50
P2	KSHV*-Associated Primary Effusion Lymphoma	M	59.8	White/Non-Hispanic	7	87	75	<50
P3	KSHV*-Associated Primary Effusion Lymphoma	M	30	Black/Non-Hispanic	11	160	210	<50
P4	KSHV*-Associated Primary Effusion Lymphoma	M	32	Black/Non-Hispanic	11	39	146	25
P5	**KSHV*-Associated Primary Effusion Lymphoma**	**M**	**28**	**Black/Non-Hispanic**	**2**	**1**	**27**	**<50**
P6	KSHV*-Associated Primary Effusion Lymphoma	M	52.3	White/Non-Hispanic	7	200	786	<50
P7	Metastatic Undifferentiated Adenocarcinoma	M	60.7	White/Non-Hispanic	>15	14	167	<50
P8	Lymphoma Post Bone Marrow transplant	M	37	White/Hispanic	>3	Not known	104	21

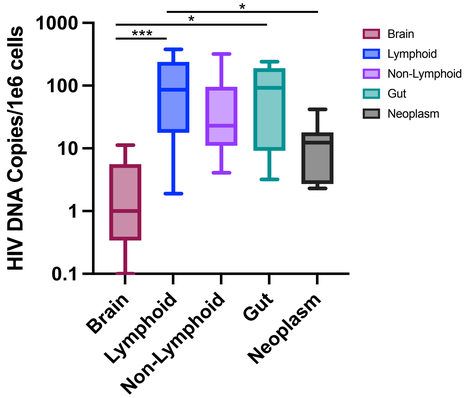
*** KSHV = Kaposi Sarcoma Herpes Virus**

Local Immune Pressures Shape the HIV Proviral Landscape in Anatomic Compartments

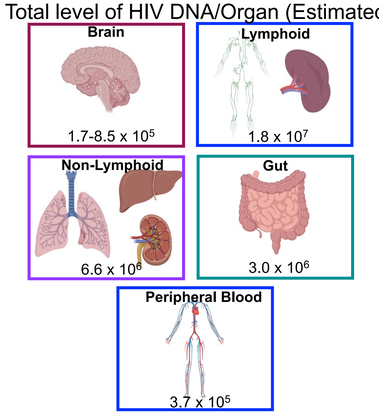
Results

Figure 1. HIV proviruses are widely and differentially distributed in anatomic compartments

1a. Concentration of HIV infected Cells in Tissues



1b. Total level of HIV DNA/Organ (Estimated)



- All individuals (n=8) with presence of HIV associated disease, variable degrees of immune recovery, prolonged ART, and undetectable plasma viremia by commercial assay.
- Single copy assay revealed wide distribution of HIV DNA levels between tissue compartments with lowest copy number in brain (0 – 11.25 copies/1e6 cells) and highest in lymphoid tissues (1.9 – 358 copies/1e6 cells).
- HIV proviruses were also found in non-lymphoid tissue (4.10 – 320 copies/1e6 cells) and cancer tissues (2.3 – 42 copies/1e6 cells).

Figure 2. HIV proviral populations in P5

P5 was a 28 yo man with HIV risk men who have sex with men, diagnosed with HIV 6 y prior to expiration who had been non-adherent with ART and diagnosed with KS (skin, GI, pulmonary, lymph nodes), subsequently with primary effusion lymphoma; CD4 =30 cells/µl. He underwent doxorubicin therapy without improvement followed by six cycles of cytotoxic/biological modifier therapy (EPOCH-R) and lenalidomide with improvement of PEL, regression of KS, but no immune reconstitution, with CD4= 27 cells/µl at expiration, viral RNA <50 c/ml. He developed CMV encephalitis and colitis, progressive pulmonary decline despite ventilation.

At autopsy, he was noted to have diffuse alveolar damage but no acute inflammatory infiltrates were noted. Although KS lesions were present, little evidence of *active* KS was present (HHV-8 negative). Anatomic evidence of immunodepletion. He expired from progressive respiratory failure with widespread alveolar damage, but no acute infectious complication

2a. High variability in LTR/*gag* ratios

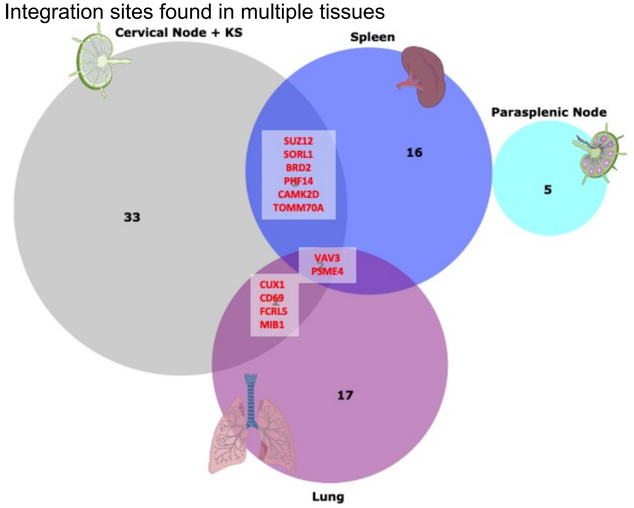
Tissue	LTR/gag ratio	% gag deleted provirus
☐ Cervical Node and KS	**3.31**	39.6%
▣ Lung	**3.39**	41.08%
● Spleen	**5.33**	62.5%
Liver	**7.52**	73.42%
★ Parasplenic Node	**8.79**	77.24%
△ Bladder and KS	**15.91**	87.43%

- LTR/gag ratios largely varied across 6 tissues (3.31. – 15.91)

2b. Intermingled proviral populations by phylogenetic analysis (left)

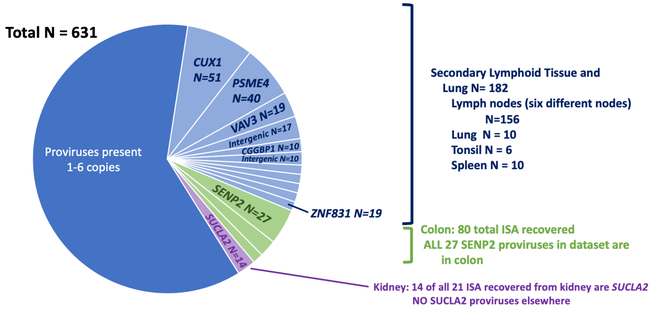
- Neighbor-joining phylogenetic analysis revealed sequences were generally well-mixed among tissues, although distinct populations with strong (>85%, "*") bootstrap support were identified both within a tissue and across lymphoid and nonlymphoid tissue, including tissues with KS lesions evident.

2c. Integration sites found in multiple tissues



- 631 integration sites collected across >15 tissues, 36 integration sites found in multiple tissues
- Significant difference in intra-tissue diversity (alpha diversity) between neoplastic and non-neoplastic (p=0.03) and in inter-tissue diversity (Bray-Curtis analyses) between lymphoid vs non-lymphoid tissues (p=0.002).

2d. Clonal distribution is not uniform



- SENP2 and SUCLA2 clones identified only in colon and kidney, respectively.

Conclusions:

- HIV proviruses are widely distributed in lymphoid and non-lymphoid anatomic compartments individuals with comorbid neoplasms.
- IPDA analyses of autopsy material are limited by high shearing indices, and low HIV concentration in tissues such as central nervous system. Multiplexed LTR/*gag* assays are a quick and useful approach to quantify the total proviral population, as well as frequency of *gag*-deleted proviruses in anatomic compartments.
- Clonal expansion contributes to the proviral landscape, even after extensive cytotoxic chemotherapy, and in the absence of immune reconstitution.
- Unique sequences without hypermutations are mixed across tissues suggesting the lack of ongoing replication during ART.
- Significant differences in LTR/*gag* ratios and proportions of hypermutations but intermingled proviral populations across tissues suggest differential **local immune pressures in shaping the HIV proviral landscape.**

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