# *Anatomical Sites in FIND 2.0 Decedents Harbor Compartmentalized, Transcriptionally Intact HIV-1 Env*



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## BACKGROUND

HIV-1 persistence poses a significant barrier to HIV eradication. Findings from the largest post-mortem study (**FIND 2.0**), which utilized minimally invasive tissue sampling in **38 virally suppressed individuals** on antiretroviral therapy (ART) (**VS**; <400 RNA copies/mL), and **22 unsuppressed (VU) individuals** showed the presence of proviral DNA in all **11 anatomically distinct compartments**. We report the detailed genotypic characterization of full-length HIV-1 envelope glycoproteins (*env*/ Env) from this cohort.

## METHODS

Full-length HIV-1 *env* was PCR amplified from genomic DNA (gDNA) from bone marrow aspirate (BM) and trephine (BMT), brain (Br), bronchoalveolar lavage (BAL), cervicovaginal lavage (CVL), kidney (Ki), lymph nodes (LN), liver (Li), lung (Lu), spleen (Sp), and whole blood (WB) compartments. Following population-based sequencing using the Illumina MiSeq platform HIV-1 *env*/Env sequences were analyzed for subtype, minority variants, premature stop codons, hypermutations, intact rev response elements (RRE), and coreceptor tropism. Viral compartmentalization was assessed by comparing sequence identity, coreceptor tropism prediction, V3 loop variants, and phylogenetic clustering of each *env*/ Env per compartment/ decedent.

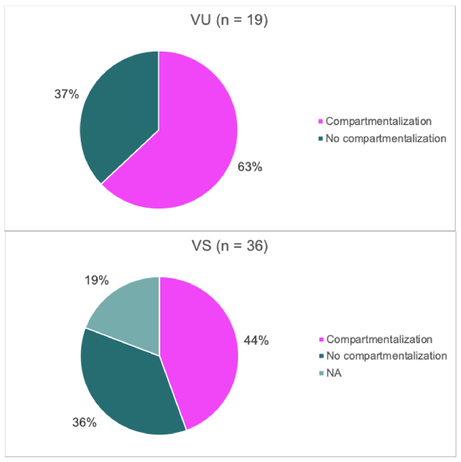



**Figure 2: Proportion of FIND 2.0 decedents that displayed viral compartmentalization, indicated by viremic status.**

**Intact** proviral HIV-1 ***env*/Env** sequences were detected in almost every compartment of both **VS (58%) and VU (37%) decedents**, *except for the liver* in the VU group.

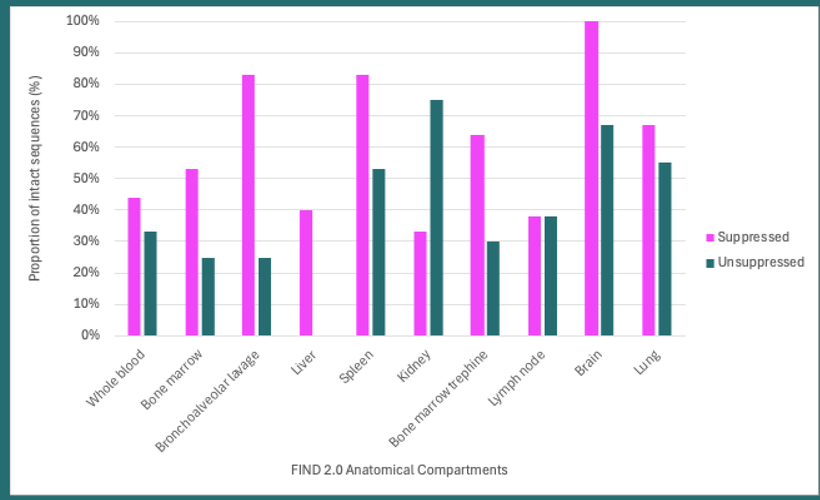



**Figure 1: Intact proviral HIV-1 env/Env sequences in FIND 2.0 decedents, based on the absence of hypermutations and premature stop codons, and presence of intact RRE regions. See Table 1.**

## RESULTS

Of 521 DNA samples, 242 (46%) *env* amplified yielding 218 quality sequences: 127 from 36 VS, 91 from 19 VU (**Table 1**). Phylogenetic clustering confirmed all were infected with HIV-1 subtype C, with sequences clustering by patient. Higher proportions of intact *env*/Env sequences were detected in the VS group compared to the VU group, when accounting for the absence of hypermutations and premature stop codons, and intact RRE regions (**Table 1, Figure 1**).

Minority variants were detected in 81% of *env*, with greater diversity in VU decedents. CCR5-tropic viruses predominated in both groups (92% VS; 80% VU), while CXCR4-tropic variants were more common in VU decedents, particularly in Br, Li, and WB. Minor variant V3 loop sequence analysis showed mixtures of CCR5/CXCR4-utilizing viruses in some compartments, while single tropism sequences were found in others. As per the criteria used, compartmentalization was identified in 44% VS and 63% VU decedents (**Figure 2**).

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## CONCLUSIONS

- These findings offer new insights into the dynamic and complex proviral HIV-1 populations across distinct anatomical compartments, largely independent of ART status. While we demonstrated generally higher proviral DNA burdens in the compartments of VU decedents, there was a smaller, but still significant proviral DNA burden in VS decedents.
- Although we highlighted the persistence of diverse, transcriptionally intact proviral sequences in the viral reservoirs across the anatomical compartments of both decedent groups, there did appear to be a difference between the groups based on the classification of the defect.
- Regarding viral tropism, CCR5-utilizing viruses predominated the majority variants of both decedent groups, and the emergence of CXCR4-utilizing viruses was more common in VU decedents, particularly for the brain, liver, and whole blood compartments.
- We observed different degrees of viral compartmentalization in both decedent groups, but this was higher in the VU decedents.

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**Table 1: Number of successfully sequenced HIV-1 *env* amplicons and intact sequences for each anatomical compartment from the FIND 2.0 suppressed and unsuppressed decedents.**

| | Suppressed (n = 38) | | | | Unsuppressed (n = 22) | | | |
|---|---|---|---|---|---|---|---|---|
| **Compartment** | **Full-length *env* NGS** | **No G-A hypermutations** | **No premature stop codons** | **No RRE defects** | **Full-length *env* NGS** | **No G-A hypermutations** | **No premature stop codons** | **No RRE defects** |
| **Whole blood** | 18 | 10 | 11 | 10 | 15 | 15 | 14 | 6 |
| **Bone marrow** | 17 | 13 | 12 | 10 | 12 | 10 | 9 | 4 |
| **Bronchoalveolar lavage** | 6 | 5 | 5 | 5 | 4 | 4 | 1 | 3 |
| **Liver** | 5 | 5 | 5 | 2 | 5 | 5 | 2 | 2 |
| **Spleen** | 18 | 18 | 17 | 15 | 15 | 15 | 14 | 9 |
| **Kidney** | 9 | 6 | 7 | 4 | 8 | 7 | 7 | 6 |
| **Bone marrow trephine** | 22 | 20 | 19 | 15 | 10 | 8 | 6 | 6 |
| **Lymph node** | 13 | 10 | 8 | 8 | 8 | 8 | 8 | 3 |
| **Brain** | 1 | 1 | 1 | 1 | 3 | 3 | 3 | 2 |
| **Lung** | 18 | 15 | 15 | 12 | 11 | 10 | 10 | 6 |