# Different T-Cell Subsets contain Intact Proviruses in Blood and Lymph Node during ART

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Antiretroviral therapy (ART) for the treatment of HIV-1 is highly successful in suppressing viral replication, reducing the level of virions in the blood, and increasing the life expectancy of infected individuals. Despite this, ART does not cure HIV-infected individuals, as HIV-1 remains latent in resting memory CD4+ T-cells. Latent HIV-1 proviruses are not targeted by ART or the host immune system. However, latent replication-competent HIV-1 proviruses persist as viral load rapidly rebounds if ART is interrupted. A thorough understanding of the distribution of replication-competent HIV-1 within different CD4+ T-cells and anatomic compartments is required for future eradication studies.
Here, we applied near full-length sequencing to determine the potential replication-competency of HIV-1 proviruses isolated from different CD4+ T-cell subsets in peripheral blood and lymph node from participants on long-term ART.

We sequenced near full-length HIV-1 proviruses from naïve (NV), central (CM), transitional (TM), and effector (EM) memory CD4+ T-cells from the peripheral blood (PB, n = 13) and lymph node (LN, n = 5) of participants on long-term ART (3-17 years).

Full-Length Individual Proviral Sequencing (FLIPS)

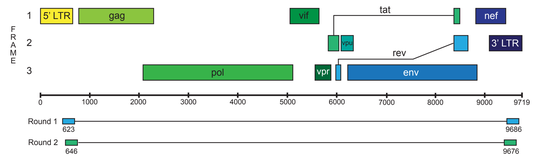



Figure 1. The FLIPS assay amplifies single, near full length (9 kb) HIV-1 proviruses isolated from CD4+ T-cell subsets using nested PCR. Amplified proviruses are sequenced by Next Generation Sequencing on the Illumina MiSeq Platform and assembled *de novo*.

Table 1. Number of peripheral blood cells and sequences obtained for each participant.

| Peripheral blood | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Naïve | | | Central Memory | | | Tranisitional Memory | | | Effector Memory | | |
| Participant | Defective | Intact | # Cells analyzed | Defective | Intact | # Cells analyzed | Defective | Intact | # Cells analyzed | Defective | Intact | # Cells analyzed |
| 2302 | 15 | 4 | 3,287,933 | 35 | 0 | 704,122 | 31 | 3 | 820,287 | 32 | 6 | 728,025 |
| 2115 | - | - | - | 28 | 0 | 2,294,141 | 29 | 0 | 859,259 | 22 | 1 | 1,888,889 |
| 2275 | - | - | - | 3 | 0 | 251,852 | 21 | 0 | 1,057,688 | 33 | 3 | 1,330,640 |
| 2046 | 0 | 0 | 6,465 | 24 | 0 | 190,647 | 1 | 0 | 4,048 | 23 | 2 | 153,236 |
| 2452 | 35 | 2 | 641,975 | 34 | 0 | 908,333 | 37 | 0 | 225,734 | 33 | 3 | 459,055 |
| 2026 | 26 | 0 | 152,727 | 1 | 0 | 47,685 | 10 | 1 | 133,448 | 32 | 1 | 51,046 |
| 2469 | 1 | 0 | 1,824,159 | 33 | 0 | 1,459,476 | 37 | 0 | 960,696 | 34 | 1 | 500,022 |
| 2278 | 9 | 0 | 17,291,324 | 45 | 0 | 4,564,677 | 30 | 0 | 3,295,980 | 37 | 1 | 1,606,036 |
| 2286 | 13 | 0 | 906,846 | 51 | 0 | 1,997,611 | 42 | 0 | 740,607 | 27 | 0 | 929,653 |
| 2303 | 44 | 6 | 7,433,615 | 27 | 1 | 2,994,562 | 40 | 4 | 1,008,786 | 31 | 10 | 1,068,985 |
| 1292 | 32 | 2 | 1,697,256 | 52 | 1 | 434,237 | 36 | 0 | 418,886 | 59 | 0 | 979,955 |
| 2470 | 27 | 2 | 2,035,499 | 23 | 0 | 1,638,093 | 27 | 0 | 1,286,067 | 26 | 1 | 1,037,773 |
| 2241 | 17 | 3 | 1,896,296 | 24 | 0 | 1,178,519 | 25 | 0 | 955,485 | 25 | 1 | 1,325,583 |
| Total | 219 | 19 | 37,174,095 | 380 | 2 | 18,663,954 | 366 | 8 | 11,766,971 | 414 | 30 | 12,058,897 |

Table 2. Number of lymph node cells and sequences obtained for each participant.

| Lymph node | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Naïve | | | Central Memory | | | Tranisitional Memory | | | Effector Memory | | |
| Participant | Defective | Intact | # Cells analyzed | Defective | Intact | # Cells analyzed | Defective | Intact | # Cells analyzed | Defective | Intact | # Cells analyzed |
| 2452 | 33 | 12 | 505,113 | 49 | 0 | 376,485 | 36 | 2 | 667,096 | - | - | - |
| 2303 | 33 | 0 | 3,973,533 | 23 | 1 | 2,736,534 | 34 | 0 | 1,393,968 | 36 | 0 | 1,303,924 |
| 1292 | 34 | 4 | 1,524,198 | 34 | 0 | 205,822 | 24 | 1 | 510,678 | 15 | 0 | 79,765 |
| 2470 | 2 | 0 | 360,237 | 33 | 1 | 784,607 | 18 | 0 | 434,902 | 14 | 0 | 142,501 |
| 2241 | 3 | 0 | 196,155 | 1 | 0 | 47,811 | 30 | 1 | 183,466 | - | - | - |
| Total | 105 | 16 | 6,559,236 | 140 | 2 | 4,151,259 | 142 | 4 | 3,190,111 | 65 | 0 | 1,526,191 |

In total, we sequenced 1900 proviruses. Of these, 4.5% were intact and potentially replication-competent.

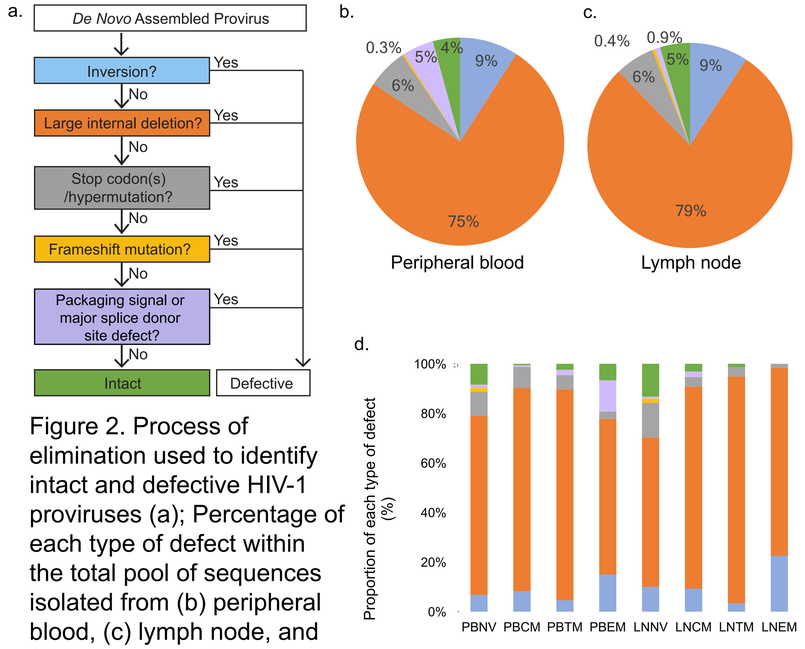



Figure 2. Process of elimination used to identify intact and defective HIV-1 proviruses (a); Percentage of each type of defect within the total pool of sequences isolated from (b) peripheral blood, (c) lymph node, and (d) individual cell subsets.

The frequency of infection with any HIV-1 genome (intact and defective) differed across the T-cell subsets in peripheral blood and lymph node ($P<0.001$).

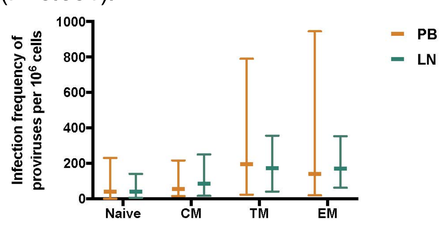



Figure 3. Proviral infection frequency within T-cell subsets from peripheral blood and lymph node.

The infection frequency of genetically intact proviruses also differed across the T-cell subsets in peripheral blood ($P<0.001$) and lymph node ($P=0.02$). Overall, the infection frequencies of intact proviruses within the two anatomical sites were similar ($P=0.67$).

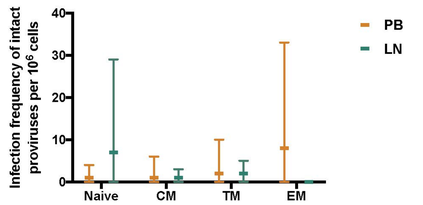



Figure 4. Infection frequency of intact HIV-1 proviruses within T-cell subsets from peripheral blood and lymph node.




Figure 5. Phylogenetic tree and sequence representation of sequences obtained for participant 2026. Stars indicate intact sequences.

## Conclusions

- The distribution of genetically intact proviruses within T-cell subsets in blood do not reflect their distribution in lymph nodes.
- In lymphoid tissues, the frequency of intact proviruses is highest in less differentiated cells such as NV cells, while in blood the frequency is highest in more differentiated EM cells.
- Tissue-based NV T-cells may act as progenitors of the total reservoir during ART, whereas in the periphery this reservoir is maintained within the EM T-cell population, perhaps by clonal proliferation.

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