Proviral Landscape in Neonates with in utero HIV-1 Receiving Very Early ART in IMPAACT P1115

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BACKGROUND

Data on the proviral landscape in neonates with in utero HIV-1 infection are limited. We performed near full-length single HIV-1 genome sequencing (nFLSGS) to analyze the proviral landscape and estimate intact proviral loads among 25 neonates with in utero HIV-1 who initiated very early ART in IMPAACT P1115 (NCT02140255)[1,2,3].

METHODS

Study Population

440 neonates in Cohort 1 of IMPAACT P1115 pre-emptively initiated ART within 48 hours of birth.

In utero HIV-1 infection was confirmed in 36 neonates, with 34 continuing on-study.

Eligibility Criteria

nFLSGS was performed on neonates with:

- HIV-1 DNA loads >20 copies/10^6 (c/10^6) peripheral blood mononuclear cells (PBMCs)
- Sufficient remnant genomic DNA

Droplet Digital PCR (ddPCR)

Total HIV-1 DNA from PBMCs measured by ddPCR was used to standardize the HIV-1 DNA input for nFLSGS.

Near Full-Length Single Genome Sequencing

A nFL outer 9kb followed by a nFL inner 9kb were performed limiting dilution to ensure single genome amplification.

Sequencing using Illumina MiSeq was performed at the Massachusetts General Hospital CCIB DNA Core.

Viral Genome Bioinformatics Analysis

Genomes were classified using HIVSeqinR[3] (v2.7.1) as **intact**, **defective**, or **hypermutated**

Manual evaluation was required for subtypes A1 and AE.

Quantification of Intact Proviral Load

Intact HIV-1 proviral load was calculated as $\left[\frac{I}{C}\right] \times e$.

I = number of intact HIV-1 proviral genomes obtained via nFLSGS
C = total cellular equivalents analyzed in standardized input of HIV-1 DNA
e = efficiency correction constant

When intact proviral genomes were not detected, data were calculated as 0.5 intact proviral genomes per cell equivalents tested without target identification.

Intact HIV-1 DNA load was normalized to c/10^6 PBMCs.

Phylogenetic Analyses

Sequences were aligned with MAFFT (v7) and the maximum likelihood tree was built using IQ-TREE (v2).

Sequences for HIV-1 subtypes B (HXB2), A1, AE and C were included for reference.

Statistical Methods

A two-sided signed rank test was performed for analysis of intact HIV-1 DNA load at birth and 2 weeks post-birth.

The proviral landscape at birth in neonates with in utero HIV-1 includes a **complex mix** of **intact**, **defective**, and **hypermutated** **genomes**, indicating **ongoing HIV-1 replication in fetal cells.**

RESULTS

- 25 of 34 neonates met criteria for analysis; n=21 at birth, n=18 at 2 weeks post-birth, n=14 at both timepoints.
- Median proviral genomes (Q1, Q3) [min, max] is 5 (3, 7) [1, 11] at birth and 5.5 (2.3. 10) [1, 20] 2 weeks post-birth.

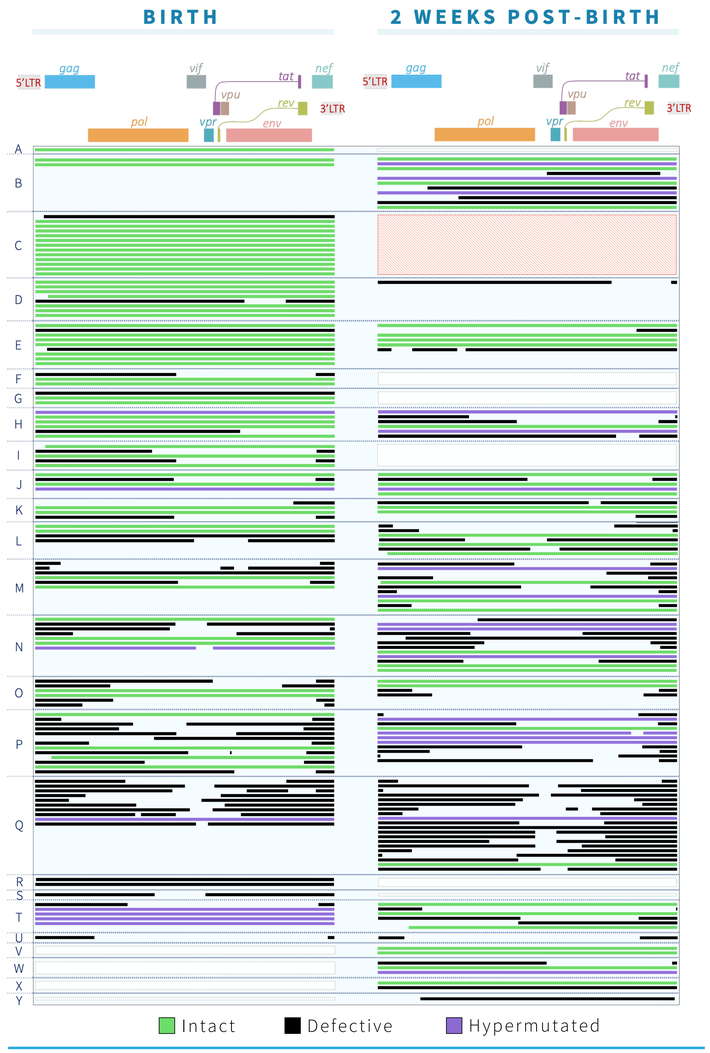

Figure 1. Proviral landscape analyses at birth (left; 114 genomes from *n*=21) and 2 weeks post-birth (right; 113 genomes from *n*=18), sorted by percent intact proviral genomes at birth. White boxes indicate insufficient volume for nFLSGS (*n*=4 at birth; *n*=3 at 2 weeks post-birth). Red hatched boxes indicate <20 cpm HIV-1 DNA at a given timepoint (*n*=1 at 2 weeks post-birth).

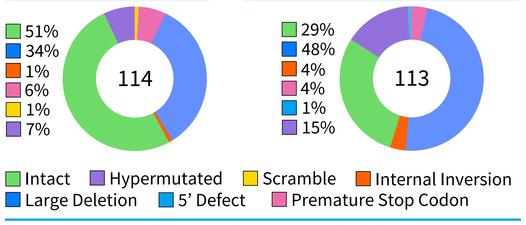

Figure 2. Distribution of intact and defective proviral genomes identified at birth (114 genomes) and 2 weeks post-birth (113 genomes).

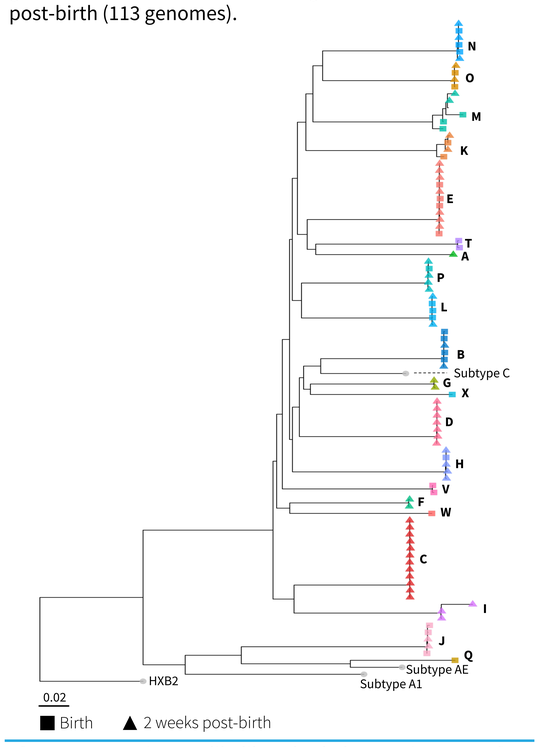

Figure 3. Maximum likelihood phylogenetic tree of 91 intact HIV-1 proviral genomes at birth (58 genomes) and 2 weeks post-birth (33 genomes), showing participant-specific clustering (*n*=21). Intacts were not detected in neonates R, S, U, and Y.

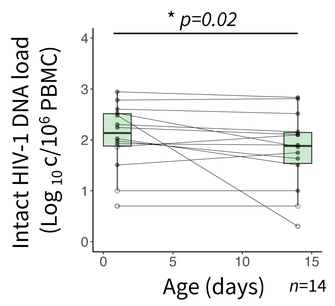

Figure 4. Intact HIV-1 DNA load from birth to 2 weeks post-birth. Median intact proviral load (Q1, Q3) [min, max] is 141 (63, 352) [5, 880] at birth and 76 (27, 190) [2, 681] 2 weeks post-birth. Two-sided signed rank test performed at a 0.05 significance level. Open circles denote participants without detected intact proviral genomes.

Limitations

Only those with the minimum required proviral load for nFLSGS (>20 HIV-1 DNA c/10^6 PBMC) and sufficient remnant DNA could be studied.

Inherent inefficiency (27-30%) of 9kb nFLSGS PCR may underrepresent intact proviral genomes.

Current analyses are unable to discriminate between integrated vs unintegrated proviral genomes.

CONCLUSIONS

Intact, defective and hypermutated proviral genomes were identified at birth in neonates with in utero HIV-1, indicating ongoing HIV-1 replication in utero.

Intact proviral genomes comprise a substantial proportion of the proviral pool and share >99% identity through 2 weeks of life, supporting low diversity infection.

In the first 2 weeks of life, intact proviral loads decrease with very early ART, suggesting early clearance of infected cells harboring intact HIV-1 proviral genomes.

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