

INTACT HIV PROVIRUSES ARE DETECTABLE 7-9 YEARS LATER WHEN ART STARTS AFTER 3 MONTHS

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ABSTRACT

Background: In adults starting ART in acute infection, only 2-5% of proviruses are intact. However, no intact full length proviral sequences were detected in a cohort of early treated, long-term suppressed children. We sought to characterize proviral sequences in another cohort of early treated children after 6-9 years on suppressive ART.

Methods: PBMC samples from perinatally infected children in the CHER study were analyzed. Single, near full length proviral amplification and sequencing (NFL-PAS) was performed at one time point after 6-9 years of suppressive ART. Amplicons with large internal deletions were excluded (<9kb on gel electrophoresis). All amplicons ≥9kb were sequenced (Sanger and Illumina) and analysed through an 'Intactness bio-informatic pipeline' to detect indels, frameshifts, inactivating point mutations and/or hypermutations that would render the proviruses defective.

Results: In 9 children who started ART at a median age of 2.3 (range: 1.7 – 11.1) months, 738 single NFL-PAS amplicons were generated. Of these, 553 (74.9%) had large internal deletions, 175 (23.7%) had hypermutation, 3 (0.4%) had deletions in the packaging signal and major splice donor site, and 7 (1%) were intact. These 7 intact sequences were from 3 children who initiated ART after 8 months of age; of whom one had two identical and intact sequences, suggestive of a cell clone harbouring a replication-competent provirus. No intact provirus was detected in 5 children who initiated ART before 2.3 months of age.

Conclusion: Rare, intact proviruses could be detected in the blood of children who initiated ART after 2.3 months of age. The frequency of intact proviruses is similar to that reported for adults treated during early HIV infection.

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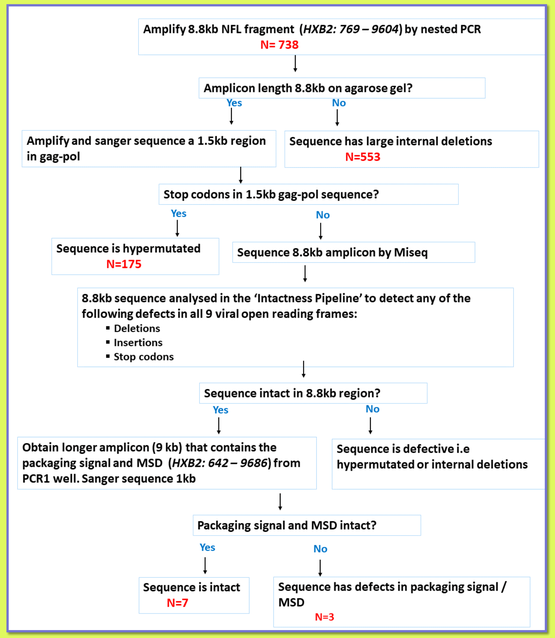
INTRODUCTION

There has been viral rebound after treatment discontinuation in almost all perinatally infected children despite early therapy, suggesting that replication competent HIV proviruses persist (1). However, in a cohort of 11 early treated, long-term suppressed children, no intact proviral sequences were detected from 162 near full length sequences (2). Most latently infected cells contain defective proviruses due to the error prone *Reverse transcriptase* enzyme and hypermutations caused by APOBEC 3G, a host restrictive factor (3,4). In adults starting ART during acute infection, only 2-5% of proviruses are genetically intact by near full length proviral sequencing (3,5). We aimed to characterize near full length proviral sequences in children from the CHER cohort after 7-9 years on ART

METHODS

9 children who initiated ART at a median of 2.3 months (range 1.7 – 11.1 months) were studied. For each child, single near full length - proviral amplification and sequencing (NFL-PAS) was performed on a PBMC sample from 7-9 years on ART.

Figure1: The process by which 7 intact near full length sequences were obtained from a total of 738 amplicons



RESULTS

Table 1: Patient characteristics and distribution of the 738 single genome amplicons

PID	Gender	Age ART start (months)	Treatment History and Longitudinal viral loads	Duration of ART at sample (years)	HIV CAD (copies/10^6 PBMC)	Number of Sequences with large internal deletions	Number of 9kb Sequences with hyper-mutations and stop codons	Number of sequences with packaging signal/MSD defects	Number Intact proviruses	Total number of single genomes
A	F	1.7	Interrupted ART after 9months; reinitiated after 3.6yr; viremic until after re-initiation	8.6	55.5	4	1	0	0	5
B	F	2.0	Continuous therapy; Delayed suppression for first 6 months on ART, good suppression thereafter	9.13	186.2	68	19	0	0	88
C	M	2.16	Interrupted on after 9.9 months on ART; reinitiated after 13 months; viremic until after re-initiation	6.9	81.9	30	4	0	0	34
D	F	2.2	Continuous therapy; Detectable viral load after 2.2yrs on ART, supressed 5 months later and good suppression thereafter	6.96	42.3	89	101	0	0	190
E	F	2.3	Interrupted after 2yr on ART; blip 4 months after interruption; reinitiated 4 months after interruption; viremic until after re-initiation	8.55	129.6	18	3	0	0	21
F	M	8.5	Continuous therapy	7.93	247.6	27	9	1	2	39
G	F	9.23	Continuous therapy	7.31	181.5	207	23	2	1	233
H	F	9.32	Continuous therapy	8.2	46.7	56	15	0	4	75
I	F	11.1	Poorly suppressed for first 2.5years on ART	8.82	86.3	54	0	0	0	54
Total	NA	Median= 2.3		Median = 8.2	Median = 86.3	553 (74.9%)	175 (23.7%)	3 (0.4%)	7 (1%)	738

Figure 2: The distribution of intact vs defective proviruses among the 9 children. 553 (74.9%) had large internal deletions, 175 (23.7%) had hypermutations, 3 (0.4%) had deletions in the packaging signal and major splice donor site, and 7 (1%) were genetically intact

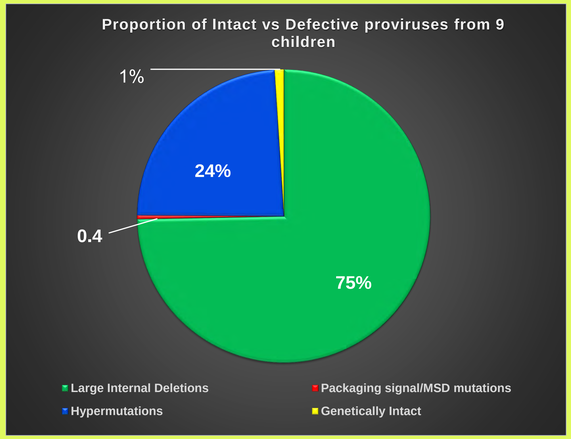
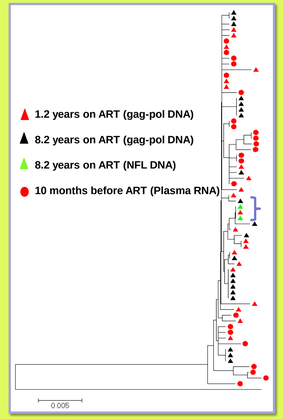


Figure 3: Neighbor joining phylogenetic tree consisting of 1.5kb gag-pol sequences (red and black symbols) and 8.8kb NFL sequences (green symbols) from patient H. Two intact NFL sequences were identical and formed a monotypic cluster (purple bracket) with a 1,5kb gag-pol DNA sequence from pre-therapy, suggesting the persistence of an intact clone that was present soon after ART was initiated



CONCLUSIONS

- **Rare, intact proviruses were detected in children who initiated ART after 2.3 months of age.**
- **We detected a 1% frequency of intact proviruses, similar to that in adults treated during early HIV infection.**
- **Although we could not sequence integration sites to prove clonality, considering the relatively diverse viral population, finding two identical near-full length intact sequences suggests a cell clone harboring a replication competent provirus.**

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