PROVIRAL LANDSCAPE IN CHILDREN PARALLELS ADULTS AND ENABLES RESERVOIR RECONSTRUCTION

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Abstract

- Near full length sequencing was performed on amplicons from PBMC of two durably suppressed children
- The proviral landscape was compared to that in durably suppressed adults using sequences obtained from the Proviral Sequence Database
- We observed similar landscapes in both groups, with common 3' deletions
- Defective proviruses were used to reconstruct an intact viral ancestor, which was identical to a recovered sequence

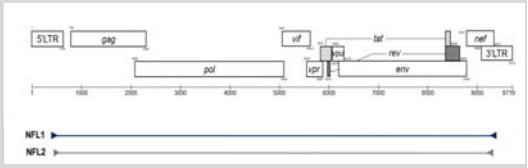
Methods

Near Full Length Sequencing

Extracted gDNA was subject to near full length (NFL) amplification using an outer NFL amplification (NFL1, 9kb), followed by a nested NFL amplification (NFL2, 9kb). Amplicons were then sequenced by MiSeq.

Analysis

Sequences were aligned using MEGA7 and checked for intactness using an intactness pipeline available on the PSD. Proviral landscapes were then compared to that in adults using sequences obtained from the PSD.

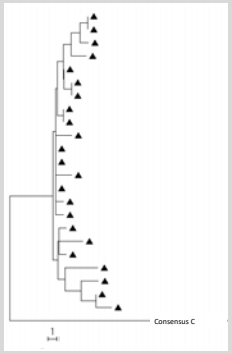


Donor Diversity

A. ZA-011 – 0.7% in P6PRRT

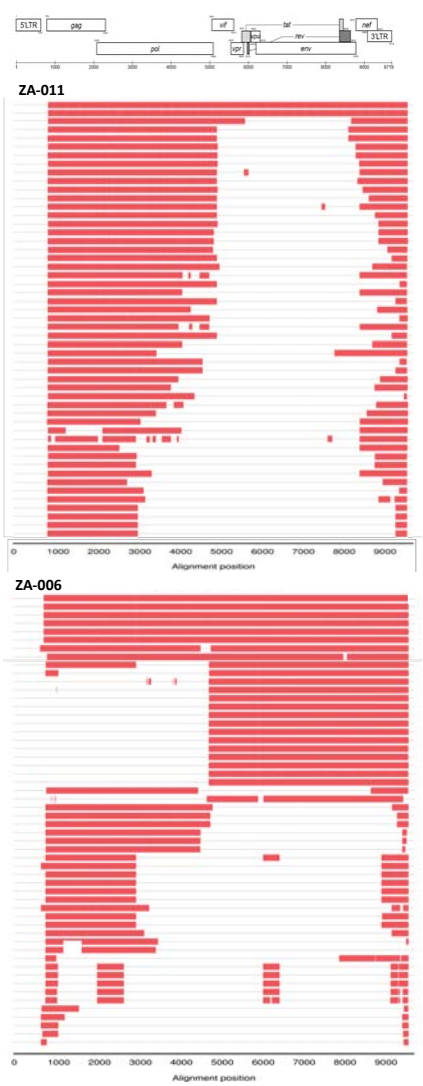


B. ZA-006 – 0.3% in P6PRRT

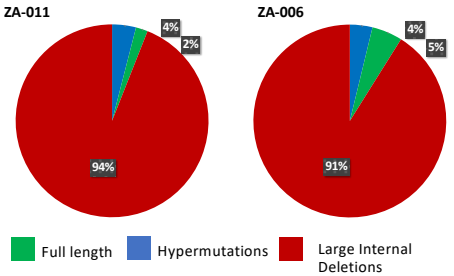


Results – Near Full Length Amplification and Miseq Sequencing

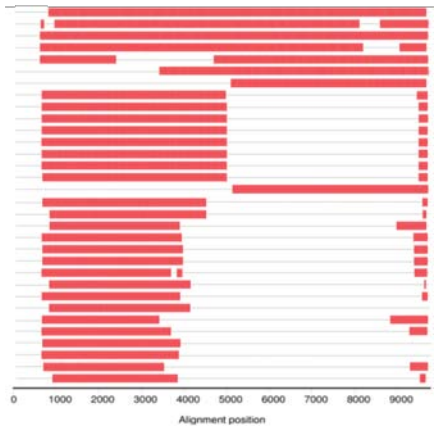
A. Proviral landscape virogram of ZA-011 and ZA-006



B. Proportion of full length vs deleted proviruses



C. Representative proviral landscape virogram of adults

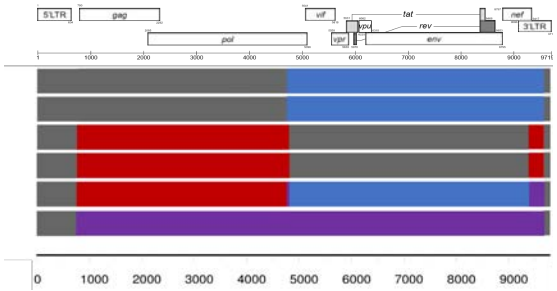


D. Donor Characteristics

	n	Age at Sampling (years)[a]	Duration of Suppression (years)[a]	Viral Load at Sampling
Children[b]	11	8.5 (6.95-9.30)	7.32 (5.06-7.89)	<40
Adults[c]	33	49.0 (27.0-76.0)	4.58 (0.67-15.92	<40

[a] Median (Range) values given
[b] Van Zyl et al., *J Clin Invest*. 2017
[c] Bruner et al., *Nat Med*. 2017, Hiene et al., *Cell Reports*. 2017, Ho et al., Cell. 2013

Reconstruction of proviral ancestor and comparison to recovered sequence



Publicly available Proviral Sequence Database



Conclusions

- Despite known differences between children and adult immune repertoire, we observe similar HIV-1 proviral landscape in both durably suppressed groups
- Majority of HIV-1 provoiruses contain large 3' deletions, including *env/tat/rev,* supporting the hypothesis that lack of proviral expression may be associated with proviral persistence
- From defective proviruses with non-overlapping deletions and identical sequence, we reconstructed the sequence of the intact viral ancestor
- We recovered the exact intact proviral sequence as the reconstructed sequence, providing proof of concept of this method
- Characterizing the genetics of the reservoir in early-treated individuals can help guide the design of therapeutic interventions for HIV remission.

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