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Assessment of HIV-1 DNA by Single-Genome Sequencing in Children on Suppressive ART

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Background and Objective

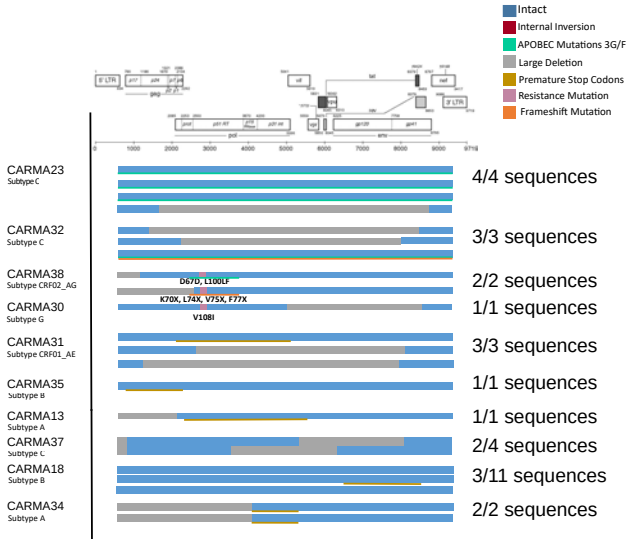
Perinatally HIV-1 infected children on early suppressive ART (PaHIV) represent an important population in the era of cure. Reservoir characteristics can determine criteria and cut-offs in future trials. We present unique HIV-1 DNA single genome sequencing (SGS) data derived from cell-associated HIV-1 DNA (CA-DNA) in PaHIV from a multicentre cross-sectional study (CARMA – **C**hild and **A**dolescent **R**eservoir **M**easurements on early suppressive **A**RT). We describe the presence of intact proviruses, defective genomes and ART resistance associated mutations (RAMs).

Methods

Single HIV-1 genome sequences were obtained by almost full-length whole genome PCR [1] in limiting dilution, followed by a 4-fragment touchdown nested PCR using an in-house protocol, creating overlapping fragments of ~3,000bp, and analysis on an agarose gel. The fragments were sequenced using the Nextera®XT DNA kit and the MiSeq (Illumina). An in-house bioinformatics pipeline with open source software was used for *de novo* assembly of genomes. SMALT and LASTZ for alignment and HIVSeqinR pipeline were used to describe intact or defective viruses. The Stanford database and Geno2pheno tool were used for resistance and subtype analysis and definition of the tropism. Alignment was performed using ClustalX, followed by generation of the cladogram with Simple Phylogeny (embl-ebi, Neighbour-joining clustering method).

Results (I)

Near full-length single genome HIV-1 sequences in CARMA participants with detectable HIV-1 DNA

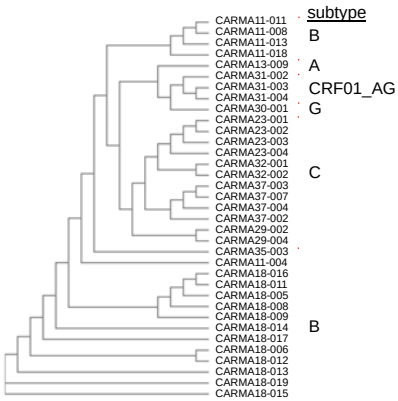


- 4-fragment nested PCR of single HIV-1 clones could successfully be sequenced and assembled into almost full-length single genome sequences
- at least one single genome HIV-1 clone could be obtained for 13 out of 34 patients with detectable HIV-1 DNA reservoir in PBMCs (10 patients are shown as an example)
  - ➢ further analysis is ongoing

Results (II)

Summary of data for 13 CARMA participants for which single genome sequences were obtained

No. in list	CA-DNA (cop./10^6 PBMC)	No of Sequences obtained	Genotype	Intact Sequence	Time to Diagnosis (months)	Baseline VL (cop./mL)
CARMA02	**112.4**	2	B		8.03	3.28E+05
CARMA11	**67.7**	5	F	1/5	5.8	5.00E+05
CARMA13	**51.5**	1	A		6.49	4.90E+05
CARMA18	**323.1**	12	B	2/12	5.02	5.00E+06
CARMA19	**41.1**	2	B		6.98	1.38E+06
CARMA23	**225.8**	4	C	3/4	2.16	8.00E+05
CARMA29	**81.9**	2	C		3.51	5.60E+03
CARMA30	**59.3**	1	G		5.31	5.00E+05
CARMA31	**108.3**	3	CRF01_AE		9.38	1.00E+06
CARMA32	**49.1**	3	C		3.08	1.48E+05
CARMA34	**260.5**	2	A		5.05	1.00E+06
CARMA35	**43.9**	1	B	1/1	4.2	5.00E+05
CARMA37	**197.0**	4	C		3.15	5.00E+05
CARMA38	**260.7**	2	CRF02_AG	1/2	14.13	1.65E+06
	total:	44				



Cladogram of almost whole genome sequences recovered in this study

summary of detected mutations in 44 sequences from 13 CARMA participants

sequence attribute	detected sequences
inferred intact	8/44
resistance mutations (IN, NRTI, NNRTI)	13/44
APOBEC3G/F mutations	13/44
deletions	12/44
premature stop codon	9/44
frame shift	3/44

- single genome HIV-1 clones could be obtained from PaHIV with HIV-1 CA-DNA as low as 40 copies/10^6 PBMCs
- genomes from the same participants cluster together (as expected)
- most common mutations were RAMs and APOBEC3G/F mutations
- majority of HIV-1 DNA single genome clones are defective

Conclusions

- intact HIV-1 genomes could be detected in PaHIV (8/44 sequences)
- APOBEC3G/F mutations and deletions were very common (one third of sequences)
- resistance mutations should be investigated in regard to ART regiment taken
- so far no clonal sequences were observed

➢ We aim to sequence at least 10 single whole genomes for all participants with detectable HIV-1 DNA to extrapolate the percentage of intact HIV-1 sequences in PBMCs from PaHIV and correlate this with the available clinical data, age at ART start and time suppressed.

Acknowledgements

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References

[1] Sharaf R, Lee GQ, Sun X, Etemad B, Aboukhater LM, Hu Z, Brumme Z, Aga E, Bosch RJ, Wen Y, Namazi G, Gao C, Acosta EP, Gandhi RT, Jacobson GM, Skiest D, Margolis DM, Mitsuyasu R, Volberding P, Connick E, Kuritzkes DR, Lederman MM, Yu XG, Lichterfeld M, Li JZ**. HIV-1 proviral landscapes distinguish posttreatment controllers from noncontrollers.** JCI, 2018 Apr; 128(9).