CONFERENCE CAHR 2020

CONGRÈS DE L'ACRV 2020

Genetic diversity of HIV proviruses persisting during cART in former viremic controllers

Hanwei Sudderuddin[1], Aniqa Shahid[1,2], F. Harrison Omondi[1,2], Natalie N. Kinloch[1,2], Bradley R. Jones[1], Rachel L. Miller[2], Olivia Tsai[2], Daniel MacMillan[1], Alicja Trocha[3], Chanson J. Brumme[1,4], Jeffrey B. Joy[1,4], Bruce D. Walker[3], Zabrina L. Brumme[1,2]

[1]British Columbia Centre for Excellence in HIV/AIDS, Vancouver, BC, Canada; [2]Faculty of Health Sciences, Simon Fraser University, Burnaby, BC, Canada; [3]Ragon Institute of MGH, MIT and Harvard, Cambridge, MA, USA; [4]Department of Medicine, University of British Columbia, Vancouver, BC, Canada

Poster: BS2.04

Presenting author: Hanwei Sudderuddin

Correspondence: hsudderuddin@cfenet.ubc.ca

I HAVE NO CONFLICT OF INTEREST

BRITISH COLUMBIA CENTRE *for* EXCELLENCE *in* HIV/AIDS

BELIEVE IN THE CURE MARTIN DELANEY COLLABORATORY

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Project overview

Background: The HIV reservoir is the main barrier to cure. Analysis of within-host proviral diversity during suppressive antiretroviral therapy (cART) in the context of within-host HIV evolutionary history provides insight into reservoir dynamics. ***However HIV controllers (individuals who naturally suppress viremia without cART) are underrepresented in such studies.***

Objective: To characterize proviral age and diversity during suppressive cART in 4 former viremic controllers using phylogenetic methods, and interpret these data in the context of within-host HIV evolutionary history and diversity pre-cART.

Approach: Reconstructing within–host HIV evolutionary history to infer proviral sequence ages

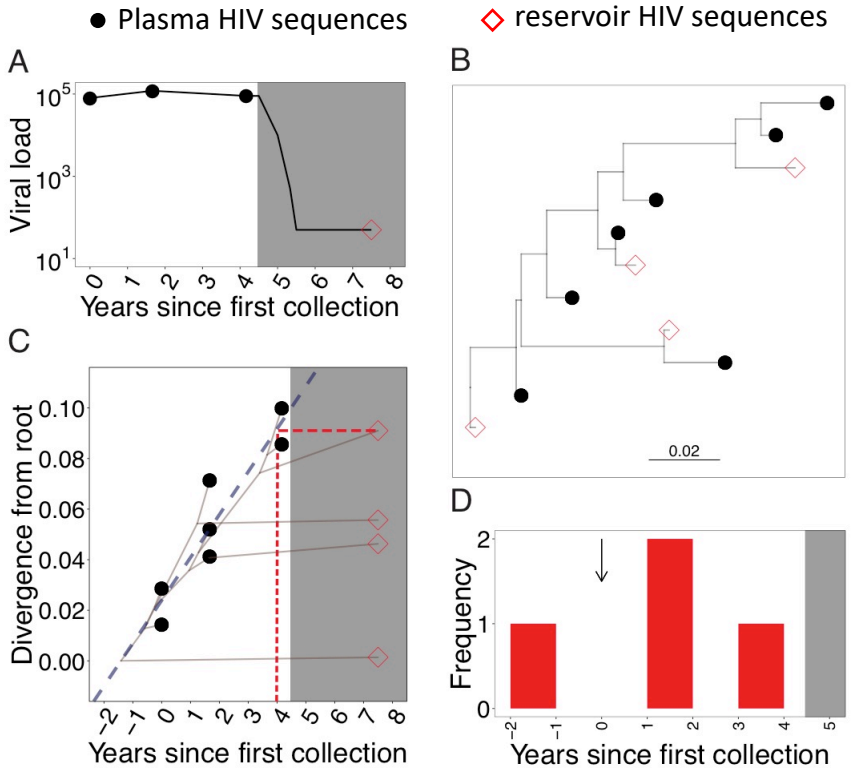


Figure adapted from Jones et al, PNAS 2018

Left: A: Hypothetical participant plasma viral load and sampling history. Shading represents cART. Single-genome sequencing of a subgenomic HIV region (nef) was performed from plasma HIV RNA at timepoints denoted by black circles; the same methods were used to characterize reservoir sequences during cART at the timepoint indicated by the red diamond. In this example, plasma HIV RNA sequences collected at baseline, 2 years and 4 years were used to infer the integration dates of proviral DNA sequences sampled during cART in year 7. ***B:*** Maximum-likelihood within-host phylogeny relating pre-cART plasma HIV RNA (black) and reservoir sequences sampled during cART (red diamonds). The root represents the most recent common ancestor (*i.e.* inferred transmitted/founder event). ***C:*** The dotted diagonal represents the linear model relating root-to-tip distances of pre-cART plasma HIV RNA sequences to their sampling dates. The linear model is used to convert root-to-tip distances of reservoir sequences (red diamonds) to their integration dates. For example, the reservoir sequence at the top right, whose divergence from the root is 0.09, is inferred to have integrated at the beginning of year 4 (dotted red line). Light brown lines trace HIV ancestor-descendant relationships. ***D:*** Histogram summarizing inferred integration dates of reservoir sequences. Arrow denotes baseline plasma sampling.

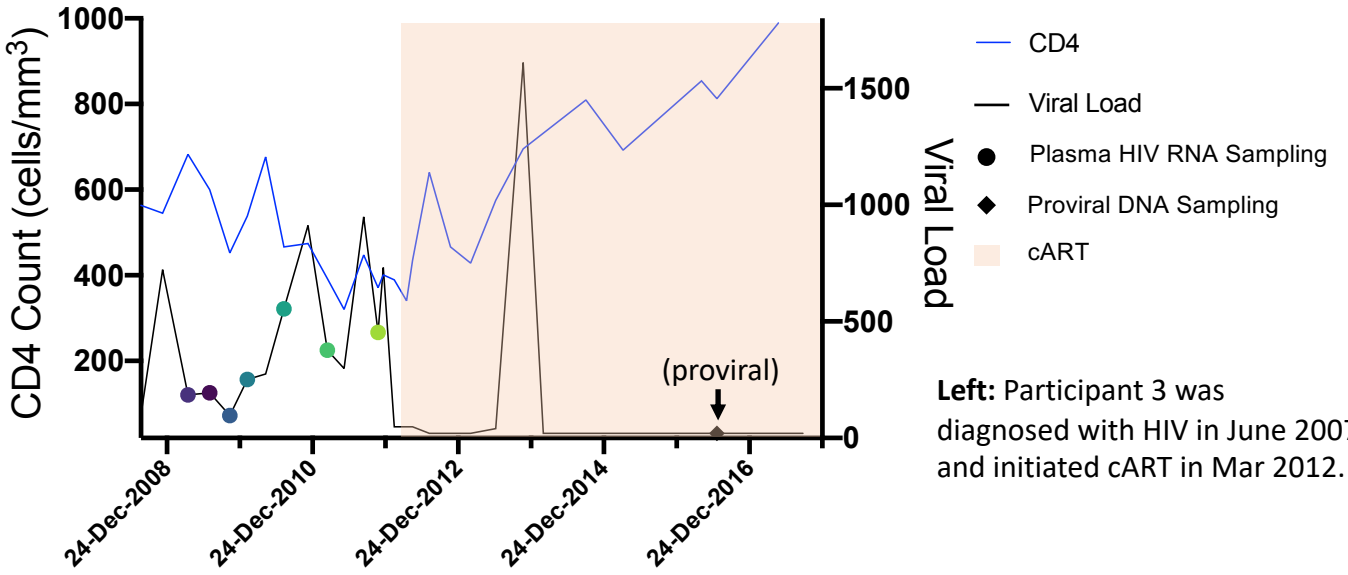
Participant and Sampling Characteristics:

Participant no.	No. of pre-cART plasma timepoints sampled	Total timespan of plasma sampling (months)	N of plasma sequences collected	cART initiation	Proviral sampling w.r.t. cART initiation (months elapsed)	No. of proviral sequences sampled	Total number of sequences
1	8	28	99	2010-09-01	24	48	147
2	9	42	67	2014-07-01	14	54	121
3	7	42	112	2012-03-01	52	24	136
4	12	71	223	2013-01-01	21	92	315

Four viremic controllers who initiated cART during chronic infection were studied. All intact, non-hypermutated sequences were analysed.

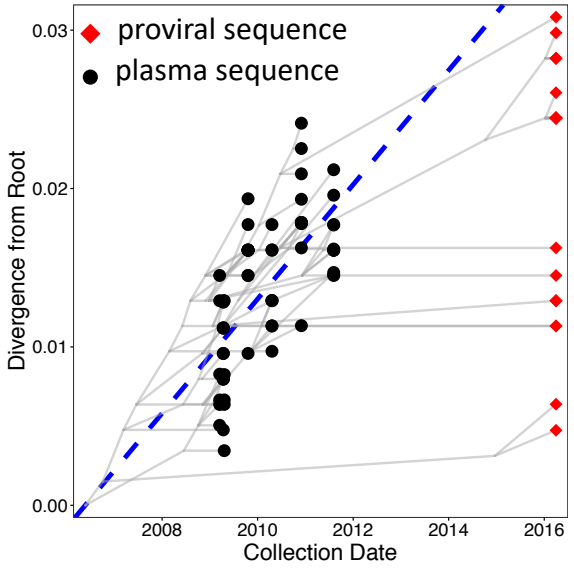
Representative findings: Participant 3 exhibits molecular clock signal and genetically heterogenous HIV reservoir

Clinical history and sampling timeline

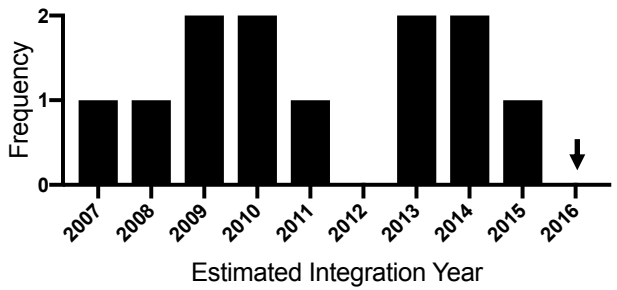


Left: Participant 3 was diagnosed with HIV in June 2007 and initiated cART in Mar 2012.

Age estimation of proviruses in the reservoir

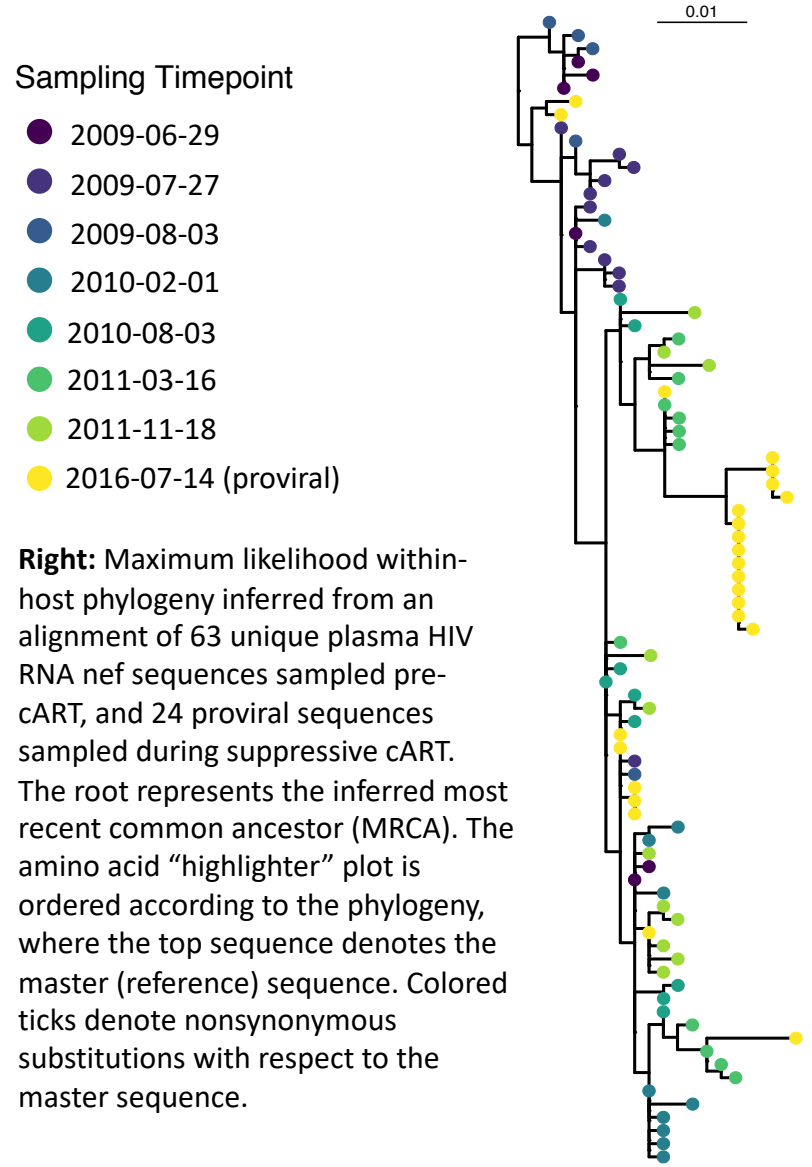


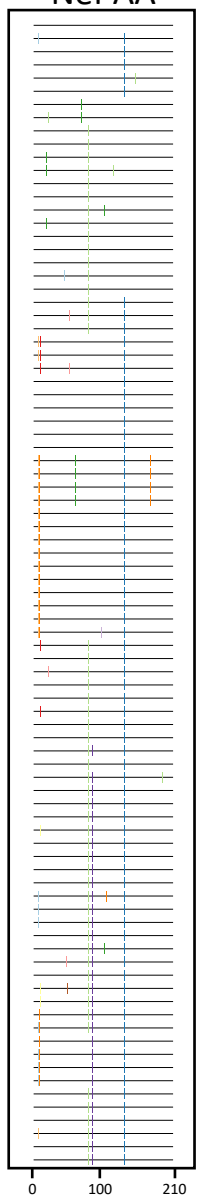
Left: HIV RNA sequences sampled pre-cART showed evidence of a molecular clock with an estimated evolutionary rate of 9.88E-06 substitutions/base/day. Estimated integration dates of proviral sequences sampled in July 2016 ranged from Dec 2007 (prior to first plasma sampling) to 2015. This is consistent with the latent reservoir being a genetically heterogenous archive of within-host HIV evolution.



Left: Inferred integration dates of unique proviral sequences. Arrow indicates when these proviruses were sampled.

Within-host HIV evolutionary reconstruction

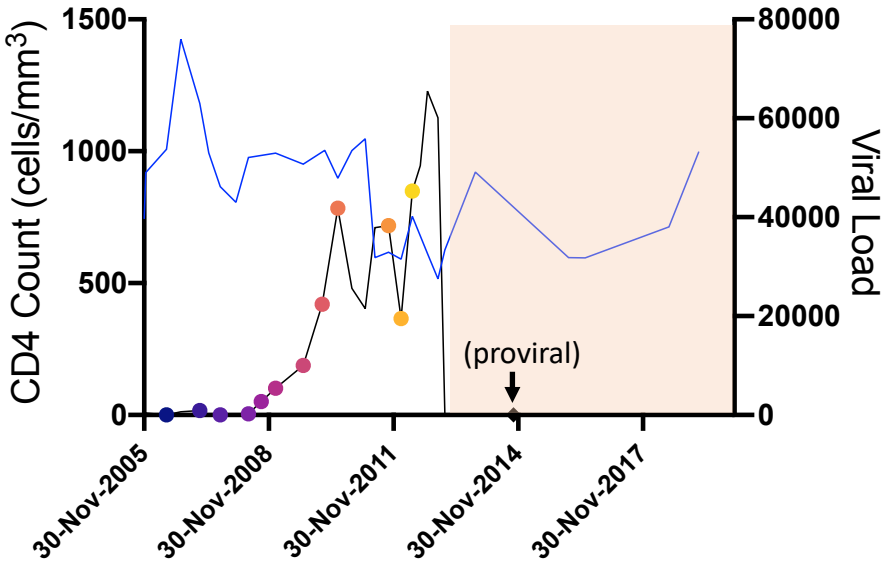


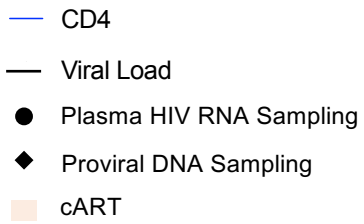


Right: Maximum likelihood within-host phylogeny inferred from an alignment of 63 unique plasma HIV RNA nef sequences sampled pre-cART, and 24 proviral sequences sampled during suppressive cART. The root represents the inferred most recent common ancestor (MRCA). The amino acid "highlighter" plot is ordered according to the phylogeny, where the top sequence denotes the master (reference) sequence. Colored ticks denote nonsynonymous substitutions with respect to the master sequence.

Participant 4's reservoir is also genetically heterogenous, but proviruses are somewhat skewed to later pre-cART dates

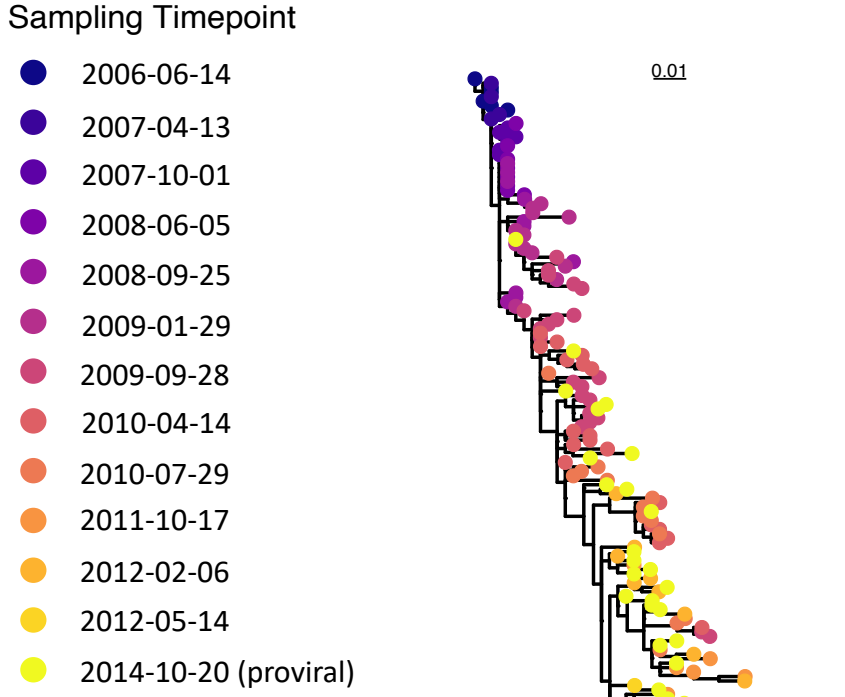
Clinical history and sampling timeline

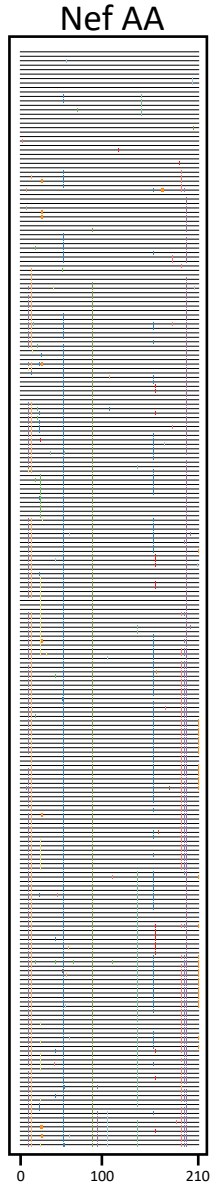




Left: Participant 4 was diagnosed with HIV in Nov 2005 and initiated suppressive cART in Jan 2013.

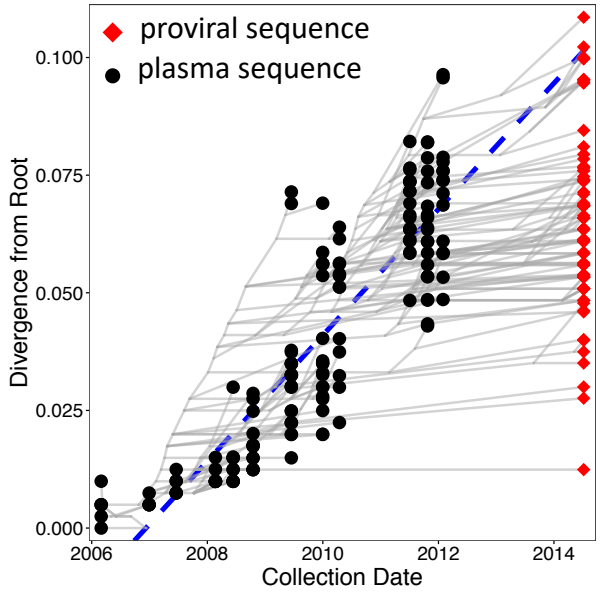
Within-host HIV evolutionary reconstruction



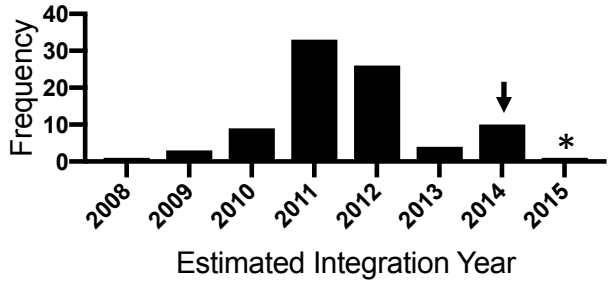


Right: Maximum likelihood phylogeny inferred from an alignment of 154 plasma (unique) and 92 proviral sequences from participant 4. The root represents the inferred MRCA. The corresponding amino acid "highlighter" plot is ordered according to the phylogeny, where the top sequence denotes the master (reference) sequence. Colored ticks denote nonsynonymous substitutions with respect to the master sequence.

Age estimation of proviruses in the reservoir



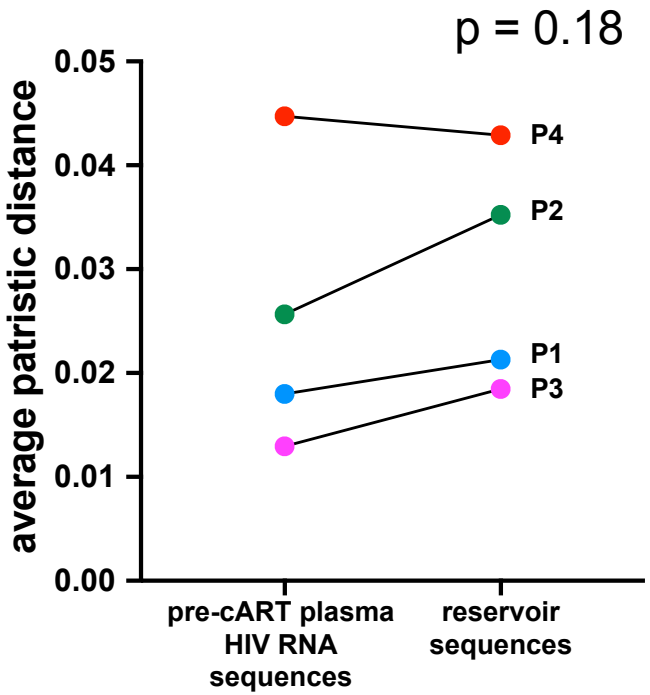
Left: HIV RNA sequences sampled pre-cART showed strong molecular clock signal with an estimated evolutionary rate of 3.67E-05 substitutions/base/day. Proviruses sampled in 2014 were genetically diverse, where the oldest was estimated to have integrated in 2008. This is consistent with the latent reservoir being a genetically heterogenous archive of within-host HIV evolution.



Left: Inferred integration dates of unique proviruses. Arrow indicates proviral sampling date.

Note the lower bound of the 95% CI for the provirus denoted by the asterisk was 2013.

HIV reservoir diversity during cART reflects longitudinal pre-cART plasma HIV diversity



Left: We compared overall HIV diversity in *unique* plasma HIV RNA sequences ever collected prior to cART, and proviral sequences sampled from the reservoir during cART.

- Briefly, for each within-host phylogeny, we computed the patristic (phylogenetic tip-to-tip) distance, in nucleotide substitutions/site, between every pair of unique HIV sequences in each sample type (pre-cART plasma HIV vs. proviruses in the reservoir). We then computed the average of these distances by sample type.
- Results indicate that the overall diversity of the reservoir is comparable to the overall diversity ever observed in HIV RNA sequences collected longitudinally prior to cART (paired t-test p=0.18)

Conclusions

- Despite natural maintenance of low viral loads during untreated HIV infection, within-host HIV evolution (evidenced by significant molecular clock signal) was observed during the pre-cART period in all four viremic controllers.
- Similar to observations in non-controllers, HIV proviruses persisting on cART in viremic controllers are genetically diverse and reflect within-host HIV evolution prior to cART.
- HIV eradication strategies must overcome this diversity.

We thank the study participants, without whom research would not be possible

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