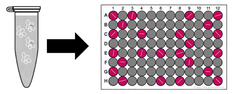
Intact HIV Genomes are Enriched in Memory T-cells with Short Half-lives

Vincent Morcilla[1,2], Charline Bacchus-Souffan[3], Katie Fisher[1,2], Xiao Qian Wang[1,2], Bethany A. Horsburgh[1,2], Timothy E. Schlub[4], Mark Fitch[5], Rebecca Hoh[6], Frederick M. Hecht[6], Jeffrey N. Martin[6], Steven G. Deeks[6], Marc K. Hellerstein[5], Joseph M. McCune[7], Peter W. Hunt[3] and Sarah Palmer[1,2]

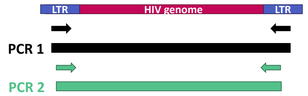
[1.] Centre for Virus Research, The Westmead Institute of Medical Research, The University of Sydney, Sydney, New South Wales, 2145, Australia, [2.] Sydney Medical School, Westmead Clinical School, Faculty of Medicine and Health, The University of Sydney, Sydney, New South Wales, 2006, Australia, [3.] Division of Experimental Medicine, Department of Medicine, University of California San Francisco, California, 94110, United States of America, [4.] Sydney School of Public Health, Faculty of Medicine and Health, The University of Sydney, Sydney, New South Wales, 2006, Australia, [5.] Department of Nutritional Sciences and Toxicology, University of California, Berkeley, California, 94720, United States of America, [6.] Division of HIV, Infectious Diseases and Global Medicine, Department of Medicine, Zuckerberg San Francisco General Hospital, University of California San Francisco, California, 94110, United States of America, [7.] Global Health Innovative Technology Solutions/HIV Frontiers, Bill & Melinda Gates Foundation, Seattle, Washington, United States of America

METHODS

Full Length Individual Proviral Sequencing[1]



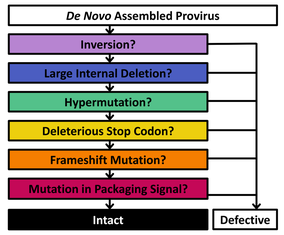
1. Limiting Dilution: Extracted DNA was diluted until 30% of the wells have HIV



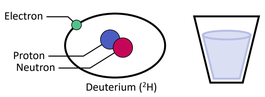
2. Nested PCR for the integrated HIV DNA using primers specific to the LTR regions to amplify ~92% of the genome



3. Positive PCR reactions are sequenced on the Illumina MiSeq platform and the HIV proviruses are assembled and aligned. These HIV proviruses are then screened for defects:



Measuring Cellular Half-lives[2]



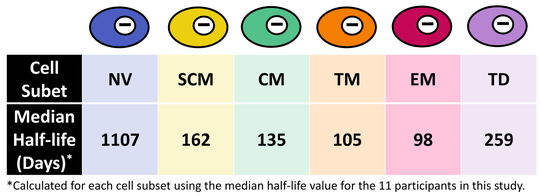
1. *In vivo* labelling with Deuterium: Participants were orally given small doses of 70% deuterated water



2. Peripheral blood mononuclear cells were extracted, isolated and sorted.



3. Deuterium enrichment in cellular genomic DNA was measured by mass spectroscopy and cellular half-lives were calculated.



Cell Subet	NV	SCM	CM	TM	EM	TD
Median Half-life (Days)*	1107	162	135	105	98	259

*Calculated for each cell subset using the median half-life value for the 11 participants in this study.

RESULTS

7.3% (94) of 1279 HIV-1 proviruses were Intact

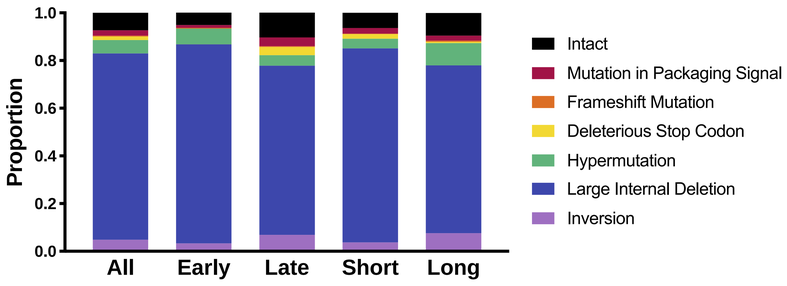


Figure 2: Percentage of intact and each type of defect within the total pool of sequences isolated from all participants, participants who initiated ART early, participants who initiated ART late, participants on short-term ART and participants on long-term ART.

Intact Sequences Concentrated in Cells with Shorter Half-lives

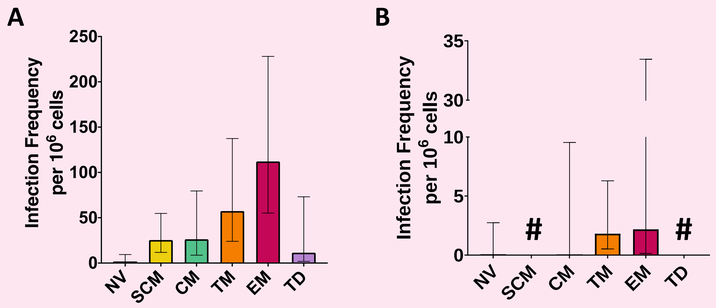


Figure 3: Infection frequency of **(A)** total provirus (intact and defective) and **(B)** intact provirus per 10^6 $CD4^+$ T-cells for NV, SCM, CM, TM, EM, and TD. #The values for SCM and TD were not depicted as their confidence intervals were outside the range of the graph due to the limited number of sequences obtained by these two subsets. Both graphs show strong evidence for a difference across subsets ($p < 0.01$) with the order **(A)** NV<CM/SCM/TD<TM<EM ($p < 0.01$) and **(B)** CM/NV/SCM/TD<TM/EM ($p < 0.01$).

Cells with Shorter Half-lives Contained More Intact Provirus

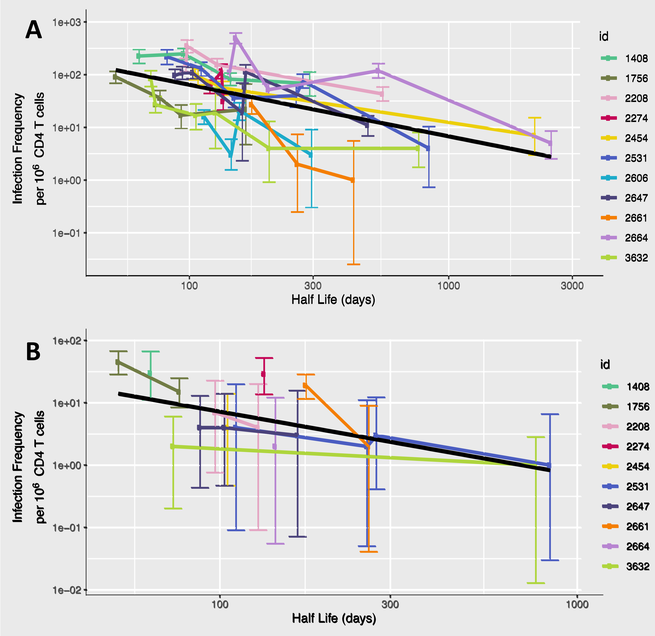


Figure 4: Mixed-effects modelling of **(A)** total infection frequency and **(B)** intact provirus infection frequency per 10^6 CD4 T-cells vs. cellular half-life. Black line depicts simple regression analysis. Two models were used for **(A)** and **(B)** which either include or exclude cellular subset in the model. For **(A)** there is strong evidence for a negative correlation between half-life and infection frequency as both models are significant ($p = 0.02$, $p < 0.001$ with and without subset information included respectively). For **(B)** there is strong evidence that the cells containing genetically intact provirus are more likely to have a shorter half life ($p = 0.001$), but this association may be driven by an association between cell subset and half life, as when adjusting for cellular subset there is no association with half life ($p = 0.18$).

INTRODUCTION

Future HIV curative therapies require a thorough understanding of the distribution of genetically intact HIV within T-cell subsets and the cellular mechanisms which maintain this reservoir. Therefore, we genetically characterized HIV genomes within T-cell subsets from participants on ART for 1.8-12.9 years and who initiated ART during the early and late stages of HIV infection.

THE PARTICIPANTS

Participant SCOPE ID	2647	2531	2664	2606	2454	2661	1408	3632	1756	2274	2208
Viral Load (copies/mL)	<40	<40	<40	<40	<40	<40	<40	<40	<40	<40	<40
Time Before Initiation of Therapy (months)	4.5	1.9	4.1	1.7	0.7	3.4	45.5	20.8	6.8	13.1	114.5
ART Duration (years)	3.4	3.4	2.7	3.5	7.1	12.9	3.1	1.8	4.1	11.8	7.1
Treatment Initiation	Early								Late		
Treatment Duration	Short			Long			Short			Long	

Figure 1: Clinical parameters of the 11 participants obtained by our collaborators. Participants were treated either within 5 months (early) or after 6 months (late) of HIV infection and were on ART for less than 4.5 years (short) or over 7 years (long).

Expansion of Identical Sequences (EIS) highest in EM and TD ($p < 0.001$)

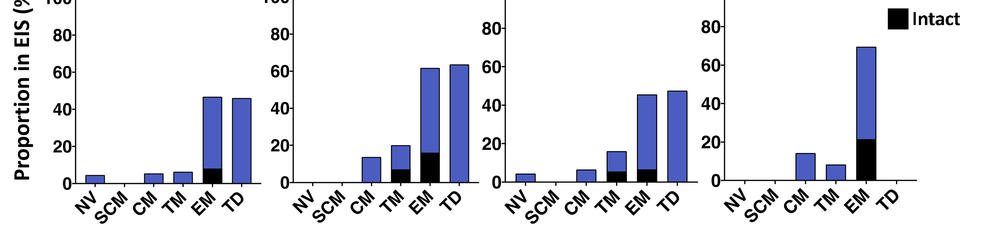


Figure 5: Proportion of defective (blue) and intact (black) sequences in each subset in an EIS for **(A)** Participants who initiated ART early **(B)** Participants who initiated ART late **(C)** Participants on short-term ART **(D)** Participants on long-term ART.

CONCLUSIONS

- A short cellular half-life could be a predictor of a higher frequency of intact proviruses.
- Both TD and EM cell subsets were marked by clusters of identical HIV genomes reflecting cellular proliferation.
- This indicates that specific cellular mechanisms such as a short half-life and greater proliferative potential, characteristics of EM T-cells, contribute to the maintenance of intact HIV.

REFERENCES

1. Hiener B, Horsburgh BA, Eden JS, Barton K, Schlub TE, Lee E, von Stockenstrom S, Odevall L, Milush JM, Liegler T, Sinclair E, Hoh R, Boritz EA, Douek D, Fromentin R, Chomont N, Deeks SG, Hecht FM, Palmer S. Identification of Genetically Intact HIV-1 Proviruses in Specific CD4+ T Cells from Effectively Treated Participants. Cell Rep. 2017 Oct 17;21(3):813-822.
2. Busch R, Neese RA, Awada M, Hayes GM, Hellerstein MK. Measurement of cell proliferation by heavy water labeling. Nat Protoc. 2007; 2: 3045–3057

ACKNOWLEDGEMENTS

We acknowledge with gratitude the participants of this study, without whom we would have never been able to perform these experiments. Immense thanks to our collaborators at the University of California San Francisco for collecting and processing these samples. This work is supported by the NHMRC, US National Institutes of Health, the University of Sydney and the Westmead Institute for Medical Research.

THE UNIVERSITY OF SYDNEY
Australian Government
National Health and Medical Research Council
Delaney AIDS Research Enterprise
DARE to find a cure
The Westmead Institute FOR MEDICAL RESEARCH