00315- INTACT PROVIRUSES FROM NAÏVE AND EFFECTOR MEMORY T-CELLS MATCH PERSISTENT VIREMIA

Katie Fisher[1,2], Bonnie Hiener[1,2], Bethany A. Horsburgh[1,2], Timothy E. Schlub[3], Eunok Lee[1,2], Vincent Morcilla[1,2], John-Sebastian Eden[1,2], Susanne von Stockenstrom[4], Jeffrey M. Milush[5], Teri Liegler[5], Rebecca Hoh[5], Remi Fromentin[6], Nicolas Chomont[6], Steven G. Deeks[5], Frederick M. Hecht[5] and Sarah Palmer[1,2]

[1.] The Westmead Institute of Medical Research, The University of Sydney; [2.] School of Life and Environmental Sciences, Faculty of Science, The University of Sydney; [3.] Sydney School of Public Health, Sydney Medical School, The University of Sydney, Sydney, New South Wales, Australia; [4.] Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden; [5.] Department of Medicine, University of California San Francisco, CA, USA; [6.] Centre de Recherche du CHUM and Department of microbiology, infectiology and immunology, Université de Montréal, Montreal, Canada

Introduction and Aim

Genetically intact, and potentially replication-competent, proviruses are a likely source for viremia during antiretroviral therapy (ART). Identifying the CD4+T-cell subsets that harbour these proviruses within different anatomic sites is important for future eradication strategies.

Participants and Methods

Naïve (NV), central (CM), transitional (TM) and effector (EM) memory CD4+ T-cells were sorted (Table 1) from the peripheral blood (PB, n=13) and lymph node (LN, n=5) of participants on long term ART (3-17 years). Near full-length HIV-1 proviruses were sequenced using the Full-Length Individual Proviral Sequencing Assay (FLIPS). Pretherapy (PT) and early on-therapy (OT) plasma RNA sequences were obtained from 9 of these participants in the p6-RT region using single-genome sequencing (SGS) (Palmer et al., 2005), and these were compared to the genetically intact proviruses obtained by FLIPS.

Table 1. Sorting Strategy for PB and LN CD4+ T cells.

Subset	Markers
Naïve	CD45RO-/CD27+/CCR7+/CD57- or CD45RA+/CD27+/CD127+/CD95-
Central Memory	CD45RO+/CCR7+/CD27+
Transitional Memory	CD45RO+/CCR7-/CD27+
Effector Memory	CD45RO+/CCR7-/CD27-

FLIPS Assay

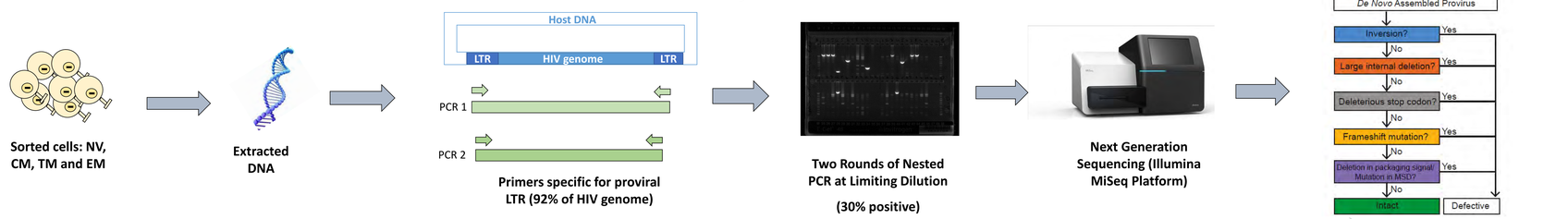

Figure 1. The Full-Length Individual Proviral Sequencing (FLIPS) assay (Hiener et al. 2017) was used to obtain near full-length HIV-1 proviruses and classify these as either genetically intact or defective.

Location of All Proviruses in PB and LN

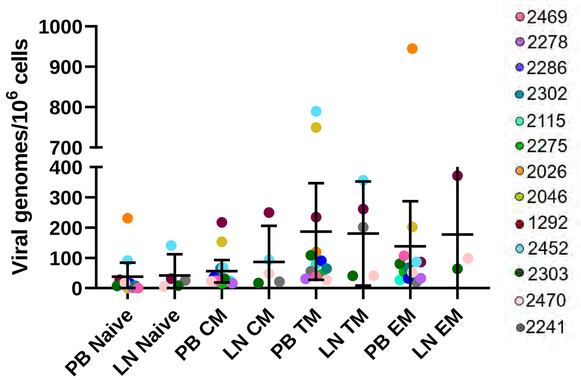

Figure 2. Infection frequency of all proviruses in PB was highest in TM and EM cells, followed by CM (P<0.05) and Naïve (P=0.02). Infection frequency of all proviruses in LN was EM>TM>CM>Naïve (all P<0.05). Total infection frequency did not differ between PB and LN (P=0.2).

Location of Intact Proviruses in PB and LN

In total, 4.2% of sequences analysed were genetically-intact. The infection frequency of genetically intact proviruses differed across T cell subsets in PB and LN (P<0.001). The intact infection frequency did not differ between PB and LN (P=0.70).

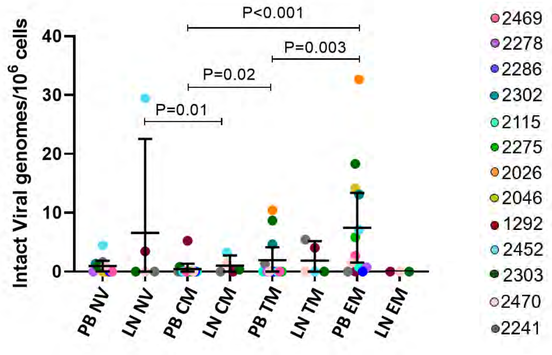

Figure 3. Infection frequency of intact proviruses in PB was highest in EM cells, followed by TM and CM. EM is also higher than NV. Infection frequency of intact proviruses in LN was NV>TM>CM>EM.

Intact Sequences from LN Naïve T cells are Genetically Unique

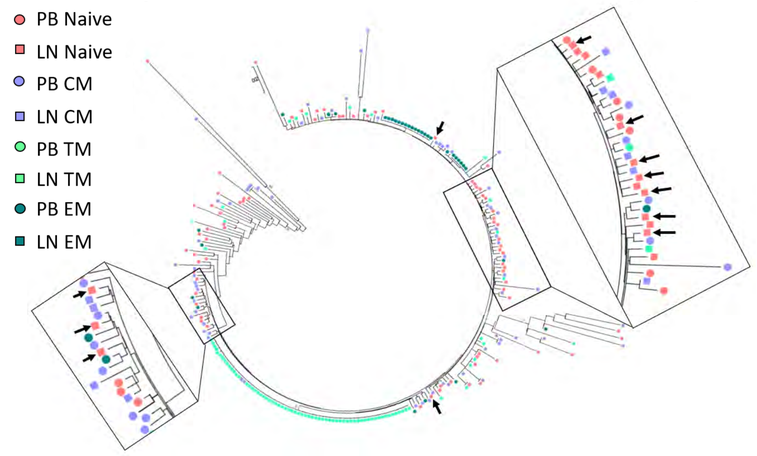

Figure 4. Phylogenetic tree of all sequences analysed from SCOPE 2452. 12/45 (26%) LN Naïve sequences were genetically intact, and all were genetically unique (black arrows).

Intact sequences from Different T cell Subsets Match Pre-Therapy and On-Therapy Plasma RNA

Genetically intact sequences from all four T cell subsets (PB and LN combined) matched PT and OT plasma-derived RNA sequences (Figure 5). Observationally, TM and EM had the highest proportion of intact sequences matching to PT RNA sequences, while EM and NV had the highest proportion matching to OT RNA sequences.

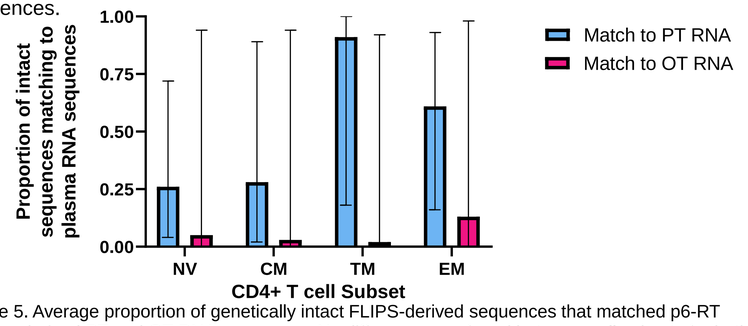

Figure 5. Average proportion of genetically intact FLIPS-derived sequences that matched p6-RT plasma-derived PT and OT RNA sequences. No difference was found between cell subsets for both PT (P=0.095) and OT (P=0.37). PB and LN sequences were combined.

Intact Proviruses Match Early On-Therapy Plasma RNA in Participants Treated During Acute/Early and Chronic Infection

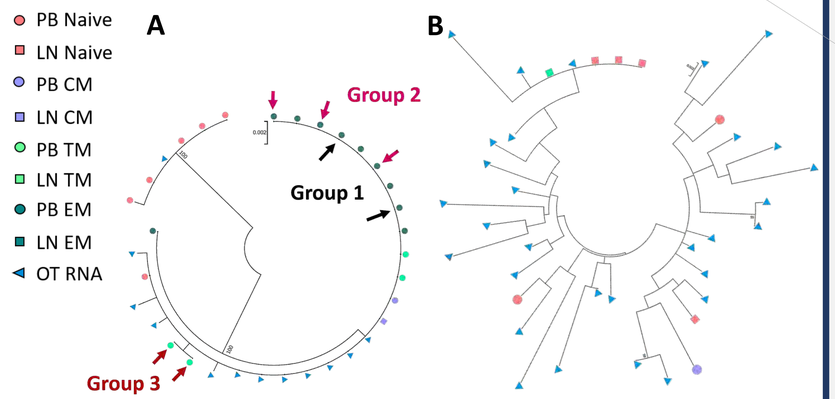

Figure 5. Phylogenetic trees of the p6-RT region of FLIPS-derived genetically intact sequences aligned with plasma-derived p6-RT sequences from (A) a participant treated during acute infection (SCOPE2303, 22 intact sequences and 12 on-therapy RNA sequences available) and (B) a participant treated during chronic infection (SCOPE1292, 8 intact sequences and 30 on-therapy RNA sequences available). Arrows and groups represent groups of sequences that were identical in the FLIPS-derived full-length sequence.

Conclusions

The distribution of intact, and likely replication-competent proviruses differs between the peripheral blood and lymph node. For the five participants with paired peripheral blood and lymph node cells available, naive cells had the highest frequency of intact proviruses in lymph node. In peripheral blood, however, the highest levels of intact genomes were found in effector memory cells.

All T cell subsets had genetically intact proviruses matching to pretherapy and early on-therapy plasma RNA p6-RT sequences. This suggests that intact proviruses are established in T cells prior to ART, especially in TM and EM T cells. All subsets may contribute to persistent viremia during ART, particularly naïve and EM T cells.

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We acknowledge with gratitude the participants of this study

HIENER, B., HORSBURGH, B. A., EDEN, J.-S., BARTON, K., SCHLUB, T. E., LEE, E., VON STOCKENSTROM, S., ODEVALL, L., MILUSH, J. M., LIEGLER, T., SINCLAIR, E., HOH, R., BORITZ, E. A., DOUEK, D., FROMENTIN, R., CHOMONT, N., DEEKS, S. G., HECHT, F. M. & PALMER, S. 2017. Identification of Genetically Intact HIV-1 Proviruses in Specific CD4+ T Cells from Effectively Treated Participants. *Cell Reports,* 21, 813-822

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