Paucity of HIV Envelope expressing cells upon latency reversal in ART suppressed PWH

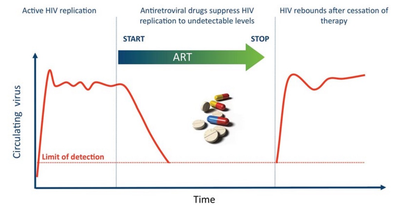
559

Jonathan Richard[1,2,#], Mehdi Benlarbi[1,2], Rémi Fromentin[1,2], Lorie Marchitto[1,2], Amélie Pagliuzza[1], Amie Albertus[3], Derek Yang[4], Jean-Pierre Routy[5], Amos B. Smith III[4], Drew Weissman[3], Edward F Kreider[3], Daniel E Kaufmann[6], Marzena Pazgier[7], Nicolas Chomont[1,2], Andrés Finzi[1,2,#]

[1]Centre de Recherche du CHUM, Montréal, Québec, Canada [2]Département de Microbiologie, Infectiologie et Immunologie, Université de Montréal, Montréal, Québec, Canada [3] Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA. [4] Department of Chemistry, School of Arts and Sciences, University of Pennsylvania, Philadelphia, PA, USA [5] Research Institute of the McGill University Health Centre, Montreal, Canada [6]Division of Infectious Diseases, Department of Medicine, Lausanne University Hospital and University of Lausanne, Lausanne, Switzerland [7]Infectious Diseases Division, Department of Medicine, Uniformed Services University of the Health Sciences, Bethesda, MD, USA [#]co-corresponding authors

BACKGROUND

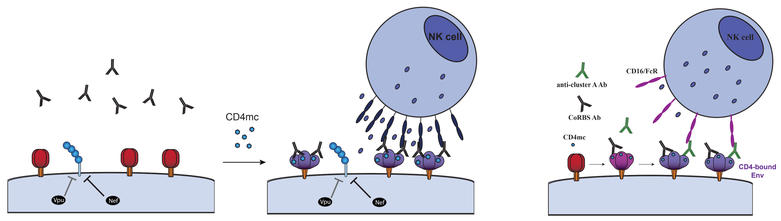
ART does not eradicate the virus



(Kulpa, Chomont, J Virus Erad,2015)

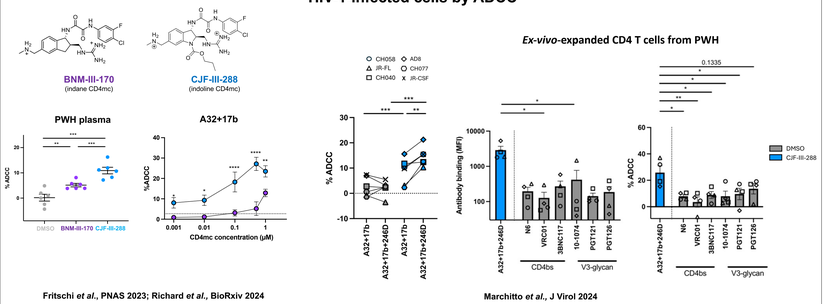
- Antiretroviral therapy (ART) effectively suppresses HIV-1 replication but fails to eradicate the virus, as HIV-1 persists in long-lived reservoirs.
  - ➢ **Therefore, novel strategies are needed to detect and eliminate these reservoirs.**
- The HIV-1 envelope glycoprotein (Env) is the only viral antigen expressed on the surface of infected cells, making it a prime target for immunotherapies.
- While current latency-reversing agents (LRAs) effectively induce p24 expression, their ability to stimulate Env expression remains unclear. Detecting Env on reactivated cells remains challenging

CD4-mimetic sensitizes HIV-1-infected cells to ADCC mediated by nnAbs



Veilllette *et al.*, J Virol 2014 | Richard *et al.*, PNAS 2015 | Richard *et al.*, EBioMedicine 2016; Anand *et al.*, JVirol 2019

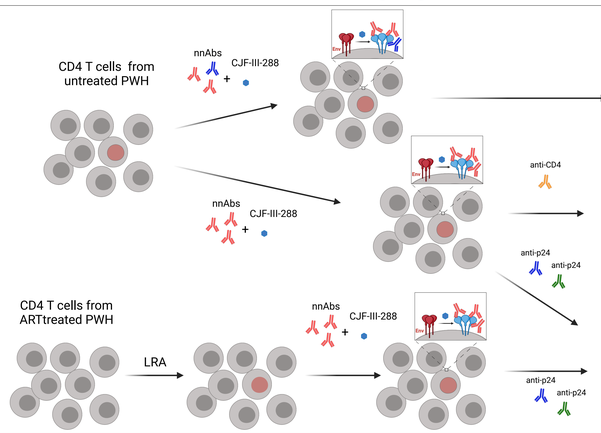
Development of a new CD4mc-nnAbs cocktail with improved capacity to recognize and eliminate HIV-1-infected cells by ADCC



Fritschi *et al.*, PNAS 2023; Richard *et al.*, BioRxiv 2024 | Marchitto *et al.*, J Virol 2024

➢ **Can we used this potent CD4mc-nnAbs cocktail to measure surface Env expression on CD4 T cells from PWH?**

METHODS



RESULTS

Detection of Env on the surface of CD4 T cells from untreated PWH

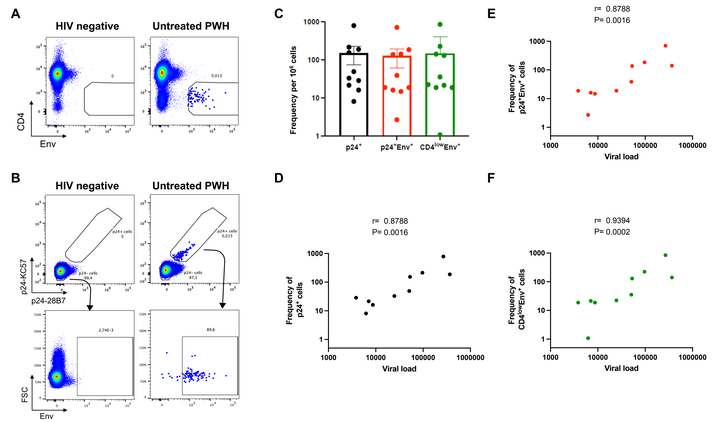


Figure 1. Gating strategies to detect (A) CD4^{low}Env^{+} and (B) p24^{+}Env^{+} cells among CD4 T cells purified from an untreated PWH. (C) Freqeuency of p24^{+}, p24^{+}Env^{+} and CD4^{low}Env^{+} cells among CD4 T cells purified from 10 untreated PWH. (D-F) Correlation between the viral load and the frequency of (D) p24^{+}, (E) p24^{+}Env^{+} and (F) CD4^{low}Env^{+} cells.

Env-expressing cells harbor HIV-1 DNA/RNA and can establish productive infection in viral outgrowth assays

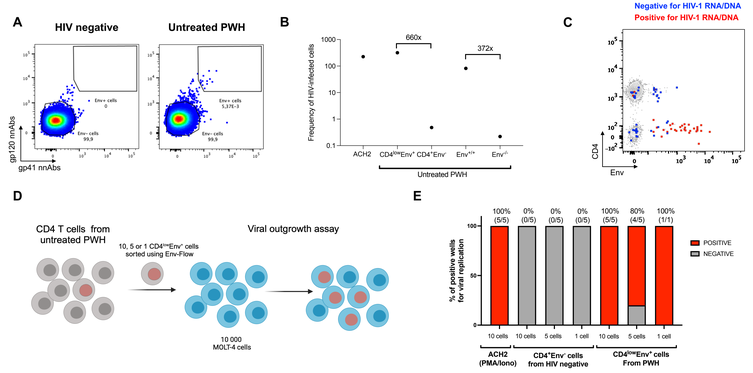


Figure 2. (A) Example of Env$^{+/+}$ cells detection from CD4 T cells purified from an untreated PWH using gp120 and gp41-specific CD4i nnAbs conjugated with distinct fluorochromes. (B) Relative frequency of HIV-1-infected cells among ACH2 (stimulated with PMA/Iono) and the indicated sorted population from CD4 T cells purified from an untreated PWH. (C) Cells from an untreated PWH were index-cell sorted in individual wells based on CD4 and Env detection. HIV-1 DNA and RNA were detected by PCR. (D-E) CD4^{low}Env^{+} cells sorted from CD4 T cells purified from an untreated PWH were co-cultured with MOLT-4 cells in a viral outgrowth assay for up to 21 days. (D) Experimental outline (E) Graph representing the percentage of wells positive or negative for viral replication.

Env-Flow demonstrates excellent sensitivity and linearity, enabling the detection of 0.6-1.1 Env+ cell per million

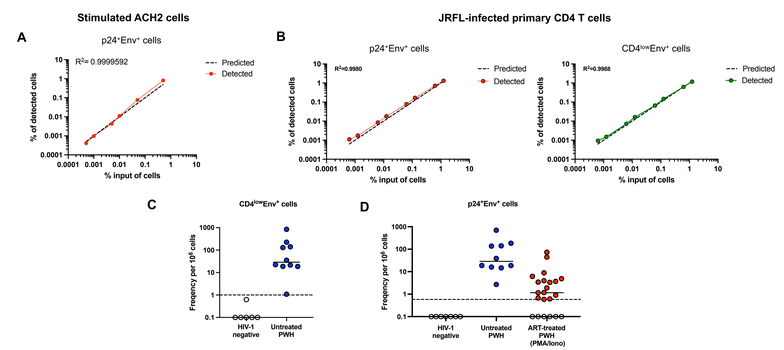


Figure 3. (A) ACH2 cells stimulated with PMA/Iono or (B) HIV-1$_{JRFL}$-infected primary CD4+ T cells were spiked into uninfected CD4 cells at different ratio. (A-B) Quantification of predicted versus detected % of p24^{+}Env^{+} or CD4^{low}Env^{+} cells using Env-Flow. (C) Frequency of CD4^{low}p24^{+} cells detected in CD4 T cells from 7 HIV-1 negative donors and 10 untreated PWH. (D) Frequency of p24^{+}Env^{+} cells detected in CD4 T cells from 7 HIV-1 negative donors, 10 untreated PWH and 22 ART-treated PWH after stimulation with PMA/Iono.

Detection of Env on the surface of reactivated CD4 T cells from ART-treated PWH

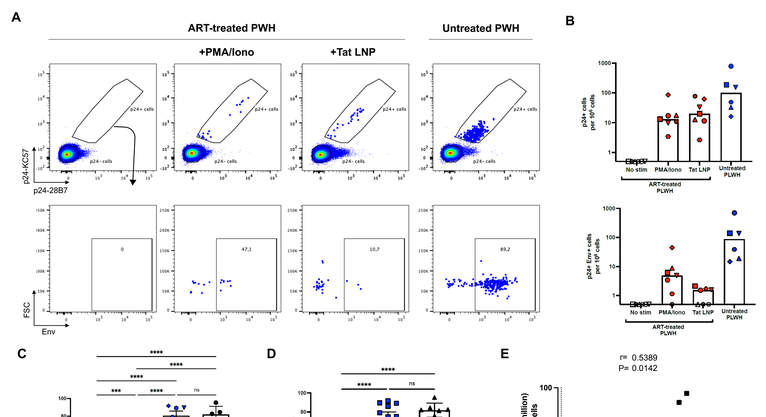


Figure 4. (A) Example of p24 and Env detection with cells from an ART-treated PWH, either unstimulated or stimulated with PMA/Iono or Tat-LNP, as well as in cells from an untreated PWH. (B) Frequency of p24^{+} and p24^{+}Env^{+} cells in samples from 7 ART-treated PWH stimulated with PMA/Iono or Tat-LNP, tested in parallel with 6 untreated PWH. (C) Percentage of Env^{+} cells among p24^{+} cells in samples from 7 ART-treated PWH stimulated with PMA/Iono or Tat-LNP, tested in parallel with 6 untreated PWH and 6 *in-vitro* infections. (D) Percentage of Env^{+} cells among p24^{+} cells in samples from 22 ART-treated PWH stimulated with PMA/Iono, tested in parallel with 10 untreated PWH and 7 *in-vitro* infections. (E) Correlation between the frequency of p24^{+}Env^{+} cells and the HIV-1 reservoir, as measured by the level of integrated HIV DNA

Reactivated cells from ART-treated PWH exhibit higher p24 levels and significantly lower Env surface expression compared to cells from viremic PWH

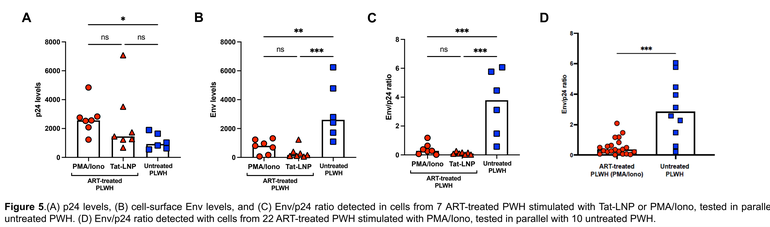


Figure 5.(A) p24 levels, (B) cell-surface Env levels, and (C) Env/p24 ratio detected in cells from 7 ART-treated PWH stimulated with Tat-LNP or PMA/Iono, tested in parallel with 6 untreated PWH. (D) Env/p24 ratio detected with cells from 22 ART-treated PWH stimulated with PMA/Iono, tested in parallel with 10 untreated PWH.

CONCLUSIONS

- **Env-Flow offers a robust tool to identify and better characterize Env-expressing in ART-treated and untreated PWH, as well as to evaluate LRAs for their ability to induce surface Env expression**
- **The frequency of Env^{+} cells in untreated PWH positively correlates with plasma viral loads.**
- **In these viremic individuals, the majority of p24^{+} cells co-express cell-surface Env.**
- **Env-expressing cells harbors HIV-1 DNA/RNA and can establish productive infection in viral outgrowth assays.**
- **In ART-treated PWH, Env expression is observed upon reactivation with PMA/Ionomycin or Tat mRNA LNP, however only a fraction of reactivated p24+ cells displays surface Env.**
  - **These cells exhibits higher p24 levels and significantly lower Env surface expression compared to cells from viremic PWH, leading to a reduced Env/p24 ratio.**

➢ **Our findings highlight the scarcity of Env expression on reactivated cells from ART-treated PWH.**

The views expressed in this presentation are those of the authors and do not reflect the official policy or position of the Uniformed Services University, US Army, the Department of Defense, or the US Government.

NIH National Institutes of Health | INNOVATION.CA CANADA FOUNDATION FOR INNOVATION / FONDATION CANADIENNE POUR L'INNOVATION | Fonds de recherche Santé Québec | CIHR IRSC Canadian Institutes of Health Research / Instituts de recherche en santé du Canada | Réseau SIDA-MI | ERASE HIV