Basic and Translational Science CROI2023

jmpicado@irsicaixa.es Javier Martinez-Picado @JavierMPicado

IrsiCaixa Institut de Recerca de la Sida · IGTP Germans Trias i Pujol Research Institute · UVIC UNIVERSITAT DE VIC · ciber · ICREA

The year of the "single-cell"

1

Oral Abstract Session-05 HIV RESERVOIRS AND CURE STRATEGIES

#135

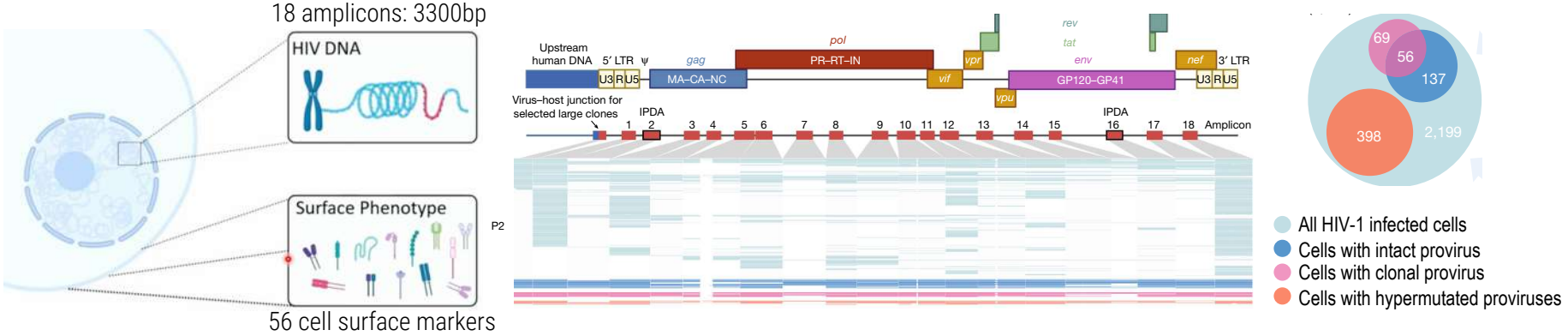
SINGLE-CELL PROTEOGENOMIC PROFILING OF HIV-1 RESERVOIR CELLS

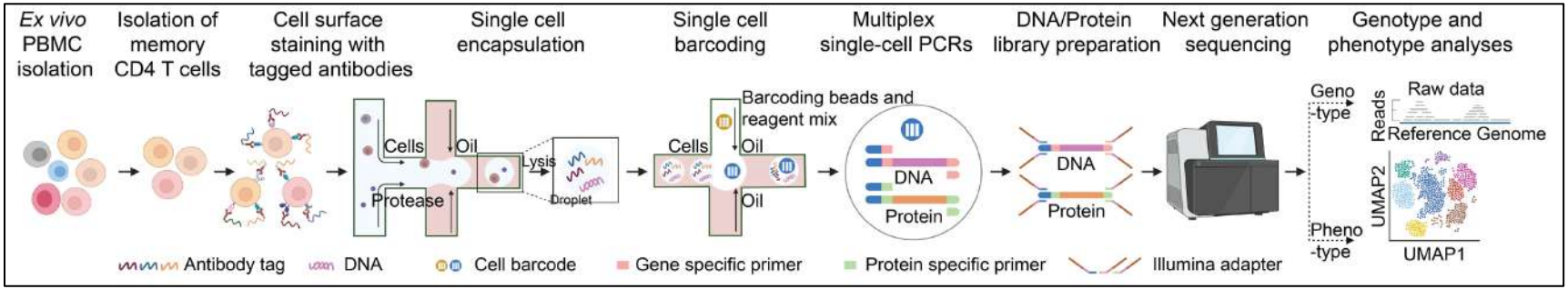
Weiwei Sun[1], Ce Gao[1], Ciputra A. Hartana[1], Matthew R. Osborn[1], Kevin B. Einkauf[1], Xiaodong Lian[1], Benjamin R. Bone[1], Nathalie Bonheur[1], Tae-Wook Chun[2], Eric S. Rosenberg[3], Bruce D. Walker[1], Xu G. Yu[1], Mathias Lichterfeld[1]

Ragon Institute of MGH, MIT and Harvard, Cambridge, MA, USA, [2]National Institute of Allergy and Infectious Diseases, Bethesda, MD, USA, [3]Massachusetts General Hospital, Cambridge, MA, USA

Phenotyping and Proviral Sequencing (PheP-Seq)

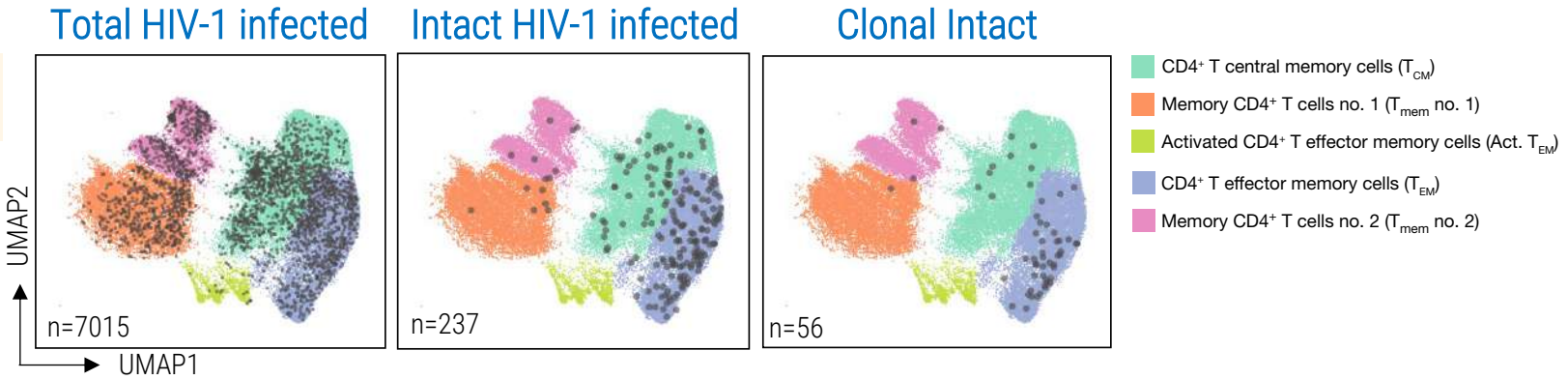
Simultaneous detection of HIV-1 proviral sequence and surface phenotype at the single-cell level

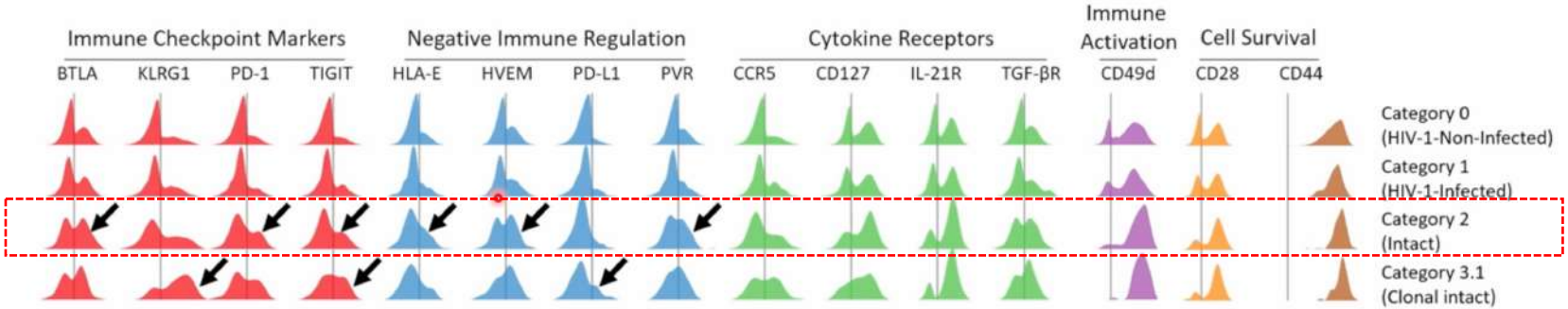




Distinc phenotypic profile in Peripheral Blood Cells

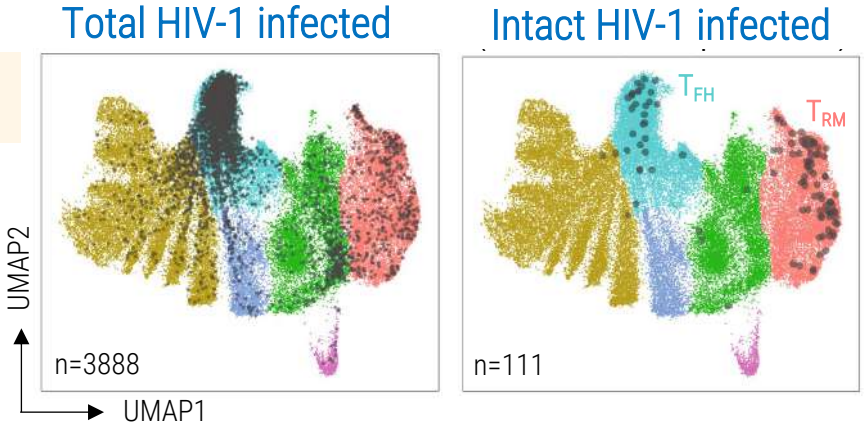
5 PWH
n =787,717 cells



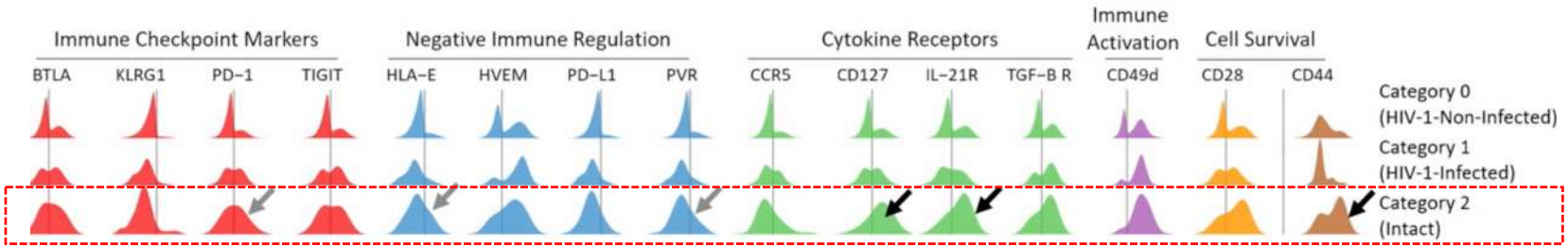


Distinc phenotypic profile in lymph node mononuclear cells

3 PWH
n =496,628 cells



- CD4⁺ T resident memory cells (T_{RM})
- CD4⁺ T follicular helper cells (T_{FH})
- Memory CD4⁺ T cells no. 1 (T_{mem} no. 1)
- Memory CD4⁺ T cells no. 2 (T_{mem} no. 2)
- Memory CD4⁺ T cells no. 3 (T_{mem} no. 3)
- Memory CD4⁺ T cells no. 4 (T_{mem} no. 4)



Conclusions

- PheP-Seq is capable of profiling the phenotypic features of single HIV-1-infected cells *ex-vivo*
- No major phenotypic differences between HIV-1-infected (defective) and non-infected cells
- After ~10 years of ART, cells harboring intact proviruses are enriched for negative immunoregulatory markers and immune checkpoint markers
- This phenotype may help reservoir cells with intact HIV-1 to avoid being exposed to and killed by host immune cells
- Data suggest that only HIV-1-infected cells with optimal adaptation to host immune activity or their specific tissue microenvironment can survice long term

→ Reservoir cells with intact HIV are subject to active immune selection pressure

Illuminating a long-term mystery

2

Oral Abstract Session-01 VIROLOGY/PATHOGENESIS

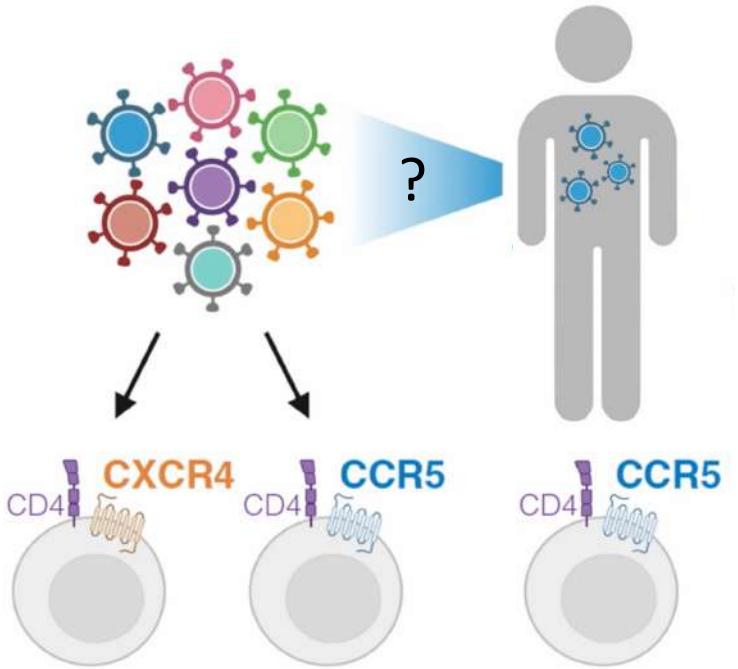
#105

HOST CELL GLYCOSYLATION DIFFERENTIALLY AFFECTS CCR5- AND CXCR4-TROPIC HIV-1 INFECTION

Hannah L. Itell[1], Julie M. Overbaugh[2]

[1]Fred Hutchinson Cancer Research Center, Seattle, WA, USA, [2]University of Washington, Seattle, WA, USA

R5-tropic HIV bottleneck during transmission



Longstanding question in the HIV field:

What are the viral and host properties that drive preferential transmission of CCR5-tropic viruses?

Wolinski, 1992; Wolfs, 1992, Zhu, 1993, Long, 2002; Keele, 2008 and many subsequent references (reviewed in Ronen, AIDS 2015)

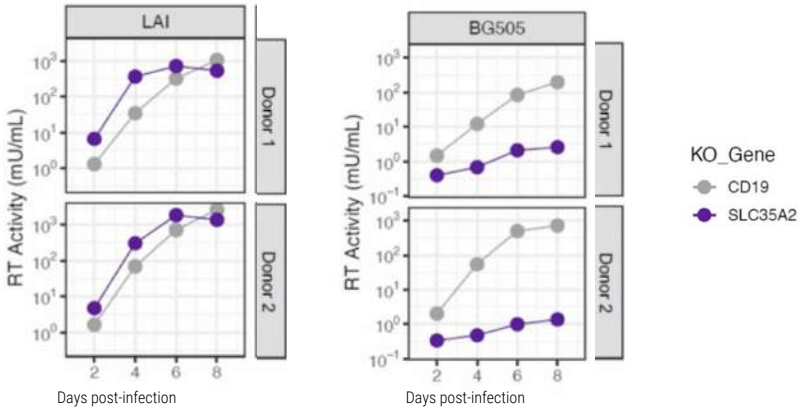
SLC35A2 is associated with R5-tropic infection

CRISPR KO screen for HIV restriction factors comparing R5-tropic *vs.* X4-tropic HIV

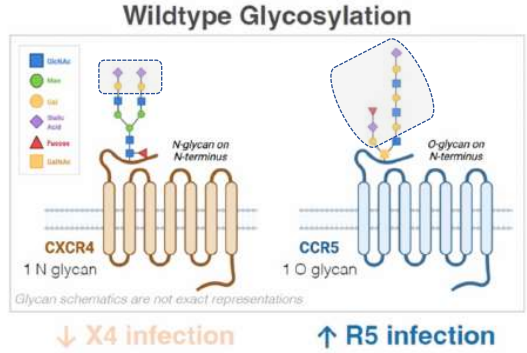
SLC35A2 KO has consistent, opposite effects on X4 *vs.* R5 HIV (primary CD4+ cells)

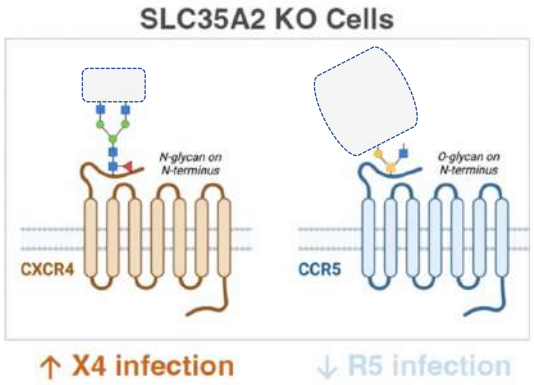
CXCR4-tropic
↑ infection

CCR5-tropic
↓ infection



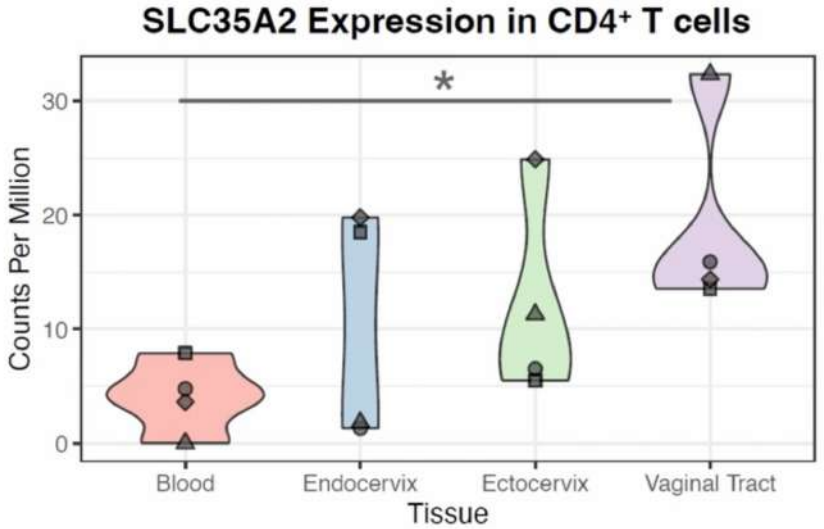
SLC35A2 encodes a transporter of UDP-galactose whose inactivation causes truncated glycans





SLC35A2 is more highly expressed in the lower Female Reproductive Tract than in blood

Bulk RNA-seq on CD4⁺ T Cells
Matched sample types for 4 donors
Davis et al., 2021 *Mucosal Immunol*



Increased SLC35A2 ➛ Increased glycosylation ➛ R5 selection at transmission sites

Conclusions

- Underappreciated role for host cell glycans on the infection of different tropic-HIV strains
- Wildtype glycosylation promotes R5 infection while hindering that of X4
- Putative role of SLC35A2, a gene required for normal glycosylation, which encodes a transporter of UDP-galactose. Its inactivation causes truncated glycans.
- Wildtype glycosylation may be even more pronounced in the genital tract due to elevated levels of SLC35A2, contributing to R5 selection at transmission sites

→ The differential impact of host cell glycosylation on X4 and R5 viruses may therefore largely drive R5 selection during HIV transmission.

Gene/Cell Therapies

Oral Abstract Session-10 IMMUNOPATHOGENESIS AND VACCINES

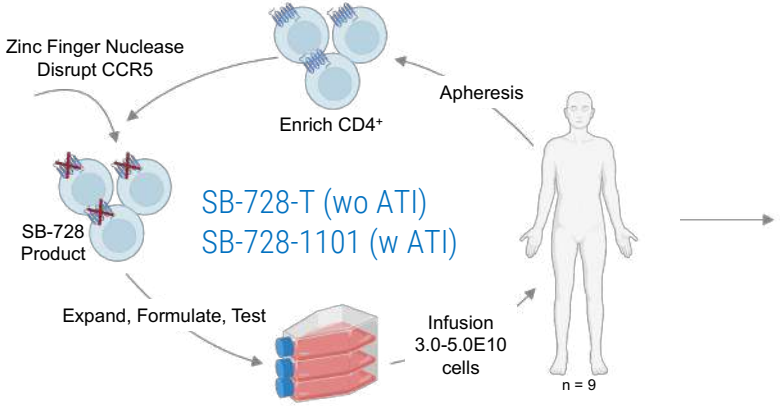
#182

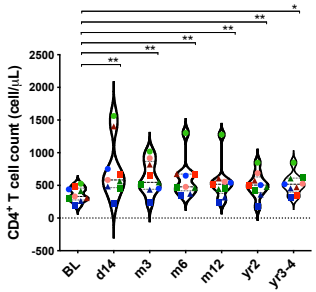
SINGLE INFUSION OF STEM LIKE CCR5-MODIFIED CD4 T CELLS PROVIDE LONG-TERM HIV CONTROL

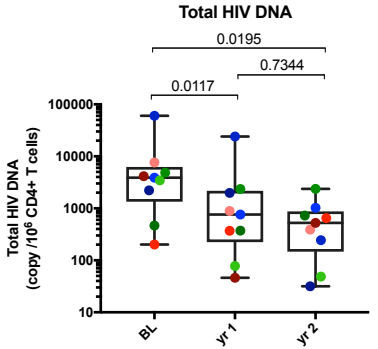
Ana B. Enriquez[1], Ashish Arunkumar Sharma[1], Joumana Zeidan[2], Gary Lee[3], Slim Fourati[1], Khader Ghneim[1], Gabriela Sanchez[1], Francesco Procopio[4], Robert Balderas[5], Nicolas Chomont[6], Dale Ando[7], Steven G. Deeks[8], Rafick P. Sékaly[1], Rémi Fromentin[9]

[1]Emory University, Atlanta, GA, USA, [2]CellCarta, Montreal, QC, Canada, [3]Senti Biosciences, San Francisco, CA, USA, [4]Lausanne University Hospital, Lausanne, Switzerland, [5]BD Biosciences, San Diego, CA, USA, [6]Université de Montréal, Montréal, Canada, [7]Consultant, Walnut Creek, CA, USA, [8]University of California San Francisco, San Francisco, CA, USA, [9]Centre de Recherche du CHUM, Montreal, QC, Canada

Autologous CCR5-modified T cells

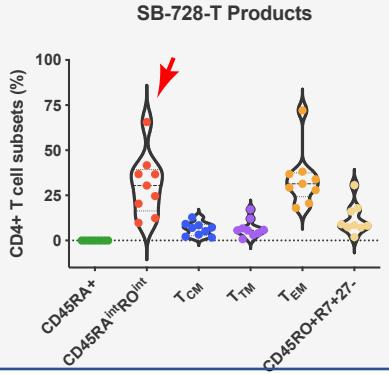


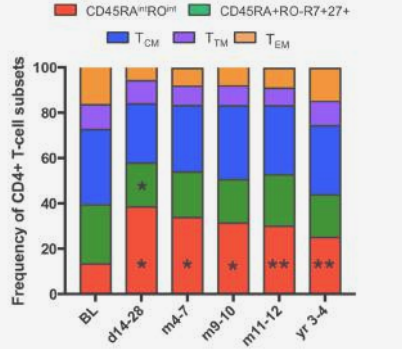




What is the phenotype of CD4 T cells?

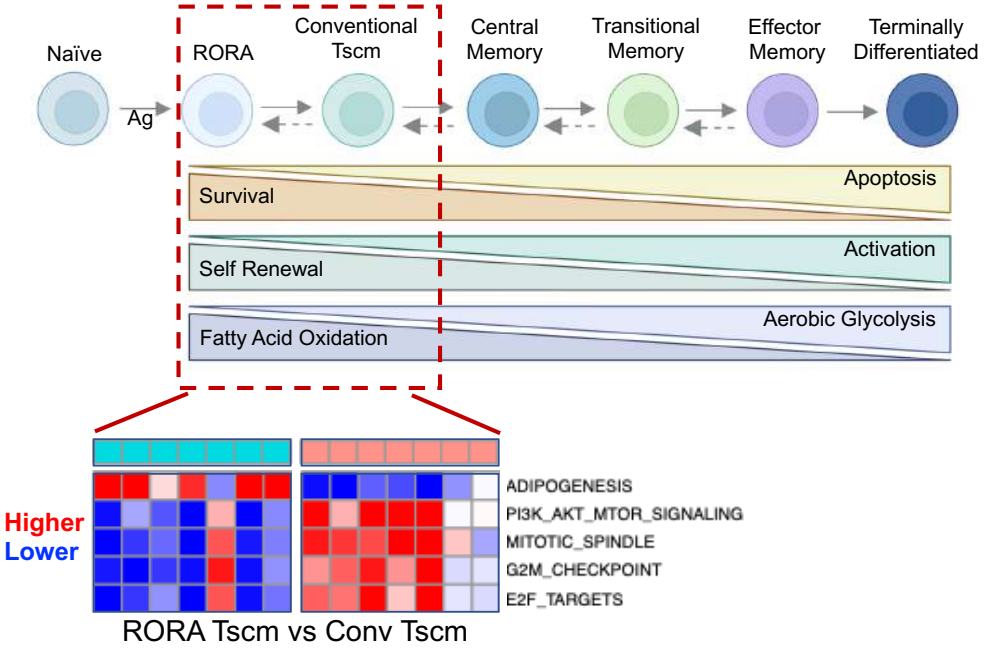
Novel CD4 CD45RA^{int}RO^{int} (RORA) population is enriched post-infusion



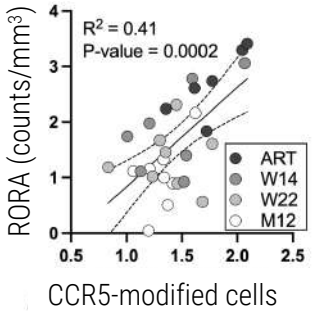


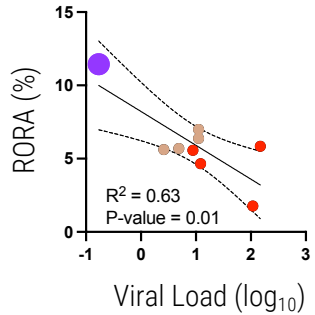
RORA cells persist up to 4 years post-infusion

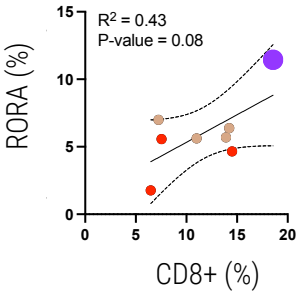
RORA cells are distinct from conventional T stem cell memory subsets

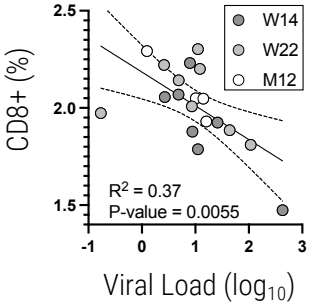


Can RORA cells provide protection in absence of ART?



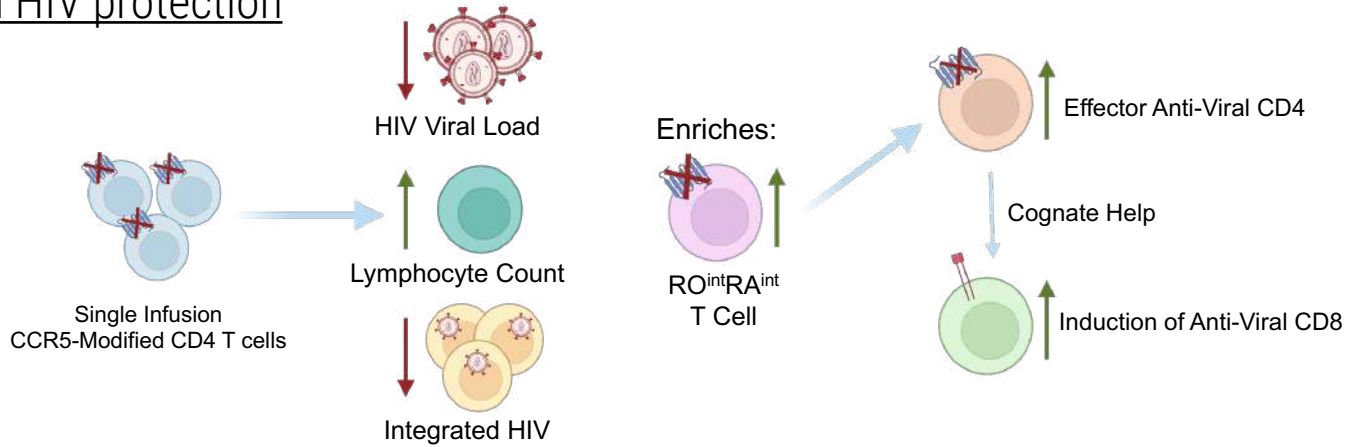






Conclusions

- Single infusion of CCR5-modified T cells enriches a novel RORA CD4 T cell population:
  - Have a stem-like phenotype (innately more “quiescent”)
  - Correlate with CCR5-modified T cells
  - Can differentiate into effector cells that correlate with reduced viral load and effector CD8 T cells (in absence of ART)
  - Confer long-term HIV protection



30th CROI 2023

Distinguishing 'self' from 'non-self' DNA

4

PLENARY-03 WEDNESDAY PLENARY SESSION

#037

HOW THE HUSH COMPLEX PROTECTS YOUR GENOME FROM RNA-DERIVED RETROELEMENTS

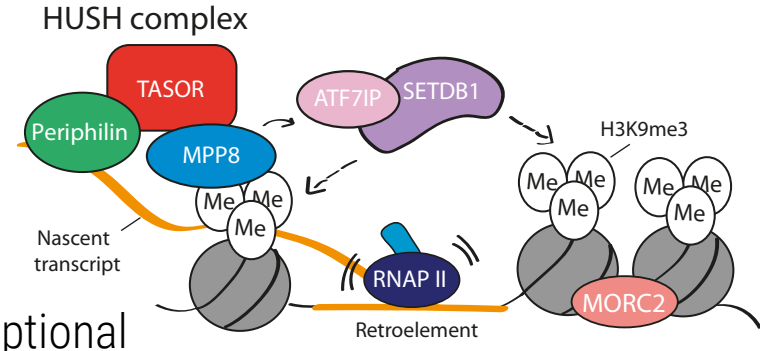
Paul J. Lehner
Cambridge University, Cambridge, United Kingdom

Retrotransposition

- Reverse Transcriptase: RNA → cDNA → subsequent genome integration
- 40% of the human genome are retroelements (retroviruses/ retrotransposons)
- Retrotransposition is therefore tolerated, but needs to be closely regulated

increases genome diversity and resilience

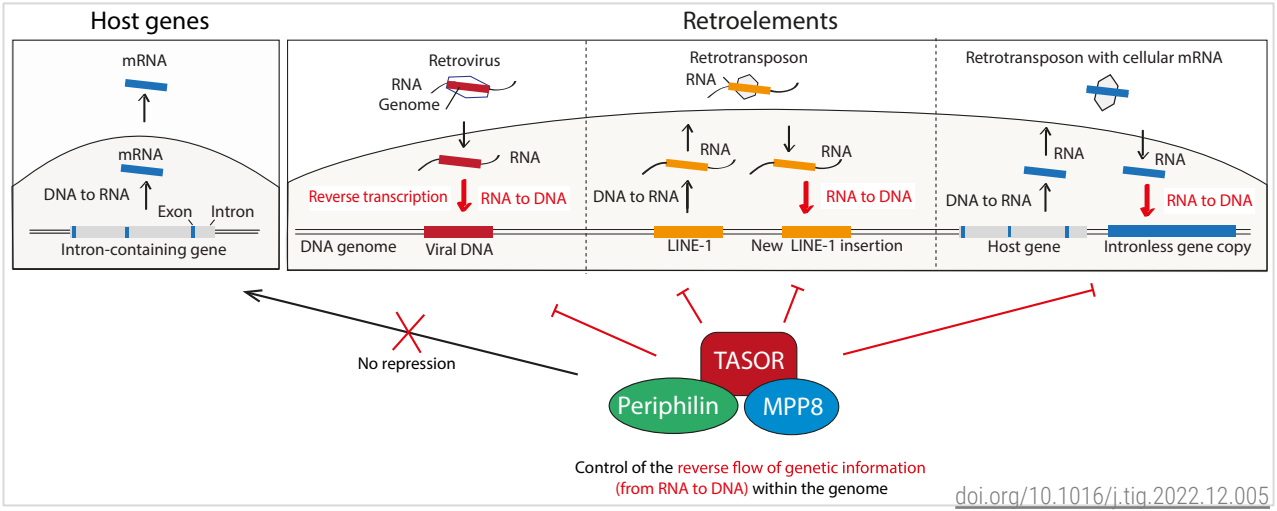
invasion from foreign, RNA-derived DNA (eg HIV)



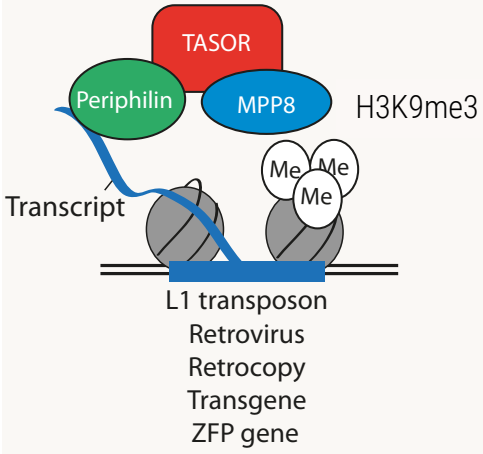
- 'HUSH' (Human Silencing Hub): epigenetic transcriptional repressor complex which identifies and silences invading transgenes
  - It defends the genome from retroelement attack from outside the cell i.e. retroviruses (including HIV) and from within the cell (LINE1 retrotransposons)

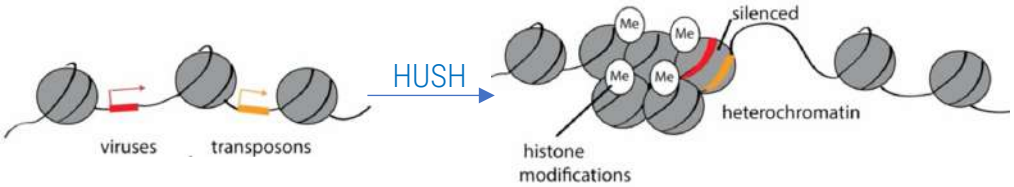
How does HUSH discriminate ‘self’ from ‘non-self’ genomic DNA?

- HUSH recognizes ‘intronless’ DNA → retroelements lack non-coding introns acting (~PAMPs)
- cDNA > ~1.5Kb, rich in adenine, and transcriptionally active



doi.org/10.1016/j.tig.2022.12.005

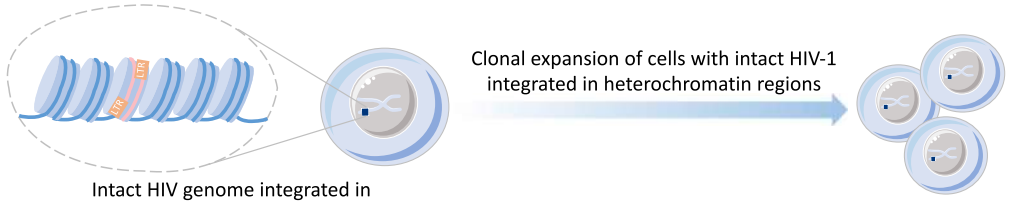




PAMP: Pathogen-Associated Molecular Pattern

Biological relevance

- Novel component of the innate immunosystem → immunosurveillance of the genome, a compartment not thought to be accessible to the immune system (no cell killing!)
- Major therapeutic potential (beyond fundamental biological importance):
  - Strategies to express genes for a wide range of purposes have, somewhat unwittingly, been a battle against HUSH
  - HUSH inhibition has the potential to dramatically improve gene expression, and to release neo-antigens for cytotoxic T-lymphocyte recognition for immunotherapy
  - HIV?
    - *Elite controllers*
    - *Long-term ART*



Enrichment of the repressive histone feature H3K9me3 at intact-HIV integration sites

Jiang *et al.* Nature 2020
Lian *et al.* Cell Host and Microbes 2023

Conclusions

- HUSH → epigenetic transcriptional repressor complex which silences invading DNA
- HUSH defends the genome from retroelement attack from outside the cell i.e. retroviruses (including HIV) and from within the cell (LINE1 retrotransposons)
- Unique RNA-dependent genome surveillance system linking transcription to epigenetic gene silencing, by recognizing 'intronless' DNA
- Major therapeutic potential: improve gene expression to release neo-antigens for cytotoxic T-lymphocyte recognition for immunotherapy, and maybe HIV …

→ HUSH: "Molecular domestication" of retroviruses and retrotransposons

30th CROI 2023

nature medicine

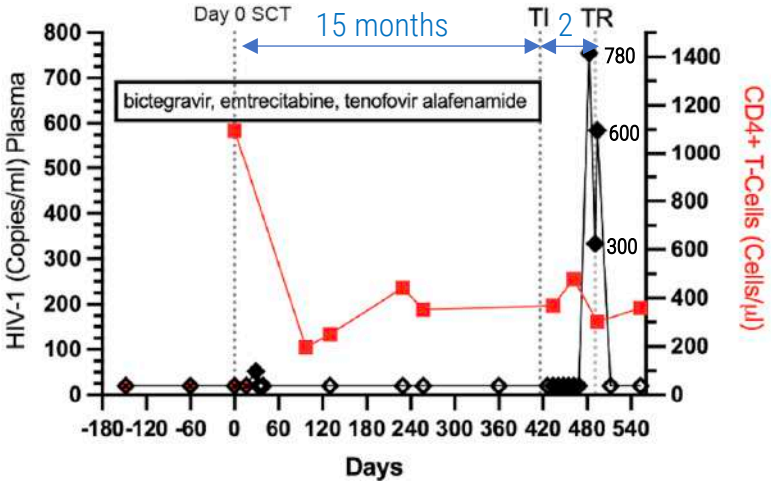
Brief Communication https://doi.org/10.1038/s41591-023-02213-x

Bonus

In-depth virological and immunological characterization of HIV-1 cure after CCR5Δ32/Δ32 allogeneic hematopoietic stem cell transplantation

#434 HIV-1 Host Reservoir Reactivation after a CCR5Δ32/32 Allogeneic Stem Cell Transplant

- AML; 10/10 match, CCR5Δ32
- Rapid full donor chimerism (in peripheral blood)
- Decrease in cell-associated DNA and RNA
- Decrease in ultrasensitive plasma viral load
- Rebounding viruses are CCR5-tropic
  - *Residual R5-tropic HIV can persist in vivo even >1 year after SCT*
  - *Non clonal reactivation*
- No increase in plasma HIV-associated antibodies
- No evidence of graft infection to date
- No data on tissues




Thank you!