Infezione da HIV:

Possibilità di eradicazione

Claudia Alteri

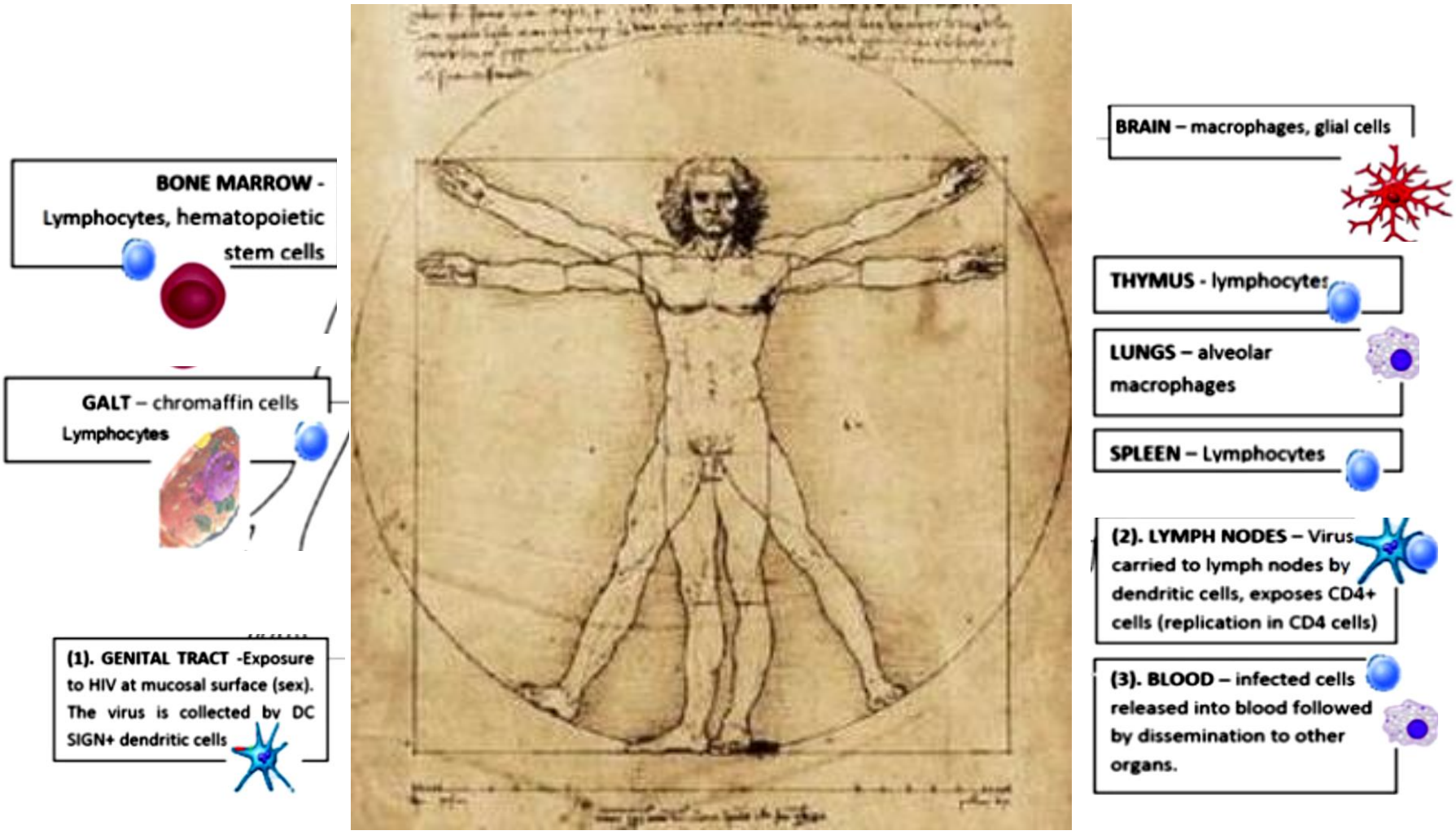
Tor Vergata

Definitions

Residual viremia: Persistent HIV-RNA measured in plasma at levels below the limit of detection of the standard clinical assay

Viral reservoir: An anatomical site or cell type in which a replication-competent form of HIV persists accumulating with more stable properties than the circulating pool of replicating virus. This HIV can persist even during effective therapy.

The HIV hiding places



HIV's hiding places. After exposure at mucosal surfaces (1), the virus is carried to the local lymph nodes (2) by dendritic cells. Fusion of dendritic cells with CD4+ T lymphocytes results in infection of the lymphocytes and viral replication in these cells. Infected CD4+ T lymphocytes are released into the blood stream (3) and disseminated to anatomical reservoirs in other organs (4) including the brain, CNS, spleen, bone marrow, thymus, lungs, kidneys, lymph nodes, and GALT with infection of associated cellular reservoirs in these organs. DC-SIGN indicates dendritic cell–specific intercellular adhesion molecule-3-grabbing nonintegrin; GALT, gut-associated lymphoid tissue

A lot of progresses has been done...

The Berlin Patient – The First and Unique Person Cured of HIV Infection

BRIEF REPORT

Long-Term Control of HIV by *CCR5* Delta32/Delta32 Stem-Cell Transplantation

Gero Hütter, M.D., Daniel Nowak, M.D., Maximilian Mossner, B.S., Susanne Ganepola, M.D., Arne Müßig, M.D., Kristina Allers, Ph.D., Thomas Schneider, M.D., Ph.D., Jörg Hofmann, Ph.D., Claudia Kücherer, M.D., Olga Blau, M.D., Igor W. Blau, M.D., Wolf K. Hofmann, M.D., and Eckhard Thiel, M.D.

N Engl J Med. 2009

The New York Times

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New Hope of a Cure for H.I.V.

Heidi Schumann for The New York Times

VIRUS-FREE Timothy Brown of San Francisco had two bone-marrow transplants to treat leukemia, and H.I.V. can no longer be detected in his body.

By ANDREW POLLACK

Published: November 28, 2011

Berlin Patient: Summary of Results From Follow-Up Tests for Viral Persistence (2011-2012)

	Testing Laboratories (Positive Results/Total Number)	Consensus	Typical Levels With ART Viral Suppression	Fold Difference
Plasma HIV RNA	2/4	?Intermittent positive ?<1 copy/mL	1-2 copy/mL	2-20
PBMC				
HIV DNA	0/4	Negative ($\leq$1 in 10^{6-7})	751/10^6 total PBMC	750-7500
HIV RNA	0/3	Negative ($\leq$1 in 10^{6-7})	66/10^6 total PBMC	66-660
Sorted cells from blood				
HIV DNA	0/1	Negative	Unknown	--
HIV RNA	0/1	Negative	Unknown	--
Peripheral CD4+ T (IUPM)	0/2	Negative ($\leq$1 IU/10^{7-9})	1/10^6 CD4+ T cell	10-1000
CSF HIV RNA	0/2	Negative	--	--
CSF cells HIV DNA	0/1	Negative	--	--
Lymph node				
HIV DNA	0/1	Negative	1-12 copies/100 ng	--
HIV RNA	0/1	Negative	$\leq$4 $\log_{10}$ copies/g	--
Rectum (biopsy or cells)				
HIV DNA	1/2	?Intermittent positive	777/10^6 total gut cells	780
HIV RNA	0/3	Negative ($\leq$1 in 10^{6-7})	21/10^6 total gut cells	21-210
Ileum (biopsy or cells)				
HIV DNA	0/1	Negative ($\leq$1 in 10^6)	415/10^6 total gut cells	415
HIV RNA	0/2	Negative ($\leq$1 in 10^6)	37/10^6 total gut cells	37

Yukl SA, et al. PLoS Pathog. 2013

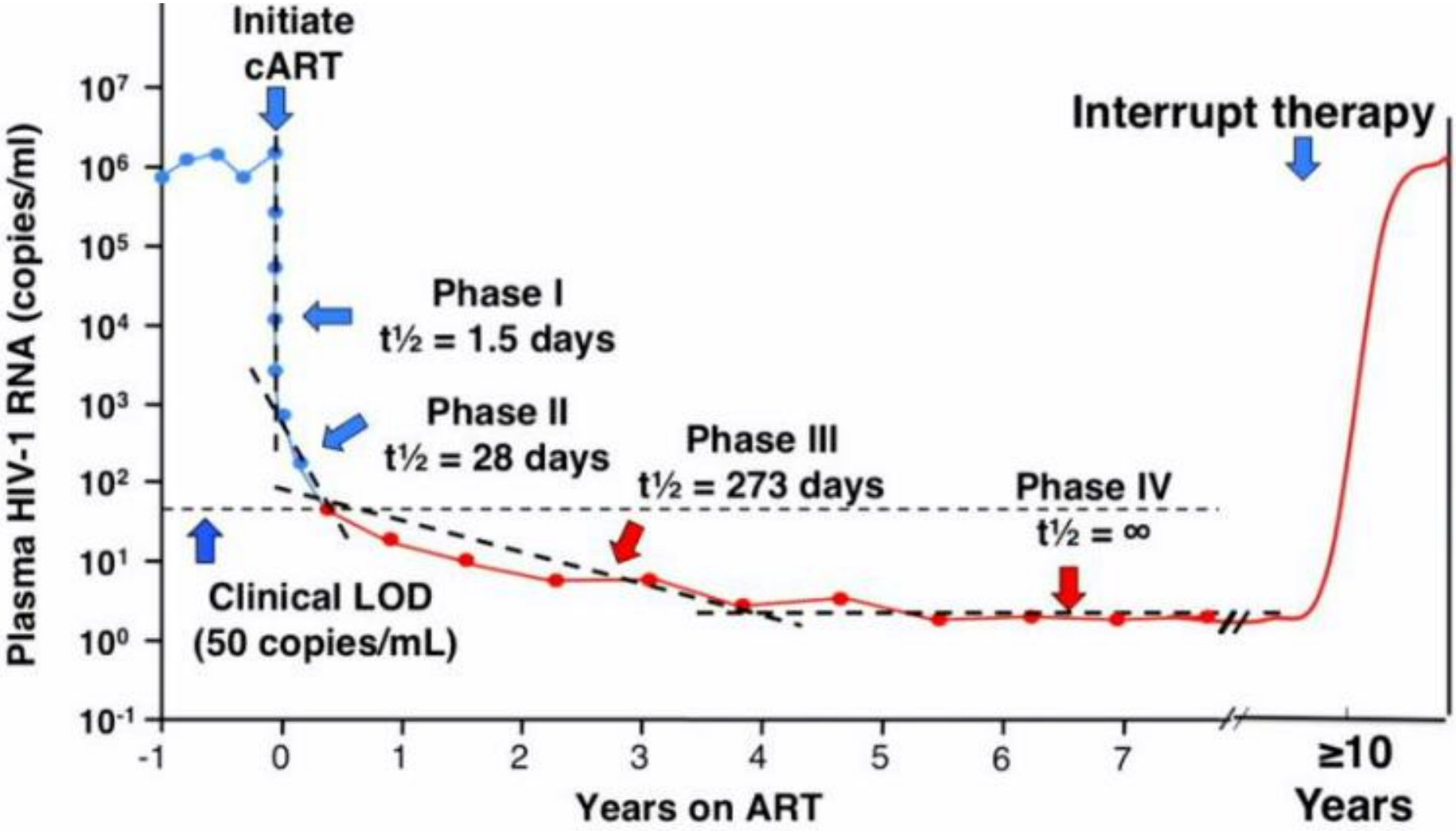
..., but much more is needed

Latent infected cells

KEPLEF-186F

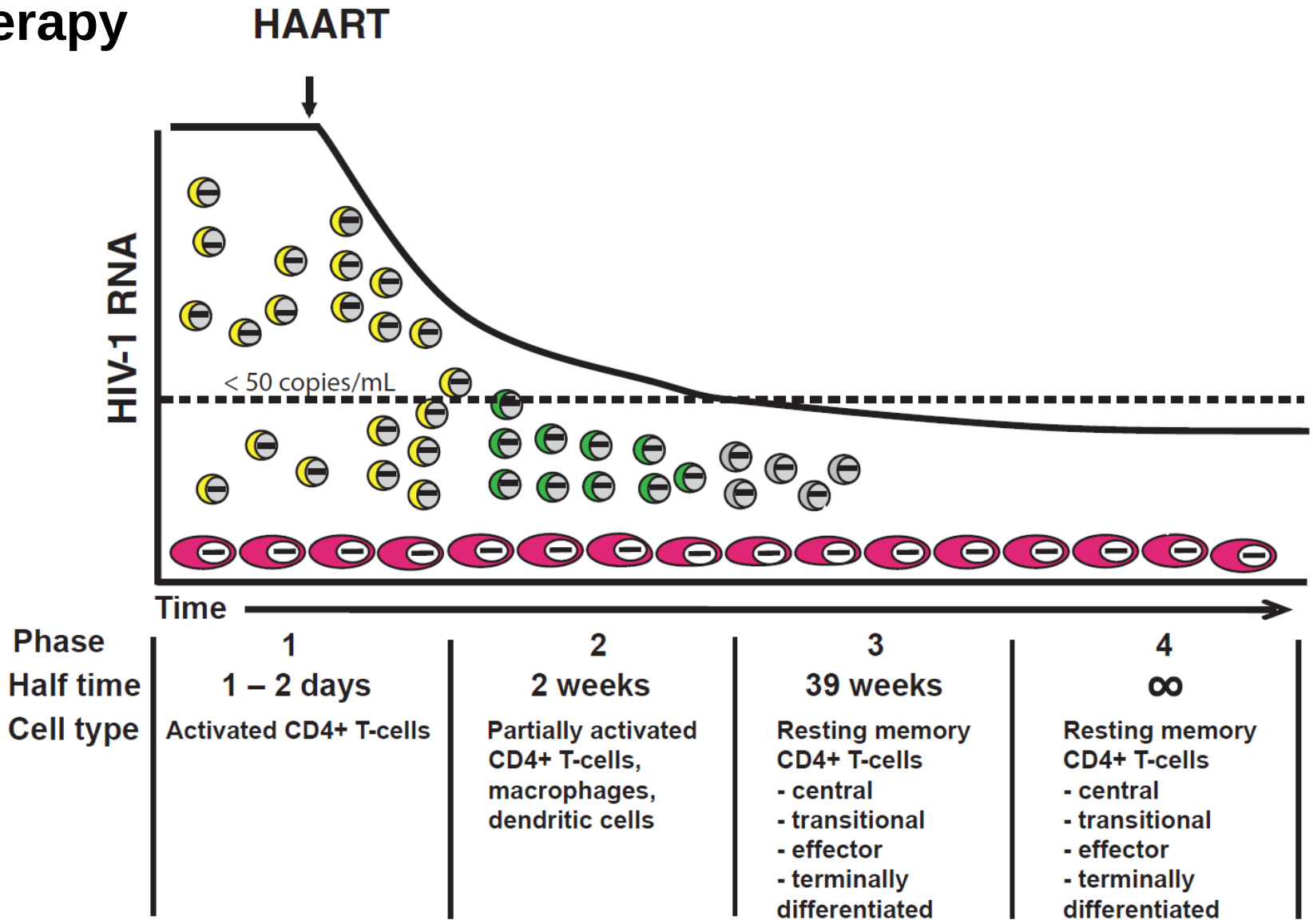
After 25-years improving therapies

HIV cure is not feasible yet



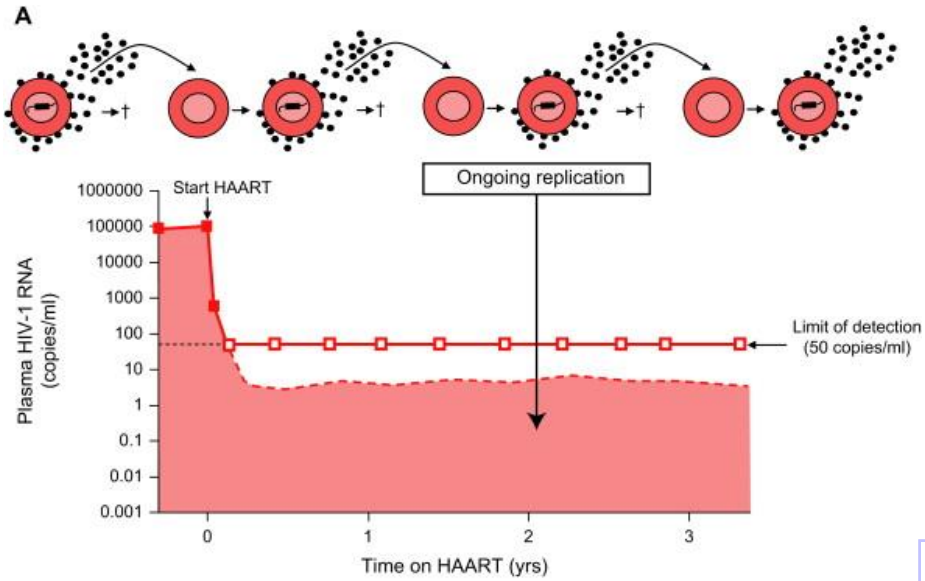
Adapted from Hilldopher et al Current HIV AIDS report 2012

Despite this profound antiviral effect, low level residual viremia persists in most patients on current antiretroviral therapy



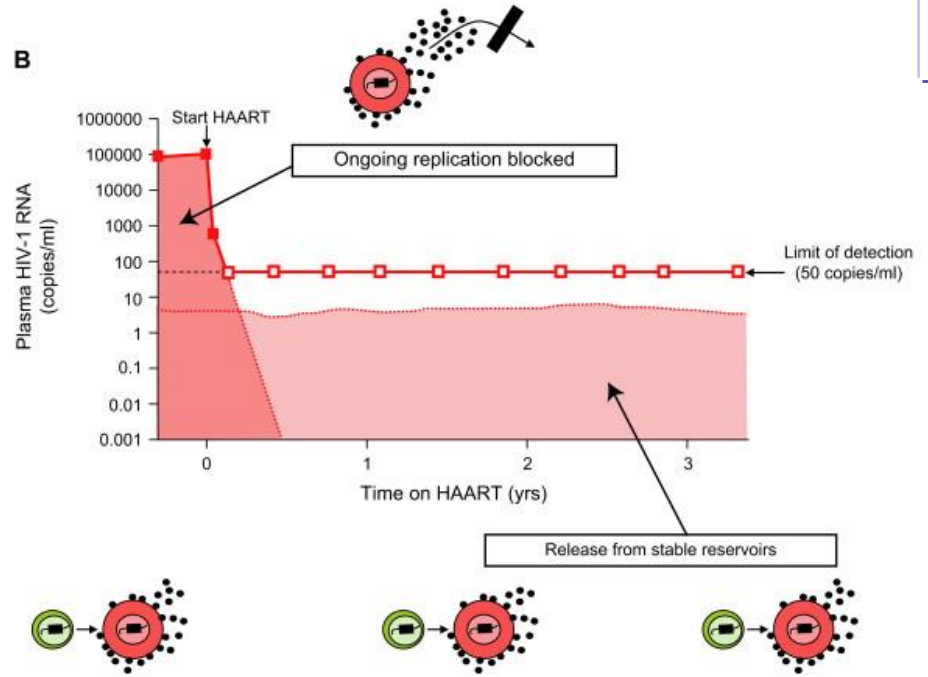
S. Palmer et al., J Int Med 2011

Where does the residual viremia came from?
Where we are?



- **Ongoing virus replication in sanctuary cellular or body compartments due to poor drug penetration or activity**

Two models to explain residual viraemia



- **Virus reactivation in latently infected cells in response to stochastic antigenic stimulation, with presence of HAART ensuring that new cells cannot be productively infected**

Published in final edited form as:
Nat Med. 2009 August ; 15(8): 893–900. doi:10.1038/nm.1972.

HIV reservoir size and persistence are driven by T cell survival and homeostatic proliferation

Nicolas Chomont[1,2,3], Mohamed El-Far[1,2,3], Petronela Ancuta[3], Lydie Trautmann[1,2,3], Francesco A Procopio[1,2,3], Bader Yassine-Diab[1,2,3], Geneviève Boucher[1], Mohamed-Rachid Boulassel[4], Georges Ghattas[5], Jason M Brenchley[6], Timothy W Schacker[7], Brenna J Hill[8], Daniel C Douek[8], Jean-Pierre Routy[4,9], Elias K Haddad[1,2,3,9], and Rafick-Pierre Sékaly[1,2,3,9,10,11]

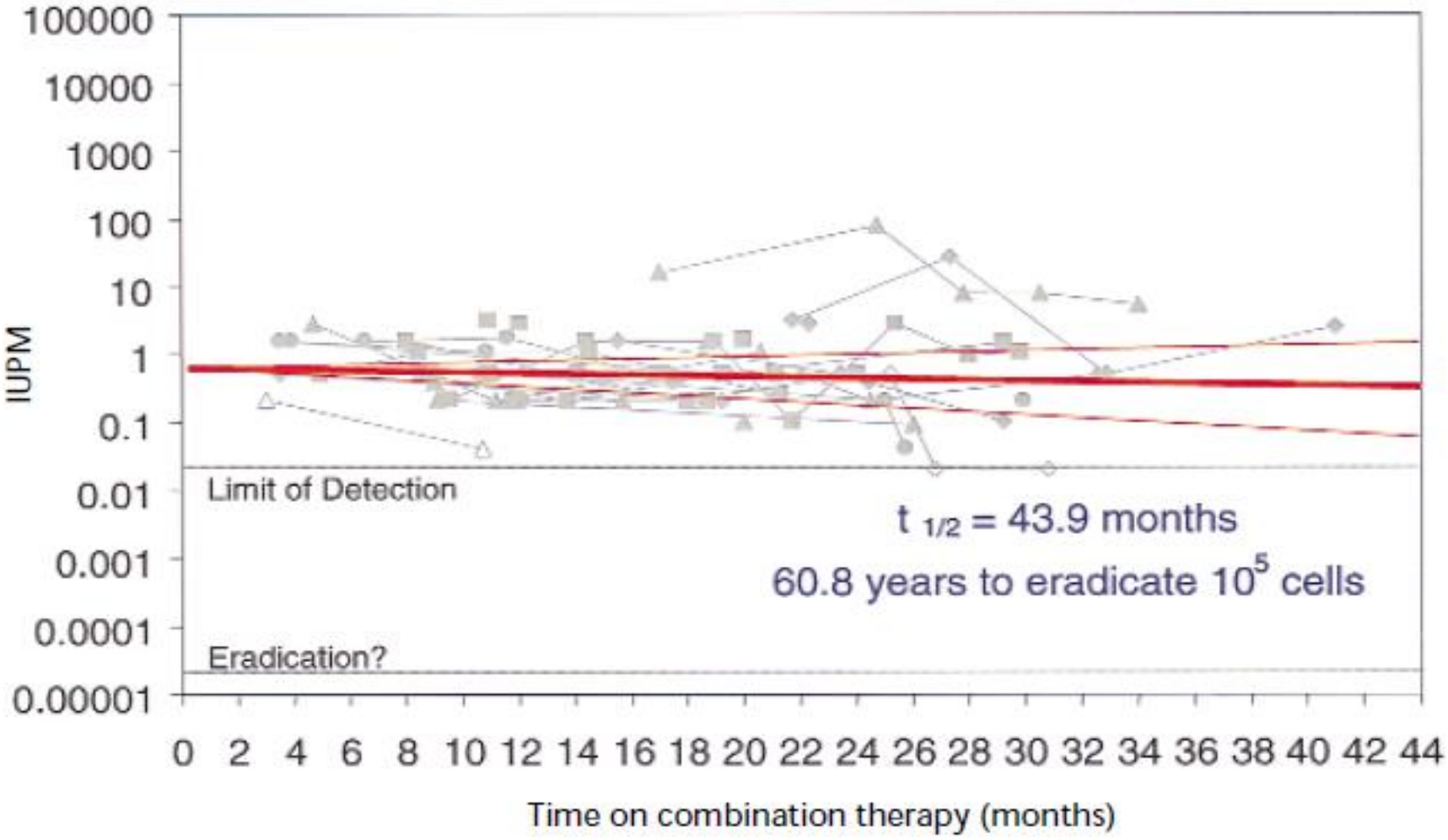
By measuring HIV DNA levels in sorted CD4+ T cell subsets, Chomont et al **showed that the majority of the reservoir resides in central memory(T_{CM}) and transitional memory (T_{TM}) CD4+ T cells.**

The relative contribution of T_{CM} and T_{TM} to the **reservoir varied from one patient to another.**

Patients with high CD4+ cell counts had their reservoir mainly located in T_{CM}, although T_{TM} constituted the main reservoir in patients with low CD4+ cells. The T_{CM} reservoir was maintained through T cell survival, and the T_{TM} reservoir through homeostatic proliferation involving interleukin-7 (IL-7).

This work also showed that the naïve CD4+ T cell subset contribution to the reservoir was marginal (1.9%).

Long half-life of the latent reservoir

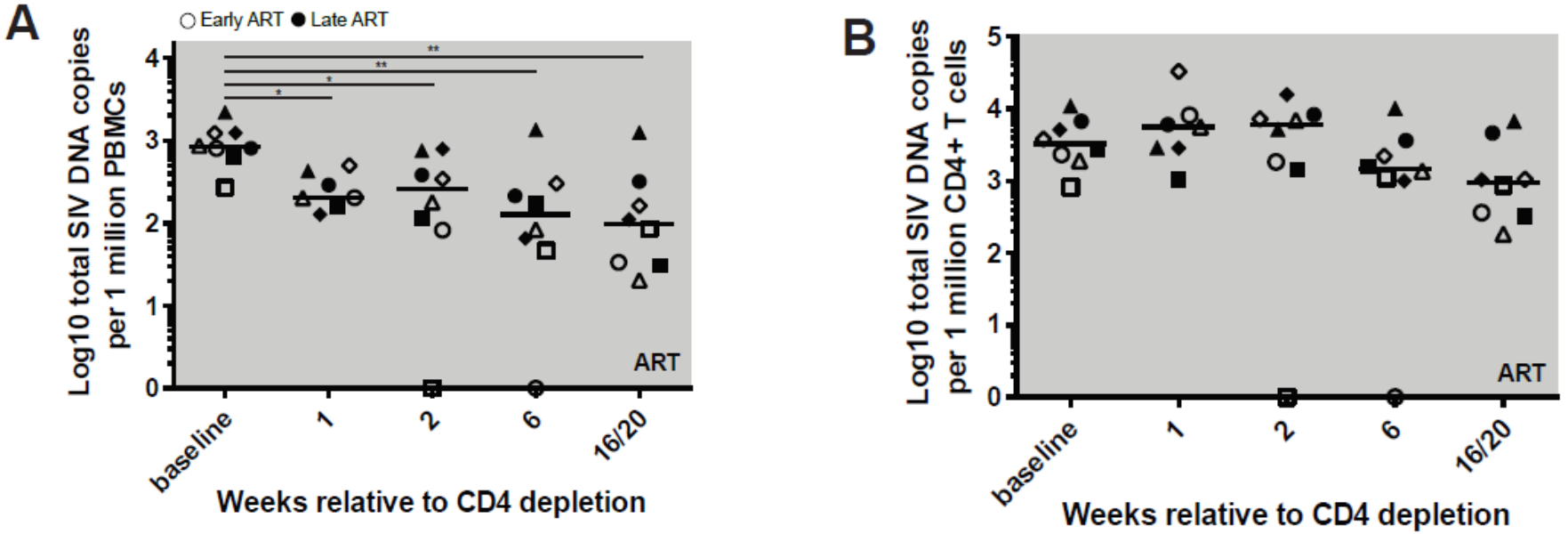


Finzi D, Blankson J, Siliciano JD et al. Latent infection of CD4+ T cells provides a mechanism for lifelong persistence of HIV-1, even in patients on effective combination therapy.; Nat Med. 1999 May;5(5):512-7.

Antibody-mediated CD4 depletion induces homeostatic CD4+ T cell proliferation without detectable virus reactivation in ART-treated SIV-infected macaques

Nitasha A Kumar[1], Julia B McBrien[1], Diane G Carnathan[1], Maud Mavigner[3], Cameron Mattingly, Erick R White[1], Federico Viviano[1], Steve E Bosinger[1,2], Ann Chahroudi[3], Guido Silvestri[1,2], Mirko Paiardini[1,2], Thomas H Vanderford[1,*]

JVI Accepted Manuscript Posted Online 5 September 2018
J. Virol. doi:10.1128/JVI.01235-18



After administration of the CD4R1 antibody, we observed a CD4+ T-cell depletion of 65-89% in peripheral blood and 20-50% in lymph nodes, followed by a significant increase in CD4+ T-cell proliferation during CD4+ T-cell reconstitution.

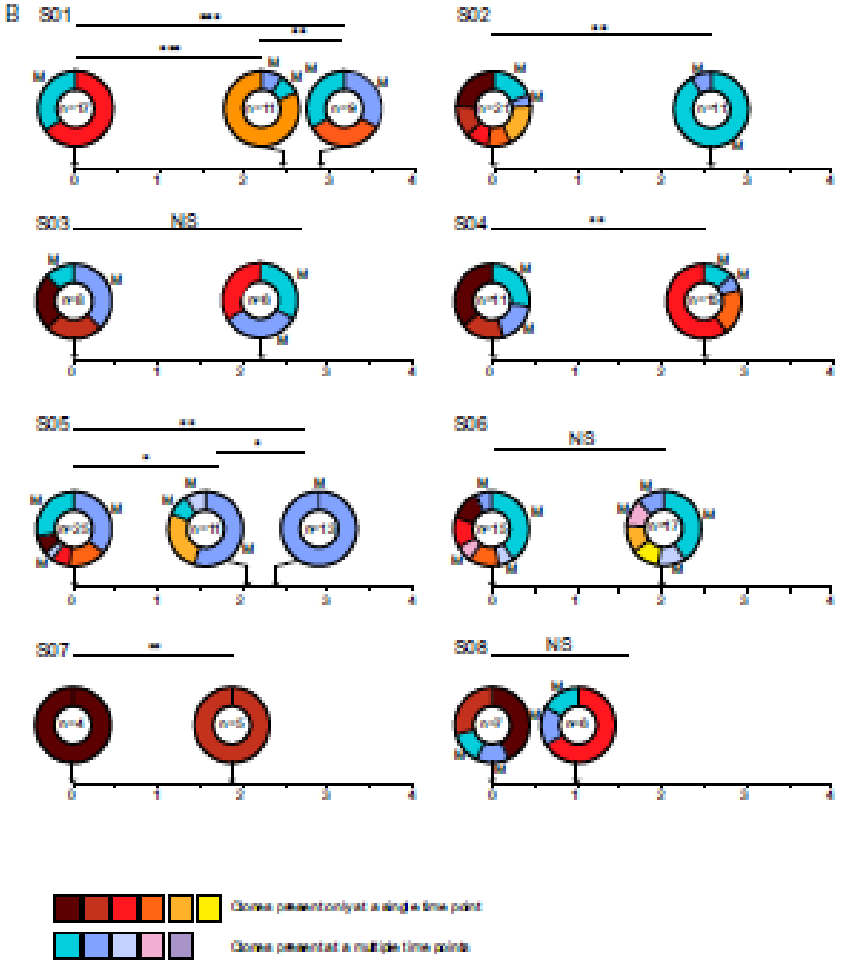
Expanded cellular clones carrying replication-competent HIV-1 persist, wax, and wane

2018

Zheng Wang[a], Evelyn E. Gurule[a], Timothy P. Brennan[a], Jeffrey M. Gerold[b], Kyungyoon J. Kwon[a], Nina N. Hosmane[a], Mithra R. Kumar[a], Subul A. Beg[a], Adam A. Capoferri[a], Stuart C. Ray[a], Ya-Chi Ho[c], Alison L. Hill[b], Janet D. Siliciano[a], and Robert F. Siliciano[a,d,1]

Contributed by Robert F. Siliciano, January 24, 2018 (sent for review December 4, 2017; reviewed by Douglas F. Nixon and Rafick Sekaly)

The latent reservoir for HIV-1 in resting CD4$^+$ T cells is a major barrier to cure. Several lines of evidence suggest that the latent reservoir is maintained through cellular proliferation. Analysis of this proliferative process is complicated by the fact that most infected cells carry defective proviruses. Additional complications are that stimuli that drive T cell proliferation can also induce virus production from latently infected cells and productively infected cells have a short in vivo half-life. In this ex vivo study, we show that latently infected cells containing replication-competent HIV-1 can proliferate in response to T cell receptor agonists or cytokines that are known to induce homeostatic proliferation and that this can occur without virus production. Some cells that have proliferated in response to these stimuli can survive for 7 d while retaining the ability to produce virus. This finding supports the hypothesis that both antigen-driven and cytokine-induced proliferation may contribute to the stability of the latent reservoir. Sequencing of replication-competent proviruses isolated from patients at different time points confirmed the presence of expanded clones and demonstrated that while some clones harboring replication-competent virus persist longitudinally on a scale of years, others wax and wane. A similar pattern is observed in longitudinal sampling of residual viremia in patients. The observed patterns are not consistent with a continuous, cell-autonomous, proliferative process related to the HIV-1 integration site. The fact that the latent reservoir can be maintained, in part, by cellular proliferation without viral reactivation poses challenges to cure.



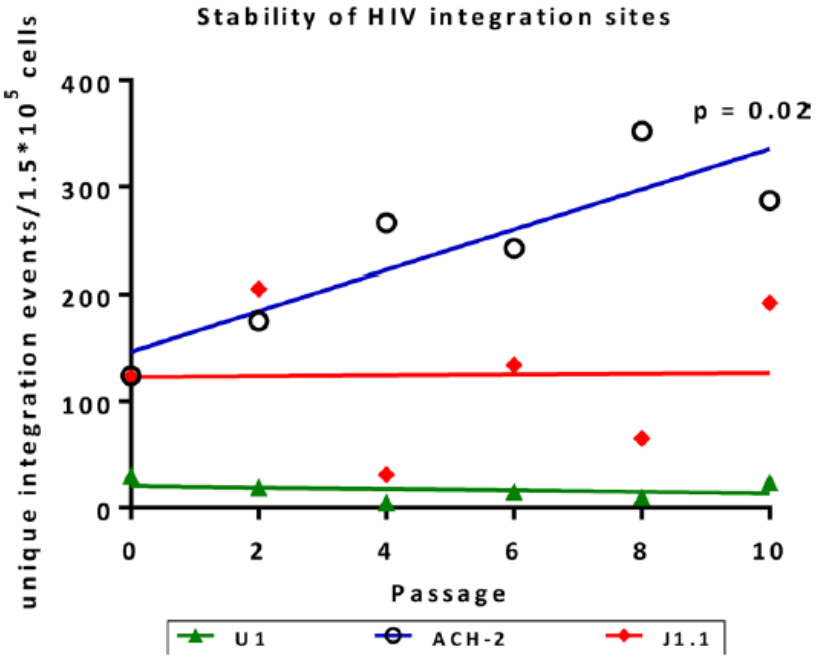
Symons *et al. Retrovirology (2017) 14:2*
DOI 10.1186/s12977-016-0325-2

Retrovirology

RESEARCH Open Access

HIV integration sites in latently infected cell lines: evidence of ongoing replication

Jori Symons[1†], Abha Chopra[2†], Eva Malantinkova[3], Ward De Spiegelaere[3], Shay Leary[2], Don Cooper[2], Chike O. Abana[4], Ajantha Rhodes[1], Simin D. Rezaei[1], Linos Vandekerckhove[3], Simon Mallal[2,4], Sharon R. Lewin[1,5†] and Paul U. Cameron[1,5*†]



Conclusion: Cell lines latently infected with intact HIV demonstrated multiple unique HIV integration sites indicating that these cell lines are not clonal and in the ACH-2 cell line there was evidence of low level virus replication. These findings have implications for the use of latently infected cell lines as models of HIV latency and for the use of these cells as standards.

Ongoing replication vs Homeostatic proliferation in LN: unresolved question

Published in final edited form as:
Nature. 2016 February 4; 530(7588): 51–56. doi:10.1038/nature16933.

- **Persistent HIV-1 replication maintains the tissue reservoir during therapy**

Ramon Lorenzo-Redondo[#],

- Ongoing HIV Replication During ART Reconsidered

Mary F. Kearney,[1] Ann Wiegand,[1] Wei Shao,[2] William R. McManus,[1] Michael J. Bale,[1] Brian Luke,[2] Frank Maldarelli,[1] John W. Mellors,[3] and John M. Coffin[4]

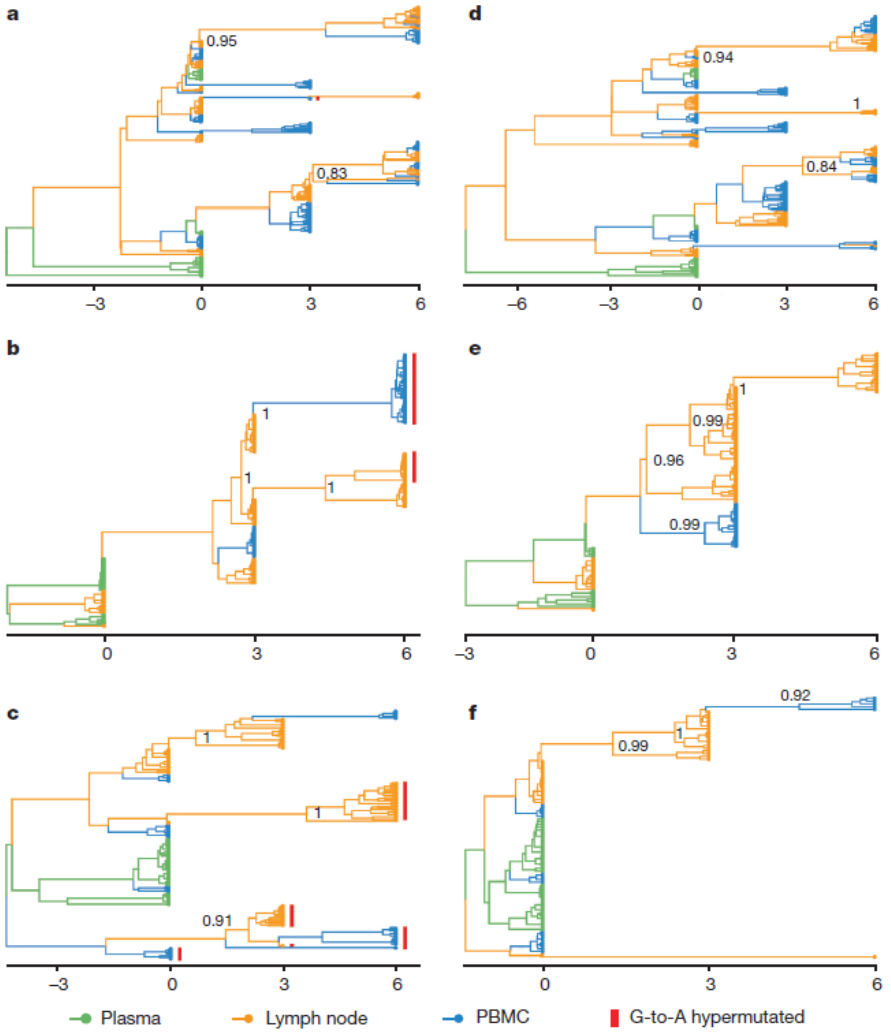
Open Forum Infectious Diseases

PERSPECTIVES

The low drug concentration causes virus evolution and trafficking between tissue compartments in patients with undetectable levels of virus in their bloodstream

Main Compartment (blood) -> high drug concentration

Sanctuary site (lymphoid tissue) -> low drug concentration



Modified from Lorenzo-Redondo, et al Nature 2016

Ongoing replication vs Homeostatic proliferation in LN: unresolved question

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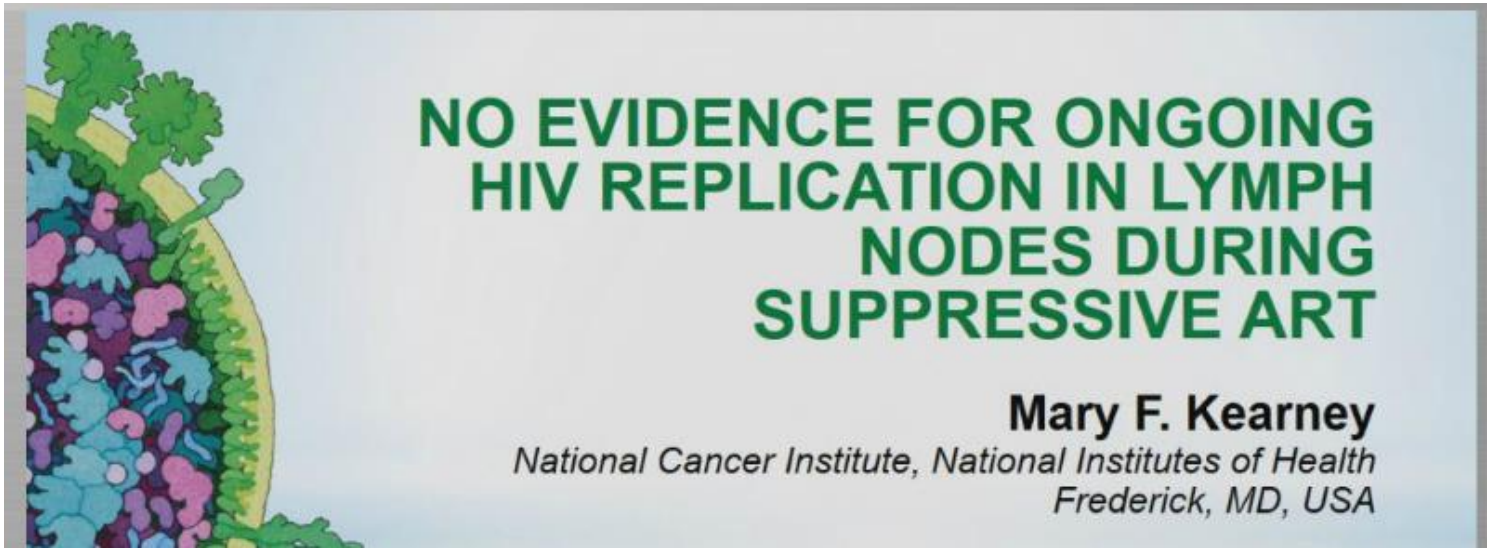
Open Forum Infectious Diseases

PERSPECTIVES

CROI 2018



NO EVIDENCE FOR ONGOING HIV REPLICATION IN LYMPH NODES DURING SUPPRESSIVE ART

Mary F. Kearney
National Cancer Institute, National Institutes of Health
Frederick, MD, USA

- Analyzed HIV-1 proviral genetics in pre-ART and after 2-13 years on suppressive ART in paired lymph node and peripheral blood samples
- Analyzed HIV-1 integration sites in paired lymph node and peripheral blood samples
- Analyzed HIV-1 RNA expression levels in single cells in paired lymph node and peripheral blood samples

CROI 2018

NO EVIDENCE FOR ONGOING HIV REPLICATION IN LYMPH NODES DURING SUPPRESSIVE ART

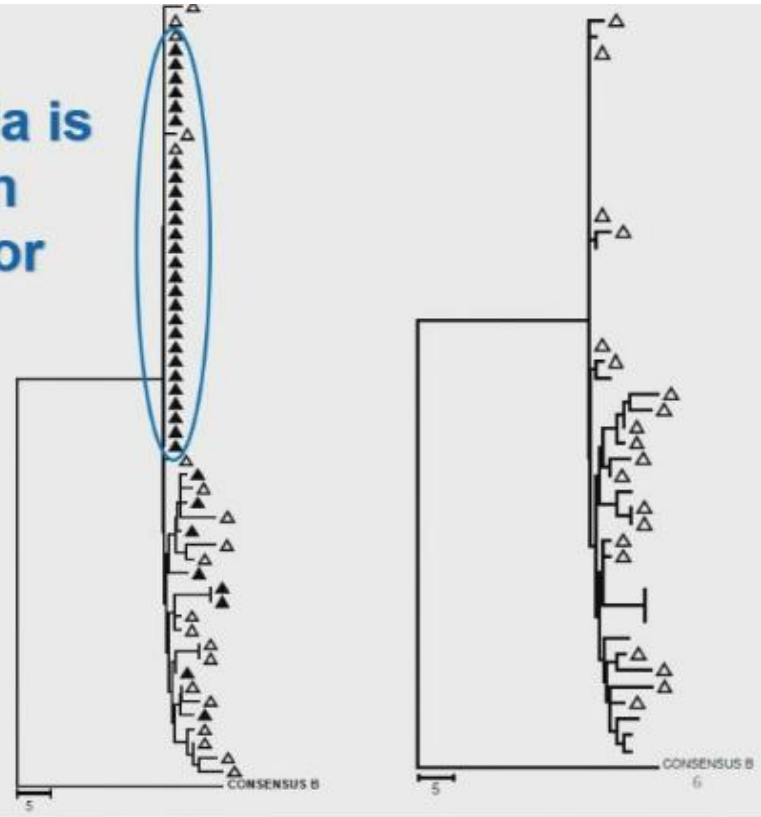
Mary F. Kearney
National Cancer Institute, National Institutes of Health
Frederick, MD, USA

No Detectable HIV-1 Replication When Viremia is Suppressed on ART in Peripheral Blood (PB) or Lymph Node (LN)

(PID 1683 - 5.5yrs on ART)

△ Pre-Therapy PB Proviral DNA

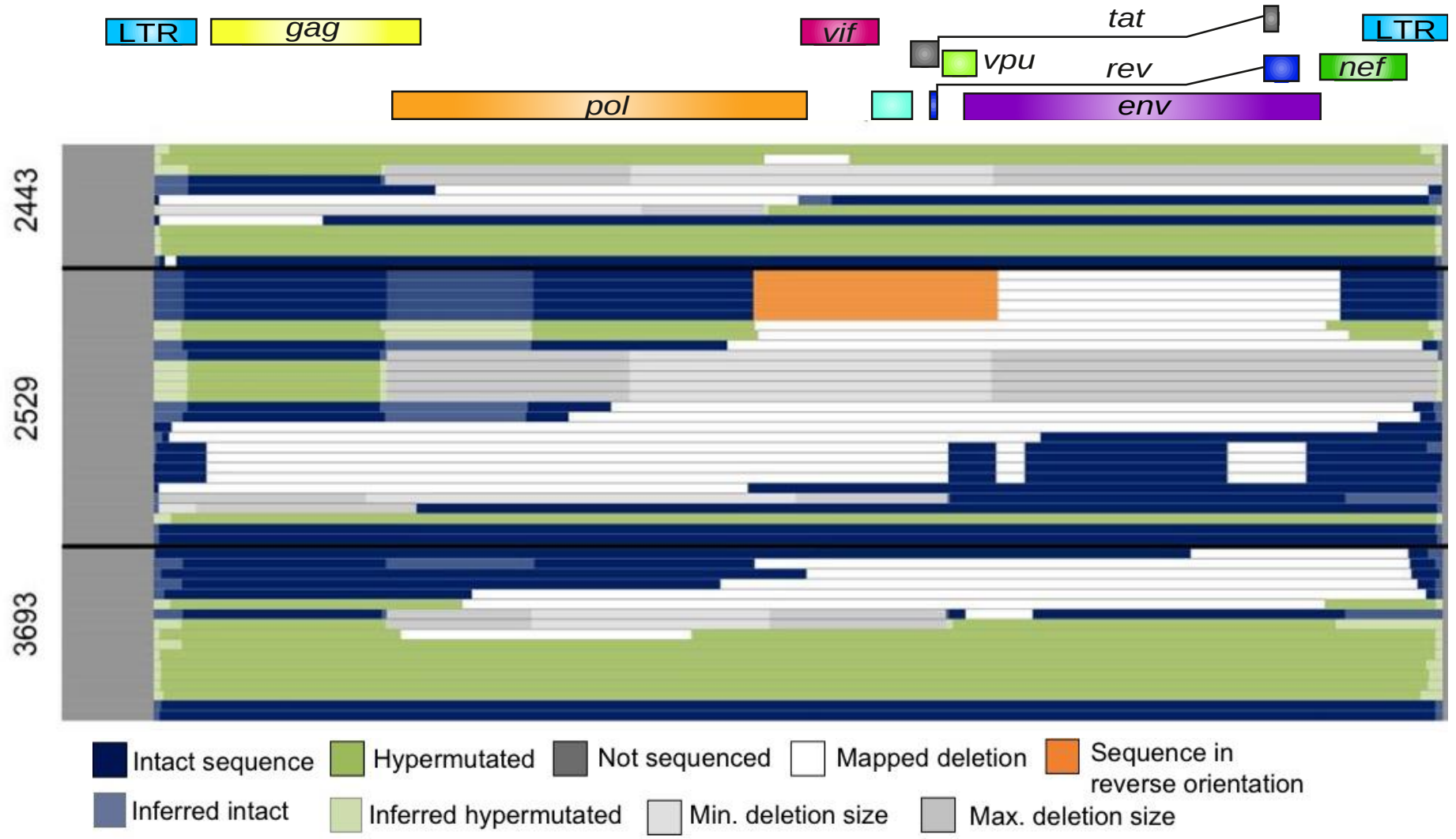
▲ On-Therapy PB Proviral DNA



- **Where can we find replication competent provirus? Where we are**

Rapid accumulation of defective proviruses…

as early as two to three weeks after infection to make up over 93% of all proviruses, regardless of how early ART is initiated



Bruner et al, Nat Med 2016

- The vast majority of proviruses that persist on ART are defective. Of the minority that are intact (~2%), the fractions that are latent or replicative competent are not known.

- These “ZOMBIE” proviruses (Imamichi, H. et al., International AIDS Conference, 2014) lack the ability to produce intact viruses but can inflict harm by producing foreign nucleic acids and proteins. Persistence of these proviruses may explain the persistent seropositivity to HIV-1 and persistent immune activation seen in patients with "undetectable" virus.

Quan et al. Virology Journal 2014, 11:177
http://www.virologyj.com/content/11/1/177

VIROLOGY JOURNAL

RESEARCH Open Access

Identification of an env-defective HIV-1 mutant capable of spontaneous reversion to a wild-type phenotype in certain T-cell lines

Yudong Quan[1], Hongtao Xu[1], Victor G Kramer[1], Yingshan Han[1], Richard D Sloan[1] and Mark A Wainberg[1,2,3*]

Abstract

Background: Attempts to eradicate HIV from cellular reservoirs are vital but depend on a clear understanding of how viral variants are transmitted and survive in the different cell types that constitute such reservoirs. Mutations in the env gene of HIV may be able to exert a differential influence on viral transmission ability in regard to cell-free and cell-associated viral forms.

Methods: The ability of HIV containing an env G367R mutation in cell-free and cell-associated viruses to cause infection and to revert to wild-type was measured using several T cell lines. To determine factors that might potentially influence the reversion of G367R, we studied each of entry inhibitors, inhibitors of cellular endocytosis, and modulators of cell growth and activation.

Results: We demonstrate that an HIV-1 variant containing a G367R substitution within the CD4 binding site of gp120 was non-infectious as free virus in culture but was infectious when infected cells were co-cultured with certain T cell lines or when cells were transfected by a relevant proviral plasmid. Differences in viral infectivity by cell-associated G367R viruses were determined by the type of target cell employed, regardless which type of donor cell was used. Reversion was slowed or inhibited by entry inhibitors and by inhibitors of cellular endocytosis. Interleukin 2 was able to block G367R reversion in only one of the T cell lines studied but not in the other, while phorbol 12-myristate 13-acetate (PMA) inhibited G367R reversion in all the T cell lines.

Conclusions: Env-defective HIV may have a different phenotype as cell-free versus cell-associated virus. The persistence of defective forms can potentially lead to the emergence of virulent forms. The heterogeneity of cell types that constitute the HIV reservoir can contribute to viral variability, even among similar types of cells. This is the first demonstration of a mutation in the HIV envelope, i.e. G367R, that can compromise infection by cell-free virus but less severely by cell-associated virus and that does so in a cell type-dependent manner.

Keywords: Defective virus, Reversion, HIV, Cell-associated transmission

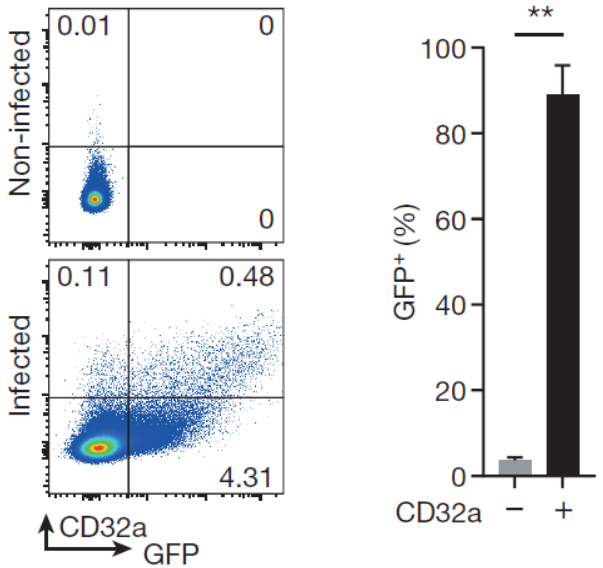
LETTER

doi:10.1038/nature21710

CD32a is a marker of a CD4 T-cell HIV reservoir harbouring replication-competent proviruses

Benjamin Descours[1]*, Gaël Petitjean[1]*, José-Luis López-Zaragoza[2,3,4], Timothée Bruel[2,5], Raoul Raffel[1], Christina Psomas[6], Jacques Reynes[6], Christine Lacabaratz[2,3,4], Yves Levy[2,3,4], Olivier Schwartz[2,5], Jean Daniel Lelievre[2,3,4] & Monsef Benkirane[1]

CD32a expression in PBMCs pool from 3 different HIV-1 positive patients



Nature 2017

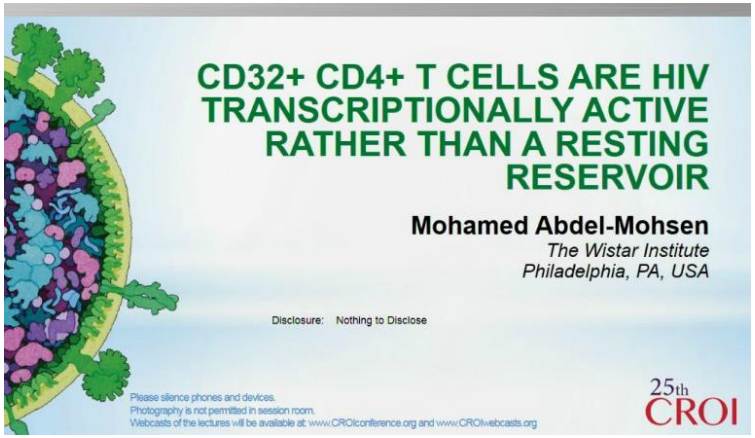
SCIENCE TRANSLATIONAL MEDICINE | RESEARCH ARTICLE

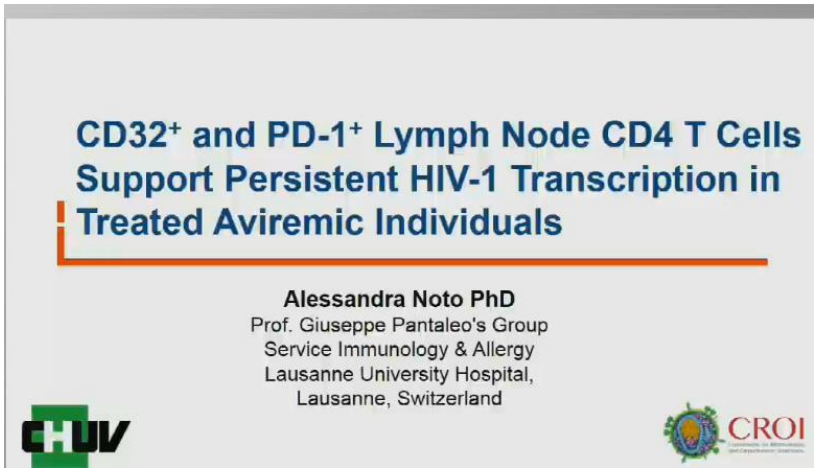
HIV

2018

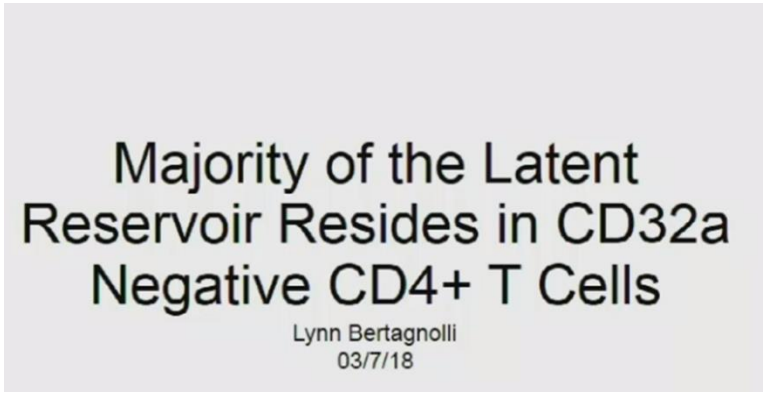
CD32 is expressed on cells with transcriptionally active HIV but does not enrich for HIV DNA in resting T cells

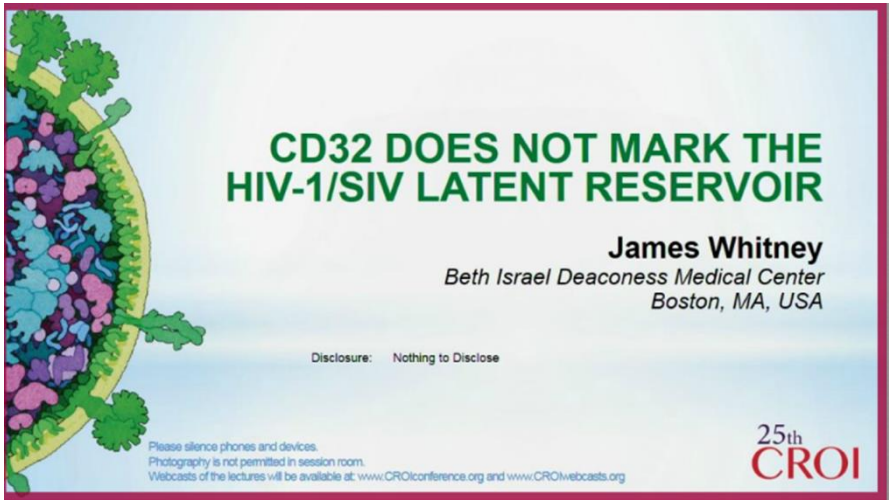
Mohamed Abdel-Mohsen,[1]* Leticia Kuri-Cervantes,[2]* Judith Grau-Exposito,[3]* Adam M. Spivak,[4]





CROI 2018





CROI 2018

Hematopoietic stem and progenitor cells are a unique functional HIV reservoir 374LB

Thomas D. Zaikos[1], Valeri H. Terry[2], Nadia T. Sebastian Kettinger[3,4], Andrew Neevel[2], James Riddell IV[5], Dale Bixby[6], Norman Markowitz[7], Frances Taschuk[2], Adewunmi Onafuwa-Nuga[2], Lucy A. McNamara[1], and Kathleen L. Collins[1,2,3]

[1]Department of Microbiology and Immunology, [2]Department of Internal Medicine, [3]Program in Cellular and Molecular Biology, [4]Medical Scientist Training Program, [5]Division of Infectious Disease, Department of Internal Medicine, [6]Division of Oncology, Department of Internal Medicine University of Michigan, Ann Arbor, MI, USA, and [7]Division of Infectious Diseases, Henry Ford Hospital, Detroit, MI, USA

Figure 1. Donor inclusion and summary of study cohort sub-groups

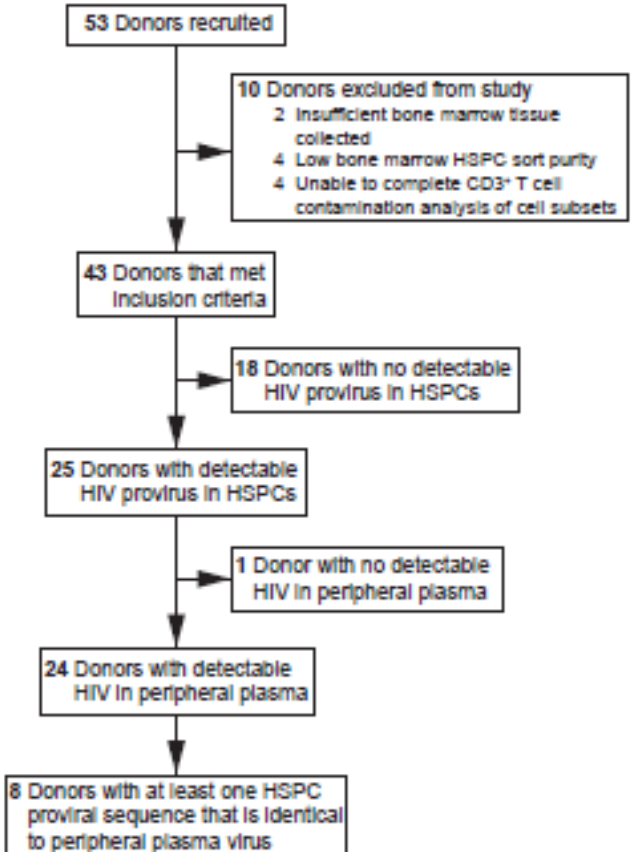
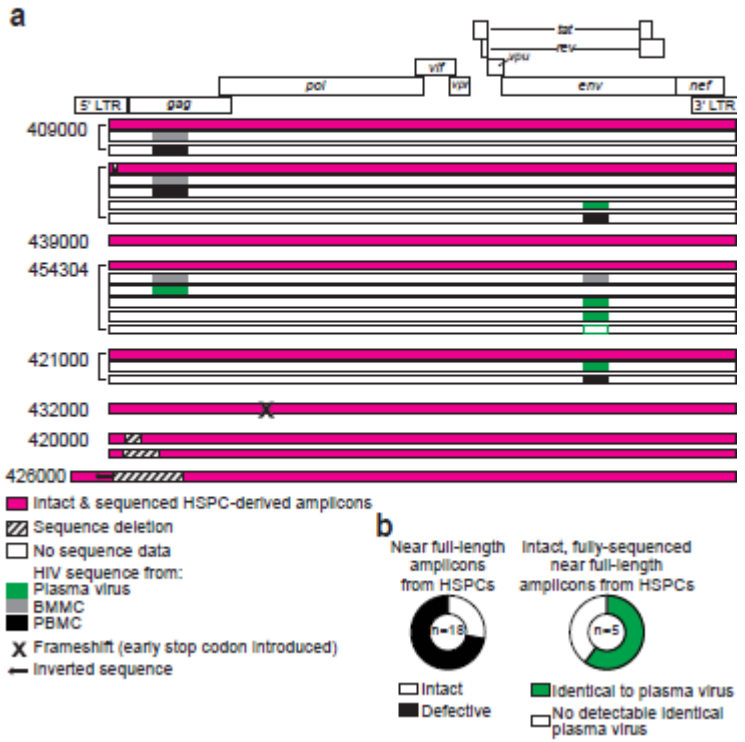


Figure 3. Residual plasma virus is often derived from groups of clusters of HSPC-associated identical proviral genomes (CHIPs)

Figure 5. HSPCs contain intact, near full-length HIV genomes, which are represented in residual plasma virus at a high rate



- Macrophages as Viral Tissue Reservoirs

CVI
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MINIREVIEW

Gastrointestinal Tract and the Mucosal Macrophage Reservoir in HIV Infection

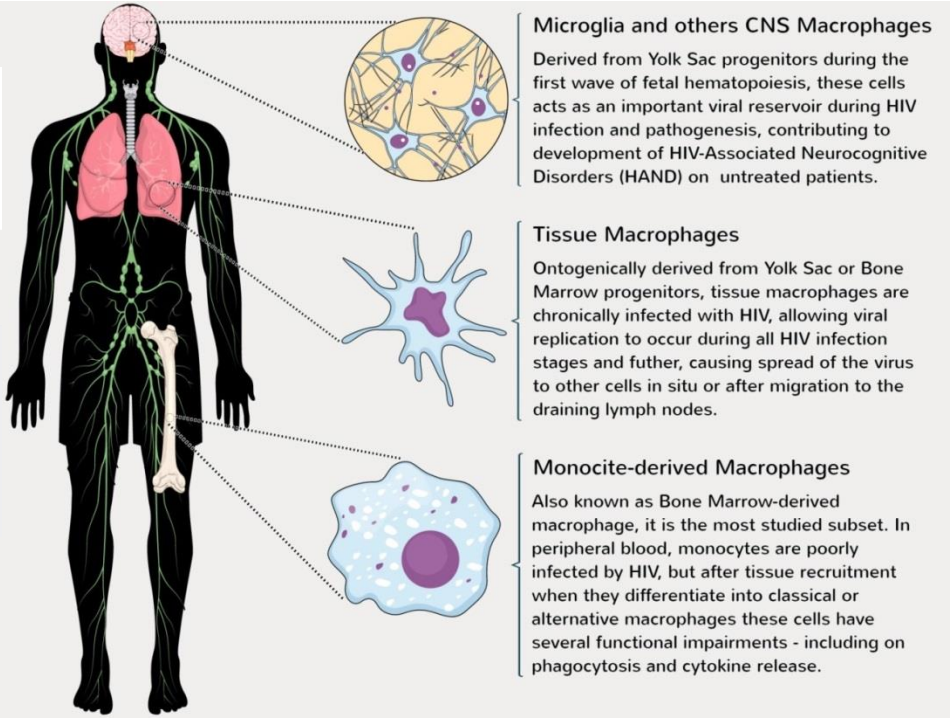
Dallas Brown, Joseph J. Mattapallil
Uniformed Services University of the Health Sciences, Bethesda, Maryland, USA

Macrophages sustain HIV replication in vivo independently of T cells

Jenna B. Honeycutt,[1] Angela Wahl,[1] Caroline Baker,[1] Rae Ann Spagnuolo,[1] John Foster,[1] Oksana Zakharova,[1] Stephen Wietgrefe,[2] Carolina Caro-Vegas,[3] Victoria Madden,[4] Garrett Sharpe,[3] Ashley T. Haase,[2] Joseph J. Eron,[1] and J. Victor Garcia[1]

The Journal of Clinical Investigation 2016

To determine whether tissue macrophages are productively infected, we used 3 different but complementary humanized mouse models. Two of these models (bone marrow/liver/thymus [BLT] mice and T cell–only mice [ToM]) have been previously described, and the third model was generated by reconstituting immunodeficient mice with human CD34$^+$ hematopoietic stem cells that were devoid of human T cells (myeloid-only mice [MoM]) to specifically evaluate HIV replication in this population. Using MoM, we demonstrated that macrophages can sustain HIV replication in the absence of T cells; HIV-infected macrophages are distributed in various tissues including the brain; replication-competent virus can be rescued ex vivo from infected macrophages; and infected macrophages can establish de novo infection. Together, these results demonstrate that macrophages represent a genuine target for HIV infection in vivo that can sustain and transmit infection.



Microglia and others CNS Macrophages

Derived from Yolk Sac progenitors during the first wave of fetal hematopoiesis, these cells acts as an important viral reservoir during HIV infection and pathogenesis, contributing to development of HIV-Associated Neurocognitive Disorders (HAND) on untreated patients.

Tissue Macrophages

Ontogenically derived from Yolk Sac or Bone Marrow progenitors, tissue macrophages are chronically infected with HIV, allowing viral replication to occur during all HIV infection stages and futher, causing spread of the virus to other cells in situ or after migration to the draining lymph nodes.

Monocite-derived Macrophages

Also known as Bone Marrow-derived macrophage, it is the most studied subset. In peripheral blood, monocytes are poorly infected by HIV, but after tissue recruitment when they differentiate into classical or alternative macrophages these cells have several functional impairments - including on phagocytosis and cytokine release.

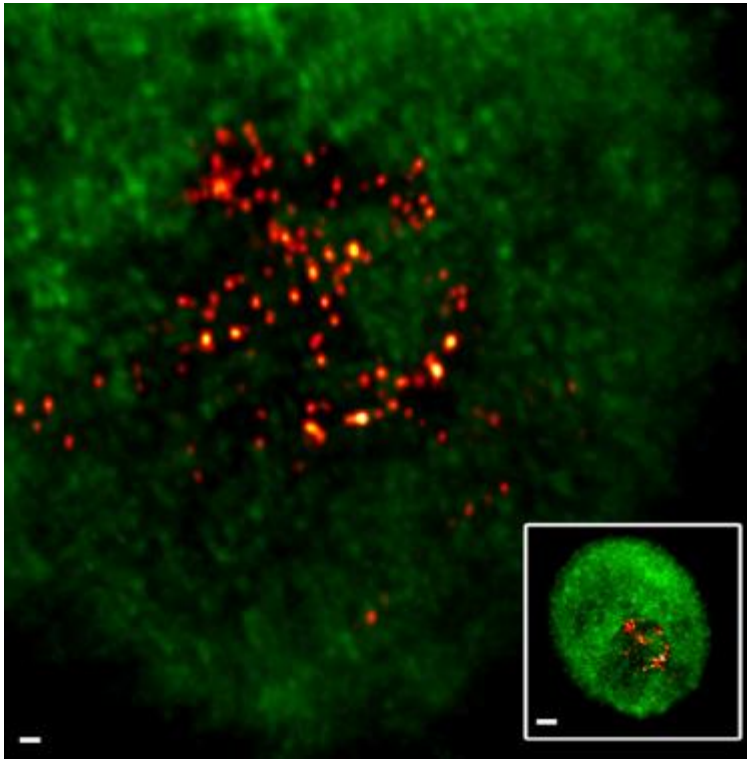
Modified from Leonardo J. Galvão-Lima, Braz J Inf Dis 2017

- And allow the viral spread to CD4+T cell

- in vitro infection of MDM leads to accumulation of infectious particles in a surface-connected vesicular compartment termed the virus-containing compartment (VCC) (Deneka et al., 2007; Jouve et al., 2007; Welsch et al., 2007)

- Infectious virus may be stored within the VCC for extended periods (Sharova et al., 2005) and then transferred rapidly to contacting CD4+ T cells (Giese and Marsh, 2014; Gousset et al., 2008; Groot et al., 2008).

- human CD169+ macrophages efficiently capture blood- or lymph-borne retroviruses in spleen and lymph nodes



Retroviruses use CD169-mediated trans-infection of permissive lymphocytes to establish infection

Xaver Sewald,[1*] Mark S. Ladinsky,[2†] Pradeep D. Uchil,[1†] Jagadish Beloor,[3] Ruoxi Pi,[1] Christin Herrmann,[1] Nasim Motamedi,[4‡] Thomas T. Murooka,[5] Michael A. Brehm,[6] Dale L. Greiner,[6] Leonard D. Shultz,[7] Thorsten R. Mempel,[5] Pamela J. Bjorkman,[2] Priti Kumar,[3*] Walther Mothes[1*]

Sciencexpress

2015

AMERICAN SOCIETY FOR MICROBIOLOGY | mBio®

2017

Mechanisms of CNS Viral Seeding by HIV$^+$ CD14$^+$ CD16$^+$ Monocytes: Establishment and Reseeding of Viral Reservoirs Contributing to HIV-Associated Neurocognitive Disorders

Mike Veenstra,[a] Rosiris León-Rivera,[a] Ming Li,[b] Lucio Gama,[b,c] Janice E. Clements,[b] Joan W. Berman[a,d]

IMPORTANCE HIV infects different tissue compartments of the body, including the central nervous system (CNS). This leads to establishment of viral reservoirs within the CNS that mediate neuroinflammation and neuronal damage, contributing to cognitive impairment. Our goal was to examine the mechanisms of transmigration of cells that contribute to HIV infection of the CNS and to continued replenishment of CNS viral reservoirs, to establish potential therapeutic targets. We found that an HIV-infected subset of monocytes, mature HIV$^+$ CD14$^+$ CD16$^+$ monocytes, preferentially transmigrates across the blood-brain barrier. This was mediated, in part, by increased junctional proteins JAM-A and ALCAM and chemokine receptor CCR2. We

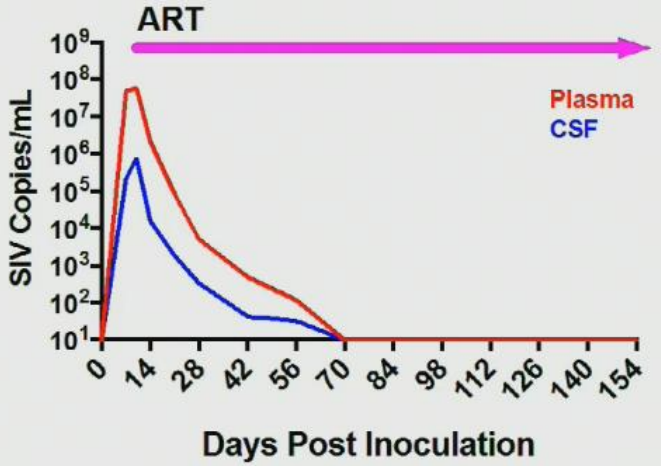
- **The HIV-1 sanctuary: new insights**

CROI 2018

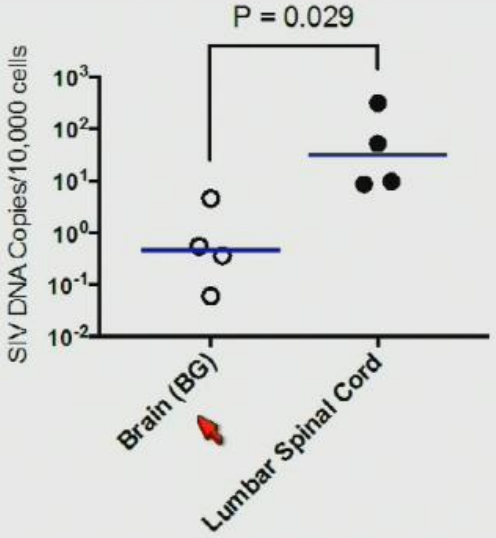
SIV REBOUND IN THE SPINAL CORD AFTER STOPPING ART: A NOVEL CNS RESERVOIR

Joseph Mankowski
Johns Hopkins University School of Medicine
Baltimore, MD, USA

SIV + ART: Suppression in Plasma and CSF



SIV DNA Levels: Brain vs Spinal Cord

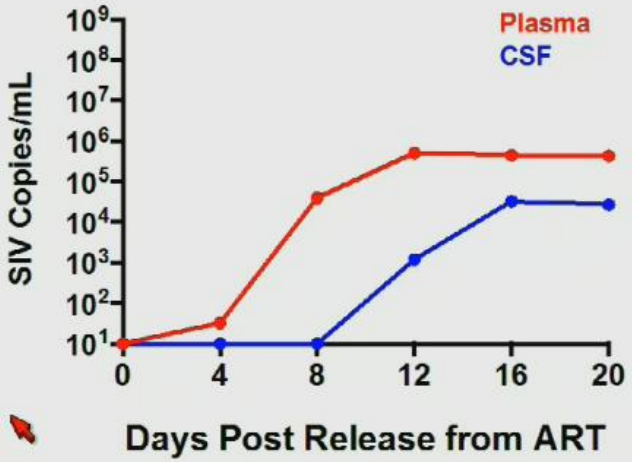


CROI 2018

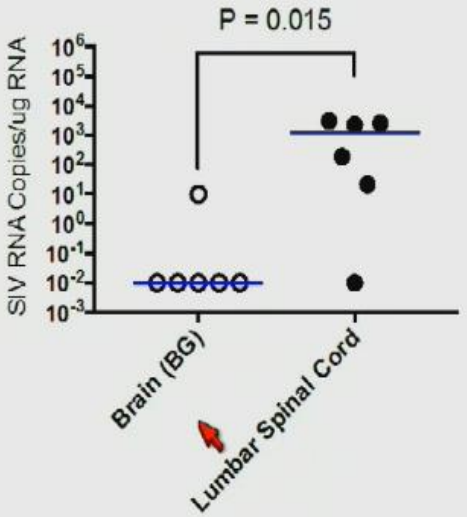
SIV REBOUND IN THE SPINAL CORD AFTER STOPPING ART: A NOVEL CNS RESERVOIR

Joseph Mankowski
Johns Hopkins University School of Medicine
Baltimore, MD, USA

SIV Rebound after Stopping ART: Plasma vs CSF



SIV RNA Levels: Brain vs Spinal Cord



PULMONARY MUCOSAL T CELLS AS POTENTIAL HIV RESERVOIRS DURING LONG-TERM ART

CROI 2018

Author(s):

Syim Salahuddin[1], Omar Farnos[2], Ron Olivenstein[3], Aurelie Le Page[1], Amelie Pagliuzza[4], Christina de Castro[1], Jean Bourbeau[3], Petronela Ancuta[4], Bertrand Lebouché[1], Jean-Pierre Routy[1], Nicolas Chomont[4], Cecilia Costiniuk[1], Mohammad-Ali Jenabian[2]

Cells from bronchoalveolar lavage (BAL), obtained by bronchoscopy, and matched peripheral blood samples were collected from n=16 HIV+ individuals without respiratory symptoms and under long-term suppressive ART (undetectable plasma viral load and CD4 count higher than 350 cells/mm3 for at least 3 years). T-cell subsets were characterized by flow cytometry, and total and integrated HIV DNA were assessed by ultrasensitive PCR. Paired t-test was used in statistical analyses.

In virally suppressed HIV+ adults, the lungs contain higher levels of HIV DNA and higher frequencies of various T cell subsets known as preferential HIV reservoirs including CCR6+ and CD32a+ CD4 T cells as well as activated DN T cells when compared to peripheral blood. This particular distribution of mucosal T cells could contribute to the preferential persistence of HIV reservoirs within the lungs.

HIV RNA and proviral HIV DNA can be detected in semen after 6 months of antiretroviral therapy although HIV RNA is undetectable in blood

Table 2. HIV RNA in blood plasma and seminal plasma and HIV DNA in PBMCs and seminal cells after six month ART

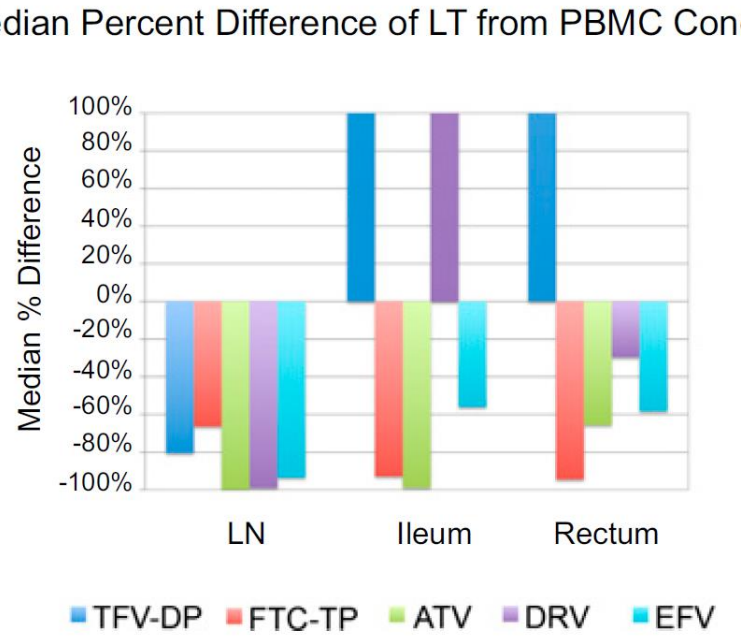
	HIV RNA copies/mL		Total HIV DNA copies/10^6 cells		Integrated HIV DNA copies/10^6 cells		2LTR circular HIV DNA copies/10^6 cells	
Patient	Blood plasma	Seminal plasma	PBMCs	Seminal cells	PBMCs	Seminal cells	PBMCs	Seminal cells
1	<LDL	14,300	109.45	743.44	26.44	190.53	16.97	0.50
2	<LDL	34,900	892.14	25.64	538.57	15. 00	185.67	0.27
3	14,996	8790	43.26	687.76	16.91	389.91	1.05	3.19
4	<LDL	60,500	30.90	64.87	13.57	11.61	8.08	1.17
5	<LDL	<LDL	54.40	575.35	43.83	435.94	2.58	2.97
6	<LDL	600	337.97	121.32	19.77	97.74	6.61	17.54
7	<LDL	1500	20.21	29.70	10.01	4.60	3.54	2.79
8	<LDL	6230	44.18	83.02	9.41	23.04	29.58	10.59
9	<LDL	9900	248.87	71.14	83.9	27.70	8.6	7.95
10	<LDL	2800	19.51	27.57	8.50	15.52	2.34	2.42
11	<LDL	860	58.36	28.54	35.8	15.25	1.90	1.05
12	<LDL	4780	115.22	477.55	18.03	64.23	0.60	31.62
13	178,161	74,300	48.65	21.03	36.24	10.98	7.87	1.24
14	<LDL	<LDL	29.29	618.87	19.01	315.55	2.21	15.05
15	<LDL	618	51.94	32.84	19.16	15.07	5.70	1.86
16	<LDL	6847	409.88	684.75	57.56	101.01	20.21	34.19
17	<LDL	4098	63.00	29.08	33.28	17.55	2.53	2.25
18	<LDL	306	80.63	41.74	363.11	17.38	1.48	3.31
19	<LDL	<LDL	62.45	23.01	8.47	11.02	0.56	1.35

LDL, lower than limit of detection

Du et al Microbiol Immunol 2016

- **The HIV-1 sanctuary: the meaning of compartmentalization**

Compared with concentrations in PBMCs, the **concentration** of **TFV, FTC, ATV, DRV and EFV** was **lower in the lymphatic tissue compartment**, particularly in the **lymph node**.

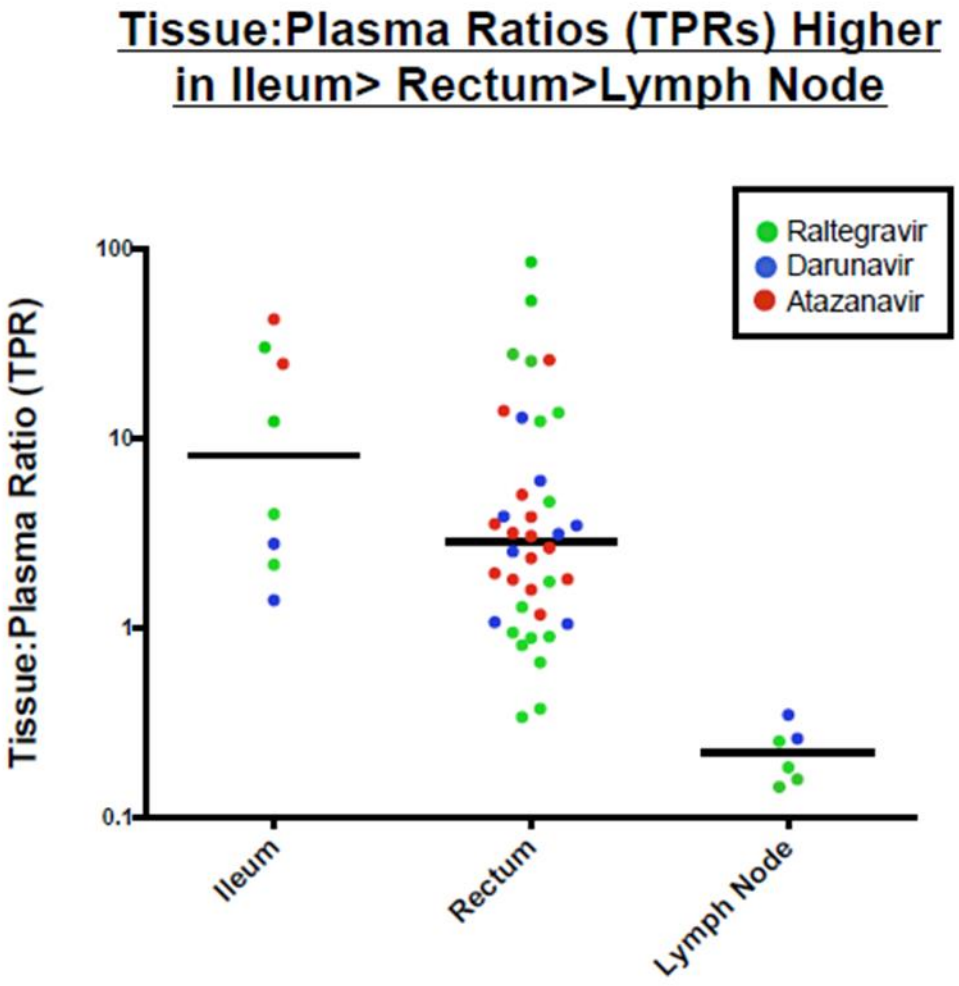


Significance

We show that HIV continues to replicate in the lymphatic tissues of some individuals taking antiretroviral regimens considered fully suppressive, based on undetectable viral loads in peripheral blood, and that one mechanism for persistent replication in lymphatic tissues is the lower concentrations of the antiretroviral drugs in those tissues compared with peripheral blood. These findings are significant because they provide a

Fletcher, PNAS 2014

INTEGRASE AND PROTEASE INHIBITOR CONCENTRATIONS IN LYMPH NODE AND GUT MUCOSAL TISSUE



- This is the first study to evaluate GALT and LN tissue concentrations in patients receiving RAL and 800 mg daily DRV.
- Tissue:plasma ratios were higher in ileum>rectum as shown previously, and lowest in lymph node.
- In a limited number of participants, concentrations of RAL were significantly lower in lymph nodes vs. GALT, supporting prior observations.
- These results support the current limited data on tissue ART drug concentrations and have potential implications on HIV cure strategies.

Lee, et al Abstr 407, CROI 2017

HIV RNA persists in rectal tissue despite rapid plasma virologic suppression with dolutegravir-based therapy

Cecile D. Lahiri[a,b], Nakita L. Brown[a,*], Kevin J. Ryan[c], Edward P. Acosta[c], Anandi N. Sheth[a,b], Cyra C. Mehta[d], Jessica Ingersoll[e] and Ighovwerha Ofotokun[a,b]

AIDS 2018, Vol 32 No 15

Virological and immunological responses to raltegravir and dolutegravir in the gut-associated lymphoid tissue of HIV-infected men and women

Michael D Weber, Elizabeth Andrews, Heather A Prince, Craig Sykes, Elias P Rosen, Camden Bay, Nicholas J Shaheen, Ryan D Madanick, Evan S Dellon, Kristina De Paris, Julie AE Nelson, Cynthia L Gay, Angela DM Kashuba

Results: 15 men and 5 women were enrolled. There was no difference in time since HIV diagnosis for those on **RTG** [9.5 (4-22) yr] and **DTG** [17 (1-24) yr] ($p = 0.6$), although time on **RTG** [5.4 (2.3-6.7) yr] was greater than **DTG** [1.0 (0.1-1.5) yr] ($P < 0.001$). Concentrations of **RTG** and **DTG** in rectal tissue (**RT**) were similar to previous reports: median tissue:plasma ratio was 11.25 for **RTG** and 0.44 for **DTG**. **RNA:DNA** ratios were [1.14 (0.18-5.10)] for the **RTG** group and [0.90 (0.30-18.87)] for the **DTG** group ($p = 0.95$). No differences ($p \geq 0.1$) between $CD4^+$ and $CD8^+$ **T** cell markers were found.

Conclusions: **RTG** produced higher tissue exposures than **DTG**, but no significant differences in **GALT HIV RNA, DNA**, or most immunologic markers were observed.

Antiviral Therapy

JOURNAL OF VIROLOGY, Mar. 2010, p. 2395–2407 Vol. 84, No. 5
0022-538X/10/$12.00 doi:10.1128/JVI.01863-09

Compartmentalization and Clonal Amplification of HIV-1 Variants in the Cerebrospinal Fluid during Primary Infection[▽]

Gretja Schnell,[1] Richard W. Price,[2] Ronald Swanstrom,[1,3]* and Serena Spudich[2]

PLOS | PATHOGENS

2015

Compartmentalized Replication of R5 T Cell-Tropic HIV-1 in the Central Nervous System Early in the Course of Infection

Christa Buckheit Sturdevant[1¤a], Sarah B. Joseph[2], Gretja Schnell[1¤b], Richard W. Price[3], Ronald Swanstrom[1,2,4,5], Serena Spudich[6]*

Kearney *et al. Retrovirology (2015) 12:93*
DOI 10.1186/s12977-015-0212-2

RETROVIROLOGY

RESEARCH **Open Access**

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Well-mixed plasma and tissue viral populations in RT-SHIV-infected macaques implies a lack of viral replication in the tissues during antiretroviral therapy

PLOS PATHOGENS

2017

Early Antiretroviral Therapy Is Associated with Lower HIV DNA Molecular Diversity and Lower Inflammation in Cerebrospinal Fluid but Does Not Prevent the Establishment of Compartmentalized HIV DNA Populations

Michelli F. Oliveira[1]*, Antoine Chaillon[1], Masato Nakazawa[1], Milenka Vargas[1], Scott L. Letendre[1,2], Matthew C. Strain[1], Ronald J. Ellis[2,3], Sheldon Morris[1], Susan J. Little[1], Davey M. Smith[1,4], Sara Gianella[1]*

Even when antiretroviral therapy (ART) is started early after infection, HIV DNA might persist in the central nervous system (CNS), possibly contributing to inflammation, brain damage and neurocognitive impairment. Paired blood and cerebrospinal fluid (CSF) were collected from 16 HIV-infected individuals on suppressive ART: 9 participants started ART <4 months of the estimated date of infection (EDI) ("early ART"), and 7 participants started ART >14 months after EDI ("late ART"). For each participant, neurocognitive functioning was measured by Global Deficit Score (GDS). HIV DNA levels were measured in peripheral blood mononuclear cells (PBMCs) and CSF cell pellets by droplet digital (dd)PCR. Soluble markers of inflammation (sCD163, IL-6, MCP-1, TNF-α) and neuronal damage (neurofilament light [NFL]) were measured in blood and CSF supernatant by immunoassays. HIV-1 partial *C2V3 env* deep sequencing data (Roche 454) were obtained for 8 paired PBMC and CSF specimens and used for phylogenetic and compartmentalization analysis. Median exposure to ART at the time of sampling was 2.6 years (IQR: 2.2–3.7) and did not differ between groups. We observed that early ART was significantly associated with lower molecular diversity of HIV DNA in CSF ($p<0.05$), and lower IL-6 levels in CSF ($p = 0.02$), but no difference for GDS, NFL, or HIV DNA detectability compared to late ART. Compartmentalization of HIV DNA populations between CSF and blood was detected in 6 out of 8 participants with available paired HIV DNA sequences (2 from early and 4 from late ART group). Phylogenetic analysis confirmed the presence of monophyletic HIV DNA populations within the CSF in 7 participants, and the same population was repeatedly sampled over a 5 months period in one participant with longitudinal sampling. Such compartmentalized provirus in the CNS needs to be considered for the design of future eradication strategies and might contribute to the neuropathogenesis of HIV.

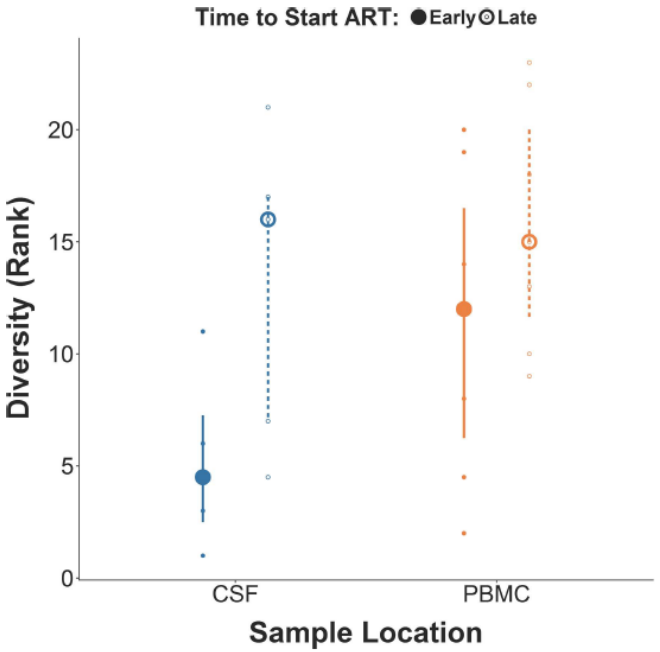


Fig 1. Comparison of molecular diversity for HIV DNA (partial *env* gene) in CSF cells and PBMC between early ART versus late ART groups. Mann Whitney comparison between early ART versus late

AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Virology

2016

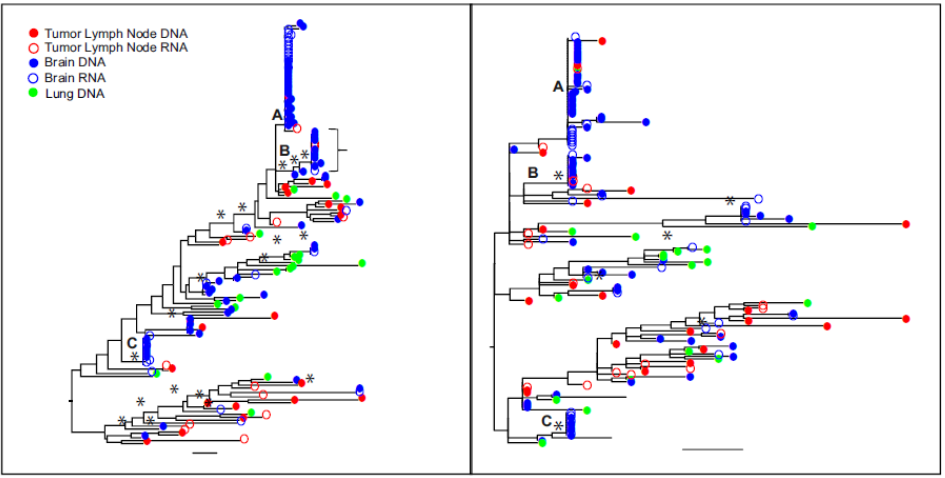
HIV Maintains an Evolving and Dispersed Population in Multiple Tissues during Suppressive Combined Antiretroviral Therapy in Individuals with Cancer

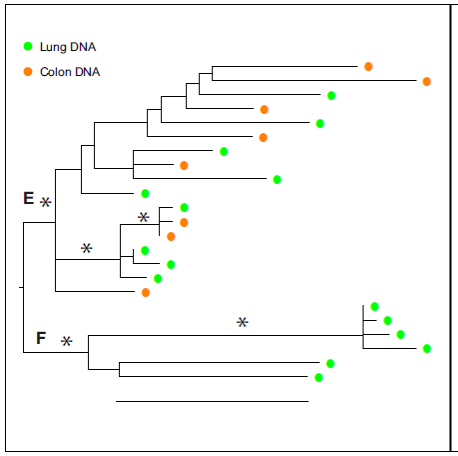
Rebecca Rose,[a] Susanna L. Lamers,[a] David J. Nolan,[a,b] Ekaterina Maidji,[c] N. R. Faria,[d] Oliver G. Pybus,[d] James J. Dollar,[b] Samuel A. Marunlak,[b] Andrew C. McAvoy,[b] Marco Salemi,[b] Cheryl A. Stoddart,[c] Elyse J. Singer,[e] Michael S. McGrath[f,g]

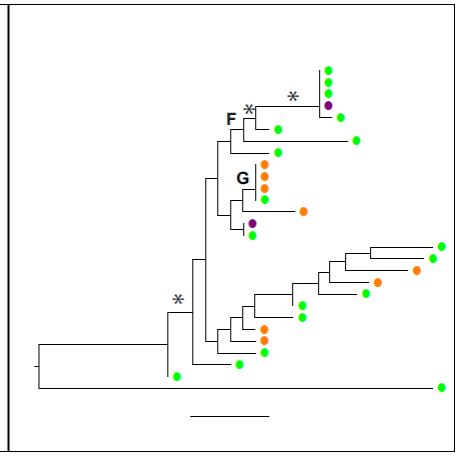
ABSTRACT

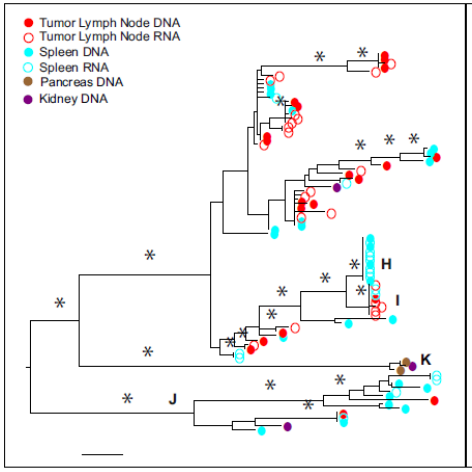
While combined antiretroviral therapy (cART) can result in undetectable plasma viral loads, it does not eradicate HIV infection. Furthermore, HIV-infected individuals while on cART remain at an increased risk of developing serious comorbidities, such as cancer, neurological disease, and atherosclerosis, suggesting that during cART, tissue-based HIV may contribute to such pathologies. We obtained DNA and RNA *env*, *nef*, and *pol* sequences using single-genome sequencing from postmortem tissues of three HIV^+ cART-treated ($cART^+$) individuals with undetectable viral load and metastatic cancer at death and performed time-scaled Bayesian evolutionary analyses. We used a sensitive *in situ* hybridization technique to visualize HIV *gag-pol* mRNA transcripts in cerebellum and lymph node tissues from one patient. Tissue-associated virus evolved at similar rates in $cART^+$ and cART-naive ($cART^-$) patients. Phylogenetic trees were characterized by two distinct features: (i) branching patterns consistent with constant viral evolution and dispersal among tissues and (ii) very recently derived clades containing both DNA and RNA sequences from multiple tissues. Rapid expansion of virus near death corresponded to wide-spread metastasis. HIV RNA^+ cells clustered in cerebellum tissue but were dispersed in lymph node tissue, mirroring the evolutionary patterns observed for that patient. Activated, infiltrating macrophages were associated with HIV RNA. Our data provide evidence that tissues serve as a sanctuary for wild-type HIV during cART and suggest the importance of macrophages as an alternative reservoir and mechanism of virus spread.

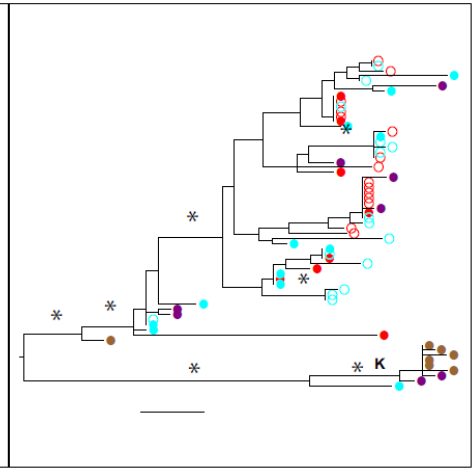
Dispersal of viral populations among tissue and evidence of activated macrophages surrounding HIV-1 expressing cells

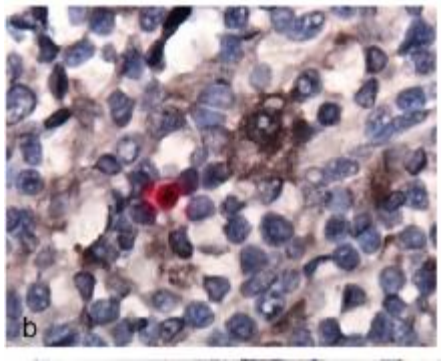


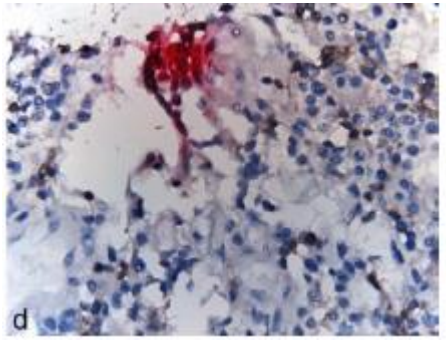












Rose R et al., 2016

Journal of NeuroVirology
https://doi.org/10.1007/s13365-018-0633-5

2018

Ultradeep single-molecule real-time sequencing of HIV envelope reveals complete compartmentalization of highly macrophage-tropic R5 proviral variants in brain and CXCR4-using variants in immune and peripheral tissues

Robin L. Brese[1] · Maria Paz Gonzalez-Perez[1] · Matthew Koch[1] · Olivia O'Connell[1] · Katherine Luzuriaga[1] · Mohan Somasundaran[1] · Paul R. Clapham[1] · James Jarad Dollar[2] · David J Nolan[2] · Rebecca Rose[2] · Susanna L. Lamers[2]

Despite combined antiretroviral therapy (cART), HIV+ patients still develop neurological disorders, which may be due to persistent HIV infection and selective evolution in brain tissues. Single-molecule real-time (SMRT) sequencing technology offers an improved opportunity to study the relationship among HIV isolates in the brain and lymphoid tissues because it is capable of generating thousands of long sequence reads in a single run. Here, we used SMRT sequencing to generate ~ 50,000 high-quality full-length HIV envelope sequences (> 2200 bp) from seven autopsy tissues from an HIV+/cART+ subject, including three brain and four non-brain sites. Sanger sequencing was used for comparison with SMRT data and to clone functional pseudoviruses for in vitro tropism assays. and 99.9%) was also performed. All brain sequences clustered exclusive of any non-brain sequences at all thresholds; however, frontal lobe sequences clustered independently of occipital and parietal lobes. Translated sequences revealed potentially functional differences between brain and non-brain sequences in the location of putative N-linked glycosylation sites (N-sites), V1 length, V3 charge, and the number of V4 N-sites. All brain sequences were predicted to use the CCR5 co-receptor, while most non-brain sequences were predicted to use CXCR4 co-receptor. Tropism results were confirmed by in vitro infection assays. The study is the first to use a SMRT sequencing approach to study HIV compartmentalization in tissues and supports other reports of limited trafficking between brain and non-brain sequences during cART. Due to the long sequence length, we could observe changes along the entire envelope gene, likely caused by differential selective pressure in the brain that may contribute to neurological disease.

1. Brain derived-viruses were compartmentalized from virus in tissue outside the brain. Lymph nodes, blood, lung and colon sequences were interspersed.

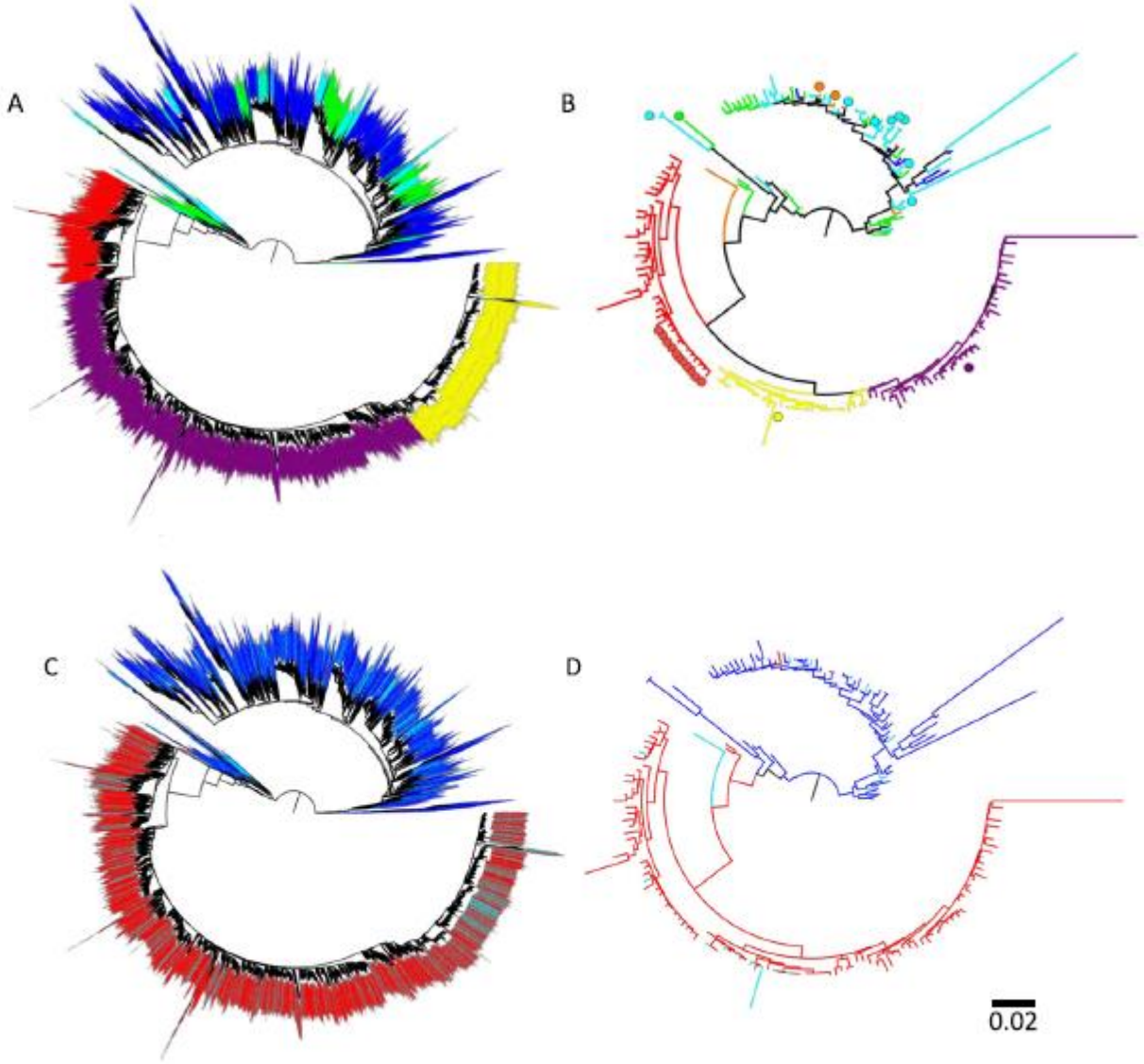


Fig. 1 Maximum-likelihood tree using the HQCS (**a**, **c**) and HQCS10 (**b**) datasets. Branches are scaled in substitutions/site according to the bar at the bottom of each tree. **a**, **b** Branches are colored to indicate the tissue of origin as follows: dark blue = blood, orange = colon, aqua = lung, green = lymph node, red = frontal lobe, yellow = occipital lobe, purple = parietal lobe. **b** Molecular clones are indicated with circles. **c** R5 and X4 variants estimated using WebPSSM x4r5 algorithm. Branches are colored as follows: blue = R5, red = X4, aqua = undetermined

1. ENV from brain tissues conferred high level of infectivity to macrophages in contrast to colon, lymph nodes, lungs. Complete exclusion of X4 variants in brain

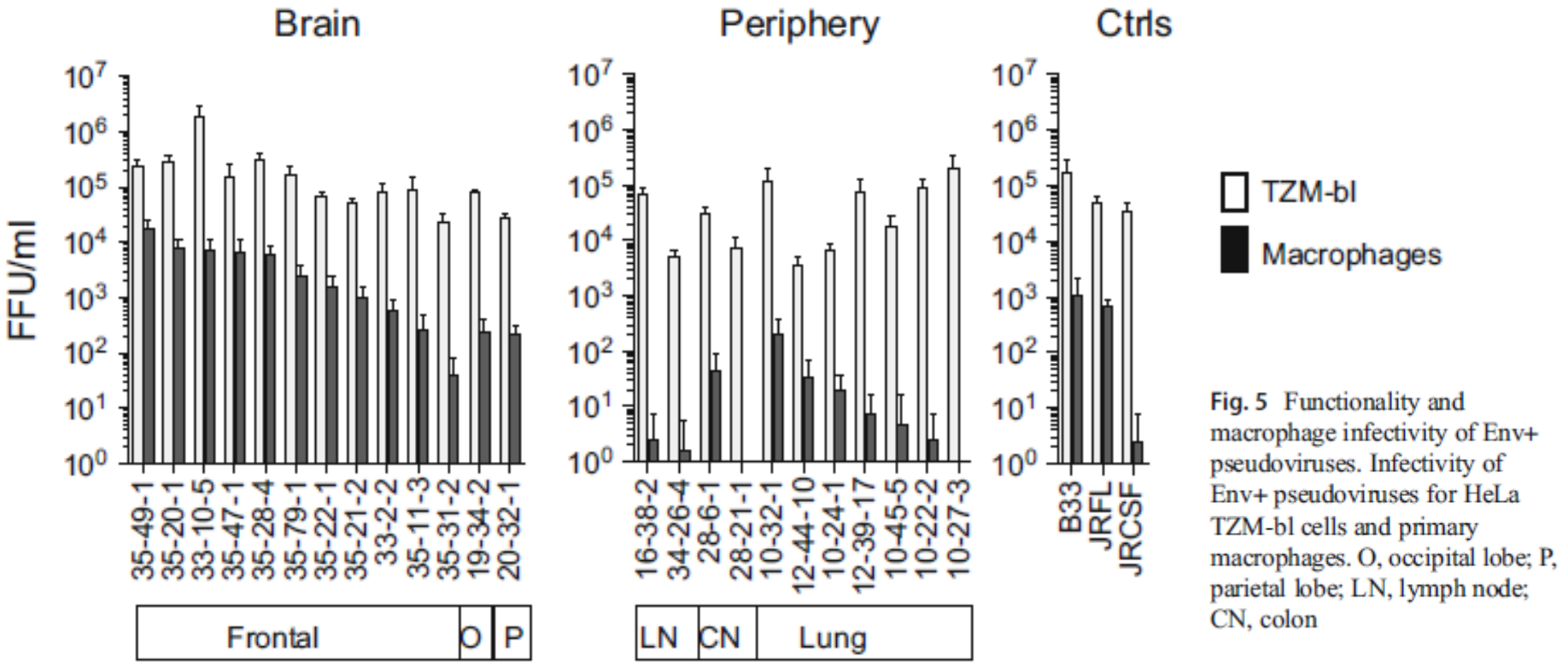
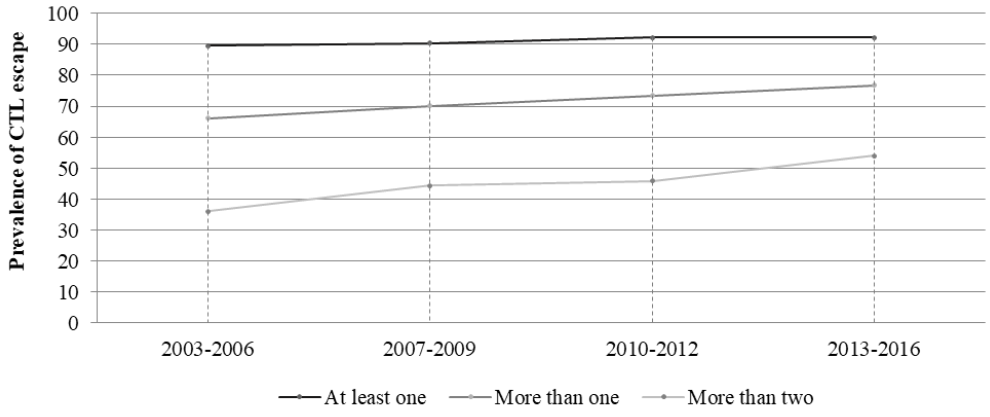


Fig. 5 Functionality and macrophage infectivity of Env+ pseudoviruses. Infectivity of Env+ pseudoviruses for HeLa TZM-bl cells and primary macrophages. O, occipital lobe; P, parietal lobe; LN, lymph node; CN, colon

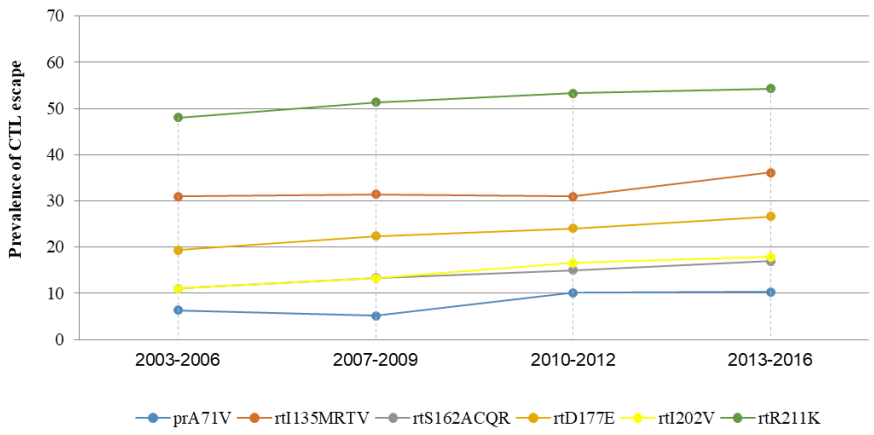
Factors that may influence compartmentalization, transcriptional potential, virus spread

- Physical isolation of a particular tissue/cell type
- Local concentrations of antiviral drugs and/or drug resistance
- Altered requirements for target cell entry and recognition (ENV variability, altered transcriptional potential, CTL escape…)

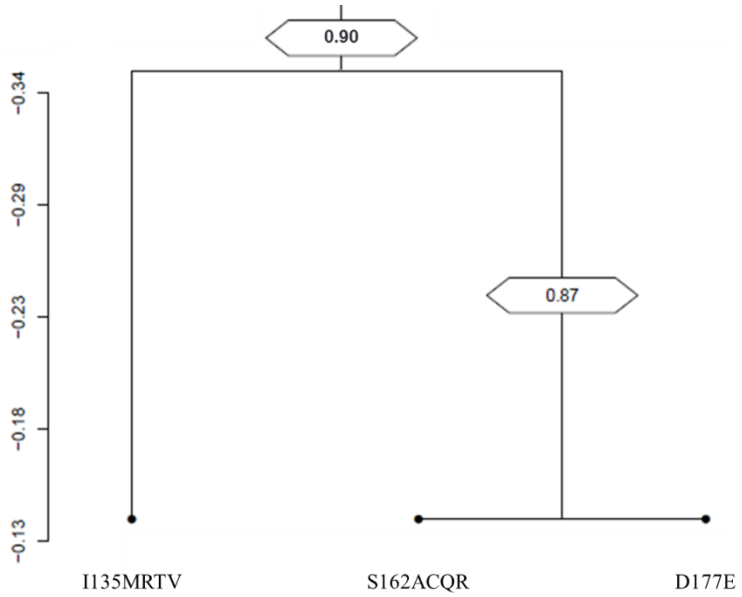
HIV-1 B subtype genetic evolution and impact on its pathogenic potential over the years 2003-2016



P-value	0.03	<0.0001	<0.0001

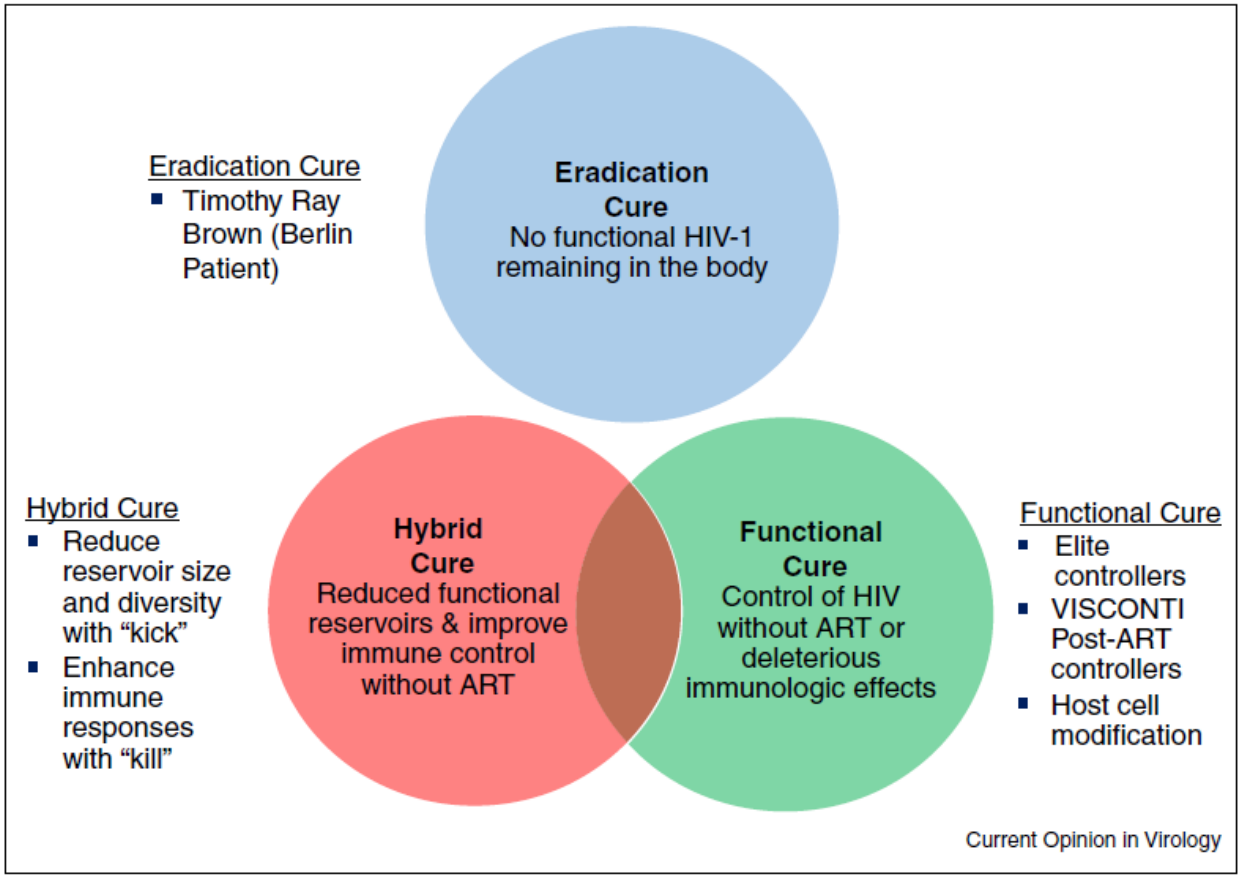


P-value	<0.0001	0.036	0.001	0.001	<0.0001	0.015



Alteri et al., Submitted

In this scenario, a hybrid cure allowing the reduction in the size and diversity of the latent reservoir could be considered



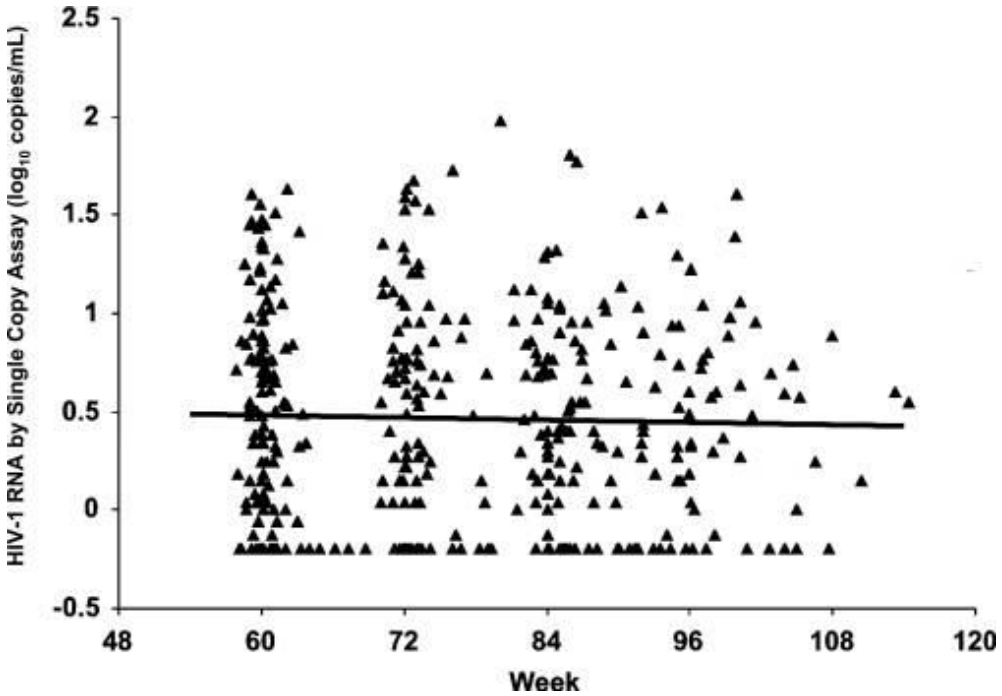
Cillo & Mellors, Current opinion in virology 2016

Markers for HIV-1 persistence:

Residual viremia

Single copy assay results revealed that >80% of patients on initial antiretroviral therapy for 60 wks had persistent viremia of one copy/ml or more with an overall median of 3.1 (range 1-49) copies/ml.

HIV-1 RNA Levels Over 50 wks of Suppressive Antiretroviral Therapy



Longitudinal studies revealed no significant decline in the level of viremia between 60 and 110 wks of suppressive antiretroviral therapy. These data suggest that the persistent viremia on current antiretroviral therapy is derived, at least in part, from long-lived cells that are infected prior to initiation of therapy.

Maldarelli et al., Plos Pathog 2007

Several studies demonstrated that an increased risk of virological failure remains even in responder patients with very low copies of viral replication during ART.

- Lambert-Niclot S, et al. Analysis and impact of ultra sensitive viral load on virological failure in 3 protease inhibitor monotherapy trials as simplification regimen (ANRS and IMEA Studies). IAS 2017, abstract MOPEB0318.
- Gianotti N, et al., Refining criteria for selecting candidates for a safe lopinavir/ritonavir or darunavir/ritonavir monotherapy in HIV-infected virologically suppressed patients. PLoS One 2017;12(2):e0171611.
- Gianotti et al., HIV DNA loads, plasma residual viraemia and risk of virological rebound in heavily treated, virologically suppressed HIV-infected patients. Clin Microbiol Infect. 2015 21:103.e7-103.e10.
- Álvarez Estévez M, et al. Quantification of viral loads lower than 50 copies per milliliter by use of the Cobas AmpliPrep/Cobas TaqMan HIV-1 test, version 2.0, can predict the likelihood of subsequent virological rebound to >50 copies per milliliter. J Clin Microbiol. 2013, 51:1555-7.
- Maggiolo F, et al. Ultrasensitive assessment of residual low-level HIV viremia in HAART-treated patients and risk of virological failure. JAIDS. 2012;60:473-82.
- Doyle T, et al.. Plasma HIV-1 RNA detection below 50 copies/ml and risk of virologic rebound in patients receiving highly active antiretroviral therapy. CID 2012, 54:724-32.
- Charpentier C, et al. Persistent low-level HIV-1 RNA between 20 and 50 copies/mL in antiretroviral-treated patients: associated factors and virological outcome. JAC 2012.67:2231-5.
- Lambert-Niclot S, et al. Factors associated with virological failure in HIV-1-infected patients receiving darunavir/ritonavir monotherapy. J Infect Dis. 2011, 204:1211-6.
- Bonora S, et al. 2009. Ultrasensitive assessment of residual HIV viremia in HAART-treated patients with persistently undetectable plasma HIV-RNA: a cross-sectional study. J Med Virol. 81:400–405.

Clinical Infectious Diseases

MAJOR ARTICLE

Ultrasensitive Human Immunodeficiency Virus Type 1 Viral Load as a Marker of Treatment Choice for Simplification Strategies

2018

Sidonie Lambert-Niclot,[1,2,3] Maxime Grude,[2] Jean-Luc Meynard,[4] Anne-Geneviève Marcelin,[1,2,5] Marc-Antoine Valantin,[6] Philippe Flandre,[2] Jacques Izopet,[7,8,9] Laetitia Moinot,[10,11] Vincent Bouteloup,[10,11] Vincent Calvez,[1,2,5] Christine Katlama,[1,2,6] Pierre-Marie Girard,[1,2,4] and Laurence Morand-Joubert[1,2,3]

Background. Using 3 randomized Protease inhibitor (PI) monotherapy studies: Kalesolo, Dream and Monoi, we performed a pooled-analysis. Our objective was to determine in PI monotherapy and standard tritherapy: 1) distribution of ultrasensitive viral load (USVL) at week 96 (W96); 2) factors associated with virological failure (VF) at W96 and 3) factors associated with USVL<1 copy at W96.

Methods. VF was defined as 2 consecutive measurements of Human Immunodeficiency Virus Type 1 RNA viral load>50 copies/mL and analysed in Intention-To-Treat. A logistic model was used to investigate which variables were predictive of a VF and Fisher test to investigate differences in USVL at W96.

Results. Among 609 patients, 73% were male with median age of 44.4 years (IQR 39.8-52.1), baseline CD4/CD8 ratio was 0.8 (IQR 0.6-1.10), baseline CD4 was 564.5/mm3 (IQR 422-707) and 59% presented a baseline USVL<1 copy/mL. At W96, the proportion of USVL<1 copy/mL was significantly different between PI monotherapy and standard tritherapy in pooled-analysis (65% versus 74%; p=0.04). Overall, baseline USVL<1copy/mL, tritherapy and to be a female were associated with USVL<1 copy/mL at W96 (p<0.0001, p=0.049 and p=0.006). In PI monotherapy receiving DRV/r was associated with USVL<1 copy/mL at W96 (p=0.003). Factors associated to virological succes at W96 were higher baseline CD4 (p=0.034) and baseline USVL<1 copy/mL (p=0.0005).

Conclusion. Pooled-analysis of 3 PI monotherapy trials showed better efficacy of tritherapy in terms of USVL at W96. Furthermore regarding USVL at W96, to receive LPV/r seems to be more deleterious than DRV/r. Baseline USVL impacts VF at W96 more specifically in tritherapy arm.

Keywords. HIV; antiretroviral strategy; PI/r monotherapy; residual viremia; virological marker.

Ultrasensitive HIV-1 viral load appears to be a useful virological marker to RV and VF at W96 for patients under PI/r monotherapy as well as for those under triple therapy

Table 4. Factors Associated With Virological Success (Viral Load < 50 Copies/mL) at W96

Multivariate Analysis			
Overall			
Variable	OR	95% CI	*P* Value
CD4 cell count (per increase of 100)	1.09	1.0–1.2	.034
USVL < 1 cp *vs* ≥ 1 cp at D0	1.92	1.3–2.8	.0005
Monotherapy group			
CD4 cell count (per increase of 100)	1.22	1.1–1.4	.002
Triple therapy group			
USVL < 1 cp *vs* ≥ 1 cp at D0	2.23	1.3–3.7	.002

Abbreviations: CI, confidence interval; OR, odds ratio; USVL, ultrasensitive viral load.

Table 5. Factors Associated With an Ultrasensitive Viral Load < 1 Copy/mL at W96

Multivariate Analysis			
Overall			
Variable	OR	95% CI	*P* Value
Female vs male	2.07	1.2–3.5	.006
Tri vs Mono	1.51	1.0–2.3	.049
USVL < 1 cp vs ≥ 1 cp at D0	2.31	1.5–3.5	<.0001
Monotherapy group			
Female *vs* male	2.35	1.2–4.7	.02
DRV vs LPV	1.93	1.1–3.5	.03
USVL < 1 cp vs ≥ 1 cp at D0	2.86	1.6–5.1	.0004
Triple therapy group			
USVL < 1 cp vs ≥ 1 cp at D0	2.0	1.1–3.6	.02

Abbreviations: CI, confidence interval; DRV, darunavir; LPV, lopinavir; OR, odds ratio; USVL, ultrasensitive viral load.

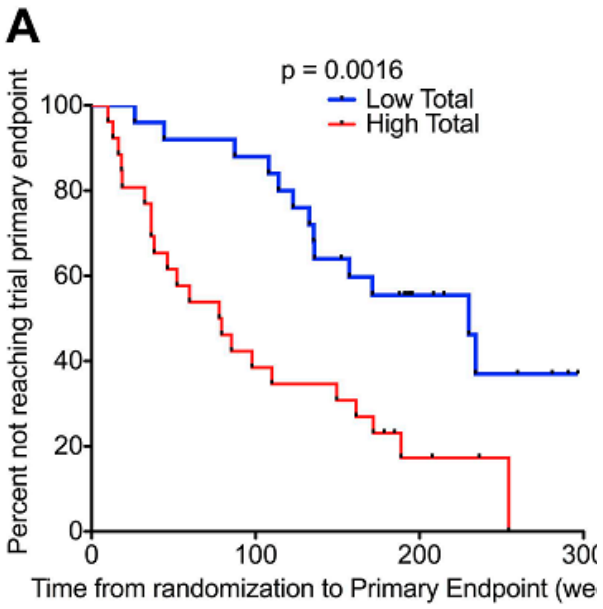
Lambert-Niclot et al., CID 2018

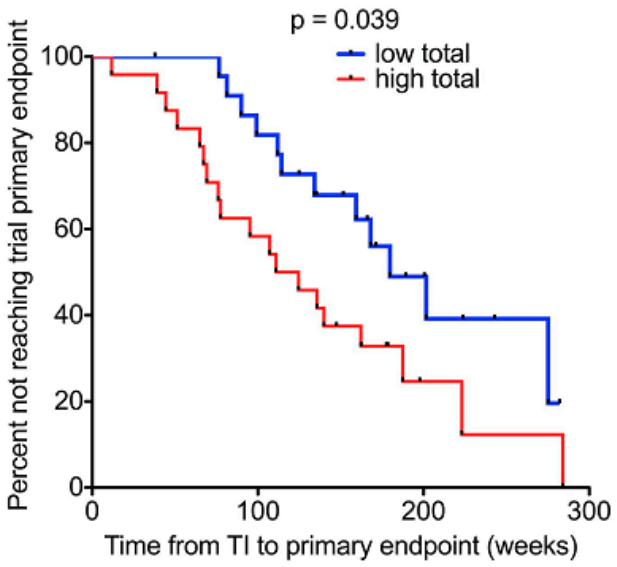
Markers for HIV-1 persistence:

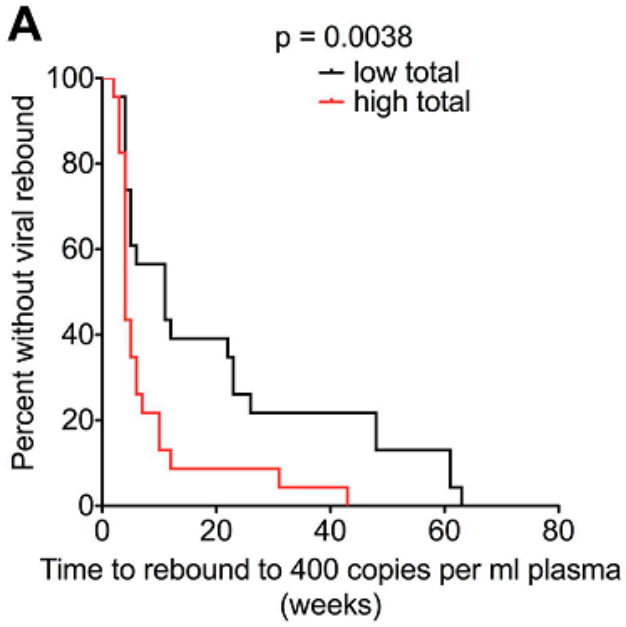
Total HIV-DNA

In particular, HIV-1 DNA may be a strong predictor of :

- clinical progression (CD4 <350) in absence of cART.
- clinical progression (CD4 <350) following treatment interruption.
- time to viral rebound.







Williams, eLife 2014

Total HIV DNA is associated with other easier-to-measure parameters in patients under successful therapy

- **Pre-therapy plasma HIV RNA**
  - *Hocqueloux, JAC 2013; Lambert-Niclot, PLoS ONE 2012*
- **Residual viremia, even when simply classified as detectable vs. undetectable**
  - *Chun, JID 2011; Lambert-Niclot, PLoS ONE 2012; Mexas, AIDS ; Parisi et al., JCM 2012; Falasca et al., JAIDS 2015; Parisi, CMI 2015.*
- **Nadir CD4 counts**
  - *Watanabe, BMCID 2011; Lambert-Niclot, PLoS ONE 2012*
- **Duration of suppression of plasma HIV RNA**
  - *Watanabe, BMCID 2011*
- **Earlier treatment start**
  - *Hocqueloux, JAC 2013*

Pre-ART HIV-1 DNA in CD4+T-cells correlates with baseline viro-immunologic status and outcome in patients under first-line ART

Francesca CECCHERINI-SILBERSTEIN1*, Alessandro COZZI LEPRI2, Claudia ALTERI14 , Esther MERLINI3, Matteo SURDO1,4, Giulia MARCHETTI3, Maria Rosaria CAPOBIANCHI55 , Andrea DE LUCA6, Nicola GIANOTTI7, Pierluigi VIALE8, Massimo ANDREONI1, Andrea ANTINORI56 , Carlo Federico PERNO5,9, Antonella D'ARMINIO MONFORTE3 7 on behalf of ICONA Foundation Study Group†

Abstract

Objectives: We evaluated the association between pre-ART HIV-DNA and HIV infected participants' characteristics at baseline as well as with their response to first-line ART.

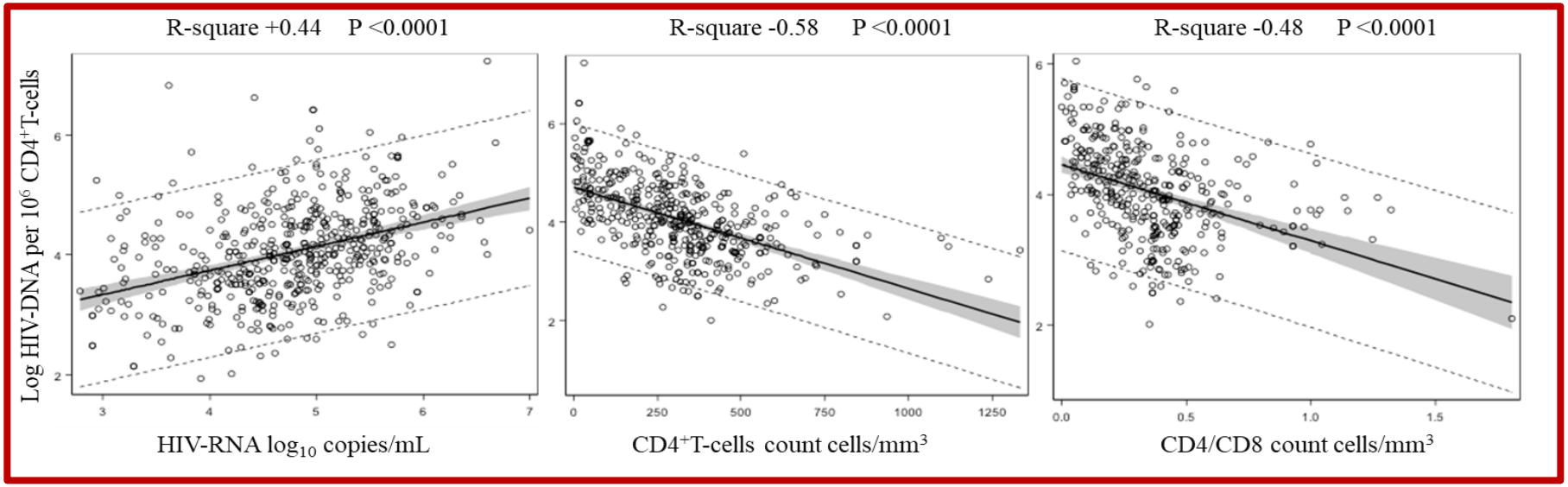
Methods: 433 patients of the ICONA-cohort, starting first-line ART after year 2000 were analysed. Pre-ART HIV-DNA was quantified with a modified COBAS TaqMan-HIV-1 Test, and normalized by CD4+ T-cells. Linear correlation between pre-ART HIV-DNA and other continuous markers (HIV-RNA, CD4 count, markers of inflammation and coagulation) at baseline was evaluated by means of Pearson correlation coefficient and linear regression-model. Survival-analyses and Cox regression models were used to study the association between pre-ART HIV-DNA and time to viro-immuno-clinical events.

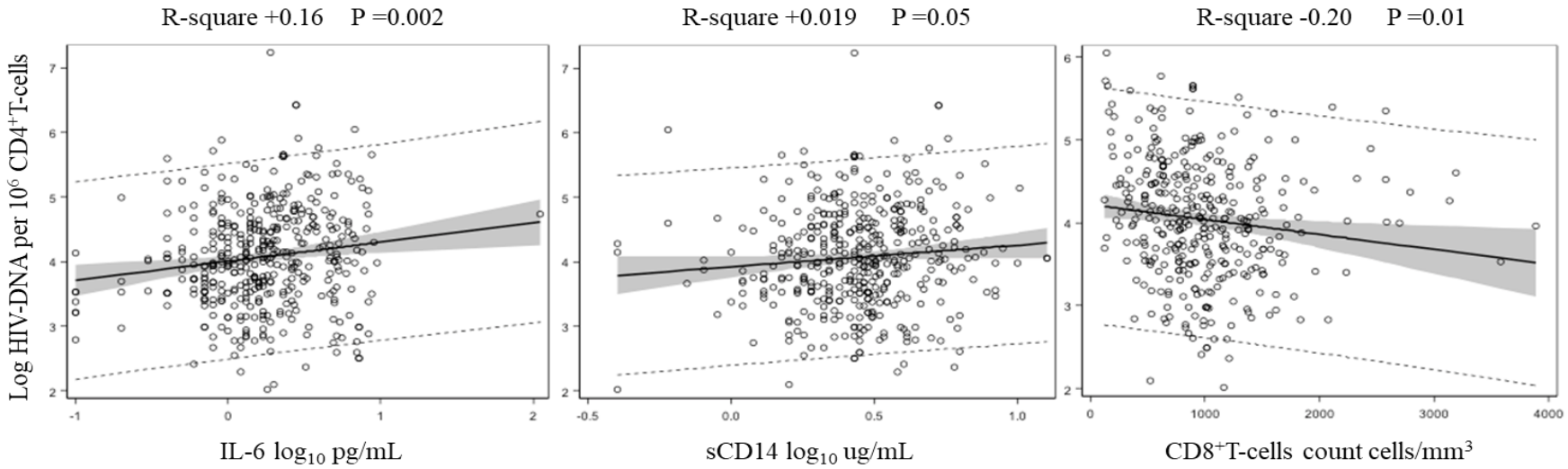
Results: Pre-ART HIV-DNA (median [IQR]: 10,702 [3,397-36,632] copies/10^6 CD4+ T-cells) was correlated with pre-ART HIV-RNA (R-square=+0.44, ($p<0.0001$)), CD4+ T-cells (-0.58, ($p<0.0001$)), CD4/CD8 ratio (-0.48, ($p<0.0001$)), while weaker correlations were observed with CD8+ T-cells (-0.20, p=0.01), IL-6 (+0.16, p=0.002) and sCD14 (+0.09, p=0.05). Patients with higher pre-ART HIV-DNA showed lower rate and delayed virological response (defined as HIV RNA≤50 copies/mL), compared with those having lower HIV-DNA (67.2% for >10,000 copies/10^6 CD4+ T-cells, 81.1% for 1,000-10,000, and 86.4% for 10-1,000,p=0.0004). Higher pre-ART HIV-DNA also correlated with increased risk of virological rebound (defined as HIV RNA>50 copies/mL) by 24-months (17.2% for >10,000, 7.4% for 1,000-10,000, 4.3% for 10-1,000, 42 p=0.0048). Adjusted hazard-ratios of all virological rebound definitions confirmed these findings ($p<0.02$).

Conclusion: Pre-ART HIV-DNA, along with HIV-RNA and CD4+ T-cells count, should be considered as a new staging marker, to better identify people at lower (or higher) risk of viral rebound following achievement of virological suppression ≤50 copies/mL.

Accepted on JAC

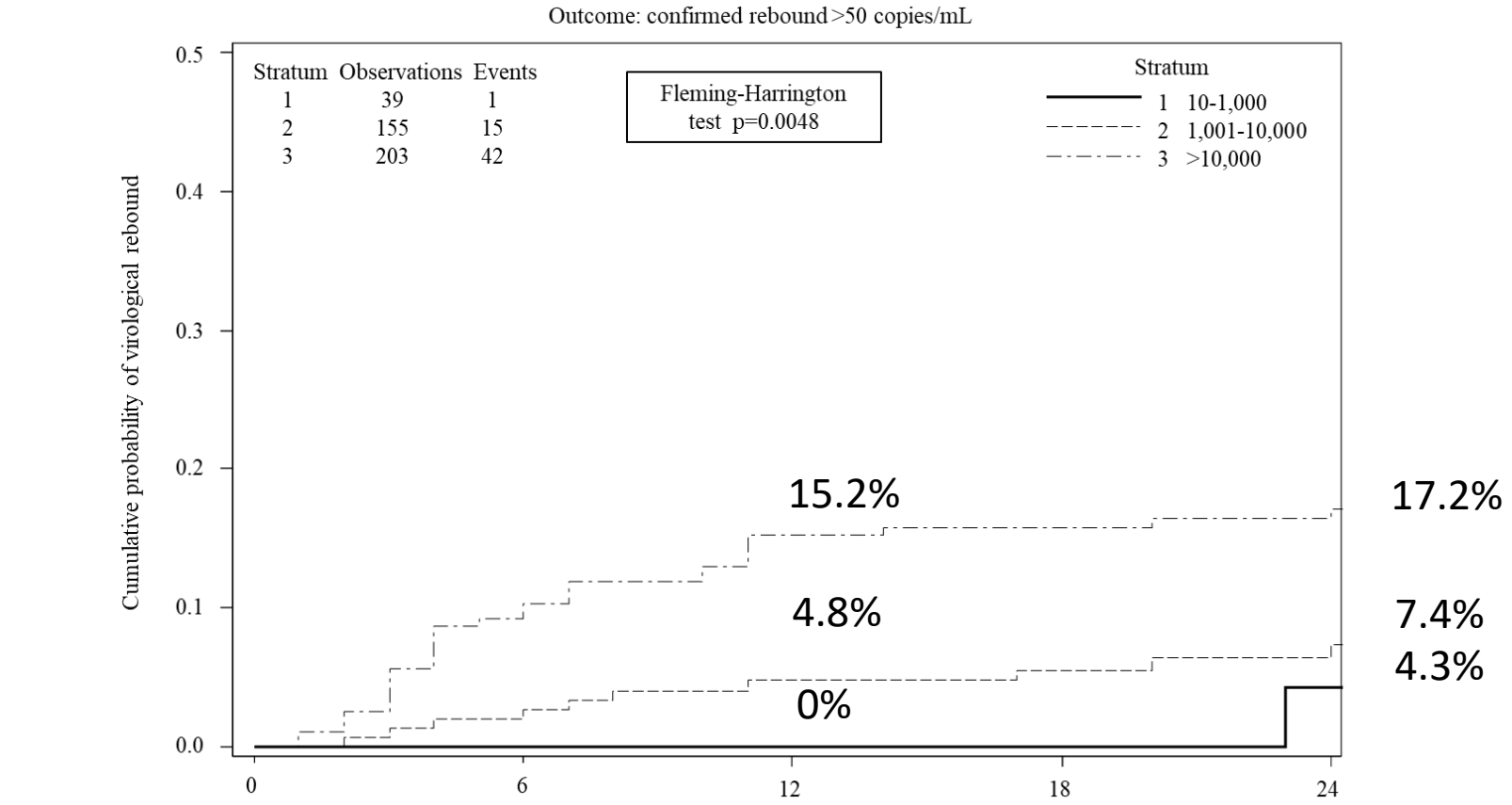
As expected, correlations between higher levels of pre-ART HIV-DNA and 1) higher plasma HIV-RNA, 2) lower CD4+ T-cells and 3) lower CD4/CD8 ratio, all collected at baseline of ART, were confirmed by significant linear correlations.





Ceccherini-Silberstein et al. , Accepted on JAC

By considering the 397 patients achieving virological suppression, **the probability of experiencing virological rebound**, defined by 2 confirmed plasma HIV-RNA >50 copies/mL, **was 12%** (95% CI: 8.6-15.5). By stratifying patients for the 3 different pre-ART HIV-DNA levels, **increasing rates of virological rebound were found by increasing pre-ART HIV-DNA.**



Ceccherini-Silberstein et al. , Accepted on JAC

Prognostic value of pre-ART HIV-DNA and pre-ART HIV-RNA on virological rebound

Looking at the risk of virological rebound in people who initially achieved virological suppression, **in the bivariable model only patients with a higher pre-ART HIV-DNA were at higher risk of virological rebound**, with all definitions of rebound (confirmed >50 copies/mL, confirmed >200 copies/mL, or single >1,000 copies/mL), independently of pre-ART HIV-RNA (HR [95% CI]: 1.96 [1.32–2.92]; HR [95% CI]: 2.94 [1.58–5.47], HR [95% CI]: 2.24 [1.36, 3.70], respectively).

In contrast, **after controlling for HIV-DNA, pre-ART HIV-RNA was not associated with the risk of viral rebound which ever was the definition**.

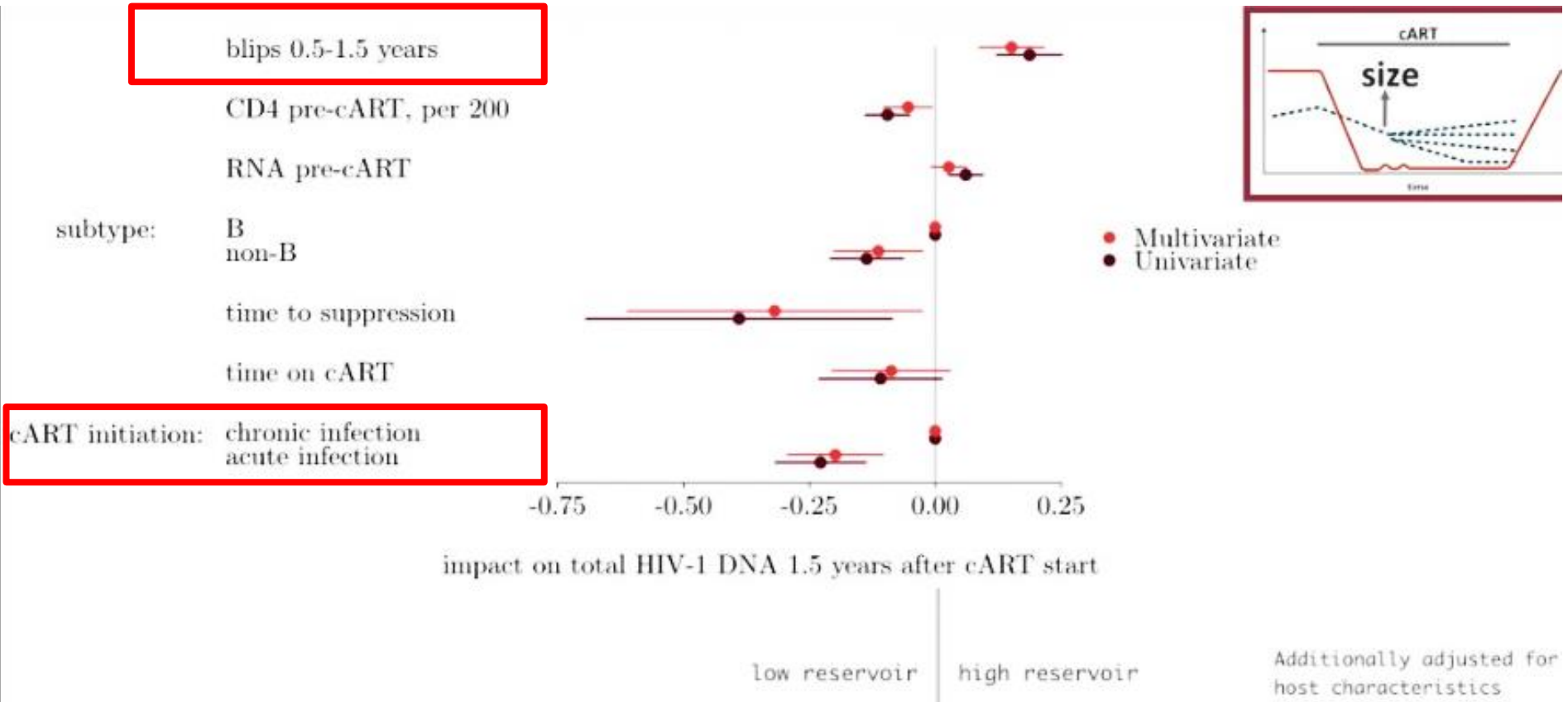
Interestingly, **in all three models with adjustment for other confounding factors, higher pre-ART HIV-DNA was confirmed to be the only factor to have a strong prognostic value for viral rebound**, whatever definition was applied for it.

Ceccherini-Silberstein et al. , Accepted on JAC

CROI
Conference on Retroviruses and Opportunistic Infections

DETERMINANTS OF HIV-1 RESERVOIR SIZE AND LONG-TERM DYNAMICS UNDER SUPPRESSIVE ART

Bachmann N, AB: LB69



Various studies have shown that the level of baseline HIV-DNA can also influence the maintenance of virological success under simplification therapy

J Med Virol. 2007 Jul;79(7):880-6.

Cellular HIV-1 DNA quantitation in patients during simplification therapy with protease inhibitor-sparing regimens.

Sarmati L[1], Parisi SG, Nicastri E, d'Ettorre G, Andreoni C, Dori L, Gatti F, Montano M, Buonomini AR, Boldrin C, Palù G, Vullo V, Andreoni M.

J Antimicrob Chemother. 2010 May;65(5):1005-7. doi: 10.1093/jac/dkq084. Epub 2010 Mar 18.

Impact of 48 week lopinavir/ritonavir monotherapy on blood cell-associated HIV-1-DNA in the MONARK trial.

Avettand-Fenoel V[1], Flandre P, Chaix ML, Ghosn J, Delaugerre C, Raffi F, Ngovan P, Cohen-Codar I, Delfraissy JF, Rouzioux C; MONARK Study Group.

HIV Clin Trials. 2013 May-Jun;14(3):120-6. doi: 10.1310/hct1403-120.

Long-term HIV-1 virologic control in patients on a dual NRTI regimen.

Prazuck T[1], Zucman D, Avettand-Fènoël V, Ducasse E, Bornarel D, Mille C, Rouzioux C, Hocqueloux L.

Role of Baseline HIV-1 DNA Level in Highly-Experienced Patients Receiving Raltegravir, Etravirine and Darunavir/Ritonavir Regimen (ANRS139 TRIO Trial)

PLOS ONE

2013

Charlotte Charpentier[1]*, Catherine Fagard[2,3], Céline Colin[2,3], Christine Katlama[4], Jean-Michel Molina[5], Christine Jacomet[6], Benoit Visseaux[1], Anne-Marie Taburet[7], Françoise Brun-Vézinet[1],

Virological Factors Associated With Outcome of Dual ETR/RAL Therapy (ANRS-163 Trial)

16TH EUROPEAN AIDS CONFERENCE October 25-27, 2017 Milan, Italy

PS6/4

Cathia Soulie, Lambert Assoumou, Sophie Sayon, Thuy Nguyen, Marc-Antoine Valantin, Virginie Ferre, Chakib Alloui, Brigitte Montes, Véronique Avettand-Fenoel, Constance Delaugerre, Diane Descamps, Esteban Martinez, Jacques Reynes, Gilles Peytavin, Dominique Costagliola, Christine Katlama, Vincent Calvez, Anne-Geneviève Marcelin.

FACTORS PREDICTING VIROLOGICAL FAILURE DURING DOLUTEGRAVIR MAINTENANCE MONOTHERAPY

Ingeborg Wijting, Sofie L. Rutsaert, Casper Rokx, David M. Burger, Elrozy Andrinopoulou[1], Linos Vandekerckhove, Bart Rijnders

CROI Conference on Retroviruses and Opportunistic Infections

The level of baseline HIV-DNA is also associated with the level of HIV-DNA, residual viremia and/or the presence of viral blips after therapy simplification

J Acquir Immune Defic Syndr. 2016 May 1;72(1):46-51. doi: 10.1097/QAI.0000000000000966.

NRTI Sparing Therapy in Virologically Controlled HIV-1 Infected Subjects: Results of a Controlled, Randomized Trial (Probe).

Maggiolo F[1], Di Filippo E, Valenti D, Serna Ortega PA, Callegaro A.

Author information

In AtLaS-M trial…for each log increase of BL HIV-1 DNA the odds of detectable residual viremia at W96 increased by 2.47.

Table 4 BL HIV-1 DNA as predictor of W96 residual viremia

			W96 undetectable/detectable		
	B	OR	confidence Interval 95 %		p
BL log HIV-1 DNA	0.903	2.47	1.17	5.19	0.017

The AtLaS-M trial showed that in virologically suppressed patients on atazanavir/ritonavir (ATV/r) with 2 NRTIs, switching to a dual therapy with ATV/r plus lamivudine (3TC) had superior efficacy as compared to continuing the previous triple therapy. **This sub-study was designed to evaluate the impact at 96 weeks of the dual therapy versus the triple therapy on the HIV-1 cellular reservoir as reflected by the quantification of the blood-associated HIV-1 DNA levels.**

CROI 2017 seattle

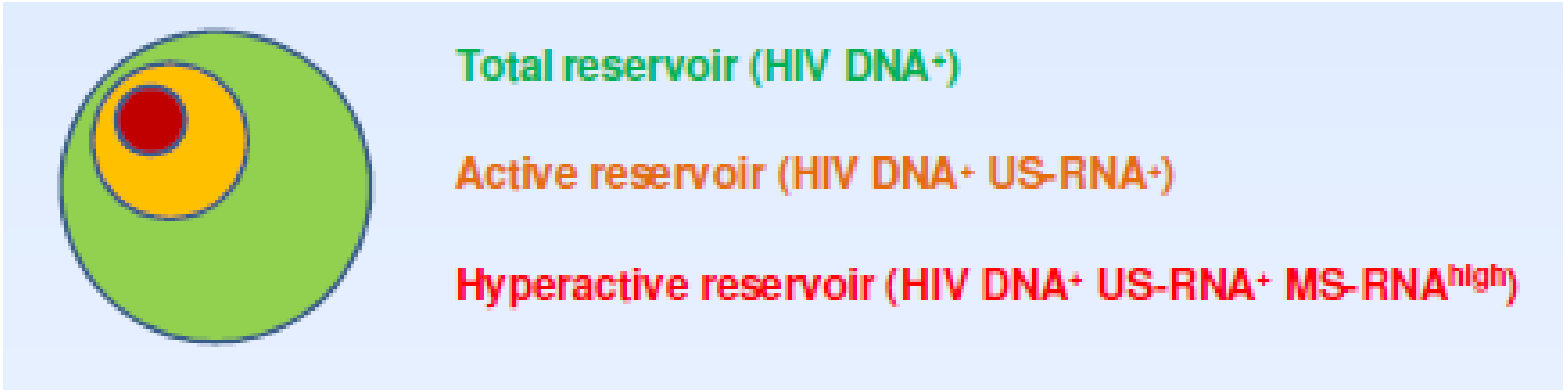
February 13-16, 2017

Lombardi et al., 2017 Abstr445

Markers for HIV-1 persistence:

CA HIV-RNA

Viral transcriptional activity as future marker for clinical progression and therapy monitoring (?)

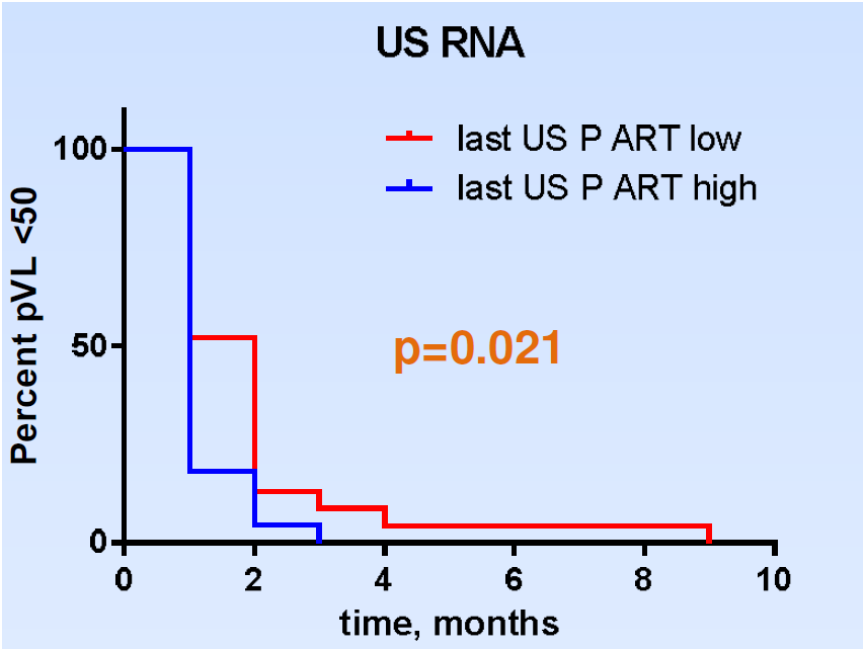


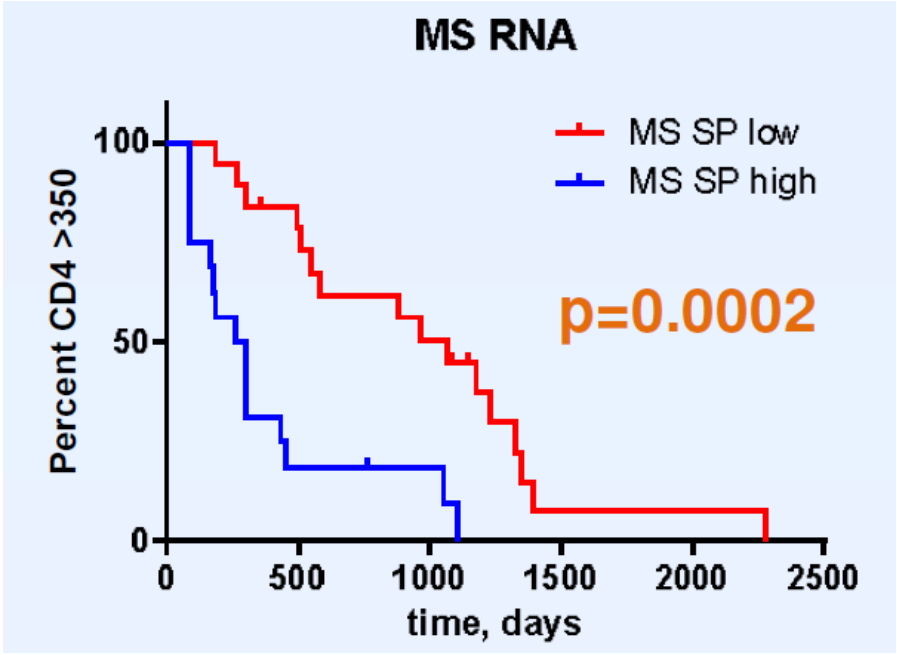
Modified from Pasternak, Abstr N. 286 CROI 2017

Cell-Associated HIV RNA Predicts Post-Treatment

HIV Control and CD4+ T-cell Loss

Cell-associated HIV-1 US RNA was the sole predictor of the duration of post-treatment virological control after the interruption of early ART, whereas MS RNA independently predicted subsequent CD4+ T-cell loss.





Pasternak, Abstr N. 286 CROI 2017

- **The spotlight entry of CRISPR/Cas9 technology**

Multiplex Genome Engineering Using CRISPR/Cas Systems

Le Cong[1,2,*], F. Ann Ran[1,4,*], David Cox[1,3], Shuailiang Lin[1,5], Robert Barretto[6], Naomi Habib[1], Patrick D. Hsu[1,4], Xuebing Wu[7], Wenyan Jiang[8], Luciano A. Marraffini[8], and Feng Zhang[1,†]

Science 2013

RNA-directed gene editing specifically eradicates latent and prevents new HIV-1 infection

Wenhui Hu[a,1,2], Rafal Kaminski[a,1], Fan Yang[a], Yonggang Zhang[a], Laura Cosentino[a], Fang Li[a], Biao Luo[b], David Alvarez-Carbonell[c], Yoelvis Garcia-Mesa[c], Jonathan Karn[c], Xianming Mo[d], and Kamel Khalili[a,2]

PNAS 2014

Genome editing strategies: potential tools for eradicating HIV-1/AIDS

Kamel Khalili*, Jennifer Gordon, Laura Cosentino, and Wenhui Hu*

J Neurovirology 2015

- **The spotlight entry of CRISPR/Cas9 technology**

Multiplex Genome Engineering Using CRISPR/Cas Systems

Le Cong[1,2,*], F. Ann Ran[1,4,*], David Cox[1,3], Shuailiang Lin[1,5], Robert Barretto[6], Naomi Habib[1], Patrick D. Hsu[1,4], Xuebing Wu[7], Wenyan Jiang[8], Luciano A. Marraffini[8], and Feng Zhang[1,†]

Science 2013

The type II prokaryotic CRISPR (clustered regularly interspaced short palindromic repeats)/Cas nuclease adaptive immune system has been shown to facilitate RNA-guided site-specific DNA cleavage.

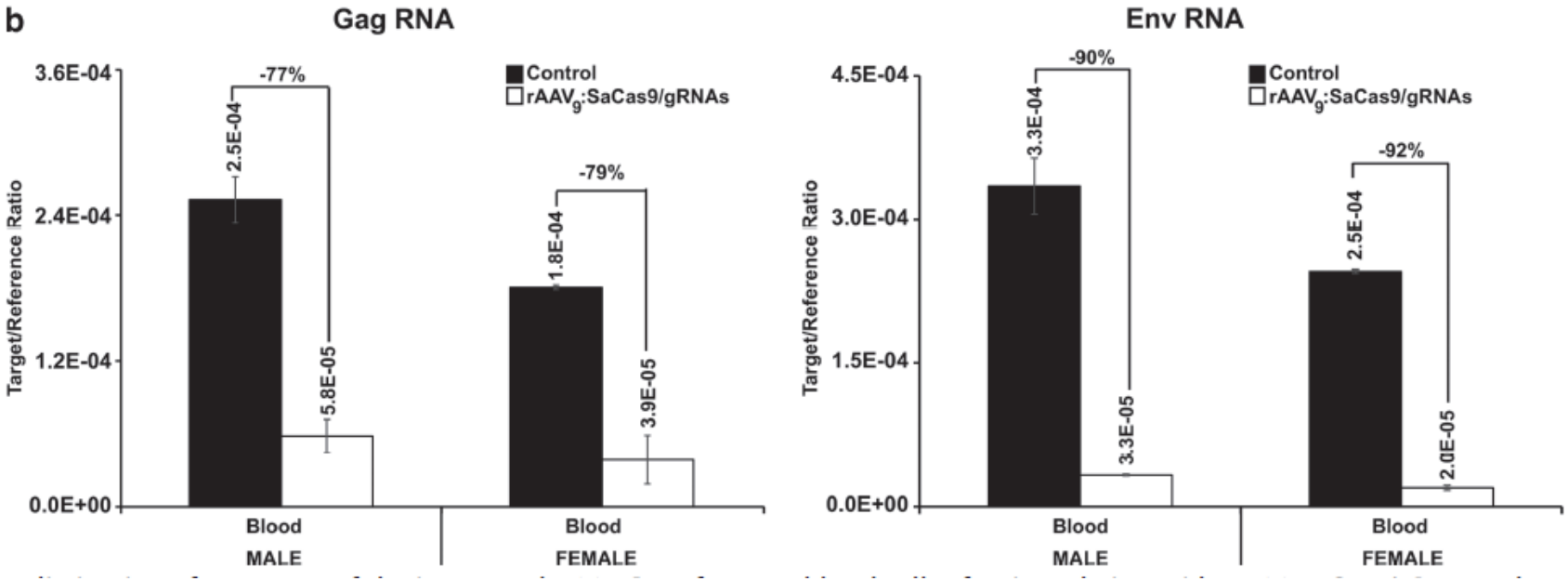
We engineered two different type II CRISPR/Cas systems and demonstrate that Cas9 nucleases can be directed by short RNAs to induce precise cleavage at endogenous genomic loci in human and mouse cells. Cas9 can also be converted into a nicking enzyme to facilitate homology-directed repair with minimal mutagenic activity.

SHORT COMMUNICATION

Excision of HIV-1 DNA by gene editing: a proof-of-concept *in vivo* study

R Kaminski[1], R Bella[2], C Yin[1], J Otte[1], P Ferrante[2], HE Gendelman[3], H Li[4], R Booze[4], J Gordon[1], W Hu[1] and K Khalili[1]

Crispr/Cas9 inoculation resulted in the cleavage of integrated HIV-1 DNA and substantially decreased the level of viral gene expression in circulating blood lymphocytes



Gene Therapy 2016

SCIENTIFIC REPORTS

OPEN

A combinational CRISPR/Cas9 gene-editing approach can halt HIV replication and prevent viral escape

Received: 27 September 2016
Accepted: 30 December 2016

Robert Jan Lebbink, Dorien C. M. de Jong, Femke Wolters, Elisabeth M. Kruse, Petra M. van Ham, Emmanuel J. H. J. Wiertz & Monique Nijhuis

HIV presents one of the highest evolutionary rates ever detected and combination antiretroviral therapy is needed to overcome the plasticity of the virus population and control viral replication. Conventional treatments lack the ability to clear the latent reservoir, which remains the major obstacle towards a cure. Novel strategies, such as CRISPR/Cas9 gRNA-based genome-editing, can permanently disrupt the HIV genome. However, HIV genome-editing may accelerate viral escape, questioning the feasibility of the approach. Here, we demonstrate that CRISPR/Cas9 targeting of single HIV loci, only partially inhibits HIV replication and facilitates rapid viral escape at the target site. A combinatorial approach of two strong gRNAs targeting different regions of the HIV genome can completely abrogate viral replication and prevent viral escape. Our data shows that the accelerating effect of gene-editing on viral escape can be overcome and as such gene-editing may provide a future alternative for control of HIV-infection.

SCIENTIFIC REPORTS

OPEN

A combinational CRISPR/Cas9 gene-editing approach can halt HIV replication and prevent viral escape

Received: 27 September 2016
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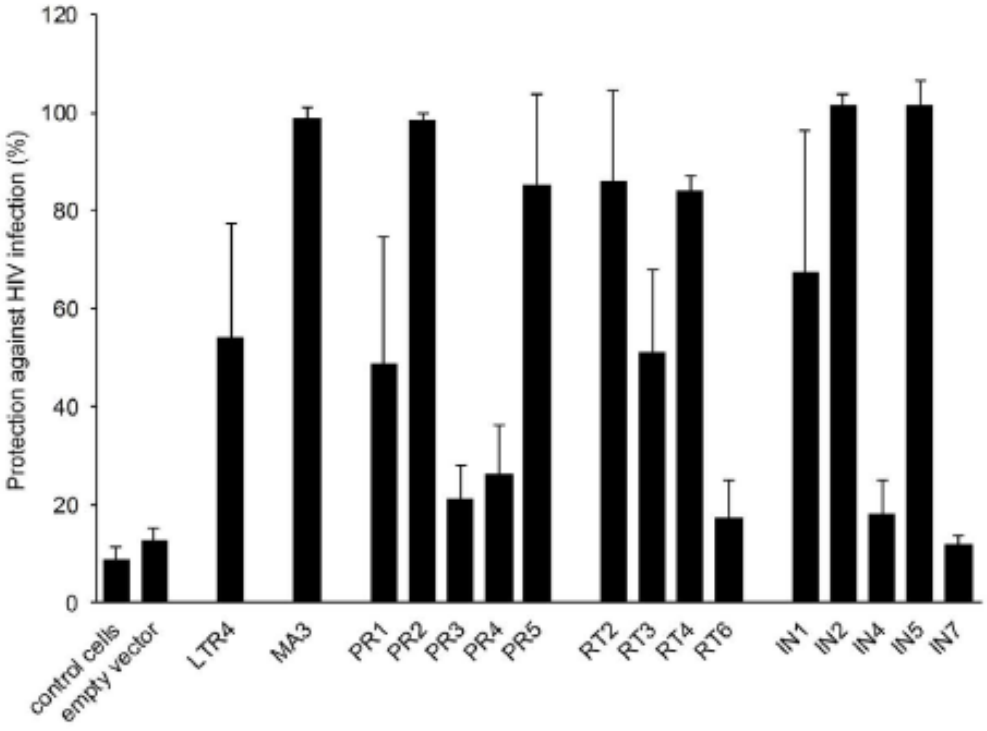
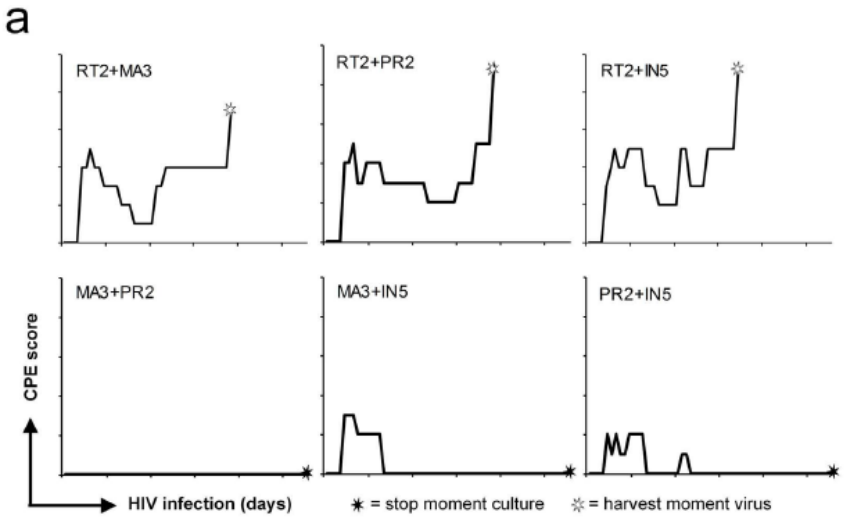
Robert Jan Lebbink, Dorien C. M. de Jong, Femke Wolters, Elisabeth M. Kruse, Petra M. van Ham, Emmanuel J. H. J. Wiertz & Monique Nijhuis





“Before moving to the clinic the CRISPR/Cas9 must solve several major issues:

- Selection of resistance
- Presence of mutant viral strains showing poor or no-cleavage at all
- Delay but no complete elimination of viral replication
- Need of a safe and effective mechanisms of delivery
- No platform for gRNA candidate evaluation
- No data about access to all tissues (Cas9/gRNA nanoparticle formulations)

From Soriano V. in Gene Therapy with CRISPR/Cas9 Coming to Age for HIV Cure AIDS Rev 2017

Conclusions

- ✓ **HIV-1 is incurable** to date because effective antiviral therapies target only replicating viruses.
- ✓ **The Berlin Patient**: a proof of concept that a "functional cure" may be possible.
- ✓ There is still debate regarding the origin of the **residual viremia.**
- ✓ The identification of **cells carrying replication competent or/ and trascriptionally active viruses** is still far

Conclusions

- ✓ **Molecular knives,** as the CRISPR/Cas9 system, can excise any genome, thus provide a great opportunity to destroy the HIV-1 genome….a promising pathway to HIV cure….

- ✓ **Treating patients as soon as possible** at the early stages of acute infection

Today still essential the construction and management of antiretroviral therapy designed taking into account a long-term strategy finalized to decrease to the lowest possible level of HIV replication and disease /comorbidity progression