Hot topics sulla patogenesi di HIV

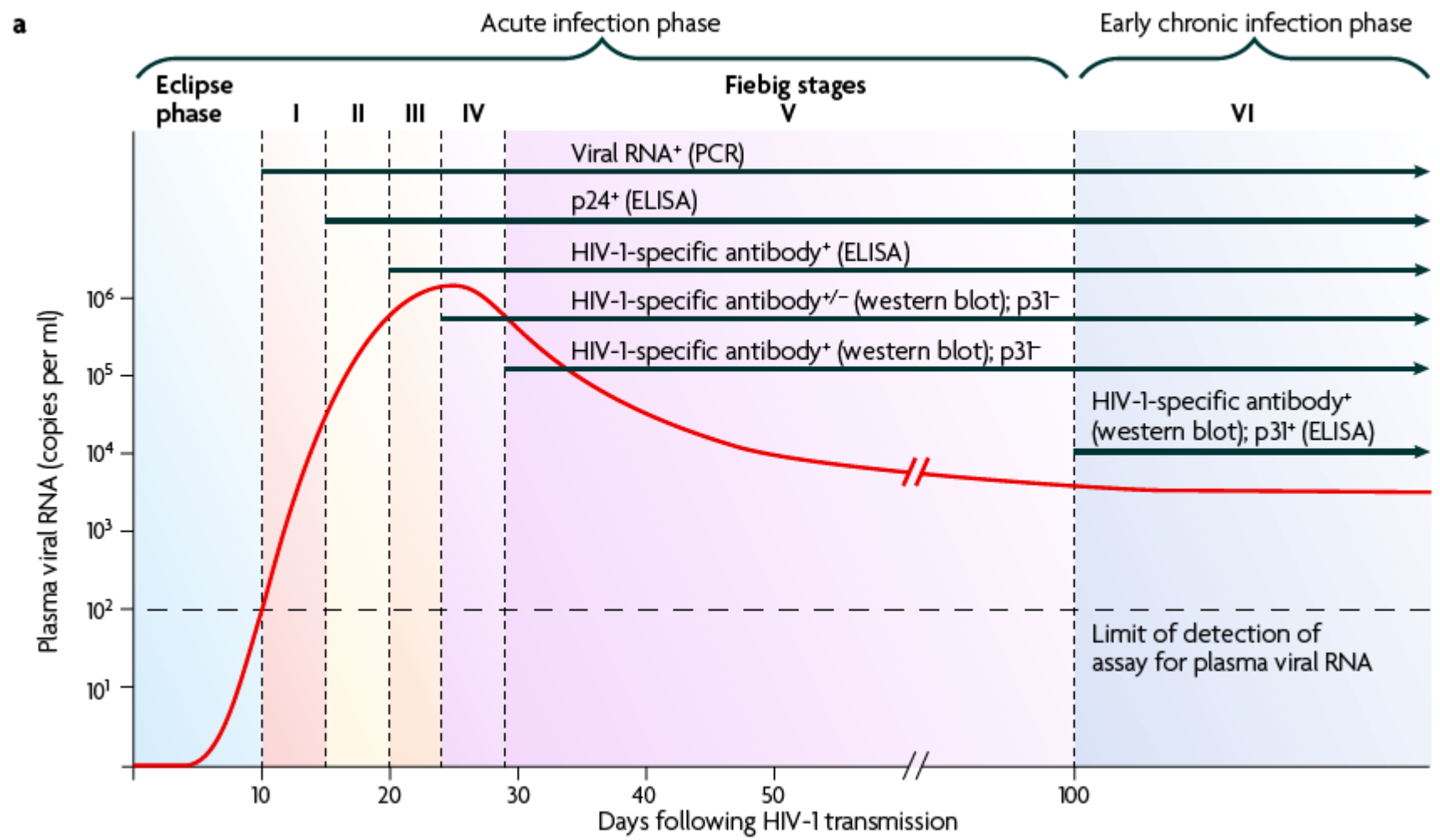
G. Tambussi

IRCSS-OSR Milano

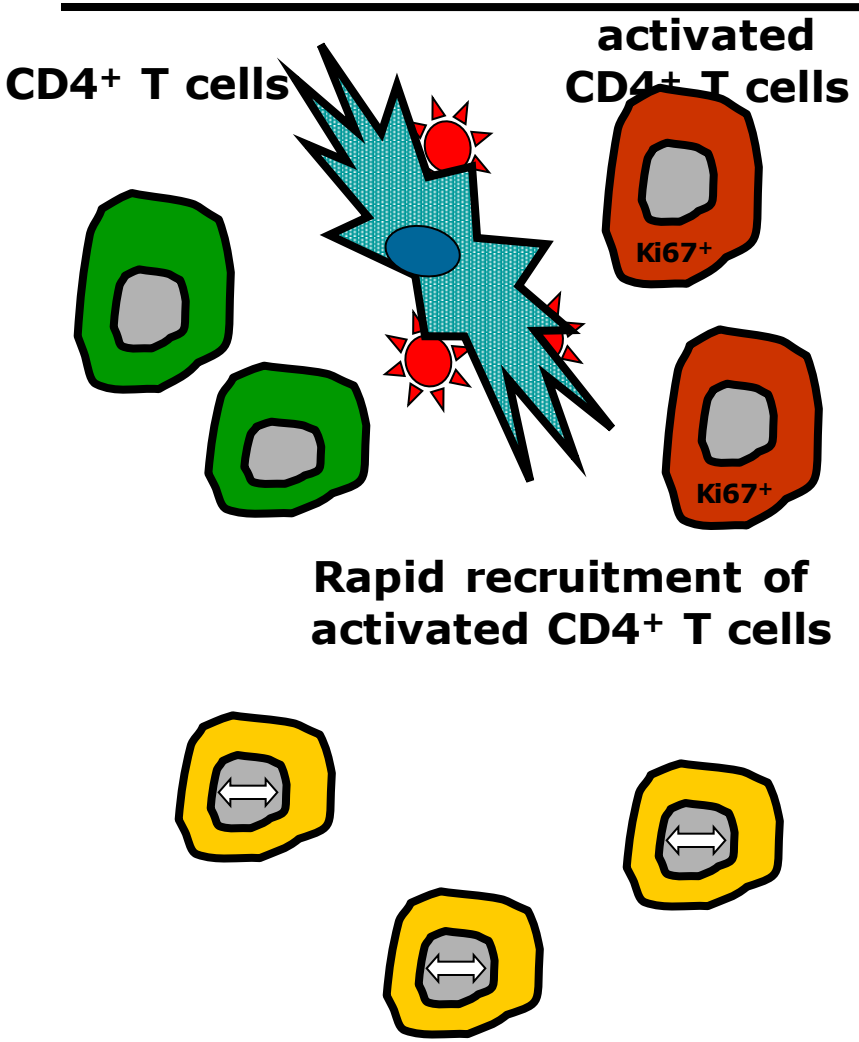
1, 2, 3, 4, 5: mate

1. Early establishment of pool of latently infected cells
2. Impairment of specific anti-HIV immune responses
3. Persistent immunoactivation (gut damage)
4. Lymphoid tissue disruption
5. …

Definition of acute HIV-1 infection

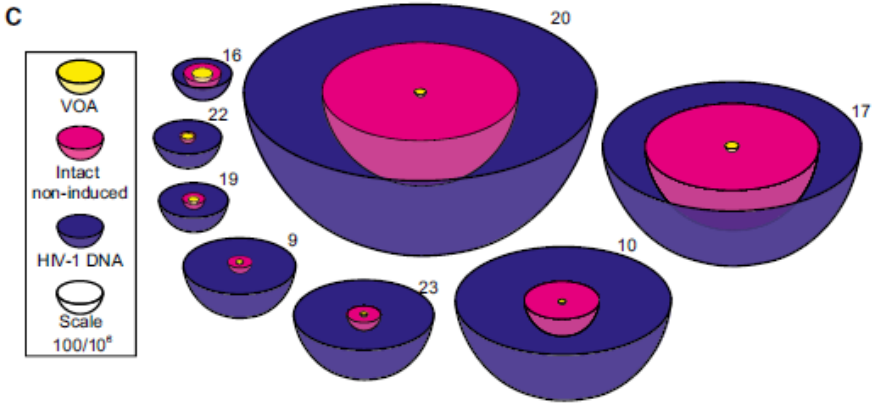


Timing of pathogenic mechanisms involved in the establishment of persistent infection *(i)*



Replication-Competent Noninduced Proviruses in the Latent Reservoir Increase Barrier to HIV-1 Cure

Ya-Chi Ho,[1] Liang Shan,[1,5] Nina N. Hosmane,[1] Jeffrey Wang,[2] Sarah B. Laskey,[1] Daniel I.S. Rosenbloom,[3] Jun Lai,[1] Joel N. Blankson,[1] Janet D. Siliciano,[1] and Robert F. Siliciano[1,4,*]



The identification of replication-competent noninduced proviruses indicates that the size of the latent reservoir—and, hence, the barrier to cure—may be up to 60-fold greater than previously estimated.

Cell *155*, 540–551, October 24, 2013

Pool of HIV latently infected cells containing replication competent virus

HIV infection and reactivation of CD4+ T Cells

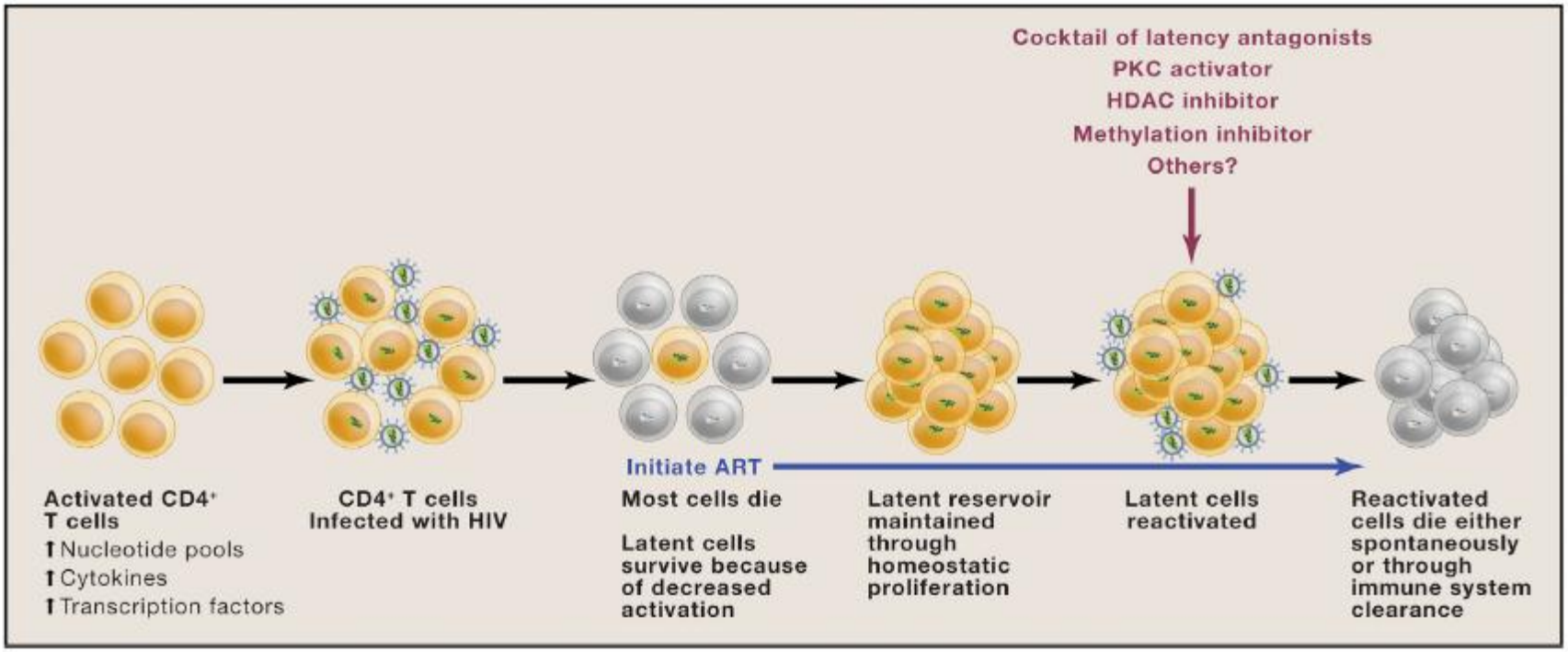


Figure 1. HIV-1 Infection and Reactivation of CD4+ T Cells

HIV-1 infects activated CD4+ T cells, which have increased nucleotide pools, cytokines, and transcription factors compared to nonactivated cells. Most of these infected cells die due to cytopathic effects of the virus or lysis by HIV-specific CTLs. Cells that revert back to a resting memory T cell survive and may undergo homeostatic proliferation. Upon initiation of ART, these latently infected cells persist. However, treatment of these cells with reactivating agents causes the cells to actively produce virus and ultimately leads to either spontaneous cell death or death through immune system clearance.

Cell *155*, October 24, 2013 ©2013 Elsevier Inc.

Contribution of T cell subset to HIV reservoir

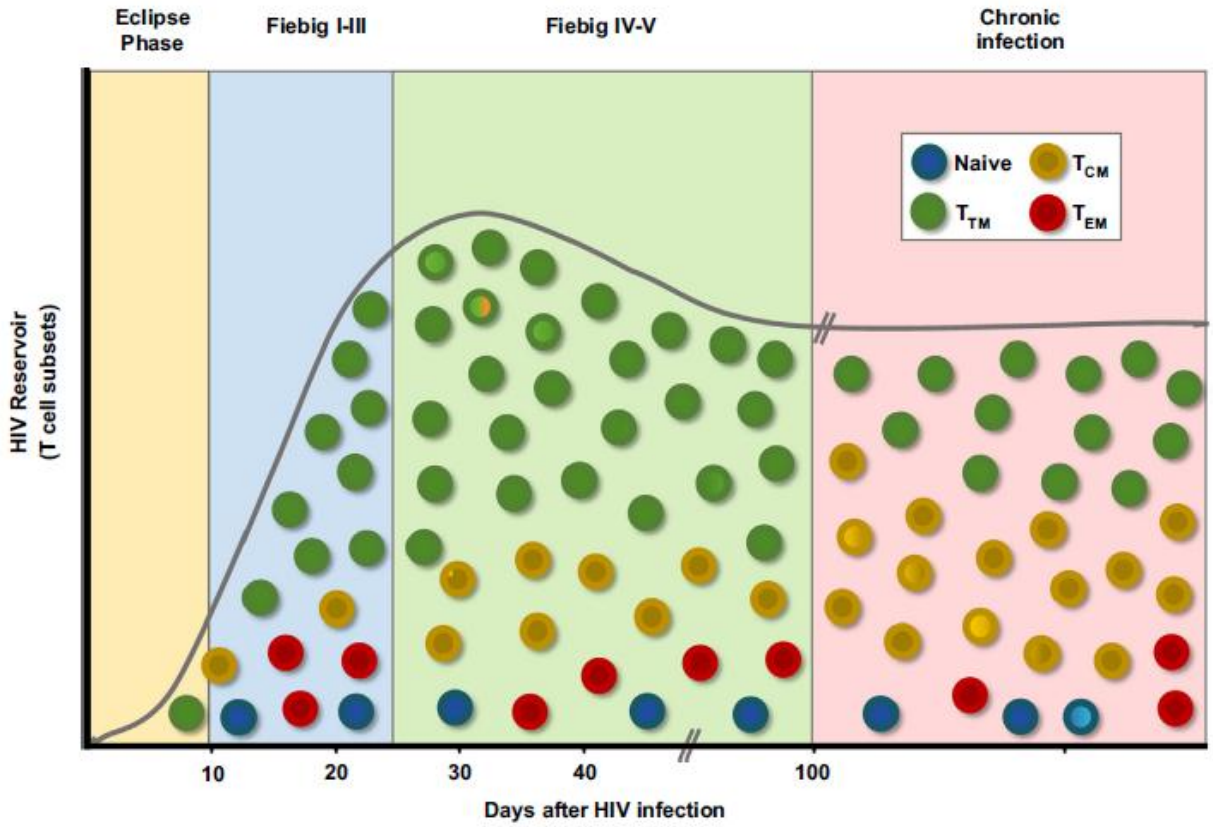


Fig. 2. Schematic representation of the HIV acute infection and the contribution of each T cell subset to the establishment of the HIV reservoir. Early treatment during Fiebig stages I–III may limit the number of infected cells and protect T_{CM} cells from infection. Treatment during Fiebig stages IV-V may decrease the contribution of long-lived T_N and T_{CM} cells to the reservoir due to low relative abundance of these cell subsets. Frequency of T_{CM} normalizes during chronic infection increasing the contribution of these cells to the reservoir.

C.P. Passaes, A. Sáez-Cirión / Virology 454-455 (2014) 340–352

1, 2, 3, 4, 5: mate

1 Early establishment of pool of latently infected cells

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3 Persistent immunoactivation (gut damage)

4 Lymphoid tissue disruption

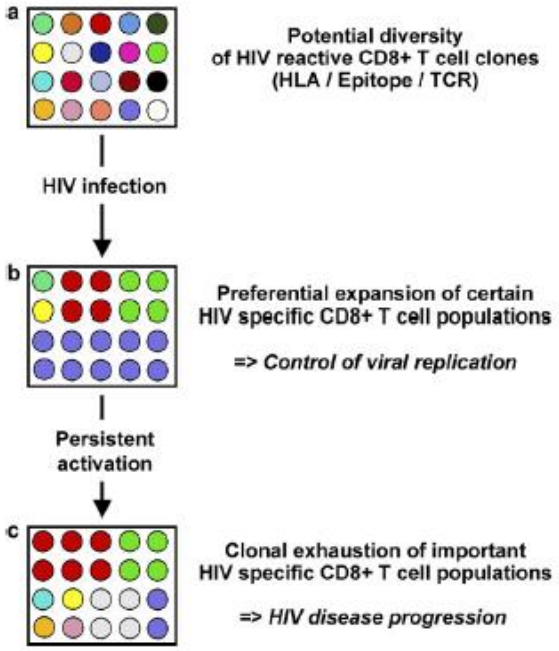
5 …

Timing of pathogenic mechanisms involved in the establishment of persistent infection *(ii)*

HIV preferentially infects HIV-specific $CD4^+$ T cells

Daniel C. Douek*†‡, Jason M. Brenchley*‡, Michael R. Betts*, David R. Ambrozak*, Brenna J. Hill*, Yukari Okamoto*, Joseph P. Casazza§, Janaki Kuruppu*, Kevin Kunstman‖, Steven Wolinsky‖, Zvi Grossman¶, Mark Dybul#, Annette Oxenius☆, David A. Price☆, Mark Connors# & Richard A. Koup*

NATURE | VOL 417 | 2 MAY 2002



V. Appay et al. / Experimental Gerontology 42 (2007) 432–437

Upregulation of CTLA-4 by HIV-specific $CD4^+$ T cells correlates with disease progression and defines a reversible immune dysfunction

Daniel E Kaufmann[1,7], Daniel G Kavanagh[1,7], Florencia Pereyra[1,2], John J Zaunders[3], Elizabeth W Mackey[1], Toshiyuki Miura[1,4], Sarah Palmer[5], Mark Brockman[1,4], Almas Rathod[1], Alicja Piechocka-Trocha[1,4], Brett Baker[1], Baogong Zhu[6], Sylvie Le Gall[1], Michael T Waring[1,4], Ryan Ahern[1], Kristin Moss[1], Anthony D Kelleher[3], John M Coffin[5], Gordon J Freeman[6], Eric S Rosenberg[1] & Bruce D Walker[1,4]

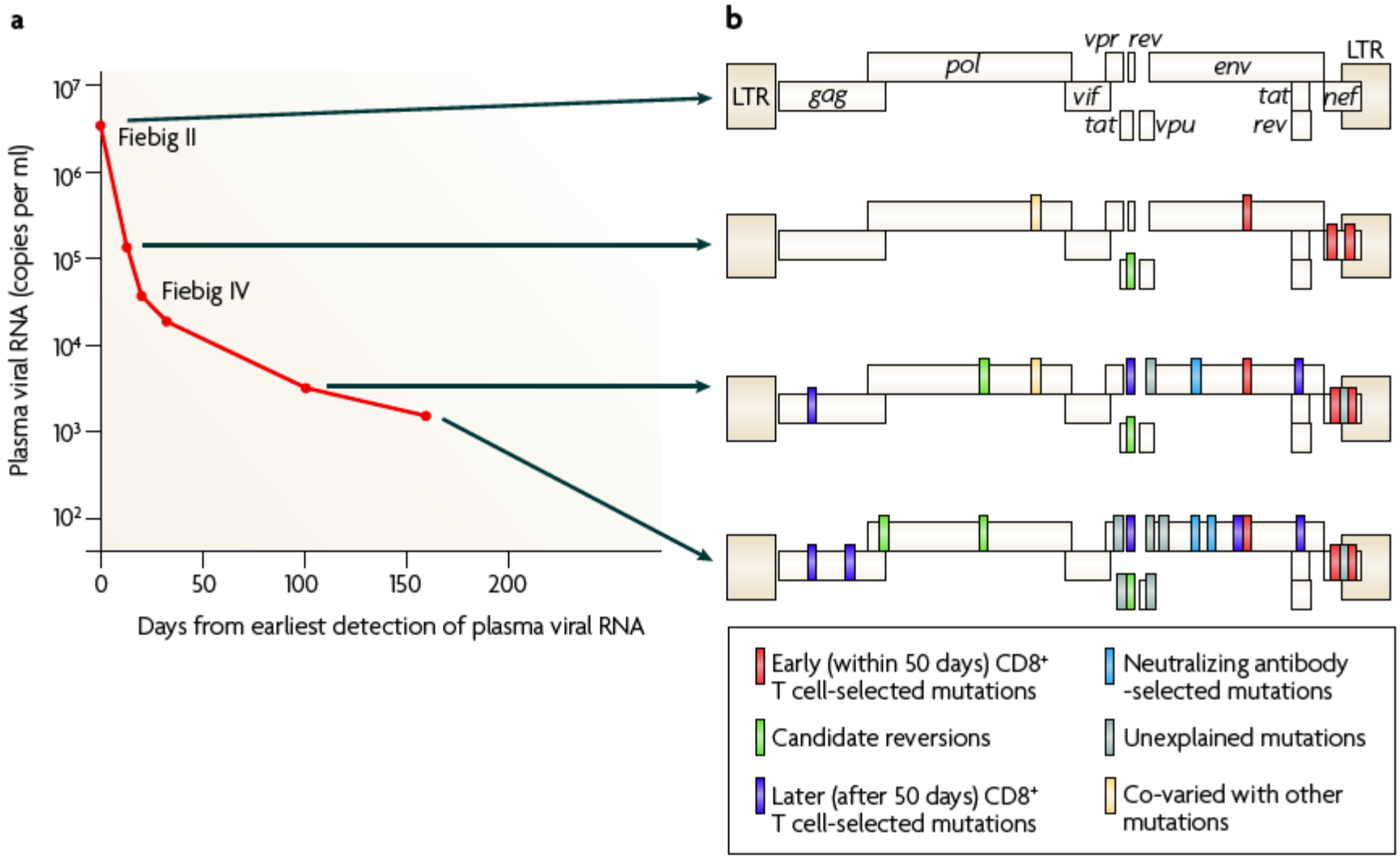
VOLUME 8 NUMBER 11 NOVEMBER 2007 NATURE IMMUNOLOGY

Programmed Death 1 Expression on HIV-Specific $CD4^+$ T Cells Is Driven by Viral Replication and Associated with T Cell Dysfunction[1]

Michelle D'Souza,* Andrew P. Fontenot,*† Doug G. Mack,* Catherine Lozupone,‡ Stephanie Dillon,* Amie Meditz,* Cara C. Wilson,*† Elizabeth Connick,* and Brent E. Palmer[2]*

The Journal of Immunology, 2007, 179: 1979–1987.

Early T cell selection of virus escape mutations in acute HIV-1 infection



1, 2, 3, 4, 5: mate

1 Early establishment of pool of latently infected cells
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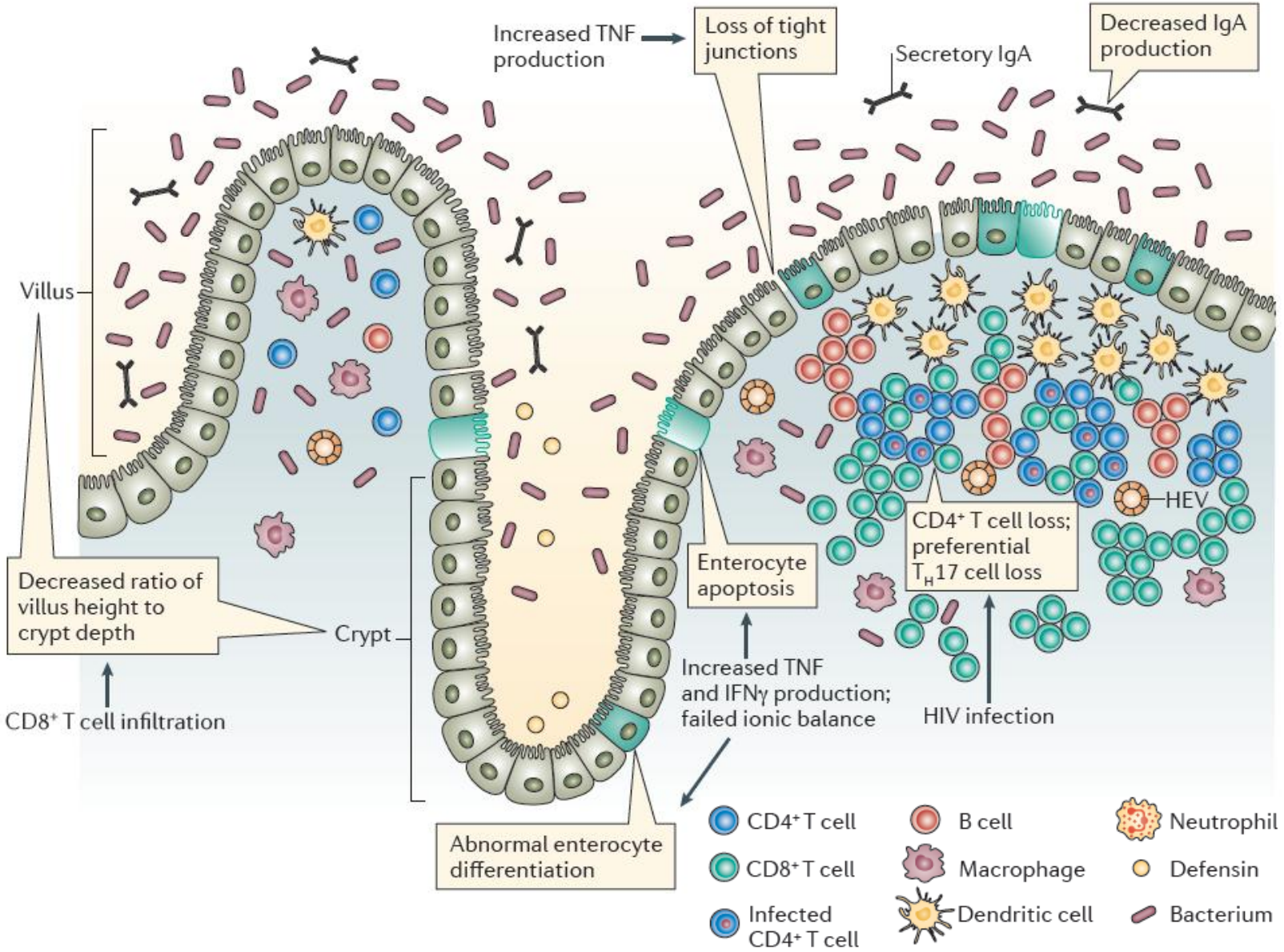
5 …

The intestinal epithelium during primary HIV infection



1, 2, 3, 4, 5: mate

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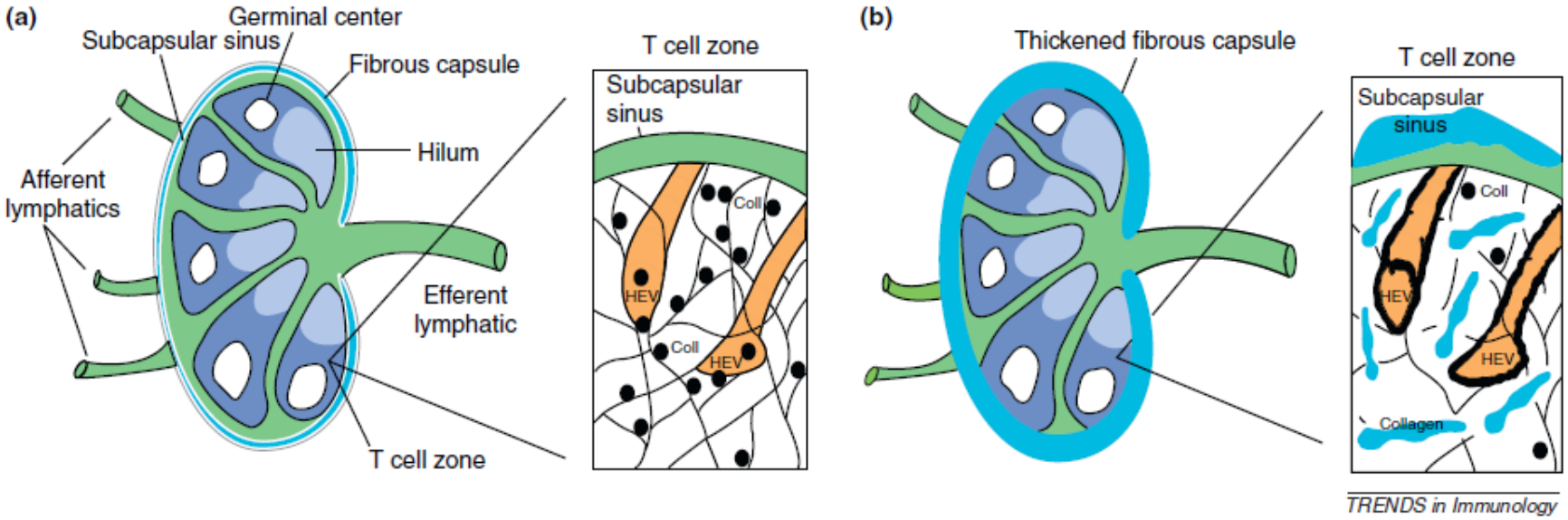
Review

Structure and function of lymphoid tissues

Lymphoid tissue structure and HIV-1 infection: life or death for T cells

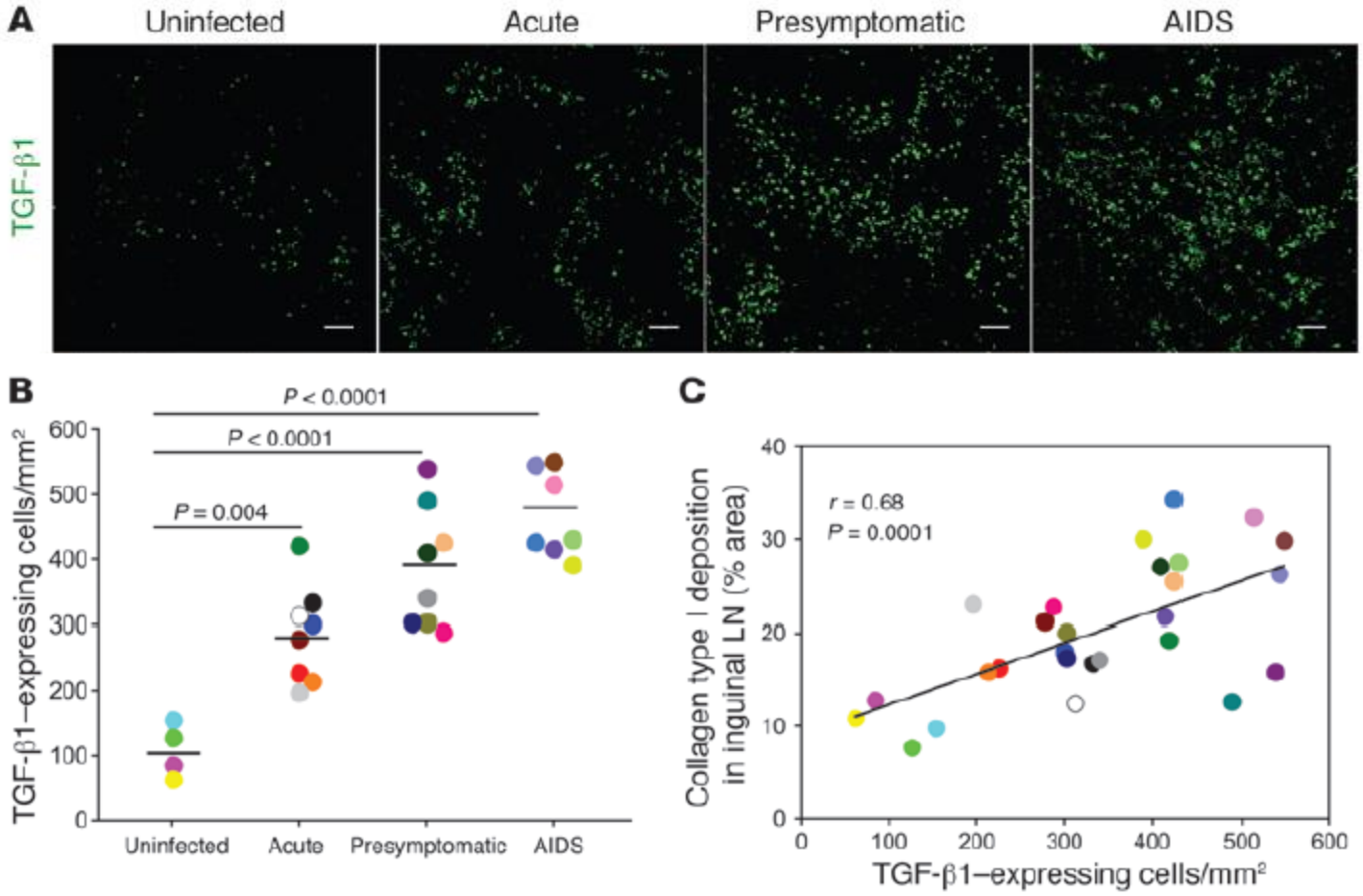
Ming Zeng[1], Ashley T. Haase[1] and Timothy W. Schacker[2]



TRENDS in Immunology

Cumulative mechanisms of lymphoid tissue fibrosis and T cell depletion in HIV-1 and SIV infections

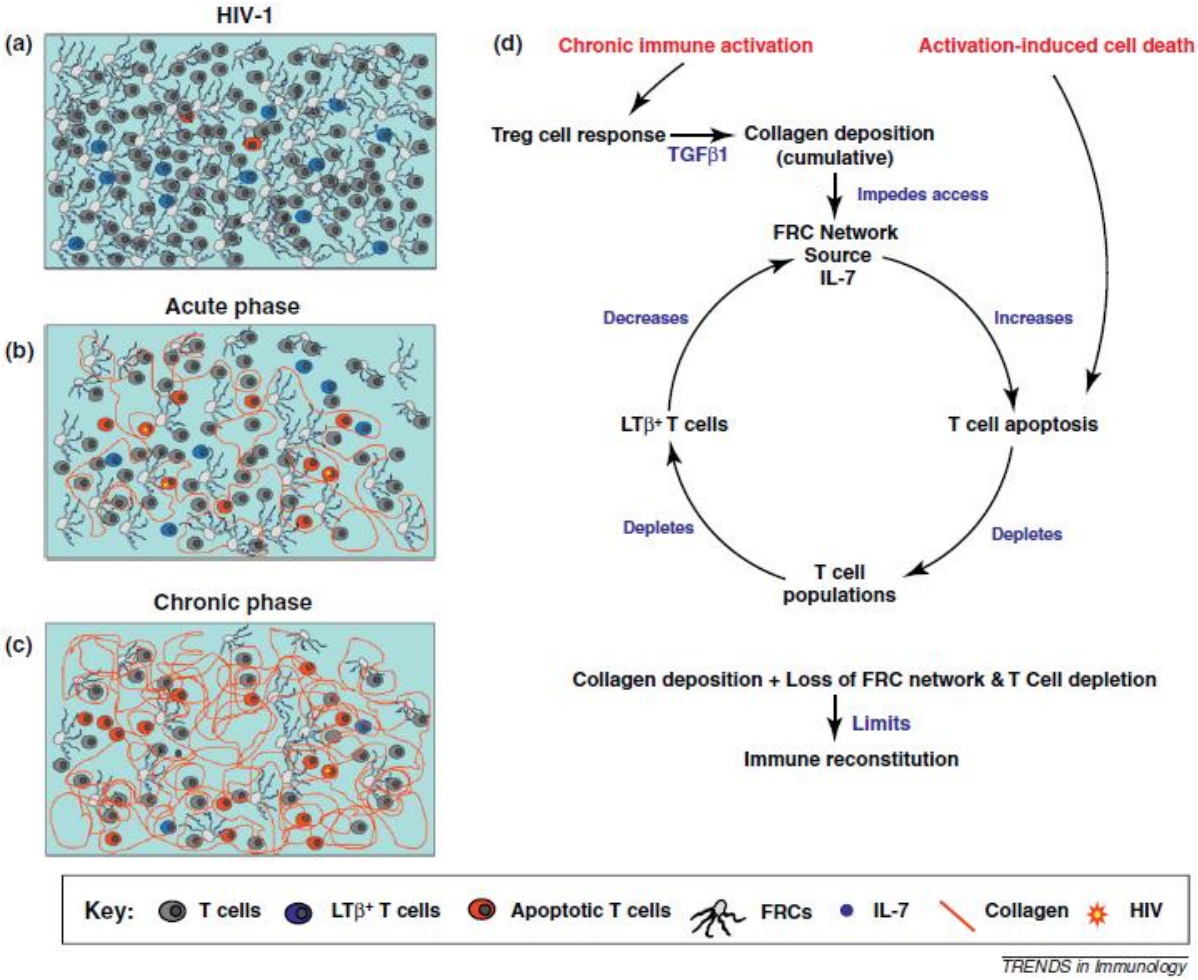
Ming Zeng,[1] Anthony J. Smith,[1] Stephen W. Wietgrefe,[1] Peter J. Southern,[1] Timothy W. Schacker,[2] Cavan S. Reilly,[3] Jacob D. Estes,[4] Gregory F. Burton,[5] Guido Silvestri,[6] Jeffrey D. Lifson,[4] John V. Carlis,[7] and Ashley T. Haase[1]



Structure and function of lymphoid tissues

Lymphoid tissue structure and HIV-1 infection: life or death for T cells

Ming Zeng[1], Ashley T. Haase[1] and Timothy W. Schacker[2]



Inadequate T follicular cell help impairs B cell immunity during HIV infection

Rafael A Cubas[1], Joseph C Mudd[2], Anne-Laure Savoye[3], Matthieu Perreau[3], Julien van Grevenynghe[1], Talibah Metcalf[1], Elizabeth Connick[4], Amie Meditz[4], Gordon J Freeman[5], Guillermo Abesada-Terk Jr[6], Jeffrey M Jacobson[7], Ari D Brooks[8], Shane Crotty[9,10], Jacob D Estes[11], Giuseppe Pantaleo[3], Michael M Lederman[2] & Elias K Haddad[1]

VOLUME 19 | NUMBER 4 | APRIL 2013 NATURE MEDICINE

Follicular helper T cells serve as the major CD4 T cell compartment for HIV-1 infection, replication, and production

Matthieu Perreau,[1] Anne-Laure Savoye,[1] Elisa De Crignis,[1] Jean-Marc Corpataux,[2] Rafael Cubas,[5] Elias K. Haddad,[5] Laurence De Leval,[3] Cecilia Graziosi,[1] and Giuseppe Pantaleo[1,4]

J. Exp. Med. 2013 Vol. 210 No. 1 143–156

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PLOS | PATHOGENS

Loss of Circulating CD4 T Cells with B Cell Helper Function during Chronic HIV Infection

Kristin L. Boswell[1], Robert Paris[1,2], Eli Boritz[3], David Ambrozak[1], Takuya Yamamoto[1], Sam Darko[3], Kaska Wloka[1], Adam Wheatley[4], Sandeep Narpala[4], Adrian McDermott[4], Mario Roederer[5], Richard Haubrich[6], Mark Connors[7], Julie Ake[2], Daniel C. Douek[3], Jerome Kim[2], Constantinos Petrovas[1]*, Richard A. Koup[1]

January 2014 | Volume 10 | Issue 1 | e1003853

Therapeutic implications

- Does therapy work?
- On which patient?
- CD4 and VL as unique and reliable parameters?
- Does combination of drugs matter?

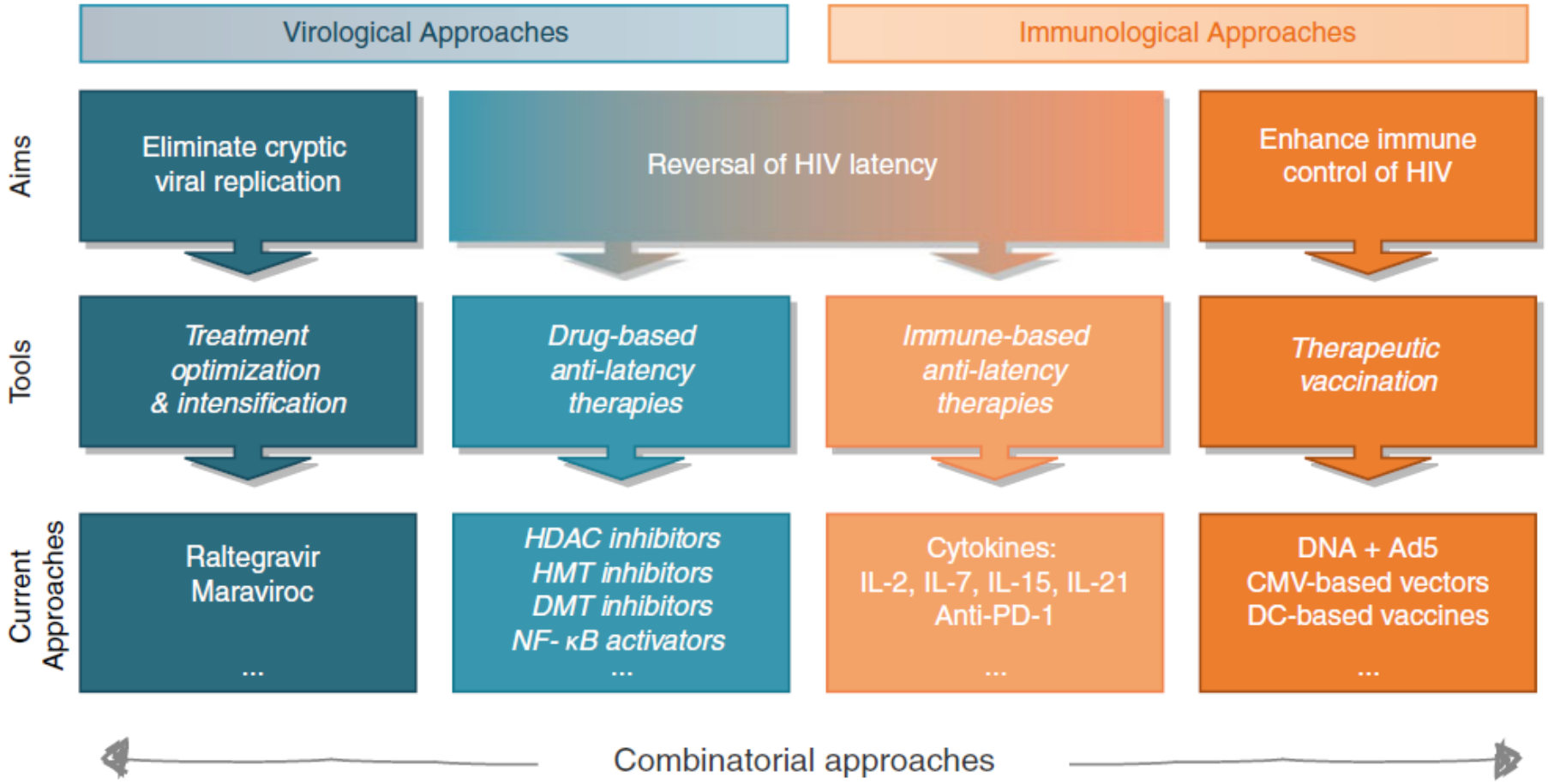
Therapeutic strategies *(i)*

	Anti-inflammatory drugs	HIV cure interventions
Phase 1	Sevelemer (anti-LPS), anti-PD1 antibody, anti-interleukin-6 antibodies, anti-interferon-α antibodies, sirolimus	Histone deacetylase inhibitors (vorinostat, panobinostat, romidepsin), disulfiram, interleukin 15, anti-PD1 antibody, sirolimus, CCR5-modified T cells and stem cells, therapeutic vaccines, neutralising antibodies
Phase 2	Treatment intensification (ART), statins, aspirin, COX-2 inhibitor, methotrexate, chloroquine/hydroxychloroquine, prebiotics/probiotics, bovine colostrum, rifaximin, aciclovir/valaciclovir, ACE inhibitors/ARBs (antifibrosis), mesalazine (anti-LPS), interleukin 7	Interleukin 7
Phase 3	None	None

LPS=lipopolysaccharide. ART=antiretroviral therapy. ACE=angiotensin-converting enzyme. ARB=angiotensin-receptor blocker.

***Table 2*: Novel therapeutic drugs in development for management of HIV disease**

Therapeutic strategies *(ii)*



Curr HIV/AIDS Rep (2013) 10:33–41

HIV Reservoir Changes in Resting CD4 Subsets in the IL7 plus ART Intensification Eramune01 Study

Manuela Pogliaghi[1,2], Sidonie Lambert[3,4], Lambert Assoumou[5], Rima Zoorob[1], Laura Papagno[1,6], Francois Lecardonnel[6], Vincent Calvez[3,4], Christine Katlama[5,7], Brigitte Autran[1], and the Eramune-01 study group[‡]

[1]UPMC Univ Paris 06, UMR-S945, Laboratory of Immunity and Infection, Paris, F-75013, France [2]Università Vita-Salute, San Raffaele Scientific Institute, Milan, Italy [3]UPMC Univ Paris 06, Inserm, UMR-S943, "Epidemiology, Therapeutic Strategies and Clinical Virology in HIV Infection", Laboratory of Virology, Paris, F-75013, France [4]AP-HP, Pitié-Salpêtrière Hospital, Department of Virology, Paris, F-75013, France [5]UPMC Univ Paris 6, Inserm, UMR-S943, "Epidemiology, Therapeutic Strategies and Clinical Virology in HIV Infection", Center of Methodology, Paris, F-75013, France [6]ORVACS, Pitié-Salpêtrière Hospital, Paris, F-75013, France [7]AP-HP, Pitié-Salpêtrière Hospital, Department of Infectious and Tropical Diseases, Paris, F-75013, France

ABSTRACT: # 877

Background:

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Methodology:

[illegible]

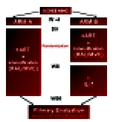
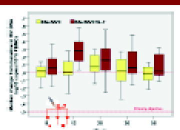
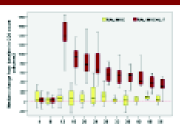
Results:

[illegible]

Conclusions:

[illegible]

BACKGROUND

STUDY DESIGN

No decrease in cell associated HIV-DNA in PBMCs from both arms

IL7 induced a massive CD4 T cell increase, mainly among TCM

We then assessed whether IL7 therapy did perturb the distribution of HIV reservoir and provoke transcriptional changes among resting CD4 T cell subsets

METHODS

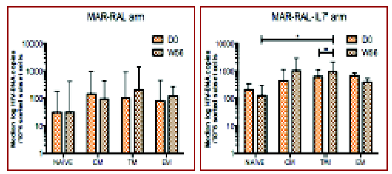
Cell sorting of resting CD25-CD69-HLADR-CD8+CD4+ subsets: N, CM, TM, EM at D0 and W56 among RAL+MVC arm (6 pts) and RAL+MVC+IL7 arm (6 pts)

Reservoir Distribution and Contribution	• HIV DNA/10^6 subset cells • HIV DNA/subset cell%/10^6 CD4 • HIV DNA /subset cells/mm3
Reservoir Inducibility	• At D6, D10, D13 HIV RNA quantified in culture supernatants and normalised for HIV DNA of each subset. • 3 different culture conditions: 1. unstimulated cells; 2. IL7 alone; 3. antiCD3+CD28+IL7+IL2+
Statistical Analysis	• Mann Whitney test for comparisons between arms (RAL-MAR vs RAL-MAR-IL7) • Wilcoxon signed rank test for comparison among arms (D0 vs W56) • Kruskal Wallis test for comparison between subsets • p value < 0.05 was considered significant
Human Transcriptome	• Human genome wide microarray Platform Lumina • Data files uploaded into Ingenuity Pathway Analysis (IPA) software. • Right tailed Fischer's exact test was used to calculate p value • Z-score to infer activation (> 2) or inhibition (< -2) of predicted transcriptional regulators

Unspliced HIV RNA transcripts from total CD4 detected by qPCR (GAG gene) at D0, W8, W12, W56 among RAL+MAR arm (6 pts) and RAL+MVC+IL7 arm (13 pts)

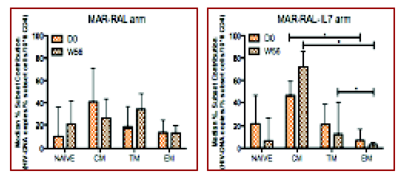
RESULTS

Reservoir Distribution (HIV DNA/10^6 cells subset)



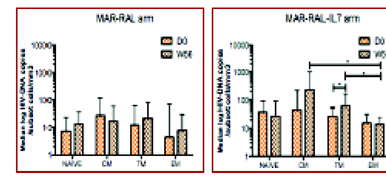
The Reservoir distribution (raw values: HIV-DNA copies/10^6 sorted subset cells) among arms did not change in RAL-MAR arm, while it increased in among the MAR-RAL-IL7 TM cells (p 0.03) that became significantly higer than in N (p 0.02).
Comparing the 2 arms, the CM HIV-DNA W56-D0 delta was significantly higher in RAL-MAR-IL7 compared to RAL-MAR (p 0.02, data not shown).

Relative subset Contribution to the HIV reservoir (HIV DNA/cell subset%/10^6 CD4)



Relative reservoir contribution of various subsets (% contribution subset/10^6 CD4) did not significantly change over time.
In the RAL-MAR-IL7 arm : higher contribution of CM than EM both at D0 and W56 (p<0.01) and higher TM contribution than EM at W56 (p0.01), with a higher W56-D0 delta in CM (p 0.01) and lower fold change EM (p 0.02) contributions than in the MAR-RAL arm (data not shown).

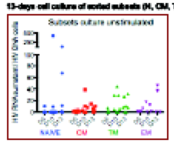
Absolute subset Contribution to the HIV Reservoir (HIV DNA/cell subset count/mm3)

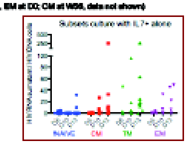


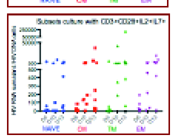
Absolute reservoir contribution values (HIV-DNA cp/subset cells/mm3) among arms did not change in RAL-MAR arm, but significantly increased in TM (p 0.03) with a trend in CM (p 0.06) in the MAR-RAL-IL7 arm. At W56 CM and TM contributed more than EM to the HIV reservoir (p 0.02, p 0.04 respectively).
In RAL-MAR-IL7, the CM and TM W56-D0 delta were significantly higher than in RAL-MAR (p 0.008, p 0.03 respectively, not shown).

In vitro viral inducibility: Cell Subset Culture

13-days cell culture of sorted subsets (N, CM, TM, EM at D0; CM at W56, data not shown)





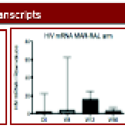


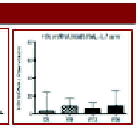
Inducibility of the HIV Reservoirs:
• HIV reservoirs were inducible in all resting CD4 subsets, with a very low spontaneous production in all subsets despite in vivo ART.
• IL-7 induced a low level HIV production at D13 mostly from memory CD4 subsets.

Ex vivo HIV mRNA transcripts

Unspliced HIV RNA transcripts from purified total CD4+ T cells:

IL7 did not induce any significant change in mRNA transcription levels over time.



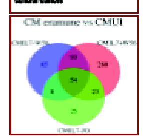


Human Genome-wide Transcriptome

- Direct comparisons between subsets did not find any differentially expressed gene among and between arms (FDR 0.05)
- Comparing sorted D0 and W56 CD4+ T CM with uninfected CD4+ T CM from healthy donors we observed:
  - no difference in RAL-MAR arm (data not shown)
  - at W56 in RAL-MAR-IL7 CM cells, the Z-score predicted significantly higher activated pathways than of healthy control (Table)

Distribution of differentially-expressed genes (FDR<0.05)

comparison between TCM from the two Eramune arms, both at D0 and W56 and from uninfected control donors



Immunovirological pathways differentially expressed in TCM from the RAL-MAR-IL7 ARM

	D0 vs CM uninfected	W56 vs CM uninfected
differentiation of lymphocytes	3,18	3,48
transcription	3,18	3,18
transcription of RNA	3,07	1,88
viral infection	[illegible]	3,01
cell death of immune cells	3,14	[illegible]
activation of blood cells		3,08
protein kinase cascade		3,03
activation of immune cells		3,07
[illegible] cascade		3,48
activation of T lymphocytes		3,43
cell death of APCs		3,43
immune response of cells		3,41
activation of leukocytes		3,48
cell movement		3,24
T cell development		3,22
apoptosis of APCs		3,28
cell death of macrophages		3,18
orientation of microtubule organizing center		3,18

CONCLUSIONS

HIV reservoirs studies in the distinct major subsets of resting CD4+ T-cells from chronically HIV-infected patients:
- After a year long intensification with RAL+MAR: No changes.
- After a year long intensification plus 3 IL7 injections, in the MAR-RAL-IL7 arm:
  - ★ cell-associated HIV-DNA significantly increased only among resting CD4+ TTM cells, with a non significant trend in CD4+ TCM, although the TCM-associated HIV-DNA at W56 was significantly higher in the MAR-RAL-IL7 arm compared to the RAL-MAR arm
  - ★ at W56 a major contribution of both CM and TM compared to EM, both in proportions and absolute values, was observed.

Transcriptome analysis. No changes in:
- HIV unspliced transcripts in total CD4 T cells, over time
- human transcriptional profiling in any resting CD4 subset from both arms between D0 and W56, despite a persistent IL7 effect on CD4 TCM compared to TCM from healthy donors. Further analysis are ongoing to assess whether these transcriptomic changes are also detectable in the other subsets.

Altogether, these findings suggest that IL7 induces some long term changes in the redistribution of the HIV reservoirs and CD4 T cell compartments, that does not simply reflect homeostatic proliferation triggered by IL7.

The EraMune-01 Study Group

Center 01 (Paris): C. Katlama, R. Tubiana, M.A. Valantin, L. Schneider, H. Stitou, B. Mory, G. Peytavin, A. Canestri, Y. Dudoit – Center 02 (Badalona): B. Clotet, B. Mothe-Pujadas, P. Coll, J. Martinez-Picado, P. Cobarsi – Center 04 (Milan): G. Tambussi, M. Pogliaghi, S. Chiappetta, L. Della Torre – Center 05 (London): M. Youle, S. Kinloch de Loës, A. Carroll, P. Byrne – Virology: V. Calvez, S. Lambert-Niclot, A. G. Marcelin – Immunology: B. Autran, L. Papagno, A. Ersaree – Methodology & Statistics: D. Costagliola, L. Assoumou, A. Delobelle, I. Peyrole – Pharmacovigilance: A. Lillo-Le Louët, V. Rougé-Gasmaleit, V. Fulda – Monitoring (Keyrus Biopharma): K. Leplatre, M. Bessadi, V. Campana, L. Régné, S. Sanchez – Project Management (ORVACS): F. Lecardonnel, L. Papagno.
We thank CYTHERIS (T. Croughs, M. Morre), ViiV Healthcare France (C. Lemarchand, L. Finkielsztejn) and MSD France (L. Haft-Ighil, F. Durand) for their support.

Immediate antiviral therapy appears to restrict resting CD4$^+$ cell HIV-1 infection without accelerating the decay of latent infection

Nancie M. Archin[a,1], Naveen K. Vaidya[b,c,1], JoAnn D. Kuruc[a], Abigail L. Liberty[a], Ann Wiegand[d], Mary F. Kearney[d], Myron S. Cohen[a], John M. Coffin[e], Ronald J. Bosch[f], Cynthia L. Gay[a], Joseph J. Eron[a], David M. Margolis[a,2], and Alan S. Perelson[b,2]

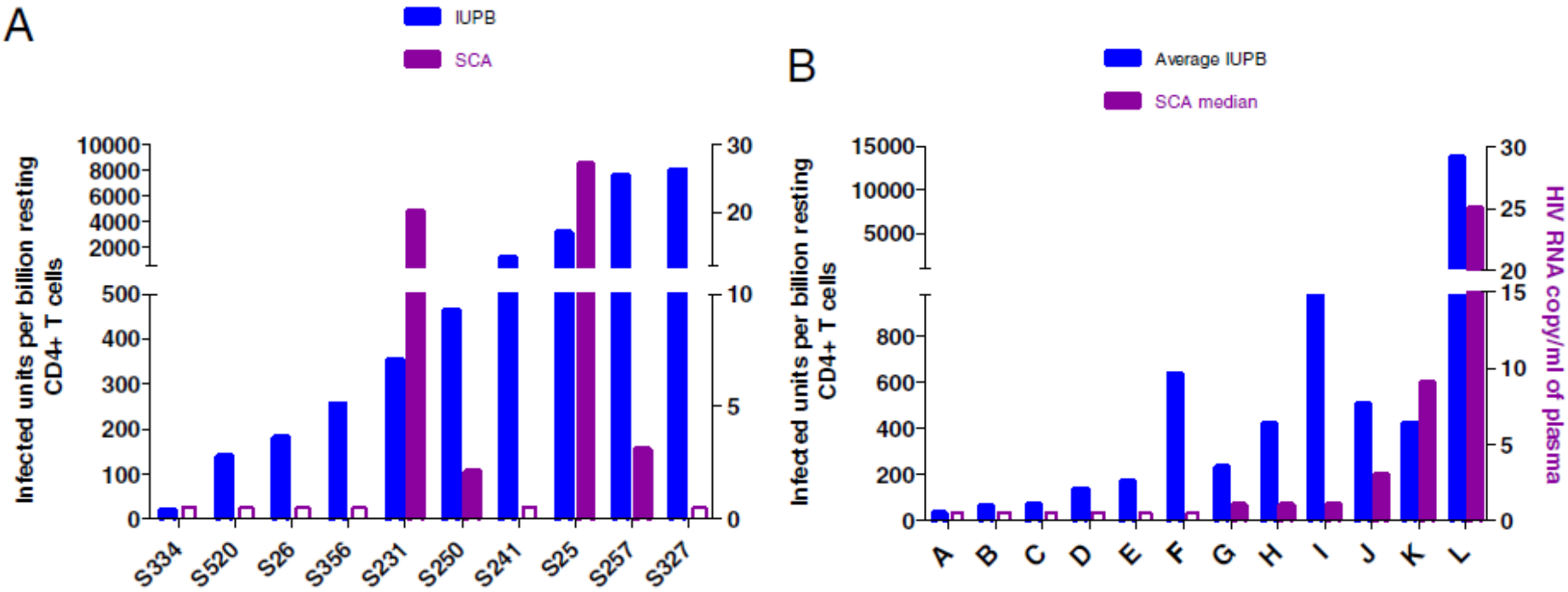


Fig. 4. Comparison of low-level viremia (single-copy assay) and RCI frequency in (*A*) acutely treated and (*B*) chronically treated HIV-1–infected patients labeled A–L.

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PLOS | PATHOGENS

Post-Treatment HIV-1 Controllers with a Long-Term Virological Remission after the Interruption of Early Initiated Antiretroviral Therapy ANRS VISCONTI Study

Asier Sáez-Cirión[1]*, Charline Bacchus[2], Laurent Hocqueloux[3], Véronique Avettand-Fenoel[4,5], Isabelle Girault[6], Camille Lecuroux[6], Valerie Potard[7,8], Pierre Versmisse[1], Adeline Melard[4], Thierry Prazuck[3], Benjamin Descours[2], Julien Guergnon[2], Jean-Paul Viard[5,9], Faroudy Boufassa[10], Olivier Lambotte[6,11], Cécile Goujard[10,11], Laurence Meyer[10,12], Dominique Costagliola[7,8,13], Alain Venet[6], Gianfranco Pancino[1], Brigitte Autran[2], Christine Rouzioux[4,5]*, the ANRS VISCONTI Study Group[¶]

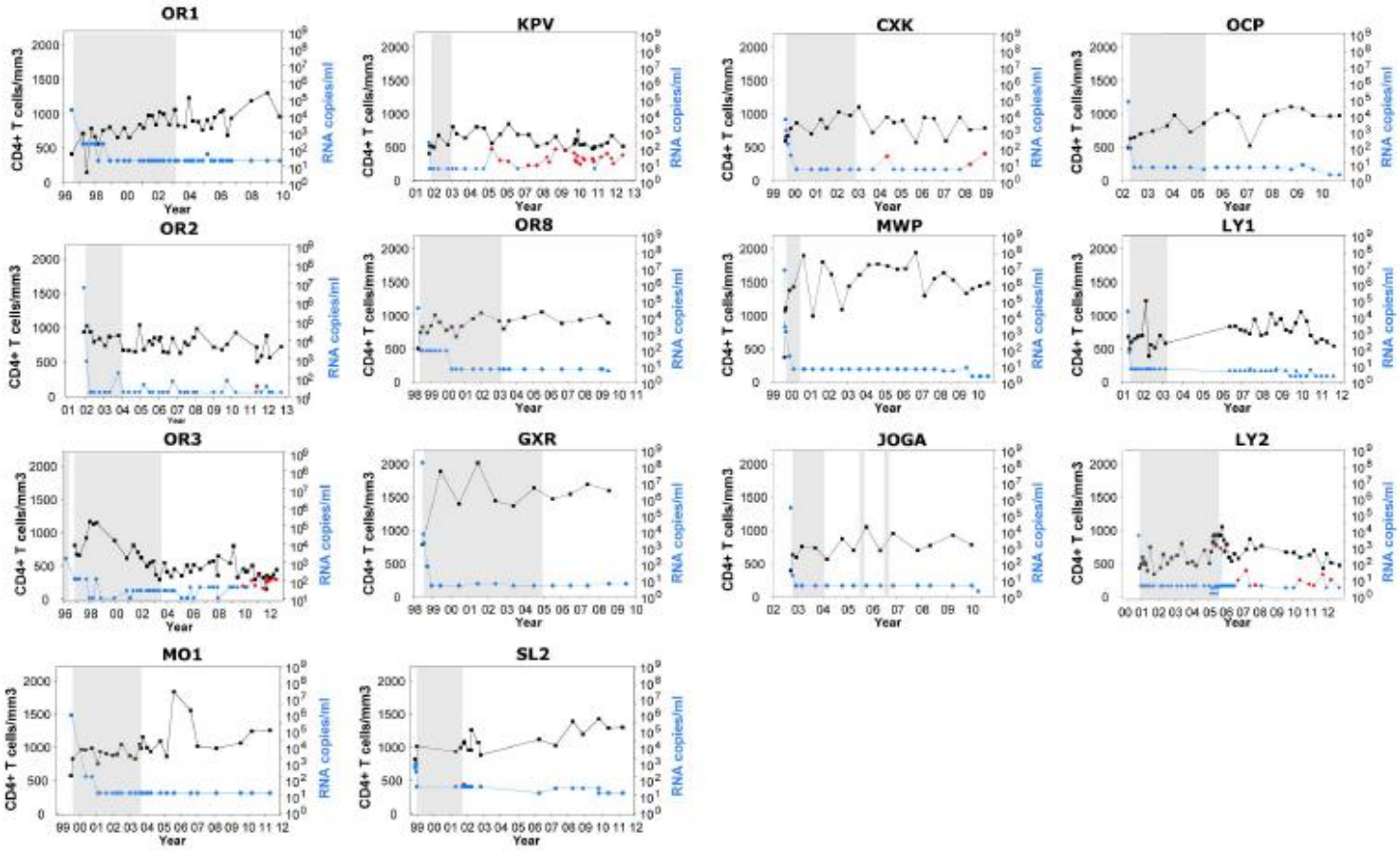

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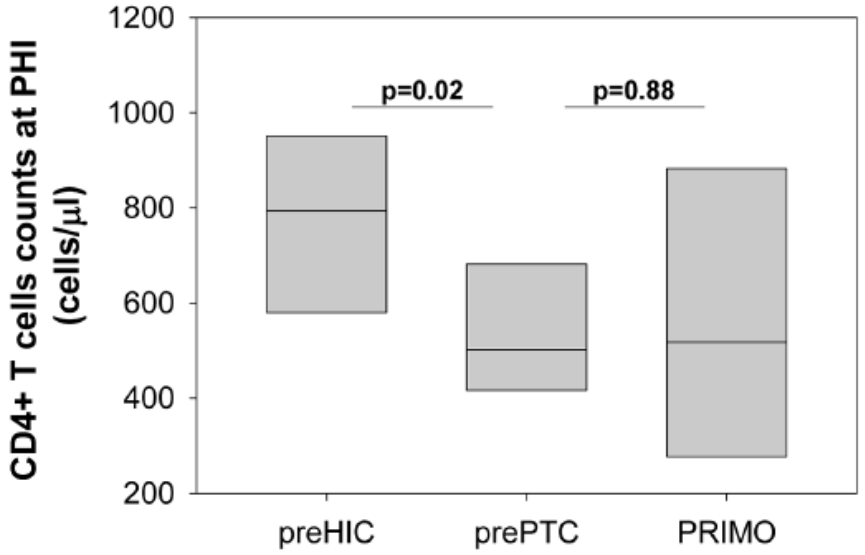
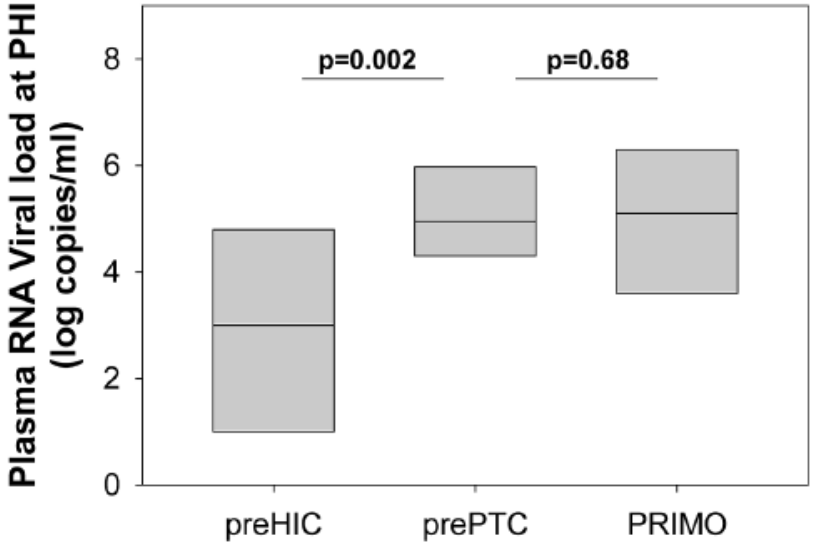
Table 1. Characteristics of PTC included in the study.

Code	Sex[1]	Year of diagnostic	PHI[2]	Fiebig	ART initiation	ART combination[3]	Time on cART (months)
OR1	M	1996	Sympt	V		2 NRTI	81
OR2	F	2001	Sympt	V		3 NRTI+PI→3 NRTI	24
OR3	F	1996	Sympt	I		2 NRTI→2 NRTI+PI	92
OR8	M	1998	Sympt	III		2 NRTI+PI→3 NRTI	60
KPV	M	2001	Sympt	V		NNRTI+2 NRTI→3NRTI	13
GXR	F	1998	Sympt	III		2 NRTI+PI	86
CXK	M	1999	Asympt	V		2 NRTI+PI	39
MWP	M	1999	Sympt	V		2 NRTI+PI	12
JOGA	F	2002	Sympt	IV		2 NRTI+PI[7]	17
OCP	M	2002	Sympt	V		2 NRTI+PI→3 NRTI	31
LY1	M	2001	Sympt	III		2 NRTI+PI→3 NRTI	23
LY2	M	2000	Asymp	V		3 NRTI	56
MO1	M	1999	Sympt	V		2 NRTI+PI→2 NRTI+NNRTI	48
SL2	M	1998	Sympt	V		3 NRTI+PI→3NRTI	34
MEDIAN		1999		V			36.5





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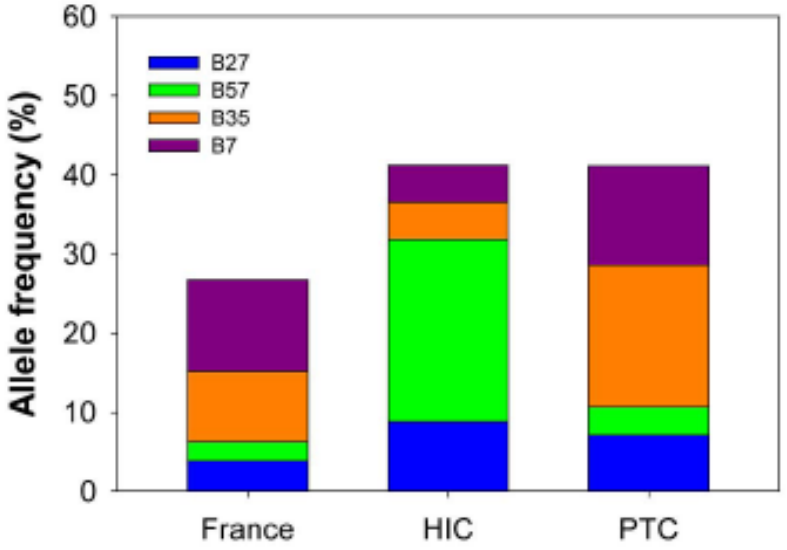


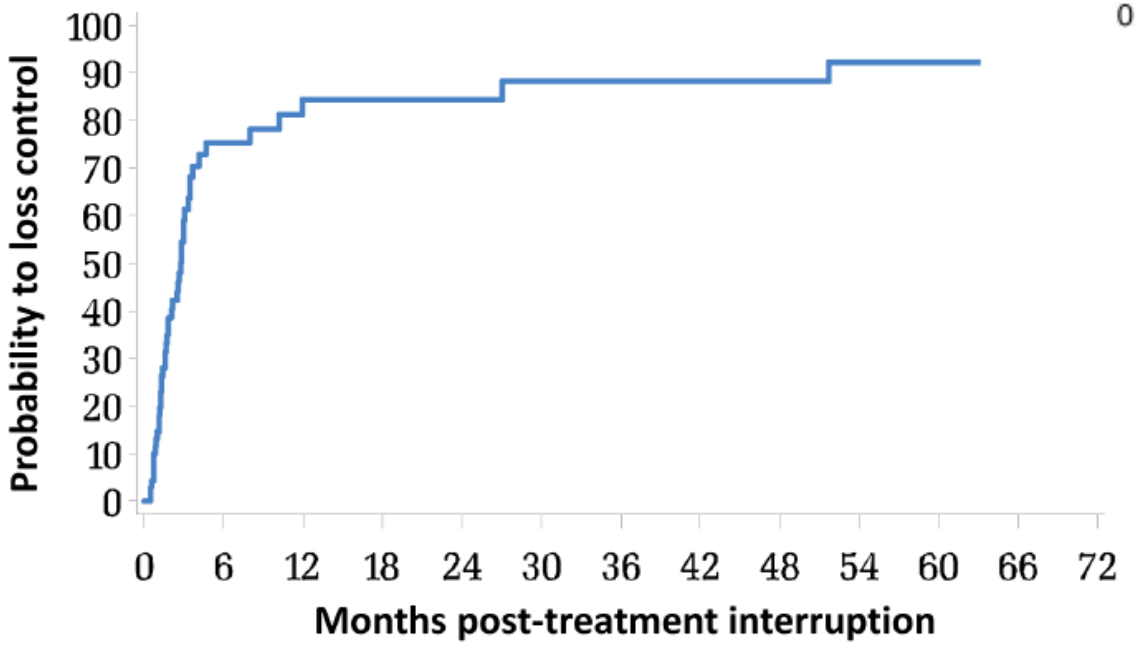


Figure 7. Interruption of long-term treatment initiated at PHI leads to a significant frequency of viremia control. Kaplan-Meier curve of the probability for patients included in the FHDH between 1997 and 2011 to lose control of viremia after interruption of a, at least, one year-long cART initiated within 6 months of HIV infection, and who had at least one viral load determination 12 months after treatment interruption (n = 74). Loss of control was defined by 2 or more viral loads above 50 RNA copies/mL or one viral load above 50 RNA copies/mL followed by resumption of cART.

Immunovirologic Control 24 Months After Interruption of Antiretroviral Therapy Initiated Close to HIV Seroconversion

Sara Lodi, PhD, MSc; Laurence Meyer, MD, PhD; Anthony D. Kelleher, PhD, MB; Magdalena Rosinska, PhD; Jade Ghosn, MD, PhD; Mette Sannes, MLT; Kholoud Porter, PhD

COMMENT

Our findings confirm the existence of PTCs, although they are rare. These individuals experienced virologic control and high $CD4^+$ levels while not receiving treatment.

The proportion of the population estimated to be PTCs at 24 months in our study (5.5%) is substantially lower than that reported by Hocqueloux et al[2] (15.6%). This difference can be explained by variations in study design. Their inclusion was restricted to individuals who were not taking cART for at least 24 months, rather than to all those at risk of losing their PTC status after cART interruption. In this manner, individuals who remained event free (ie, retained PTC status) for less than 24 months were excluded from the risk set; therefore, a selection of

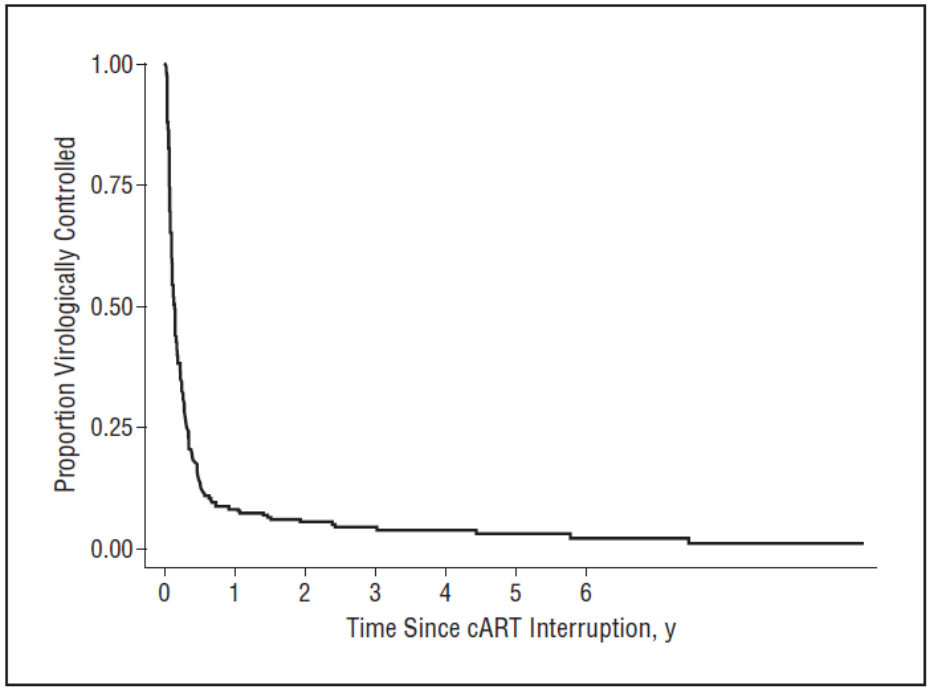
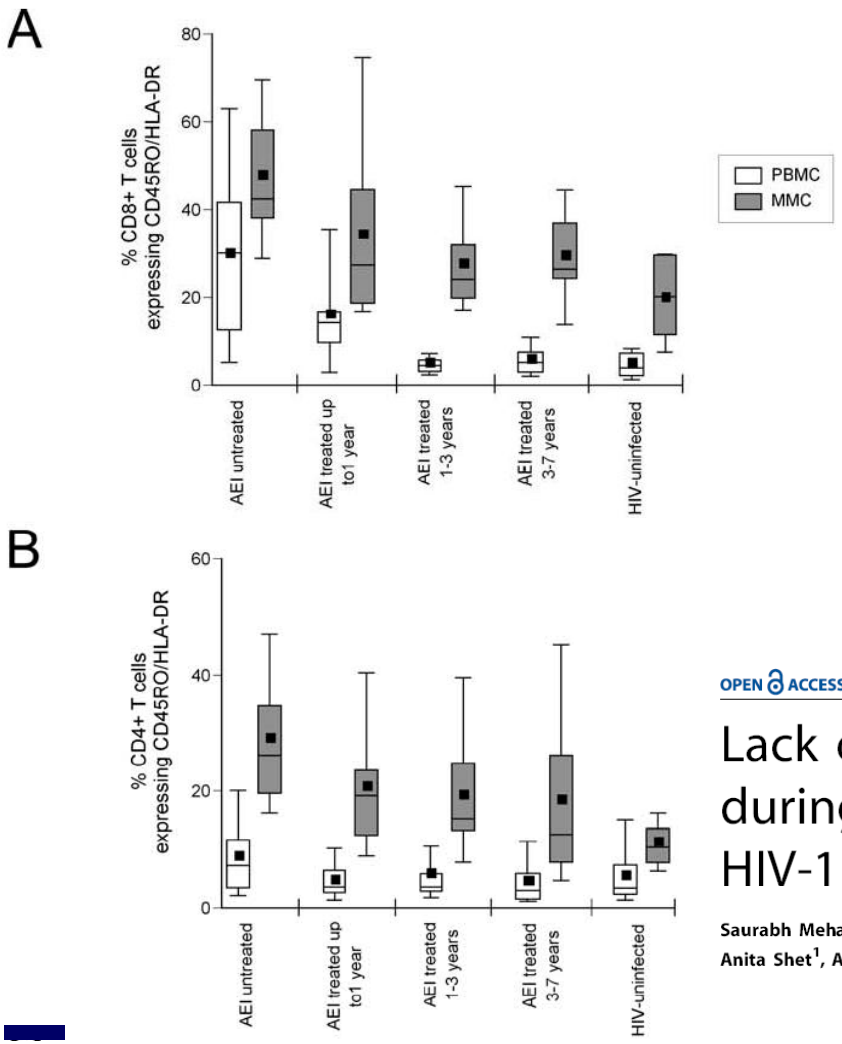
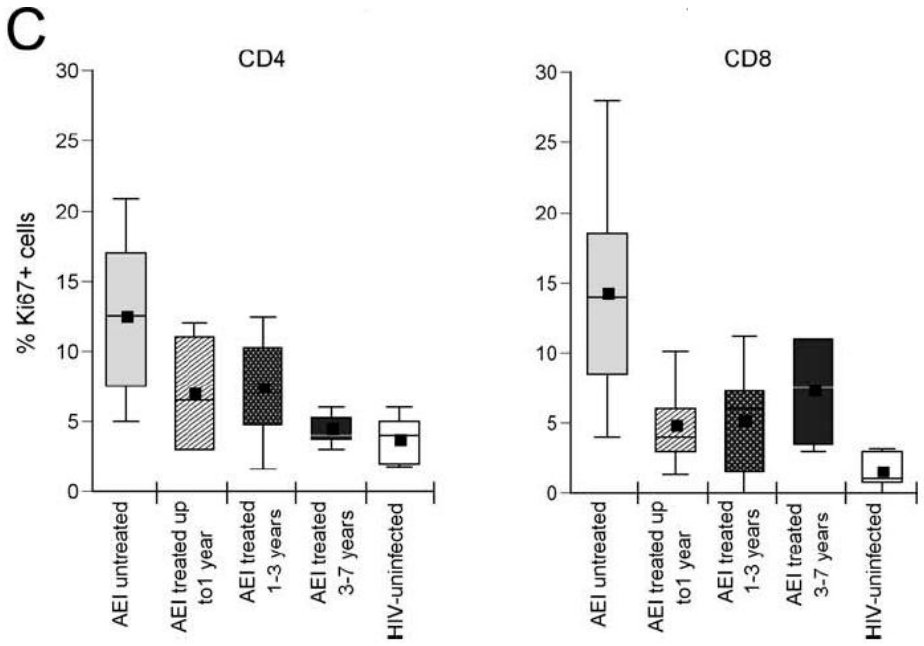


Figure. Kaplan-Meier estimate of the proportion of patients maintaining virologic control after interruption of short-course cART initiated within 3 months of HIV seroconversion. Loss of virologic control is defined as experiencing at least 2 consecutive HIV RNA levels of more than 50 copies/mL or reinitiation of cART. cART indicates combined antiretroviral therapy; and HIV, human immunodeficiency virus.

Mucosal immune reconstitution?





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PLoS MEDICINE

Lack of Mucosal Immune Reconstitution during Prolonged Treatment of Acute and Early HIV-1 Infection

Saurabh Mehandru[1], Michael A. Poles[1,2], Klara Tenner-Racz[3], Patrick Jean-Pierre[1], Victoria Manuelli[1], Peter Lopez[1], Anita Shet[1], Andrea Low[1], Hiroshi Mohri[1], Daniel Boden[1], Paul Racz[3], Martin Markowitz[1*]

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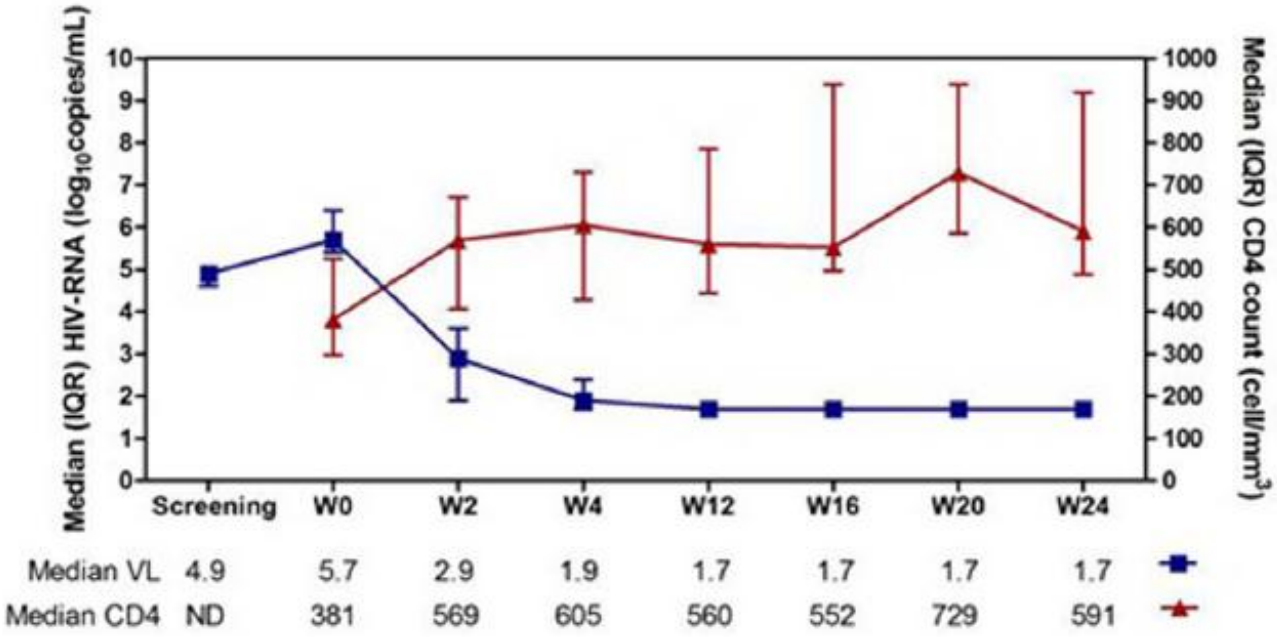
PLoS one

Impact of Multi-Targeted Antiretroviral Treatment on Gut T Cell Depletion and HIV Reservoir Seeding during Acute HIV Infection

Jintanat Ananworanich[1,2,3,4,5]*, Alexandra Schuetz[5,6], Claire Vandergeeten[7], Irini Sereti[8], Mark de Souza[5,6], Rungsun Rerknimitr[4], Robin Dewar[9], Mary Marovich[6], Frits van Griensven[10], Rafick Sekaly[7], Suteeraporn Pinyakorn[1,2,3], Nittaya Phanuphak[1,2], Rapee Trichavaroj[5], Wiriya Rutvisuttinunt[5], Nitiya Chomchey[1,2], Robert Paris[5,6], Sheila Peel[6], Victor Valcour[1,11], Frank Maldarelli[12], Nicolas Chomont[7], Nelson Michael[6], Praphan Phanuphak[1,2,3,4], Jerome H. Kim[1,5,6], on behalf of the RV254/SEARCH 010 Study Group



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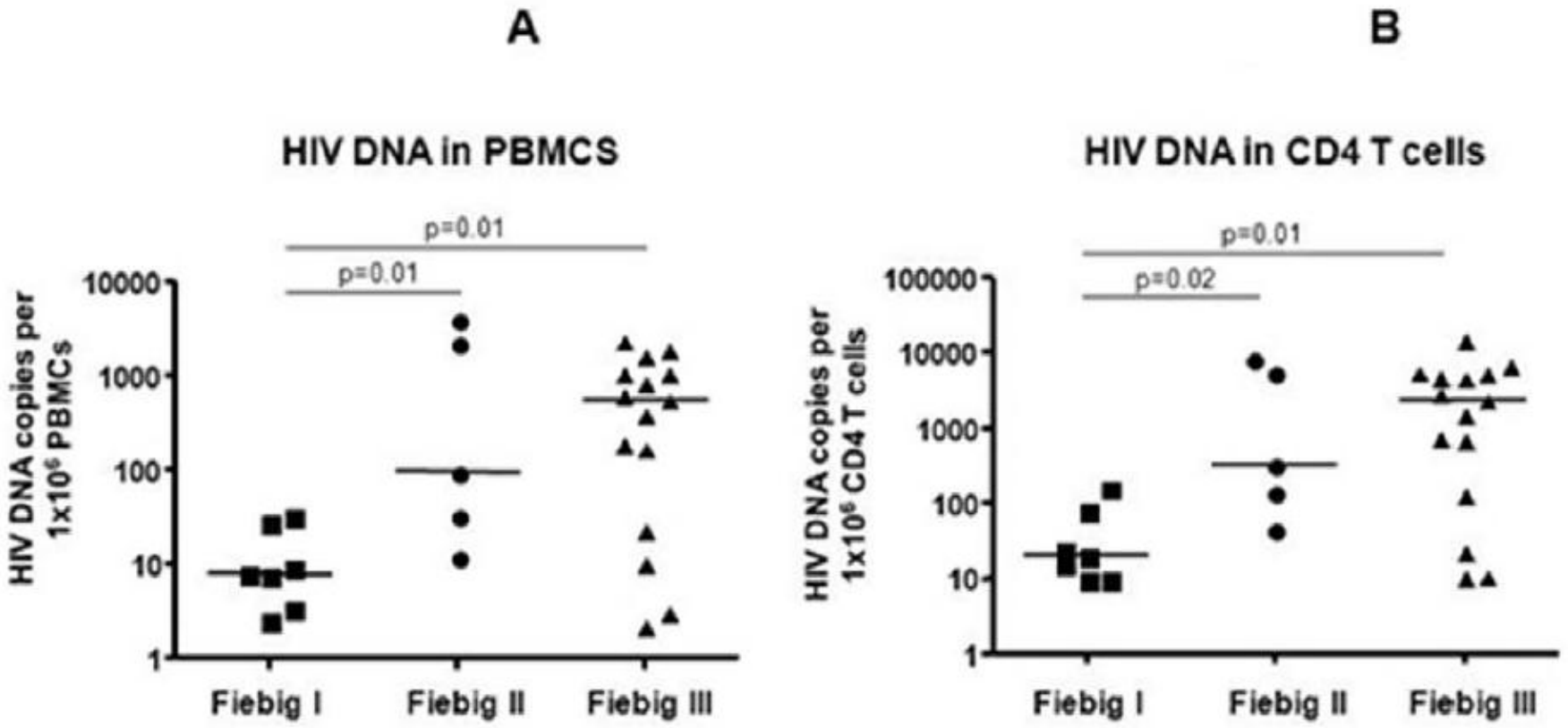

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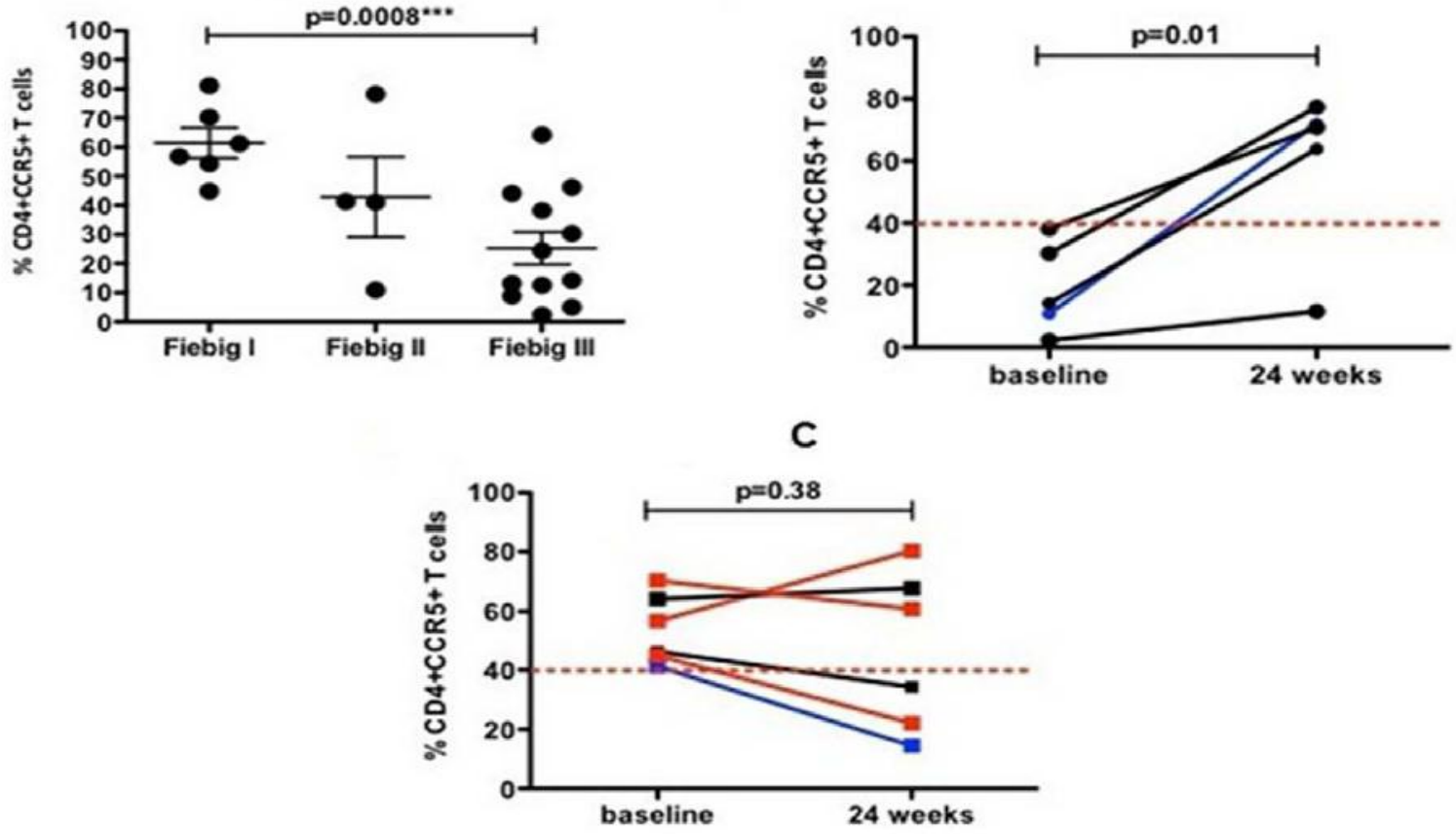

Figure 4. Frequency of CD4+CCR5+ T cells in sigmoid colon in acute-HIV infected subjects.

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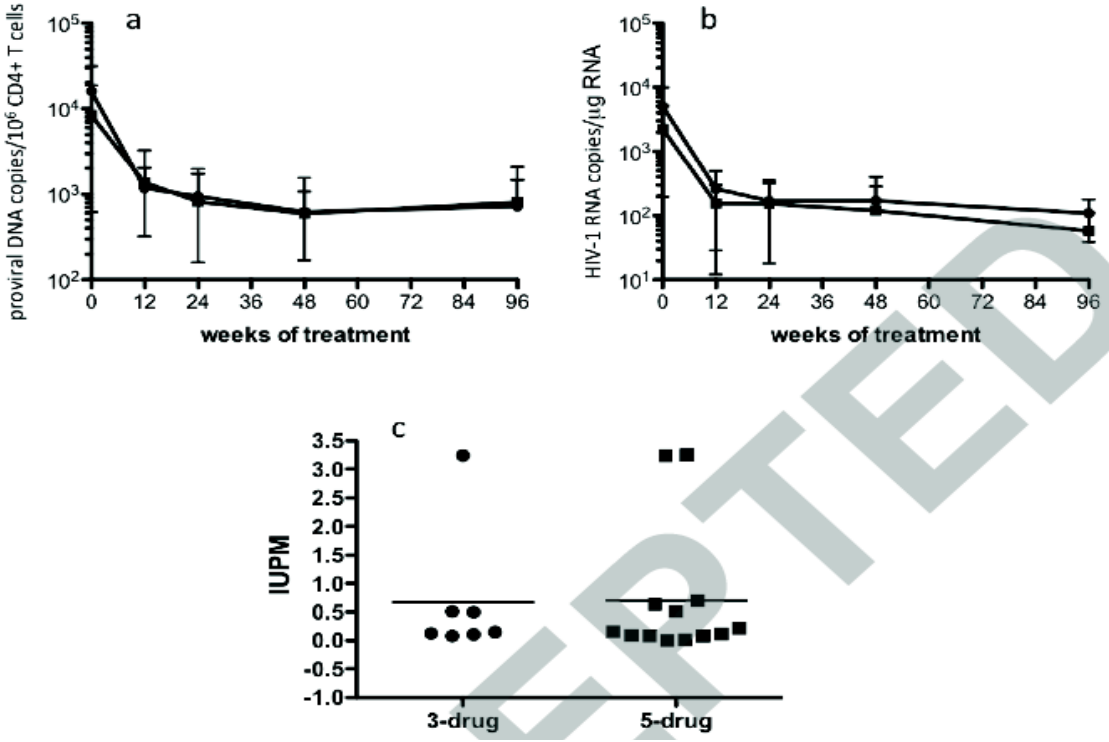
A Randomized Open-Label Study of Three- versus Five-Drug Combination Antiretroviral Therapy in Newly HIV-1 Infected Individuals

Martin Markowitz M.D.[1*], Teresa H. Evering M.D. M.S.[1], Donald Garmon N.P.[1], Marina Caskey M.D.[2], Melissa La Mar B.A.[1], Kristina Rodriguez M.P.H.[1], Vincent Sahi M.S.[1], Sarah Palmer Ph.D.[3], Nicole Prada Ph.D.[1] and Hiroshi Mohri M.D.



Conclusions: Intensified 5-drug cART initiated during early infection fails to significantly further impact virologic or immunologic responses beyond those achieved with standard 3-drug PI-based cART.

Overcoming pharmacologic sanctuaries

Theodore J. Cory[a], *Timothy W. Schacker*[b], *Mario Stevenson*[c], *and Courtney V. Fletcher*[a]

Purpose of review

Current antiretroviral treatment regimens represent significant improvements in the management of HIV-1 infection; however, these regimens have not achieved a functional or sterilizing cure. One barrier to achieving a cure may be suboptimal antiretroviral concentrations in sanctuary sites throughout the body, including the central nervous system, gut-associated lymphoid tissue, lymph nodes, and tissue macrophages. This review will focus on the problems associated with achieving effective concentrations in these restricted sanctuary sites, and potential strategies to overcome these barriers.

Recent findings

Sufficient data exist to conclude that antiretroviral drug distribution is not uniform throughout the body. Low tissue/reservoir concentrations may be associated with viral replication. Multiple means to increase drug concentrations in sanctuary sites are being investigated, including modification of currently utilized drugs, blockade of transporters and enzymes that affect drug metabolism and pharmacokinetics, and local drug administration. Accumulating data suggest these methods increase antiretroviral concentrations in reservoirs of viral replication. No method has yet resulted in the complete clearance of HIV.

Summary

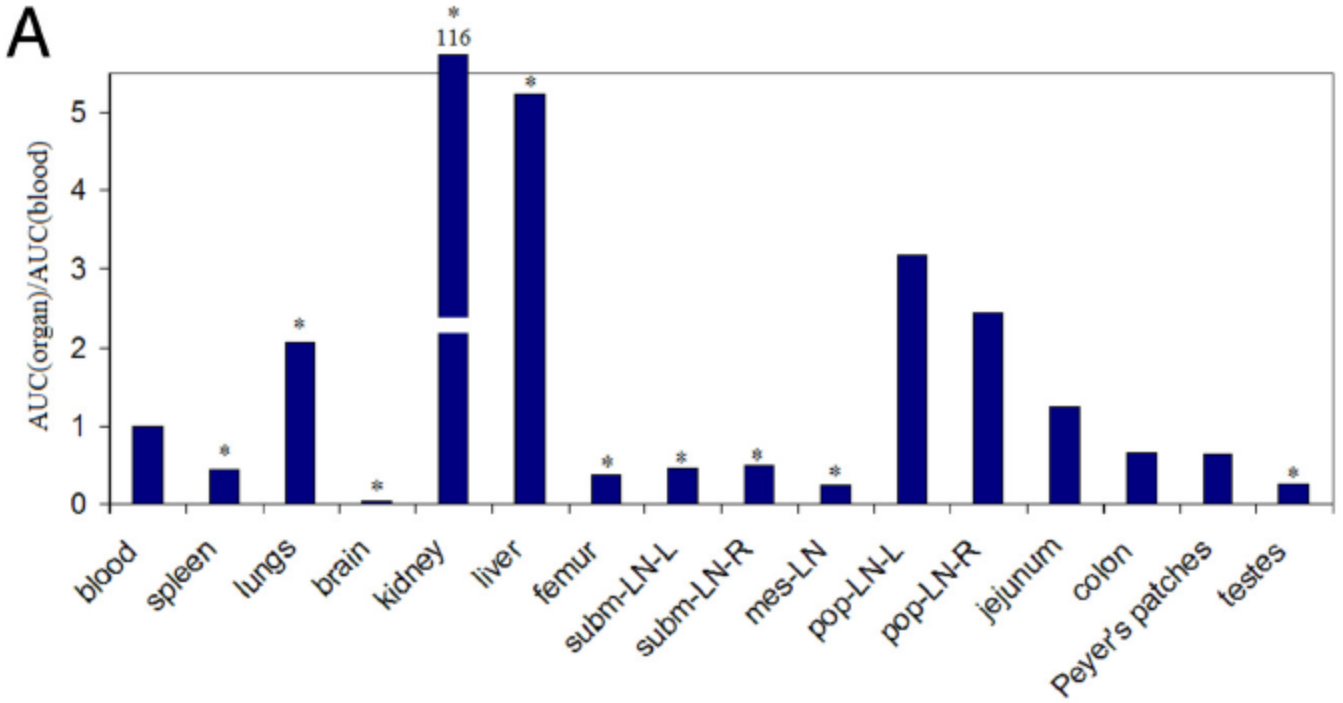
New strategies for increasing antiretroviral concentrations in predominant sites of viral replication may provide more effective means for elimination of viral sanctuaries. Additional research is necessary to optimize antiretroviral tissue distribution in order to inhibit virus replication fully, and avoid resistance and replenishment of viral reservoirs that may persist in the face of antiretroviral therapy.

Curr Opin HIV AIDS 2013, 8:190–195

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Oct. 2009, p. 4086–4095 Vol. 53, No. 10
0066-4804/09/$08.00+0 doi:10.1128/AAC.00419-09

Antiretroviral Tissue Kinetics: In Vivo Imaging Using Positron Emission Tomography▽

Michele Di Mascio,[1]* Sharat Srinivasula,[2] Abesh Bhattacharjee,[3] Lily Cheng,[4] Lucia Martiniova,[5] Peter Herscovitch,[6] Juan Lertora,[7] and Dale Kiesewetter[8]



1, 2, 3, 4, 5: mate

1 Early establishment of pool of latently infected cells
2 Impairment of specific anti-HIV immune responses
3 Persistent immunoactivation (gut damage)
4 Lymphoid tissue disruption
5 …

CROI 2014
Conference on Retroviruses and Opportunistic Infections

- ***The Role of HIV Integration Sites in Extensive Clonal Expansion of Infected Cells in Patients***

  Frank Maldarelli, Xiaolin Wu, Mary Kearney, Ling Su, Wei Shao, Shawn Hill Francesco Simonetti, Jon Spindler, John Coffin, Stephen H. Hughes

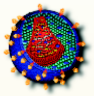
  - *DNA from PBMCs from 5 patients, randomly sheared, and linker-mediated PCR was used to amplify integration site junctions, >2000 independent integration events revealed clonal expansion of HIV infected cells*
  - *Integrations in the same orientation in a specific intron of two different genes (MKL2 and BACH2), were seen in independent clones (14 vs 12 different clones)*
  - *These data show that the expression of these genes must have been affected by these integrations in ways that made a critical contribution to the clonal expansion and/or persistence of the infected cells. Both these genes have been linked to the control of cell growth and human cancers.*

- ***Proliferation of Cells With HIV Integrated Into Regulatory Genes Is a Mechanism of Persistence***

  Thor A. Wagner, Sherry McLaughlin, Kavita Garg, Hannah Huang, Sheila Styrchak, James I. Mullins, Lisa M. Frenkel

  - *538 integration sites were determined from DNA from PBMCs from 3 participants after ~2, ~6, and ~10 years of suppressive ART*
  - *The integration site recovered most frequently (32 times) was in MDC1, which has a known role in cell cycle arrest and apoptosis. The only gene with HIV integrated into multiple sites and in multiple (2 of 3) participants was BACH2, recently identified as a tumor suppressor.*
  - *These results strongly suggest that the specific gene disrupted by HIV integration may impact cellular proliferation and survival, allowing proliferation and prolonged persistence of specific infected cells.*

The Role of HIV Integration Sites in Extensive Clonal Expansion of Infected Cells in Patients



F. Maldarelli[1], X. Wu[2], L. Su[2], W. Shao[3], S. Hill[1], F. Simonetti[1], J. Spindler[1], M. Kearney[1], J. Coffin[4], S. H. Hughes[1]

[1]HIV Drug Resistance Program, Center for Cancer Research, National Cancer Institute, Frederick, MD; [2]Laboratory of Molecular Technology, Leidos Biomedical Research, Inc., Frederick National Laboratory, Frederick MD; [3]Advanced Biomedical Computing Center, Information Systems Program, Leidos Biomed, FNL; [4]Department of Molecular Biology and Microbiology, Tufts University School of Medicine, Boston MA

Background

Despite successful suppression of HIV by combination antiretroviral therapy (cART), infected cells persist for many years in patients. Long-term control of viremia by cART reveals clonal viral genomes in the blood of most patients, implying clonal expansion of some HIV-infected cells. The mechanisms driving clonal expansion are unknown but are central to HIV persistence. To address this issue, we determined the distribution of proviral integration sites in 5 patients on successful cART using a specific and highly sensitive amplification strategy that yields large numbers of virus-host junctions, identifies their exact location, and measures the relative degree of clonal expansion.

Methodology

DNA was prepared from PBMCs from 5 patients, randomly sheared, and linker-mediated PCR was used to selectively amplify both the 5′ and 3′-LTR integration site junctions, whose sequences, as well as those of the sheared breakpoints in the host DNA, were determined by Illumina paired-end sequencing. Clonal expansion of infected cells was demonstrated by the presence of DNA fragments with exactly the same integration site and different host DNA breakpoints.

Results

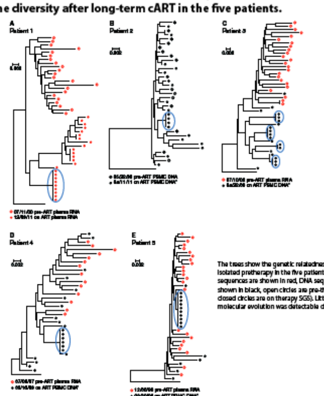
Analysis of integration site libraries comprising >2000 independent integration events revealed clonal expansion of HIV-infected cells in all patients studied. In one patient, about half of the infected PBMCs were derived from a single infected cell. Infected clones persisted in patients for at least 11 years. Integrations in the same orientation in a specific intron of two different genes (MKL2 and BACH2) were seen in independent clones from more than one patient. In one patient, we detected 669 unique sites, of which 14 were in different expanded clones derived from independent integration events in a single 3.5 kb intron of MKL2, and 12 were in a single intron of BACH2. These data show that the expression of these genes must have been affected by these integrations in ways that made a critical contribution to the clonal expansion and/or persistence of the infected cells. Both these genes have been linked to the control of cell growth and human cancers. Other genes associated with cell growth (e.g., PAK2, STAT5B, and CYTH1) in which HIV DNA integration may have contributed to clonal expansion and persistence, were also identified in more than one patient.

Table 1. Patient Demographic Data

Patient	Age at entry (y)	Gender	Race/ Ethnicity	CD4 (cells/μl)	CD4%	HIV RNA (Log_{10} copies/ml plasma)	Current ARV regimen
1*	46.5	M	Black	22	3	5.4	TDF+FTC+RTG
2	24.6	F	Black	243	13	3.9	TDF+FTC+NVP
3	51.6	M	Black	24	2	5.6	TDF+FTC+r/ATZ
4	39.2	M	White	924	39	4.2	ABC+3TC+NVP
5	43.5	M	Black	17	2	5.4	TDF+FTC+NVP

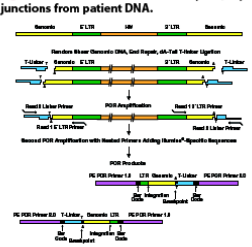
*deceased

Figure 1. A comparison of the pretherapy HIV-1 sequence diversity with the diversity after long-term cART in the five patients.



The trees show the genetic relatedness of virus isolated pretherapy in the five patients (RNA sequences are shown in red, DNA sequences are shown in black, open circles are pre-therapy SGS, closed circles are on therapy SGS). Little or no molecular evolution was detectable during therapy.

Figure 2. Methods used to selectively amplify the host/virus DNA junctions from patient DNA.

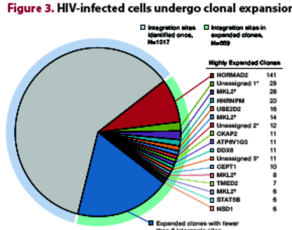


DNA was isolated from cells from the patient's blood and sheared to approximately 300-500 BP fragments (Covaris). The ends of the sheared DNAs were repaired, and a single dA added to the 3′ ends. This allows the selective ligation of a linker that is used to amplify segments that contain the ends of the viral DNA and the adjacent host sequences. Nested primers were used to increase the selective amplification of the desired fragments and to add sequences that were used for Illumina sequencing. Both ends of the amplified segments were sequenced. Differences in the breakpoints of sheared ends of the adjacent host sequences were used to show when the same integration site was present more than once in the original patient DNA.

Table 2. There is extensive clonal expansion of HIV-infected cells in five patients.

Patient	Years on therapy	Distinct integration sites	Number of expanded clones	Percent of expanded clones	Total number of sites in expanded clones	Percent of total sites in expanded clones
1	0.2	254	22	8.7	69	27.2
	4.8	80	11	13.8	27	33.8
	11.4	944	99	10.5	375	39.7
2	12.9	31	2	6.5	4	12.9
3	0	55	2	3.6	4	7.3
	7.2	355	12	3.4	176	49.6
4	12.2	228	19	8.3	83	36.4
5	14.5	125	10	8	24	19.2

Figure 3. HIV-infected cells undergo clonal expansion.

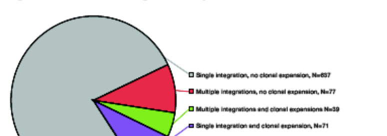


The pie chart shows the fraction of integration sites obtained once and the sites isolated more than once (those that derive from clonally expanded cells). The genes in which the viral DNA is integrated in the most highly amplified clones are shown, as is the relative degree of the amplification, based on how often we recovered the integration sites.

† These are independent integrations into the same intron of MKL2.

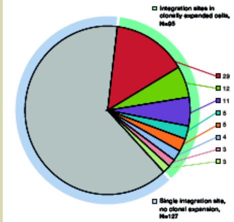
* Unassigned 1, 2, and 3 are distinct sequences.

Figure 4. Genes with integrations in patients.



The pie chart shows the distribution of the integration sites in genes for the five patients taken together. In most cases (637 out of 824), only one integration site was isolated in a given gene. However, we found genes in which there was only one integration site, but it was clonally expanded (71), genes in which there was more than one integration site, but for which we have no evidence of clonal expansion (77), and genes in which there were multiple integrations, at least some of which were clonally expanded (39).

Figure 5. Integrations that are not in any known gene.



Total integration sites that are not in genes	222
Total single integration sites that are not in genes	127
Total integration sites in expanded clones that are not in genes	95
Number of different integrations sites in expanded clones that are not in genes	18

Although most of the HIV integration sites that were identified from the patient samples were in genes, some were not. This pie chart shows what portion of the integration sites that were not in genes that were obtained only once, and what portion was associated with clonal amplification of the infected cells.

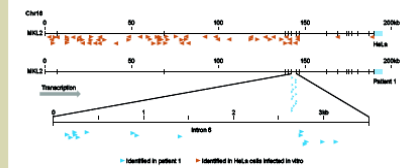
Table 3. Clones persist for >11 years in patient 1.

Gene with integration	Minimum persistence (y)	Gene description
FSIP1	11.2	fibrous sheath interacting protein 1
PARP8	11.2	poly (ADP-ribose) polymerase family, member 8
DDX6	11.2	DEAD (Asp-Glu-Ala-Asp) box polypeptide 6
STAT5B	11.2	signal transducer and activator of transcription 5B
PKP4	11.2	plakophilin 4
CTAGE5	11.2	CTAGE family, member 5 pseudogene; CTAGE family member; CTAGE family, member 4; CTAGE family, member 5
PATL2	11.2	protein associated with topoisomerase II homolog 2 (yeast)
MAP4	6.6	microtubule-associated protein 4
RBM15B	6.6	RNA binding motif protein 15B
NLRC5	6.6	NLR family, CARD domain containing 5
ATP6V1G3	6.6	ATPase, H+ transporting, lysosomal 13kDa, V1 subunit G3
PABPC1L	6.6	poly(A) binding protein, cytoplasmic 1-like
TNFSF13	6.6	TNFSF12-TNFSF13 readthrough transcript; tumor necrosis factor (ligand) superfamily, member 12; tumor necrosis factor (ligand) superfamily, member 13
ZC3H4	6.6	zinc finger CCCH-type containing 4
NAIF1	6.6	nuclear apoptosis inducing factor 1
ZCCHC11	4.8	zinc finger, CCHC domain containing 11
ZNF16	4.8	zinc finger protein 16

Table 4. Genes with ≥3 HIV integrations that are associated with clonal expansion.

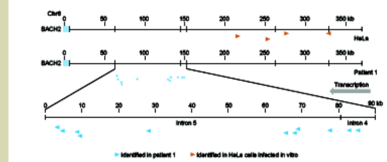
Gene	Patients with integrants	Distinct intragenic integrations (N)	Total integration sites per gene (N)	Gene designation
MKL2	1, 4	17	80	MKL/myocardin-like protein 2 Transcriptional coactivator of serum response factor
BACH2	1, 3	13	17	BTB and CNC homology 1, basic leucine zipper transcription factor 2
STAT5B	1, 3, 4, 5	6	13	Signal transducer and activator of transcription 5B
NFATC3	1, 4, 5	5	9	Nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 3
PAK2	1	4	8	p21 protein (Cdc42/Rac)-activated kinase 2
ATM	1, 3	4	5	Ataxia telangiectasia mutated Serine Theronine Kinase
HORMAD2	1, 3	3	143	HORMA domain containing 2
DDX6	1, 3	3	13	DEAD (Asp-Glu-Ala-Asp) box polypeptide 6
NSD1	1, 5	3	9	Nuclear receptor binding SET domain protein 1
MKL1	1	3	7	Megakaryoblastic leukemia (translocation) 1 transcription interacting protein
USP4	4	3	6	Ubiquitin-specific peptidase 4 (proto-oncogene)
BTBD9	1	3	5	BTB (POZ) domain containing 9
TAOK1	1	3	5	TAO kinase 1

Figure 6. Integrations in MKL2 in patient 1.



The figure shows a map of the MKL2 gene and compares the integration sites obtained in the analysis of a large library of integration sites from HeLa cells infected in vitro (~248,000 independent integration sites) with the integration sites obtained from an analysis of the integration sites in patient 1. Note that, while the in vitro library has integrations scattered throughout the gene, in the patient, all of the integration sites are in the same intron, and all are in the same orientation.

Figure 7. Integrations in BACH2 in patient 1.



The overall form of this figure is similar to Figure 6. It shows the integration sites in BACH2 in both the HeLa library and patient 1. Again, all of the integrants in the patient are in the same intron, and in the same orientation. The HeLa library has relatively few integrants in BACH2, presumably because BACH2 is not expressed in HeLa cells. We analyzed a large library of HIV integration sites in CD34+ cells. There are a number of integrants throughout the gene, in both orientations in that library (data not shown).

MKL2 and BACH2 are involved in human cancer.

MKL2

- *The transcriptional coactivators megakaryoblastic leukemia 1/2 mediate the effects of loss of the tumor suppressor deleted in liver cancer 1.* Muehlich S., Hampl V., Khalid S., Singer S., Frank N., Breuhahn K., Gudermann T., Prywes R. **Oncogene.** 2012; 31(35): 3913-23
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BACH2

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- *Identification of IGHCδ-BACH2 fusion transcripts resulting from cryptic chromosomal rearrangements of 14q32 with 6q15 in aggressive B-cell lymphoma/leukemia.* Kobayashi S., Taki T., Chinen Y., Tsutsumi Y., Ohshiro M., Kobayashi T., Matsumoto Y., Kuroda J., Horiike S., Nishida K, Taniwaki M. **Genes Chromosomes Cancer.** 2011; 50(4): 207-16.

Conclusions

- There is extensive clonal expansion of HIV-infected cells in each of the first 5 patients we analyzed; in one patient a single clone represented approximately half the infected cells in the blood.
- Clonally expanded cells can persist in patients for >11 years.
- In a substantial number of instances, the integration site plays an important role in the clonal expansion and/or persistence of the clone.

Recurrent HIV-1 Integration at the *BACH2* Locus in Resting CD4$^+$ T Cell Populations during Effective Highly Active Antiretroviral Therapy

Terumasa Ikeda, Junji Shibata, Kazuhisa Yoshimura, Atsushi Koito, and Shuzo Matsushita

Division of Clinical Retrovirology and Infectious Diseases, Center for AIDS Research, Kumamoto University, Kumamoto, Japan

The persistence of latent human immunodeficiency virus type 1 (HIV-1) has been considered one of the major obstacles for eradication of the virus in infected individuals receiving successful antiretroviral therapy. To determine the contribution of integration sites to viral latency within clinical settings, an inverse polymerase chain reaction method was used to analyze integration sites in CD4$^+$ T cells from patients showing long-term undetectable plasma viral RNA. Of 457 sites identified in 7 patients, almost all (96%) resided within transcriptional units, usually in introns of the human genome. Studies of 18 genes in which HIV-1 integrates found them to be actively expressed in resting CD4$^+$ T cells. On the other hand, integration sites in the α satellite region was also identified in some patients, albeit at low frequency. Of particular interest, HIV-1–infected cells with multiple identical integration sites were detected in longitudinal analysis of samples from 3 patients, suggesting that these cells persist for long periods and that clonal expansion may occur. Furthermore, strong integration clusters in the *BACH2* gene were observed in 2 patients (31% in patient 1 and 5% in patient 3). Our findings not only raise the possibility of biased target-site integration but also provide mechanistic insights into the long-term persistence of HIV-1.