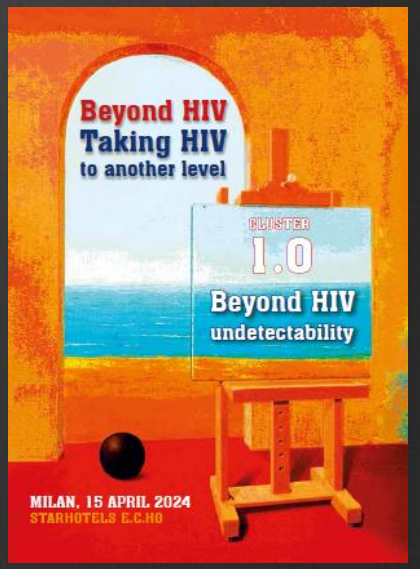
Mechanisms of suboptimal immune-reconstitution on cART

Giuseppe Nunnari
Università di Catania
ARNAS Garibaldi



Immunological Non Responders INR

The prevalence of INRs varies from 10% to 40%.

Increased risk for all-cause mortality

Regardless of the definition for poor immune response, this group of patients is exposed to an increased risk for all-cause mortality

Serrano-Villar S, Sainz T, Lee SA, Hunt PW, Sinclair E, Shacklett BL, et al. HIVinfected individuals with low CD4/CD8 ratio despite effective antiretroviral therapy exhibit altered T cell subsets, heightened CD8+ T cell activation, and increased risk of non-AIDS morbidity and mortality. PLoS Pathog. 2014;10(5):e1004078.

Kelly C, Gaskell KM, Richardson M, Klein N, Garner P,MacPherson P. Discordant immune response with antiretroviral therapy in HIV-1: a systematic review of clinical outcomes. PLoS One. 2016;11(6):e0156099.

Failure of CD4+ T-cell Recovery upon Virally-Effective cART: an Enduring Gap in the Understanding of HIV+ Immunological non-Responders

Valeria Bono, Matteo Augello, Camilla Tincati, Giulia Marchetti

Clinic of Infectious Diseases and Tropical Medicine, San Paolo Hospital, ASST Santi Paolo e Carlo, Department of Health Sciences, University of Milan, Italy

- A unique definition of INR is currently unavailable
- An appropriate immune response to cART is characterized by CD4+ T-cell counts >500 cells/μL, because the morbidity and mortality rate of these individuals has been found comparable to that of HIV-negative ones
- The Department of Health and Human Services (DHHS) attributed a more precise definition of INRs by considering those individuals with CD4+ T-cell counts between 350 and 500 cells/μL after 4-7 years of effective ART
- CD4+ T-cell or CD4/CD8 ratio?

New Microbiologica, 45, 3, 155-172 , 2022,

Definitions

- CD4 T-cell count >500 cells/μL
- CD4/CD8 ratio
- CD4 T-cell count increase following ART initiation.

Milanés-Guisado Y, Gutiérrez-Valencia A, Trujillo-Rodríguez M, Espinosa N, Viciana P, López-Cortés LF. Absolute CD4+ T cell count overstate immune recovery assessed by CD4+/CD8+ ratio in HIV-infected patients on treatment. PLoS One. 2018;13(10).

Risk factors for poor immune response

- Older age at HIV diagnosis
- Male sex
- Late ART initiation
- Lower CD4 t-cell nadir

- Milanés-Guisado Y, et al. Absolute CD4+ T cell count overstate immune recovery assessed by CD4+/CD8+ ratio in HIV-infected patients on treatment. PLoS One. 2018;13(10).
- Van Lelyveld SFL, et al. Long-term complications in patients with poor immunological recovery despite virological successful HAART in Dutch ATHENA cohort. AIDS. 2012;26(4):465–74.
- Gunda D, et al.. Prevalence and risk factors of poor immune recovery among adult HIV patients attending care and treatment centre in northwestern Tanzania following the use of highly active antiretroviral therapy: a retrospective study. BMC Res Notes. 2017;10(1):197.
- Fiseha T, Ebrahim H, Ebrahim E, Gebreweld A. CD4+ cell count recovery after initiation of antiretroviral therapy in HIV-infected Ethiopian adults. PLoS One. 2022;17(3):e0265740.

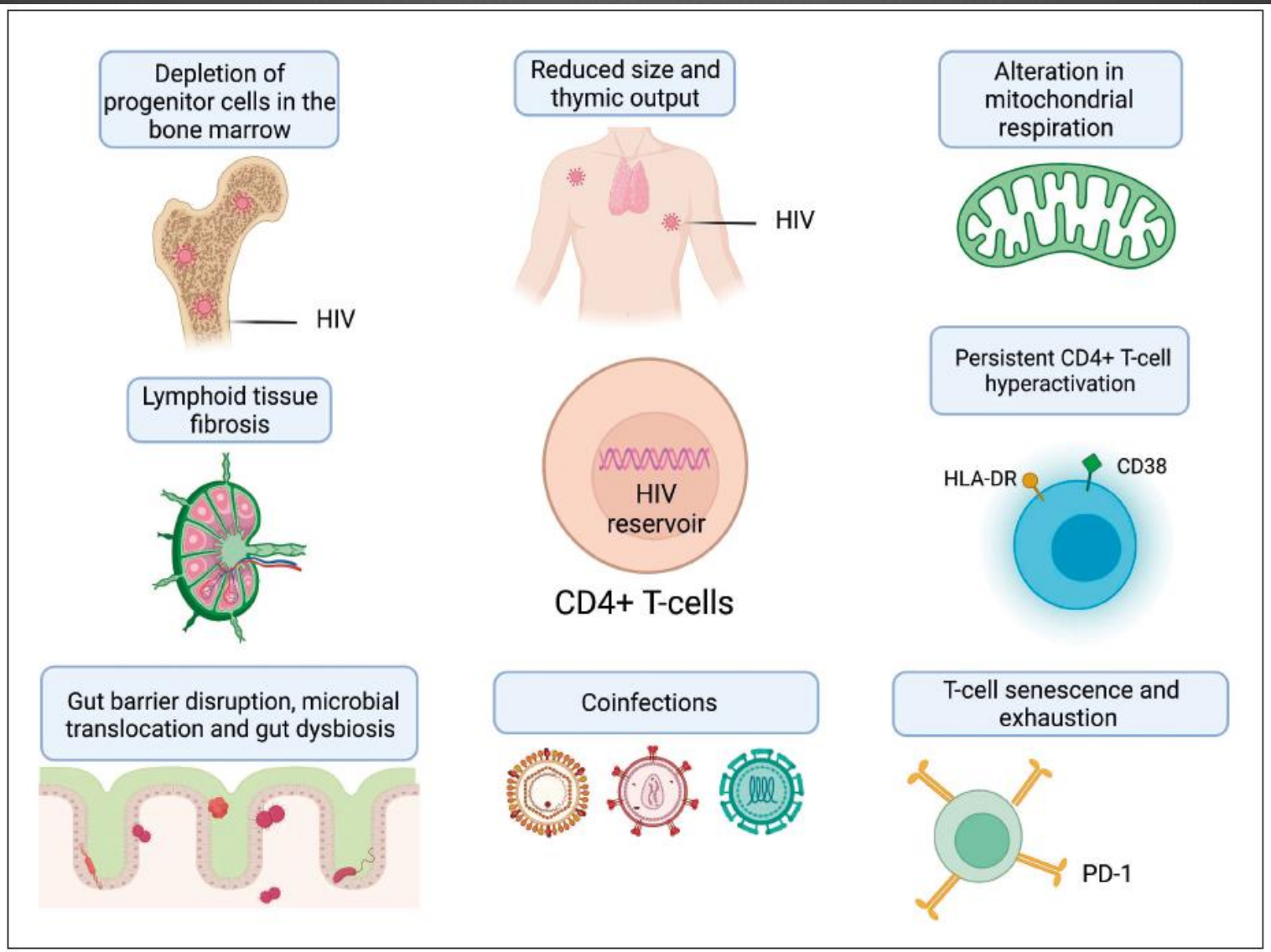


Figure 1 - *Underlying pathogenetic mechanisms of incomplete immune reconstitution in immunological non-responders. The main pathogenetic mechanisms contributing to inadequate immune reconstitution in HIV-infected individuals: bone marrow and hematopoietic progenitor cells (HPCs) depletion, thymus impairments, mitochondrial dysfunction, CD4+ T-cell hyperactivation, T-cell exhaustion, coinfections, microbial translocation, gut dysbiosis and lymphoid tissue fibrosis. Created with* BioRender.com.

New Microbiologica, 45, 3, 155-172 , 2022, ISSN 1121-7138

Bone marrow alterations Progenitor cells

- **DEPLETION** OF PROGENITOR CELLS AT THE THYMIC AND/OR PRE-THYMIC stage (Jenkins et al., 1998).
- HPC DECREASED WITH **DISEASE PROGRESSION** AND WERE ASSOCIATED WITH CD4+ T-CELL COUNTS Sauce et al., 2011
- CAPACITY OF CD34+ HPCS TO **DIFFERENTIATE INTO T-CELLS** IS SIGNIFICANTLY REDUCED
- IN INRs AS COMPARED TO FULL RESPONDERS, PRIMITIVECD34+ PROGENITOR CELLS IN INRS WAS REDUCED IN PARALLEL WITH REDUCED IL-2 PRODUCTION AND INCREASED PRODUCTION OF TNF-α
- THE **COLONY-FORMING ABILITY OFCD34+** HPCS AND HSCS FROM INRS IS MUCH LOWER THAN THAT THE COLONY-FORMING ABILITY OF THOSE FROM IRS Guo X,et al. J Acquir Immune Defic Syndr. 2016;71:467-473

frontiers | Frontiers in Immunology

TYPE Review
PUBLISHED 02 May 2023
DOI 10.3389/fimmu.2023.1152951

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OPEN ACCESS

EDITED BY
Peter Hunt,
University of California, San Francisco, United States

REVIEWED BY
Soufiel-Antoine Younes,
Emory University, United States
Julià Blanco,

Cellular and molecular insights into incomplete immune recovery in HIV/AIDS patients

Liting Yan[1,2*], Kaiju Xu[1,2], Qing Xiao[3], Lin Tuo[1,2], Tingting Luo[1,2], Shuqiang Wang[1,2], Renguo Yang[1,2], Fujie Zhang[3*] and Xingxiang Yang[1,2*]

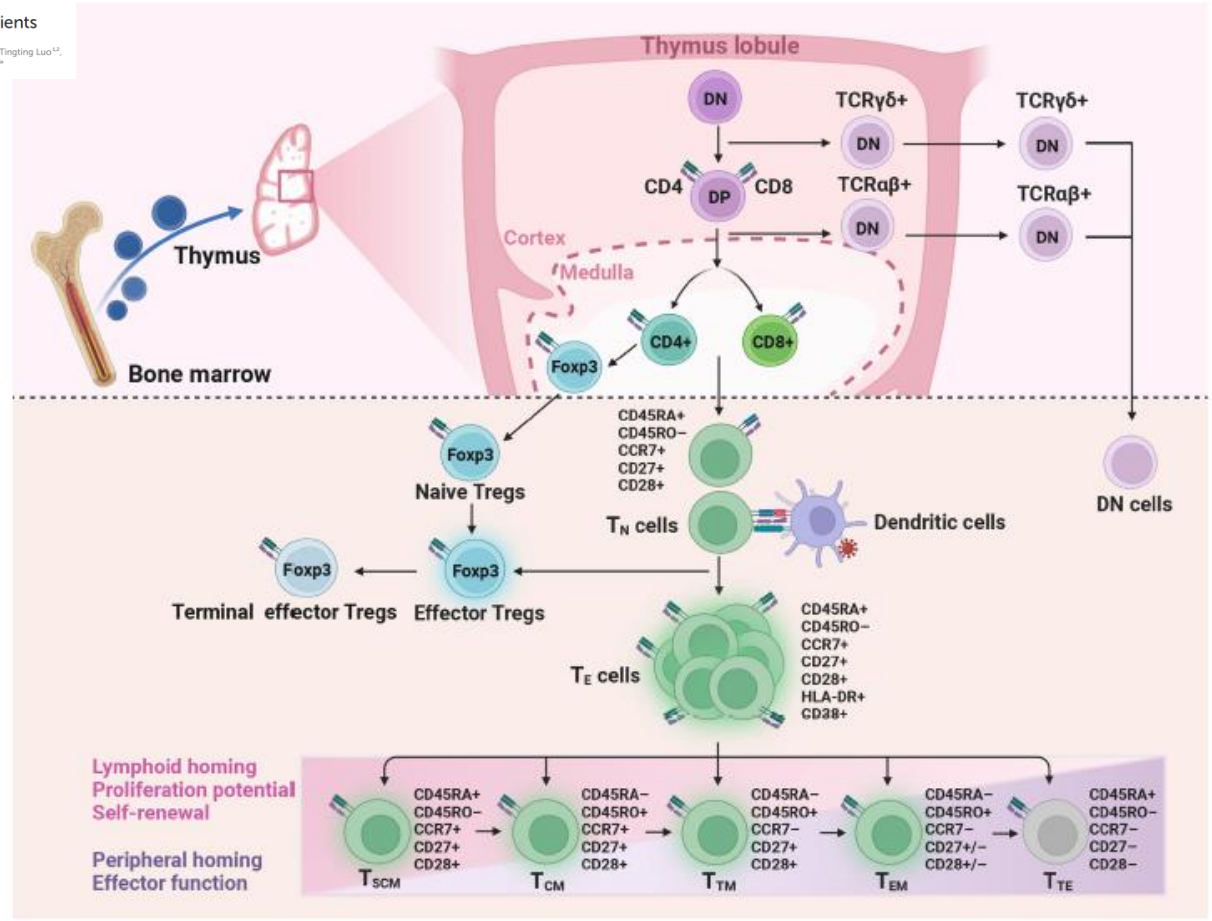


FIGURE 1
Production and differentiation of T cells. Naive $CD4^+$ and $CD8^+$ T (T_N), naive regulatory T (Tregs) cells, and double-negative (DN) T cells are released from the thymus. Then $CD4^+$ and $CD8^+$ T cells get activated and differentiate into effector (T_E) cells after encountering antigens. Only a small part of T_E develops into memory T cells, including stem-like memory (T_{SCM}), central memory (T_{CM}), transitional memory (T_{TM}), effector memory (T_{EM}), and terminal effector (T_{TE}) cells. In HIV immunological non-responders, the number of T_N cells produced by the thymus is reduced, while the proportion of activated T cells is increased. DP, double-positive; TCRαβ, T cell receptor alpha and beta; TCRγδ, T cell receptor gamma and delta; Foxp3, factor forkhead box P3.

CD3+CD4-CD8- T cells

- INRs had a low number of CD3+CD4-CD8- T cells after long-term ART and that the number of these cells positively correlated with the CD4+ T-cell count

- Low double-negative CD3(+)CD4(-)CD8(-) T cells are associated with incomplete restoration of CD4(+) T cells and higher immune activation in HIV-1 immunological non-responders. Lu X, Su B, Xia H, et al. Front Immunol. 2016;7:579.

Reduced thymic output

- Thymic biopsy?
- Therefore, thymic function is indirectly assessed by
  - T-cell receptor excision circles (TRECs)
  - Recent thymus emigrants (RTEs)
  - Naïve CD4+ T-cell counts

Lower thymic output

- INR, compared to IR, have lower levels of
  - CD4+ RTE cells
  - The numbers of sj-trecs
  - The sj/β-TREC ratios
- Indicating that lower thymic output is the main cause of incomplete immune recovery in these patient

- Shete A, et al. Incomplete functional T-cell reconstitution in immunological non-responders at one year after initiation of antiretroviral therapy possibly predisposes them to infectious diseases. Int J Infect Dis. 2019;81:114-122.
- Massanella M, et al. CD4 T-cell hyperactivation and susceptibility to cell death determine poor CD4 T-cell recovery during suppressive HAART. AIDS. 2010;24:959-968.
- Rb-Silva R, et al. Thymic function as a predictor of immunerecovery in chronically HIV-infected patients initiating antiretroviral therapy. Front Immunol. 2019;10:25.

Reduced thymic output

- INRs had a lower naïve CD4+ T-cell increase
- A lower percentage of CD4+ RTE
- A reduced thymic output may be a major mechanism of incomplete immune reconstitution

- Li T, et al. Reduced thymic output is a major mechanism of immune reconstitution failure in HIV-infected patients after longterm antiretroviral therapy. Clin Infect Dis. 2011;53(9):944-951.

Thymic SIZE matter!

- Individuals with a **large thymus** have a better immune reconstitution and a broader immunological repertoire than patients with a small thymus (Vigan. et al., 1999)
- **Thymic size** as marker of thymic function and thus CD4+ total and naive T-cell counts

ART improves thymic output

- After ART initiation, the thymic output improved significantly in HIV-1-infected adults and children, indicating that early ART initiation is essential for immune reconstitution in HIV-1-infected patients

- Castro P,, et al. Effects of different antigenic stimuli on thymic function and interleukin-7/CD127 system in patients with chronic HIV infection. J Acquir Immune Defic Syndr. 2014;66: 466-472.

- Sandgaard KS, Lewis J, Adams S, et al. Antiretroviral therapy increases thymic output in children with HIV. AIDS. 2014;28: 209-214.

Bone marrow and thymic immunological reservoir

- Is it terminally depleted?

- Is it inducible?

MAJOR ARTICLE HIV/AIDS

Effects of Recombinant Human Interleukin 7 on T-Cell Recovery and Thymic Output in HIV-Infected Patients Receiving Antiretroviral Therapy: Results of a Phase I/IIa Randomized, Placebo-Controlled, Multicenter Study

Y. Lévy,[1,2,3,a] I. Sereti,[4,a] G. Tambussi,[5] J. P. Routy,[6] J. D. Lelièvre,[1,2] J. F. Delfraissy,[4] J. M. Molina,[8] M. Fischl,[9] C. Goujard,[7] B. Rodriguez,[10] C. Rouzioux,[10] V. Avettand-Fenoël,[10] T. Croughs,[11] S. Beq,[11] M. Morre,[11] J. F. Poulin,[12] R. P. Sekaly,[12] R. Thiebaut,[13] and M. M. Lederman[14]

[1]Inserm, U955, [2]Université Paris Est, Faculté de Médecine, and [3]AP-HP, Hôpital H. Mondor–A. Chenevier, Service Hospitalier, Créteil, France; [4]National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland; [5]San Raffaele Scientific Institute, Milan, Italy; [6]McGill University Health Center, Montreal, Canada; [7]Hosp Kremlin Bicêtre, [8]Hosp St-Louis, Paris, France; [9]University of Miami School of Medicine, Florida; [10]AP-HP Hôpital Necker, Université Paris Descartes, [11]Cytheris SA, Issy-les-Moulineaux, France; [12]NIMI, Montreal, Canada; [13]INSERM U897, Bordeaux, France; and [14]Case Western Reserve University/University Hospitals/Case Medical Center, Cleveland, Ohio

Background. The immune deficiency of human immunodeficiency virus (HIV) infection is not fully corrected with ARV therapy. Interleukin-7 (IL-7) can boost CD4 T-cell counts, but optimal dosing and mechanisms of cellular increases need to be defined.

Methods. We performed a randomized placebo-controlled dose escalation (10, 20 and 30 μg/kg) trial of 3 weekly doses of recombinant human IL-7 (rhIL-7) in ARV-treated HIV-infected persons with CD4 T-cell counts between 101 and 400 cells/μL and plasma HIV levels <50 copies/mL. Toxicity, activity and the impact of rhIL-7 on immune reconstitution were monitored.

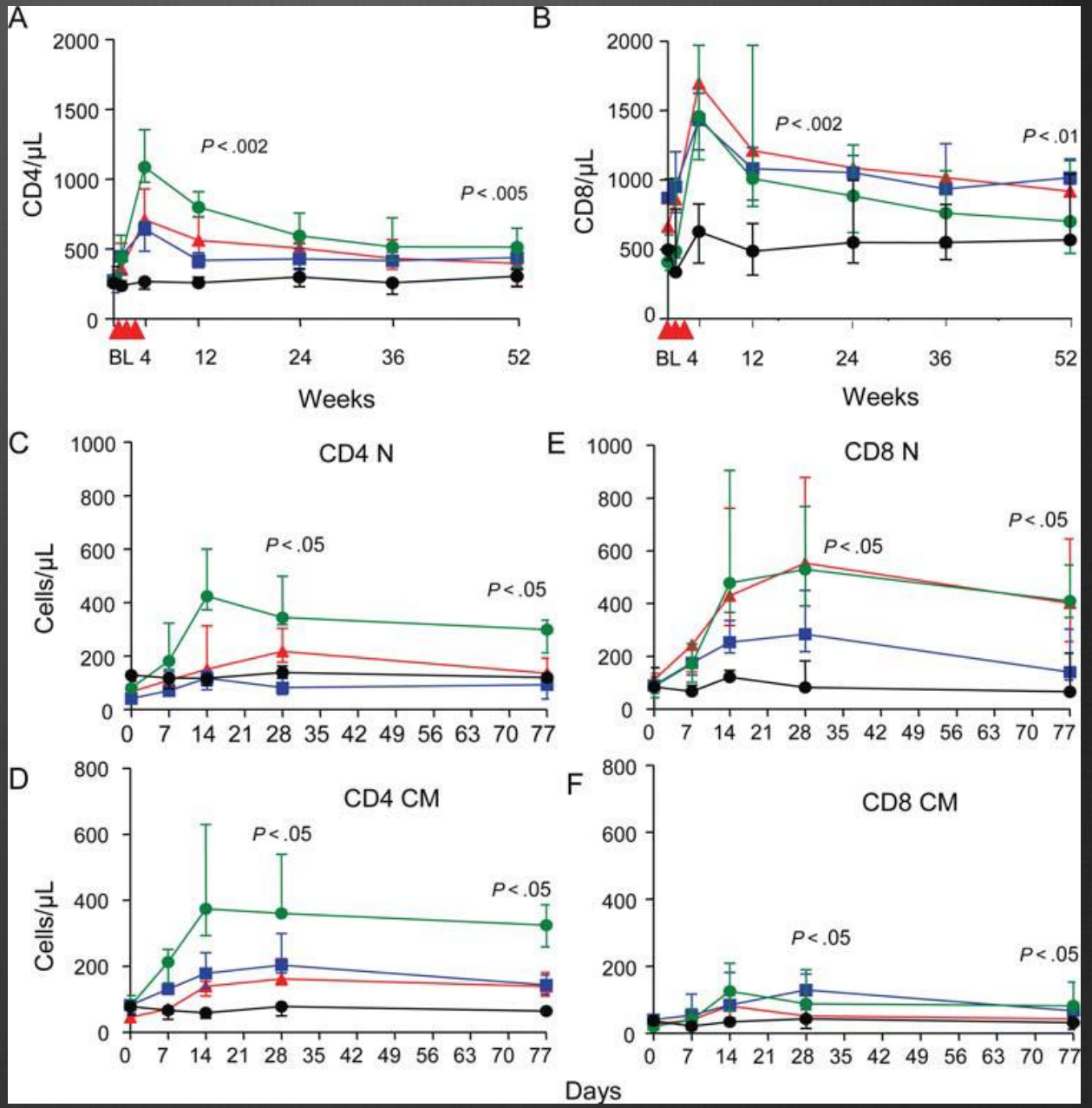
Results. Doses of rhIL-7 up to 20 μg/kg were well tolerated. CD4 increases of predominantly naive and central memory T cells were brisk (averaging 323 cells/μL at 12 weeks) and durable (up to 1 year). Increased cell cycling and transient increased bcl-2 expression were noted. Expanded cells did not have the characteristics of regulatory or activated T cells. Transient low-level HIV viremia was seen in 6 of 26 treated patients; modest increases in total levels of intracellular HIV DNA were proportional to CD4 T-cell expansions. IL-7 seemed to increase thymic output and tended to improve the T-cell receptor (TCR) repertoire in persons with low TCR diversity.

Conclusions. Three weekly doses of rhIL-7 at 20 μg/kg are well tolerated and lead to a dose-dependent CD4 T-cell increase and the broadening of TCR diversity in some subjects. These data suggest that this rhIL-7 dose could be advanced in future rhIL-7 clinical studies.

Clinical Trials Registration. NCT0047732.

CD4 100-400
VL below 50 cps/ml
32 PLWH

1) 10 pts 10, μg/kg groups
2) 12 pts 20μg/kg groups
3) 10 pts 30 μg/kg groups



Clinical Infectious Diseases 2012;55(2):291–300

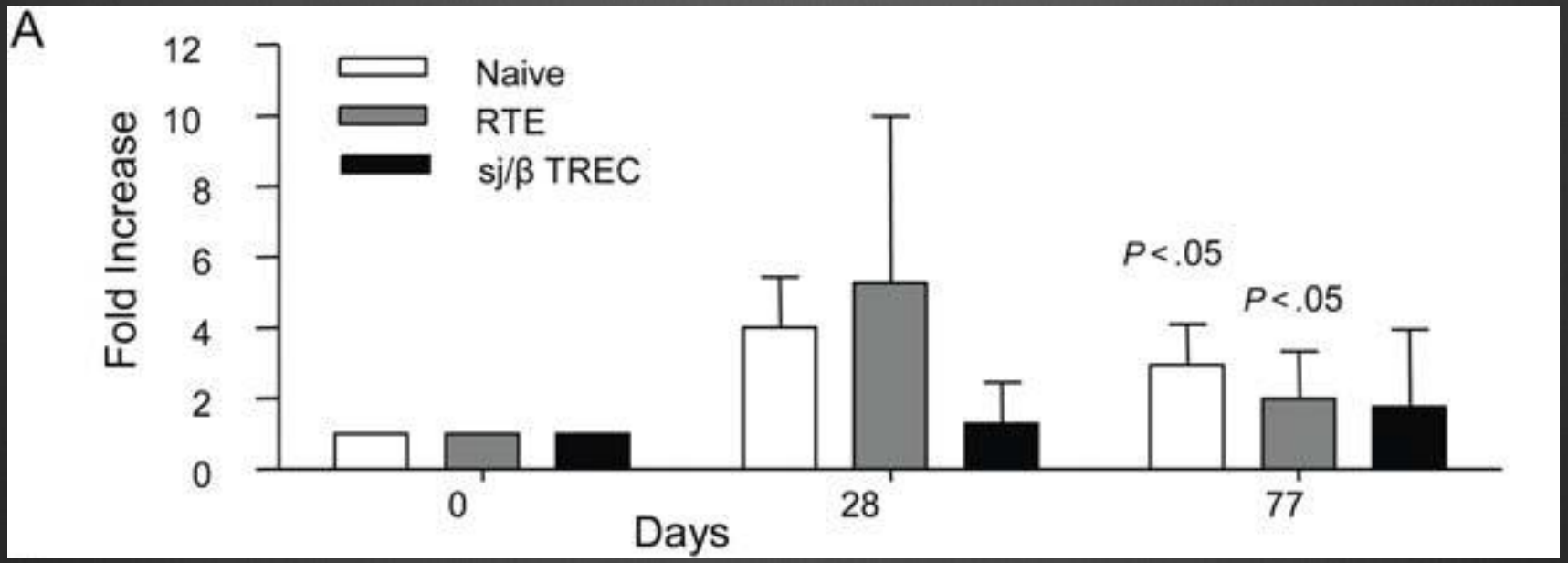


Table 2. Changes in Total Cell-Associated HIV DNA Levels

	Change in HIV DNA Level From Baseline, Median (IQR)								
	Day 0			Day 28			Week 12		
Dose Group	Copies/μL	Copies/10^6 PBMCs	Copies/10^6 CD4 T Cells	Log_{10} Copies/mL	Copies/10^6 PBMCs	Copies/10^6 CD4 T Cells	Copies/μL	Copies/10^6 PBMCs	Copies/10^6 CD4 T Cells
Placebo (n = 6)	2.75 (2.62–2.98)	2.47 (2.32–2.70)	3.37 (3.33–3.41)	3.02 (2.78–3.07)	2.67 (2.68–2.79)	3.49 (3.38–3.54)	2.77 (2.63–2.94)	2.51 (2.31–2.75)	3.35 (3.09–3.58)
rhIL-7 dose, μg/kg									
10 (n = 7)	2.89 (2.72–3.52)	2.63 (2.34–3.27)	3.65 (3.16–4.15)	3.33 (3.00–3.64)	2.90 (2.50–3.15)	3.57 (3.05–3.94)	3.36 (2.89–3.48)	3.00 (2.44–3.19)	3.79 (3.04–3.92)
20 (n = 8)	3.52 (3.00–3.70)	3.24 (2.87–3.36)	4.04 (3.59–4.22)	3.84 (3.57–4.13)	3.38 (3.00–358)	4.06 (3.77–4.15)	3.63[a] (3.23–3.87)	3.35 (2.87–3.40)	3.90 (3.62–4.10)
30 (n = 6)	2.47 (2.03–3.45)	2.29 (1.99–2.94)	3.13 (2.62–4.03)	3.15 (2.70–3.79)	2.57 (2.18–3.15)	3.16 (2.56–3.80)	2.98[a] (2.64–3.69)	2.49 (2.29–3.15)	3.12 (2.68–3.92)

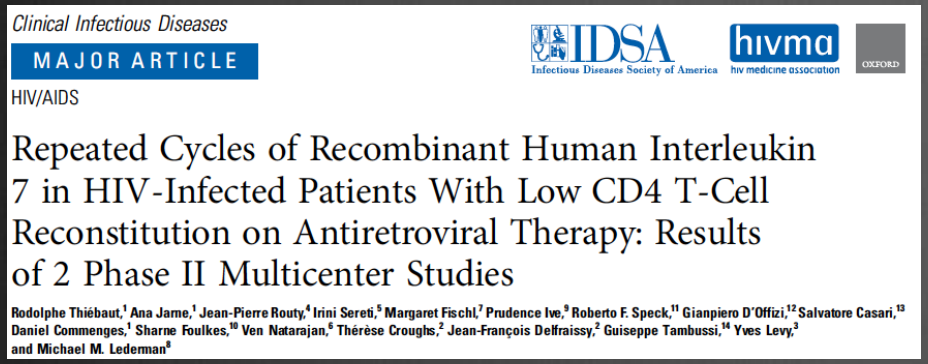
Abbreviations: HIV, human immunodeficiency virus; IQR, interquartile range; PBMCs, peripheral blood mononuclear cells; rhIL-7, recombinant human interleukin 7.

[a] Increased significantly ($P < .05$) compared with day 0 (baseline).

rIL-7 treatment

- Increase in naive and central memory CD4 T cells for one year
- Increased thymic output and/or cell survival
- An improvement in gut barrier integrity
- Decrease in inflammatory markers measured in the blood
- Minimal effect on HIV RNA and DNA

Sereti I, et al. IL-7 administration drives T cell-cycle entry and expansion in HIV-1 infection. Blood 2009; 113:6304–14.
Levy Y, et al. Enhanced T cell recovery in HIV-1-infected adults through IL-7 treatment. J Clin Invest 2009; 119:997–1007.
Levy Y, Sereti I, Tambussi G, et al. Effects of r-hIL-7 on T cell recovery and thymic output in HIV-infected patients receiving antiretroviral therapy: results of a phase I/IIa randomized, placebo controlled, multicenter study. Clin Infect Dis 2012; 55:291–300.
Thiebaut R, Drylewicz J, Lacabaratz C, Sekaly R, Lederman MM. Quantifying and predicting the effect of exogenous interleukin-7 on CD4+ T cells in HIV-1 infection. 2014; 10:e1003630.
Sereti I, Estes JD, Thompson WL, et al. Decreases in colonic and systemic inflammation in chronic HIV infection after IL-7 administration. Plos Pathog 2014; 10: e1003890.



Clinical Infectious Diseases
MAJOR ARTICLE
HIV/AIDS

Repeated Cycles of Recombinant Human Interleukin 7 in HIV-Infected Patients With Low CD4 T-Cell Reconstitution on Antiretroviral Therapy: Results of 2 Phase II Multicenter Studies

Rodolphe Thiébaut,[1] Ana Jarne,[1] Jean-Pierre Routy,[4] Irini Sereti,[5] Margaret Fischl,[7] Prudence Ive,[9] Roberto F. Speck,[11] Gianpiero D'Offizi,[12] Salvatore Casari,[13] Daniel Commenges,[1] Sharne Foulkes,[10] Ven Natarajan,[6] Thérèse Croughs,[2] Jean-François Delfraissy,[2] Guiseppe Tambussi,[14] Yves Levy,[3] and Michael M. Lederman[8]

Clinical Benefit?

Greater increases in CD4 counts

A longer time spent with count >500 cells µl (≥2 injections per cycle)

Antidrug antibodies
16-26% after the first cycle
77-82 after the second cycle

Neutralizing antibodies developed
1 patient 3 after the first cycle
and in 6 (38%) and 21 (37%) of patients after the second cycle in INSPIRE 2 and 3, respectively.

HIV RNA Blips in almost 50% of patients
HIV DNA increased

Clinical Infectious Diseases® 2016;62(9):1178–85

CD4 Naive T cells?

- Causes of reduction:
  - High Antigenic challenge
  - Immunological transformation/activation into Memory T Cells
  - Destruction

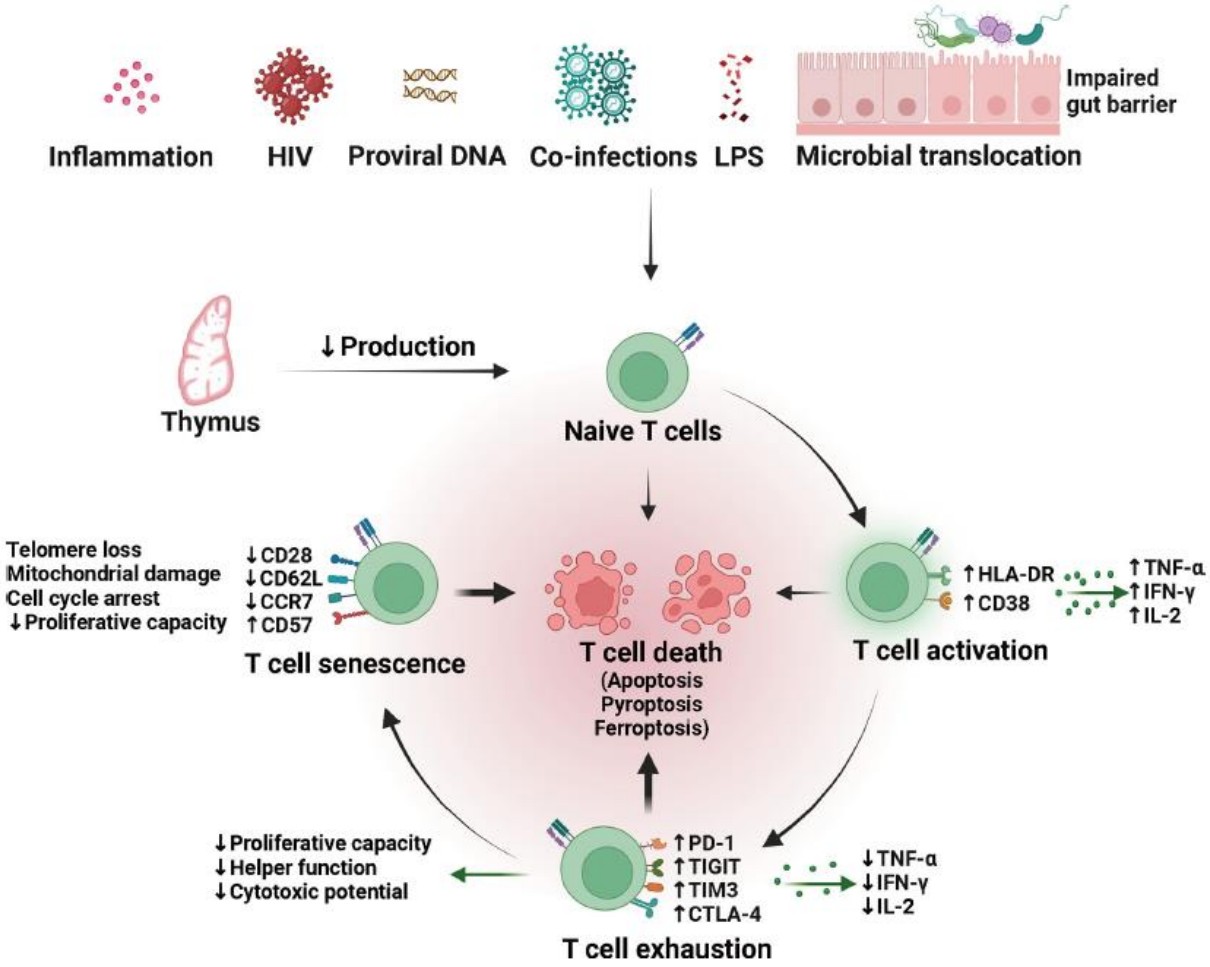


FIGURE 2
Quantity and quality alterations of T cells in HIV/AIDS patients with incomplete immune reconstitution. The naïve T (T_N) cells produced by the thymus can be activated by multiple factors, including persistent viral reservoir, low residual viremia, co-infections, microbial translocation and dysbiosis. Difficult-to-remove risk factors may lead to persistent chronic immune activation, contributing to the development of T cell exhaustion and immune senescence. Exhausted and senescent T cells are qualitatively altered and prone to death, possibly through apoptosis, pyroptosis, and ferroptosis. LPS, lipopolysaccharide; HLA-DR, human leukocyte antigen DR; TNF-α, tumor necrosis factor-alpha; IFN-γ, interferon-gamma; IL-2, interleukin-2; PD-1, programmed death-1; TIGIT, T cell immunoglobulin and ITIM domain; TIM3, T cell immunoglobulin domain and mucin domain-containing protein 3; CTLA-4, cytotoxic T lymphocyte antigen-4.

Immune activation and inflammation and mortality

- Independently associated to increased mortality
- It is only partially recovered by cART
- Despite the complete suppression of HIV replication INRs maintain a CD4+ T-cell hyperactivation comparable to that observed in patients who have not entirely achieved viral suppression

- **(Marchetti** et al., **2010)**
- **(Deeks** et al., **2004; Giorgi** et al., **1999)**

T-cell activation leads to apoptosis-pyroptosis

- Increased rate of spontaneous CD4+ T-cell apoptosis in INRs
- NLRP3 inflammasome and the caspase-1 have been found up-regulated in INRs (Bandera et al., 2018)
- via persistent immune activation and pyroptosis
- (Marchetti et al., 2006; Negredo et al., 2010; Hansjee et al., 2004),

Increased pro-inflammatory cytokines

- Increased pro-inflammatory cytokines have been observed in INRs with very low CD4+ T-cell counts
  - TNF-**α**
  - IL-1 **alpha**
  - IP-10

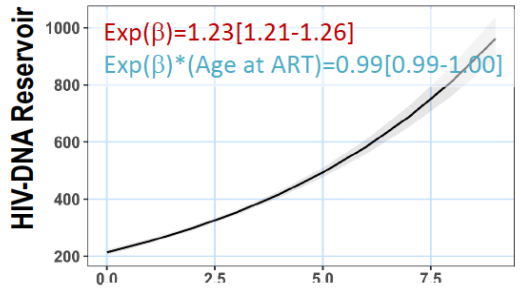
(Hunt et al., 2003; Vajpayee et al., 2009; Zhang et al., 2013).

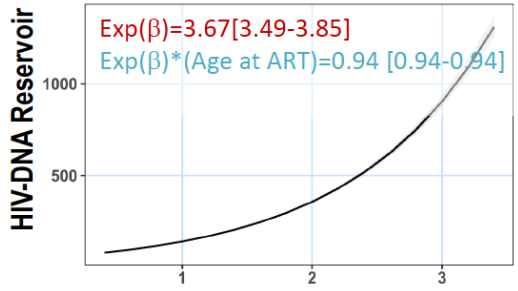
Cytokines

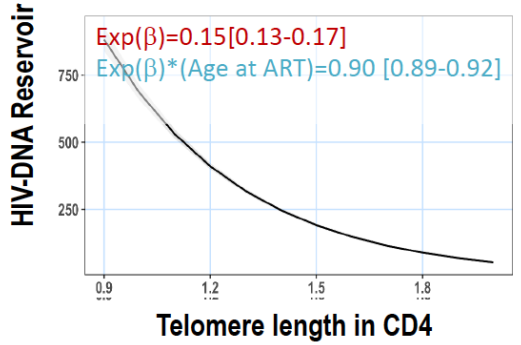
- Disrupted IL-7/IL-7R pathway as correlate of the poor immune recovery of treated PLWH**(Fry and Mackall 2005).**
- Similarly to IL-7, interleukin 2 (IL-2) and interleukin 15 (IL-15) are central regulators of T-cell proliferation, activation, and differentiation
- The production of both IL-2 and IL-15 is indeed compromised in HIV-infected individuals (Ahmad et al., 2005).
- Immune therapy?

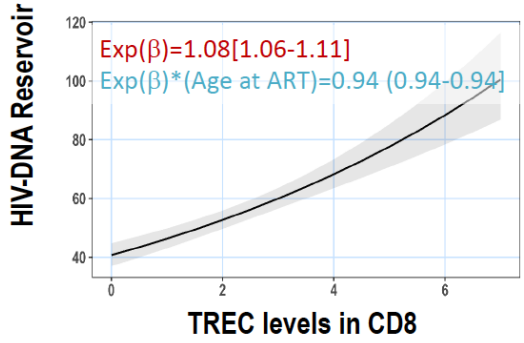
HIV < 50/ml

- HIV RNA
- HIV DNA
- HIV Proteins gp120, p24, nef, rev









These associations decrease by 1%, 10%, 6% and 6% for each month ART is delayed

Dalzini A et al. Journal of the International AIDS Society 2021, 24:e25847
https://onlinelibrary.wiley.com/doi/10.1002/jia2.25847/full | https://doi.org/10.1002/jia2.25847

JIAS JOURNAL OF THE INTERNATIONAL AIDS SOCIETY

RESEARCH ARTICLE

Size of HIV-1 reservoir is associated with telomere shortening and immunosenescence in early-treated European children with perinatally acquired HIV-1

Annalisa Dalzini[1], Giovanni Ballin[1], Sara Dominguez-Rodriguez[2], Pablo Rojo[2], Maria Raffaella Petrara[1], Caroline Foster[3], Nicola Cotugno[4,5], Alessandra Ruggiero[4], Eleni Nastouli[6], Nigel Klein[6], Stefano Rinaldi[7], Savita Pahwa[7], Paolo Rossi[4,5,8], Carlo Giaquinto[8], Paolo Palma[4,5], Anita De Rossi[1,9] and on behalf of EPIICAL Consortium[1]

[9]Corresponding author: Anita De Rossi, Section of Oncology and Immunology, Department of Surgery, Oncology and Gastroenterology, University of Padova, Via Gattamelata, 64, Padova 35128, Italy. Tel: +39 049 821 5894. (anita.derossi@unipd.it)

Abstract

Introduction: Persistence of HIV-1, causing chronic immune activation, is a key determinant of premature senescence. Early antiretroviral therapy (ART) has been associated with a reduced HIV-1 reservoir in children with perinatally acquired HIV-1 (PHIV), but its impact on the senescence process is an open question. We investigated the association between HIV-1 reservoir and biological and immune ageing profile in PHIV enrolled in the multicentre cross-sectional study CARMA (Child and Adolescent Reservoir Measurements on early suppressive ART) conducted within the EPIICAL (Early treated Perinatally HIV Infected individuals: Improving Children's Actual Life) consortium.

Methods: Between September 2017 and June 2018, CARMA enrolled 40 PHIV who started ART before 2 years of age and had undetectable viremia for at least 5 years before sampling date. Samples from 37 children with a median age of 13.8 years were available for this study. HIV-1 DNA copies on CD4 cells, relative telomere length (marker of cellular senescence) and levels of T-cell receptor rearrangement excision circle (TREC, marker of thymic output) on CD4 and CD8 cells were quantified by qPCR. Immunological profile was assessed by flow cytometry. Associations between molecular and phenotypic markers, HIV-1 reservoir and age at ART initiation were explored using a multivariable Poisson regression.

Results: Higher HIV-1 reservoir was associated ($p<0.001$) with telomere shortening (incidence rate ratio [IRR] = 0.15 [0.13–0.17]), immunosenescence (CD28$^-$CD57$^+$, IRR = 1.23 [1.21–1.26]) and immunoactivation (CD38$^+$ HLADR$^+$, IRR = 7.29 [6.58–8.09]) of CD4 cells. Late ART initiation (after 6 months of age) correlated with higher HIV-1 reservoir levels (552 [303–1001] vs. 89 [56–365] copies/10^6 CD4 cells, $p = 0.003$) and percentage of CD4 senescent cells (2.89 [1.95–6.31] vs. 1.02 [0.45–2.69, $p = 0.047$). TREC levels in CD8 cells were inversely associated with HIV-1 reservoir (IRR = 0.77 [0.76–0.79]) and were significantly lower in late treated PHIV (1128 [486–1671] vs. 2278 [1425–3314], $p = 0.042$).

Conclusions: Later ART initiation is associated with higher HIV-1 reservoir size, which correlates with increased telomere shortening and senescence of CD4 cells. Timing of ART initiation in infancy has long-term consequences on the immune and biological ageing profile of children with perinatally acquired HIV-1.

Keywords: HIV; paediatric HIV; immune ageing; telomeres; telomere length; early treatment

HIV-DNA in CD4 cells

↑ senescent CD4 cells

↓ telomere length in CD4 cells

↑ activated CD8 cells

↑ thymic output in CD8 cells

Dalzini A et al. Journal of the International AIDS Society 2021, 24:e25847

ARTICLES

nature medicine

HIV-1 infects multipotent progenitor cells causing cell death and establishing latent cellular reservoirs

Christoph C Carter[1,2,7], Adewunmi Onafuwa-Nuga[3,7], Lucy A McNamara[4,5], James Riddell IV[3], Dale Bixby[3], Michael R Savona[3,6] & Kathleen L Collins[1–4]

VIROLOGY 225, 413–418 (1996)
ARTICLE NO. 0617

SHORT COMMUNICATION

Productive and Persistent Infection of Human Thymic Epithelial Cells *in Vitro* with HIV-1

JOSÉPHINE BRAUN,* HÉLÈNE VALENTIN,*,1 MARIE-THÉRÈSE NUGEYRE,* HÉLÈNE OHAYON,† PIERRE GOUNON,† and FRANÇOISE BARRÉ-SINOUSSI*,2

JOURNAL OF VIROLOGY, Sept. 2007, p. 9693–9706 Vol. 81, No. 18
0022-538X/07/$08.00+0 doi:10.1128/JVI.00492-07
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Productive Human Immunodeficiency Virus Type 1 Infection in Peripheral Blood Predominantly Takes Place in CD4/CD8 Double-Negative T Lymphocytes

Philipp Kaiser, Beda Joos, Barbara Niederöst, Rainer Weber, Huldrych F. Günthard, Marek Fischer,* and the Swiss HIV Cohort Study

HIV-1-infected CD8+CD4+ T cells decay *in vivo* at a similar rate to infected CD4 T cells during HAART

Gareth J. Hughes[a], Alexandra Cochrane[a], Clifford Leen[c], Sheila Morris[c], Jeanne E. Bell[b] and Peter Simmonds[a]

AIDS 2008, **22**:57–65

HIV-1 DNA Is Detected in Bone Marrow Populations Containing $CD4^+$ T Cells but Is not Found in Purified $CD34^+$ Hematopoietic Progenitor Cells in Most Patients on Antiretroviral Therapy

Christine M. Durand,[1] Gabriel Ghiaur,[1] Janet D. Siliciano,[1] S. Alireza Rabi,[1] Evelyn E. Eisele,[1,3] Maria Salgado,[1] Liang Shan,[1] Jun F. Lai,[1] Hao Zhang,[4] Joseph Margolick,[4] Richard J. Jones,[2] Joel E. Gallant,[1] Richard F. Ambinder,[2] and Robert F. Siliciano[1,5]

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ARTICLES

Isolation of primary HIV-1 that target $CD8^+$ T Lymphocytes using CD8 as a receptor

KUNAL SAHA[1,2], JIANCHAO ZHANG[1], ANIL GUPTA[1], RAJNISH DAVE[1], MERON YIMEN[1] & BOUCHRA ZERHOUNI[1]

Macrophages

Among other cell types that could serve as HIV-1 reservoirs, macrophages are frequently mentioned. Early studies demonstrated HIV-1 infection of macrophages in vivo and showed that these cells are more resistant to viral cytopathic effects than are activated $CD4^+$ T cells (Gartner et al., 1986; Koenig et al., 1986). Macrophage-tropic HIV-1 variants utilize the CCR5 coreceptor (Alkhatib et al., 1996; Deng et al., 1996) but also have sequence changes in the Env protein that allow entry into cells that express low amounts of CD4, including macrophages (Schnell et al., 2011; Walter et al., 2005). Infection of macrophages is particularly prominent late in the course of infection, when most of the $CD4^+$ T cells have been lost (Igarashi et al., 2001). Although infected macrophages play a role in HIV-1 pathogenesis, it is not yet clear that these cells fit the definition proposed above for a reservoir.

Detection of HIV-1 DNA in Microglia/ Macrophages, Astrocytes and Neurons Isolated from Brain Tissue with HIV-1 Encephalitis by Laser Capture Microdissection

TOTAL HIV DNA IS A CLINICALLY RELEVANT MARKER

1) **Higher Total HIV DNA faster Porgession**

2) **Pretherapeutic Total HIV DNA Levels in PBMC is Predictive of:**
   - **Virologic**
   - **Immunologic**
   - **Clinical Responses to Antiretroviral Therapy**

3) **Time from HIV-1 Infection to Treatment Initiation Influence Total HIV DNA Decay and Levels during**

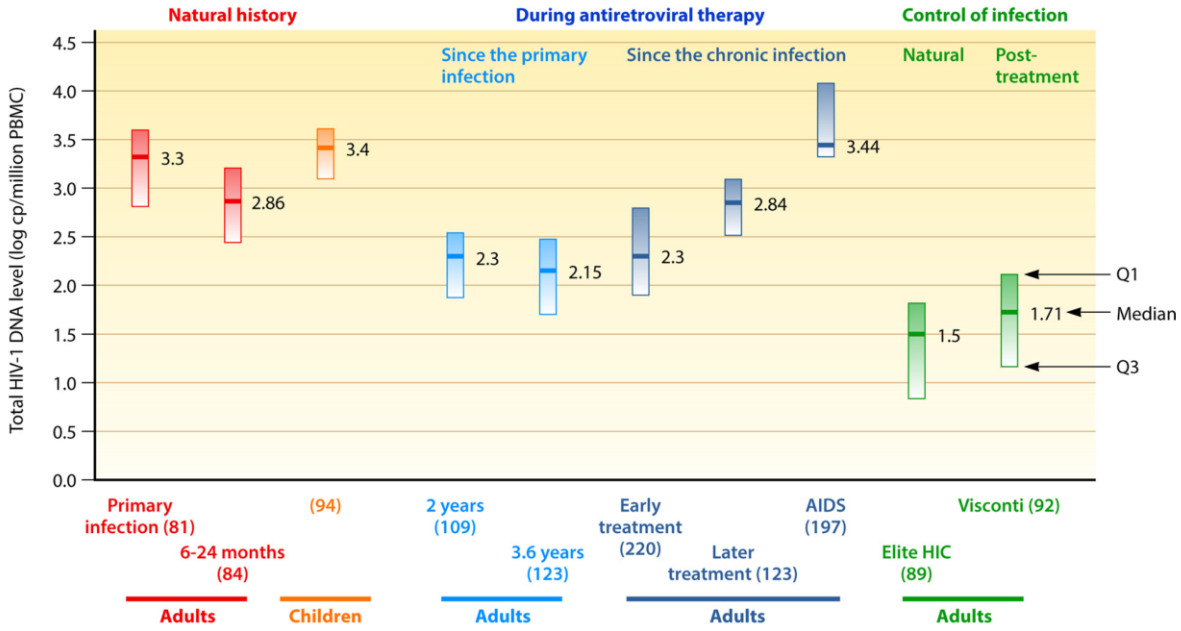
4) **Total HIV DNA Load during cART is Informative of Patient Pretherapeutic and Therapeutic History**

Clinical Microbiology Reviews

Total HIV-1 DNA, a Marker of Viral Reservoir Dynamics with Clinical Implications

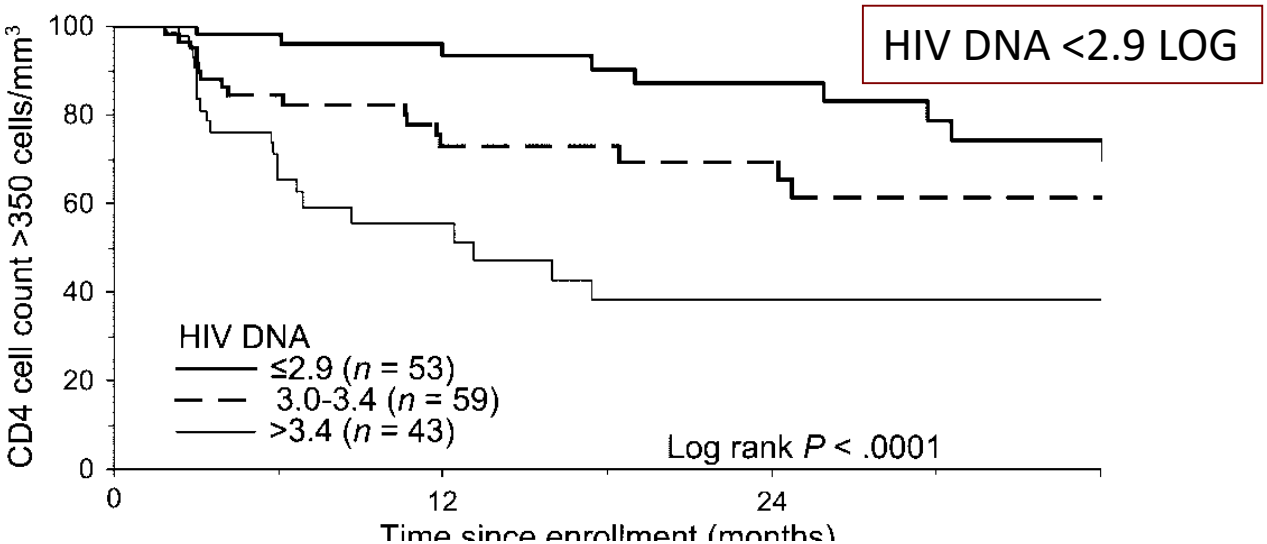
Véronique Avettand-Fènoël,[a,b] Laurent Hocqueloux,[c] Jade Ghosn,[a,d] Antoine Cheret,[a,e] Pierre Frange,[a,f,g] Adeline Melard,[a] Jean-Paul Viard,[a,d] Christine Rouzioux[a,b]

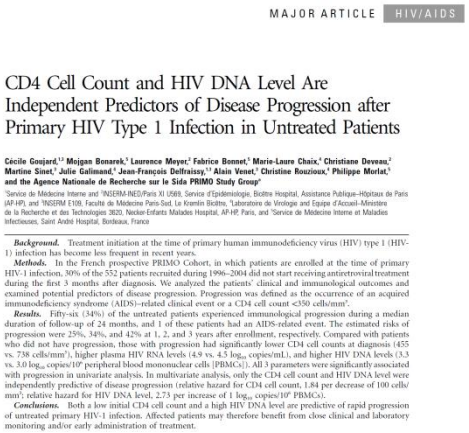
Total HIV-1 DNA, a Marker of Viral Reservoir Dynamics with Clinical Implications



TOTAL HIV DNA LOAD IS PREDICTIVE OF IMMUNOLOGIC PROGRESSION

independently of HIV RNA load and the CD4 T cell count




MAJOR ARTICLE HIV/AIDS

CD4 Cell Count and HIV DNA Level Are Independent Predictors of Disease Progression after Primary HIV Type 1 Infection in Untreated Patients

Cécile Goujard,[1,2] Mojgan Bonarek,[3] Laurence Meyer,[2] Fabrice Bonnet,[4] Marie-Laure Chaix,[4] Christiane Deveau,[2] Martine Sinet,[3] Julie Galimand,[4] Jean-François Delfraissy,[1,2] Alain Venet,[3] Christine Rouzioux,[4] Philippe Morlat,[5] and the Agence Nationale de Recherche sur le Sida PRIMO Study Group*

[1]Service de Medecine Interne and [2]INSERM-INEO/Paris XI UMR, Service d'Epidemiologie, Biothe Hospital, Assistance Publique–Hôpitaux de Paris (AP-HP), and [3]INSERM E108, Faculte de Medecine Paris-Sud, Le Kremlin Bicêtre, [4]Laboratoire de Virologie and Equipe d'Accueil–Ministere de la Recherche et des Technologies 3820, Necker-Enfants Malades Hospital, AP-HP, Paris, and [5]Service de Medecine Interne et Maladies Infectieuses, Saint André Hospital, Bordeaux, France

Background. Treatment initiation at the time of primary human immunodeficiency virus (HIV) type 1 (HIV-1) infection has become less frequent in recent years.

Methods. In the French prospective PRIMO Cohort, in which patients are enrolled at the time of primary HIV-1 infection, 30% of the 552 patients recruited during 1996–2004 did not start receiving antiretroviral treatment during the first 3 months after diagnosis. We analyzed the patients' clinical and immunological outcomes and examined potential predictors of disease progression. Progression was defined as the occurrence of an acquired immunodeficiency syndrome (AIDS)–related clinical event or a CD4 cell count <350 cells/mm^3.

Results. Fifty-six (34%) of the untreated patients experienced immunological progression during a median duration of follow-up of 24 months, and 1 of these patients had an AIDS-related event. The estimated risks of progression were 25%, 34%, and 42% at 1, 2, and 3 years after enrollment, respectively. Compared with patients who did not have progression, those with progression had significantly lower CD4 cell counts at diagnosis (455 vs. 738 cells/mm^3), higher plasma HIV RNA levels (4.9 vs. 4.5 $\log_{10}$ copies/mL), and higher HIV DNA levels (3.3 vs. 3.0 $\log_{10}$ copies/10^6 peripheral blood mononuclear cells [PBMCs]). All 3 parameters were significantly associated with progression in univariate analysis. In multivariate analysis, only the CD4 cell count and HIV DNA level were independently predictive of disease progression (relative hazard for CD4 cell count, 1.84 per decrease of 100 cells/mm^3; relative hazard for HIV DNA level, 2.73 per increase of 1 $\log_{10}$ copies/10^6 PBMCs).

Conclusions. Both a low initial CD4 cell count and a high HIV DNA level are predictive of rapid progression of untreated primary HIV-1 infection. Affected patients may therefore benefit from close clinical and laboratory monitoring and/or early administration of treatment.

Figure 2. Progression-free survival among untreated patients, according to the baseline CD4 cell count (in cells/mm3), plasma HIV RNA level (in log10 copies/mL), and HIV DNA level (in log10 copies/106 PBMCs). Kaplan-Meier estimates were made for 3 classes of CD4 cell count *(A),* HIV RNA level *(B), and HIV DNA level (C). Progression was defined as* a CD4 cell count >350 cells/mm3.

Clinical Infectious Diseases 2006; 42:709–15

Impact of HIV Type 1 DNA Levels on Spontaneous Disease Progression: A Meta-Analysis

AIDS RESEARCH AND HUMAN RETROVIRUSES
Volume 28, Number 4, 2012
© Mary Ann Liebert, Inc.
DOI: 10.1089/aid.2011.0032

Impact of HIV Type 1 DNA Levels on Spontaneous Disease Progression: A Meta-Analysis

Chrissa G. Tsiara,[1] Georgios K. Nikolopoulos,[1] Pantelis G. Bagos,[2] Cecile Goujard,[3] Terese L. Katzenstein,[4] Albert K. Minga,[5] Christine Rouzioux,[6] and Angelos Hatzakis[1]

TABLE 1. SUMMARY OF ELIGIBLE STUDIES EVALUATING THE ABILITY OF HIV-1 DNA TO PREDICT HIV-1 DISEASE PROGRESSION

Source	N[a]	Enrollment	Age (at infection or baseline)	Follow-up (median)	Sample collection after seroconversion (median)	CD4 (cells/mm^3) (median)	HIV-1 RNA (log_{10} copies/ml) (median)	HIV-1 DNA (log_{10} copies/10^6 PMBCs[b]) (median)
Rouzioux 2005	112	1988–1996	27 years (median)	75 months	3.80 months	594	4.22	2.85
Rouzioux 2005	271	1988–1996	28 years (median)	84 months	11 months	534	4.01	2.86
Kostrikis 2002	127	NA[c]	30.40 years (mean)	16 years	6.70 years (median)	320	4.25	2.65
Katzenstein 2002	90	<1989	NA	Up to 2001	Early infection (exact time not reported)	NA	NA	2.70
Minga 2008	200	1997–2006	29 years (median)	30 months	9 months	471	4.60	3.00
Goujard 2006	163	1996–2004	33 years (median)	24 months	68 days	628	4.60	3.20
Tierney 2003	111	NA	36 years (median)	NA	NA	325	4.35	2.08 (log_{10} copies/μg DNA)

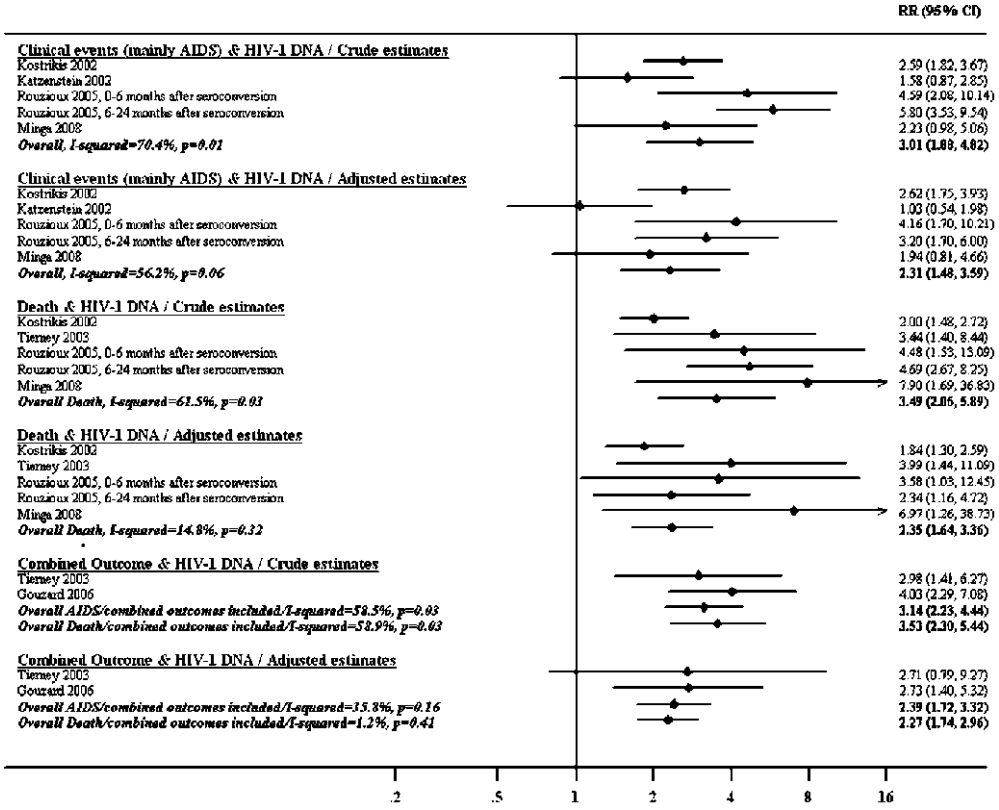
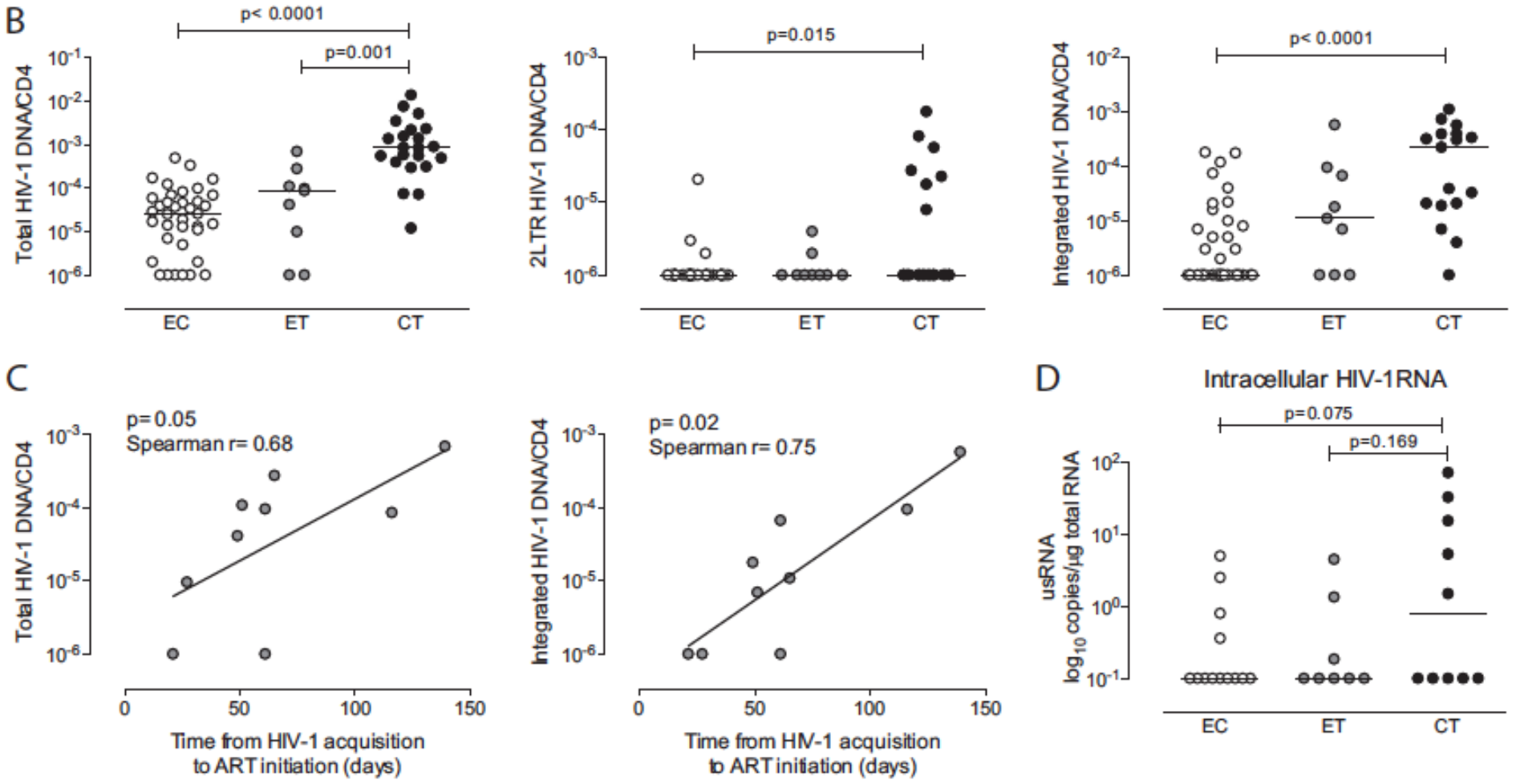


FIG. 2. The results of univariate random-effects meta-analysis concerning the ability of HIV-1 DNA to predict HIV-1 disease progression. RR, relative risk; CI, confidence interval; AIDS, acquired immunodeficiency syndrome; HIV, human immunodeficiency virus.

HIV-1 DNA was a better predictor than HIV-1 RNA

- AIDS alone
- AIDS and death

T&T
TEST & TREAT
THE SOONER THE BETTER!



BUZON ET AL. J Virol 2014; 88: 10056–10065

RESEARCH ARTICLE

Levels of HIV-1 persistence on antiretroviral therapy are not associated with markers of inflammation or activation

Rajesh T. Gandhi[1]*, Deborah K. McMahon[2], Ronald J. Bosch[3], Christina M. Lalama[3], Joshua C. Cyktor[2], Bernard J. Macatangay[2], Charles R. Rinaldo[4], Sharon A. Riddler[2], Evelyn Hogg[5], Catherine Godfrey[6], Ann C. Collier[7], Joseph J. Eron[8], John W. Mellors[2], ACTG A5321 Team[¶]

Pre ART HIV DNA CORRELATED WITH

- higher levels of plasma **HIV-1 RNA**
- **CELL ASSOCIATED HIV-RNA**
- lower pre-therapy **CD4 + T-cell counts**
- lower pre-therapy **CD4:CD8**.

On ART

- Lower on-therapy **CD4:CD8 ratios** were associated with persistently **higher HIV-1 DNA** at all on-ART time points (years 1, 4, 6±15; e.g., r = **–** 0.49 at year 1 of ART, p**<** 0.001).

PLOS Pathogens April 20, 2017

HIV DNA Time from HIV-1 Infection to Treatment Initiation Influence Total HIV DNA Decay and Levels during cART

0.01/cell

10000/millionCD4

100/millionCD4

FIEBIG

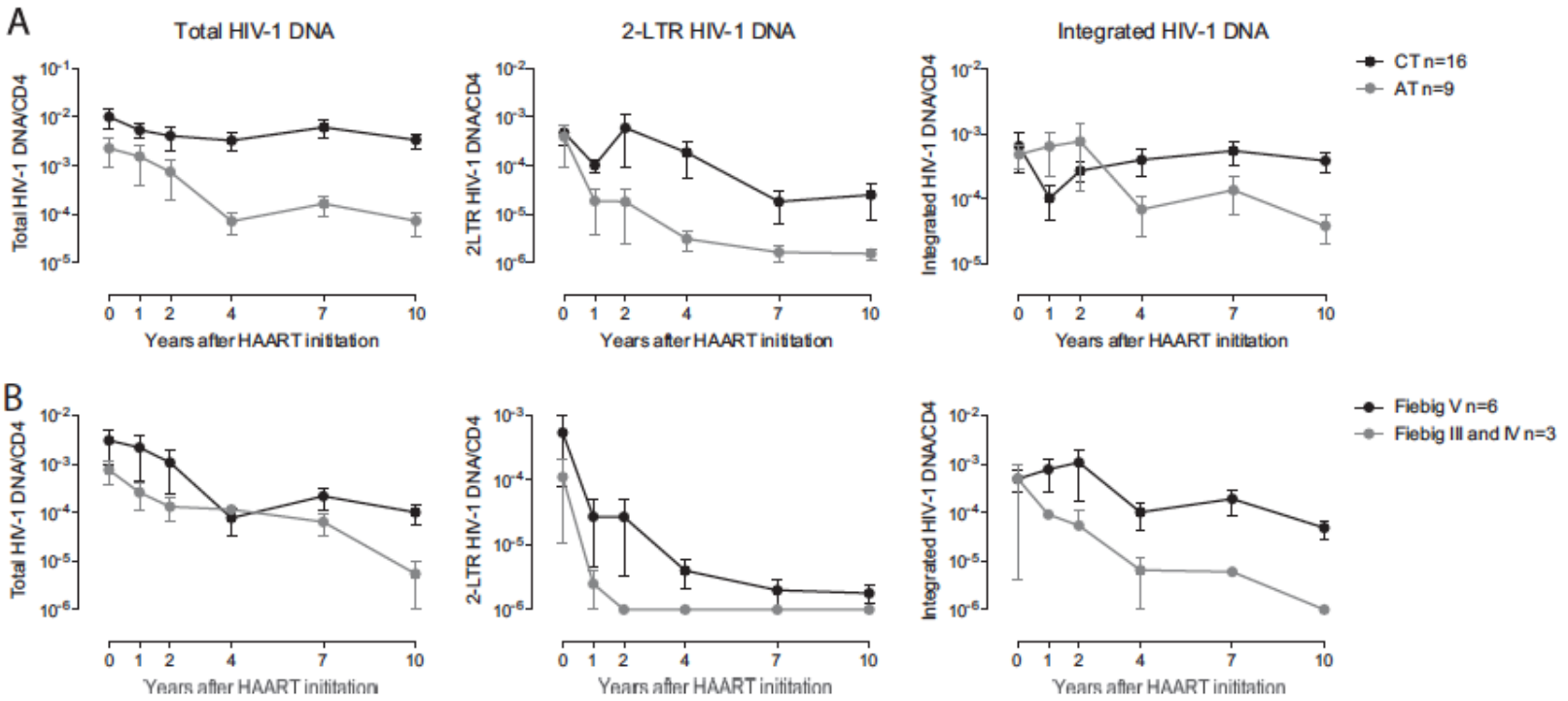


FIG 3 Antiretroviral treatment initiation in primary HIV-1 infection accelerates HIV-1 DNA decay. (A) Longitudinal evolution of copies per cell of total, 2-LTR, and integrated HIV-1 DNA in CD4 T cells from patients with antiretroviral treatment initiation in early (ET) ($n = 9$) or chronic (CT) ($n = 16$) HIV-1 infection. (B) Longitudinal decline of HIV-1 DNA after antiretroviral treatment initiation in primary HIV-1 infection, stratified according to treatment commencement before HIV-1 seroconversion (Fiebig stage III-IV) ($n = 3$) or after HIV-1 seroconversion (Fiebig stage V) ($n = 6$). Data are pointwise maximum-likelihood estimates of the means and 95% confidence interval estimates of the mean.

Long-Term Antiretroviral Treatment Initiated at Primary HIV-1 Infection Affects the Size, Composition, and Decay Kinetics of the Reservoir of HIV-1-Infected CD4 T Cells

María J. Buzon,[a,b] Enrique Martin-Gayo,[b] Florencia Pereyra,[a,b,c] Zhengyu Ouyang,[b] Hong Sun,[b,d] Jonathan Z. Li,[e] Michael Piovoso,[a] Amy Shaw,[a] Judith Dalmau,[f] Nadine Zangger,[g] Javier Martinez-Picado,[f,h] Ryan Zurakowski,[i] Xu G. Yu,[b] Amalio Telenti,[g] Bruce D. Walker,[b,c] Eric S. Rosenberg,[a] Mathias Lichterfeld[a,b]

ABSTRACT

Initiation of antiretroviral therapy during the earliest stages of HIV-1 infection may limit the seeding of a long-lasting viral reservoir, but long-term effects of early antiretroviral treatment initiation remain unknown. Here, we analyzed immunological and virological characteristics of nine patients who started antiretroviral therapy at primary HIV-1 infection and remained on suppressive treatment for >10 years: patients with similar treatment duration but initiation of suppressive therapy during chronic HIV-1 infection served as controls. We observed that independently of the timing of treatment initiation, HIV-1 DNA in CD4 T cells decayed primarily during the initial 3 to 4 years of treatment. However, in patients who started antiretroviral therapy in early infection, this decay occurred faster and was more pronounced, leading to substantially lower levels of cell-associated HIV-1 DNA after long-term treatment. Despite this smaller size, the viral CD4 T cell reservoir in persons with early treatment initiation consisted more dominantly of the long-lasting central-memory and T memory stem cells. HIV-1 specific T cell responses remained continuously detectable during antiretroviral therapy, independently of the timing of treatment initiation. Together, these data suggest that early HIV-1 treatment initiation, even when continued for >10 years, is unlikely to lead to viral eradication, but the presence of low viral reservoirs and durable HIV-1 T cell responses may make such patients good candidates for future interventional studies aiming at HIV-1 eradication and cure.

Major decline in the first 4 years

BUZON ET AL. Journal of Virology p. 10056–10065 September 2014 Volume 88 Number 17

LTNP-Long term survivors have lower levels of HIV DNA

JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 2000, p. 630–634 Vol. 38, No. 2
0095-1137/00/$04.00+0
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PCR-Based Assay To Quantify Human Immunodeficiency Virus Type 1 DNA in Peripheral Blood Mononuclear Cells

CINDY CHRISTOPHERSON,[1]* YORDA KIDANE,[1] BRIAN CONWAY,[2,3] JOHN KROWKA,[4] HAYNES SHEPPARD,[4] AND SHIRLEY KWOK[1]

Roche Molecular Systems, Inc., Alameda, California 94501[1]; University of British Columbia[2] and Viridae Clinical Sciences,[3] Vancouver, Canada; and Viral and Rickettsial Disease Laboratory, Division of Communicable Disease Control, California Department of Health Services, Berkeley, California 94704[4]

Received 30 July 1999/Returned for modification 20 September 1999/Accepted 1 November 1999

An assay that quantifies the amount of human immunodeficiency virus type 1 (HIV-1) DNA in peripheral blood mononuclear cells has been developed. PCR amplification of the HIV-1 DNA is performed in the presence of an internal quantitation standard, and colorimetric detection of the amplified product is performed with microwell plates. The copies of HIV-1 DNA are normalized to total genomic DNA input. The assay has an analytical sensitivity of 10 input copies per amplification reaction and a three-log detection range. In an analysis of sequential samples from patients on combination therapy, HIV-1 DNA was quantifiable for all individuals tested, including those with undetectable plasma HIV-1 RNA. In a separate study, a comparison of HIV-1 DNA levels was made with a group of long-term survivors and progressors. The mean HIV-1 DNA levels were lower in the long-term survivors than in the progressors (P, 0.04). The mean HIV-1 RNA levels were also lower, but the difference was not statistically significant (P, 0.164). A quantitative DNA assay will provide an additional tool to gain insight into the natural history of infection and the continued efficacy of potent antiretroviral therapies.

JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 2000, p. 630–634

TABLE 2. HIV-1 DNA and RNA levels in the circulation in long-term survivors and progressors

Group	No. of:		
	Patient	HIV-1 DNA copies/ μg of total DNA	HIV-1 RNA copies/ml
Long-term survivors	A	50	2,174
	B	33	7,263
	C	9	<200
	D	708	23,667
	E	76	434
Progressors	F	1,020	270,833
	G	537	17,305
	H	1,111	153,930
	I	548	76,688
	J	1,647	1,558,777
	K	497	58,320
	L	253	79,264
	M	2,746	2,008,893
	N	564	338,467
	O	713	Not available

LTNPs with HIV DNA loads of >1.85 log at seroconversion and a large increase in HIV DNA over time had an increased risk of losing their LTNP status

Avettand-Fènoël Goujard C, et al Clinical Microbiology Reviews. 2016 , 29 N. 4
Goujard C. et al. 2006. Clin Infect Dis **42:709** –715

Productive Infection Maintains a Dynamic Steady State of Residual Viremia in Human Immunodeficiency Virus Type 1-Infected Persons Treated with Suppressive Antiretroviral Therapy for Five Years

Diane V. Havlir,[1]* Matthew C. Strain,[2,3] Mario Clerici,[4] Caroline Ignacio,[5] Daria Trabattoni,[4] Pasquale Ferrante,[6] and Joseph K. Wong[2,5]

- **Lower baseline HIV DNA** levels were predictive of **undetectable HIV RNA load on cART**

- High HIV DNA load was associated with
  - **persistent residual HIV RNA** during cART
  - Independently of baseline HIV RNA load

JOURNAL OF VIROLOGY, Oct. 2003, p. 11212–11219

Baseline Cellular HIV DNA Load Predicts HIV DNA Decline and Residual HIV Plasma Levels during Effective Antiretroviral Therapy

Saverio Giuseppe Parisi,[a] Samantha Andreis,[a] Carlo Mengoli,[a] Renzo Scaggiante,[b] Roberto Ferretto,[c] Vinicio Manfrin,[d] Mario Cruciani,[e] Mario Giobbia,[f] Caterina Boldrin,[a] Monica Basso,[a] Massimo Andreoni,[g] Giorgio Palù,[a] and Loredana Sarmati[g]

HIV DNA DECLINE DURING ART IS ASSOCIATED WITH:

Baseline HIV DNA

Baseline HIV RNA

Increase in CD4 cells

HIV RNA load of <2.5 copies/ml

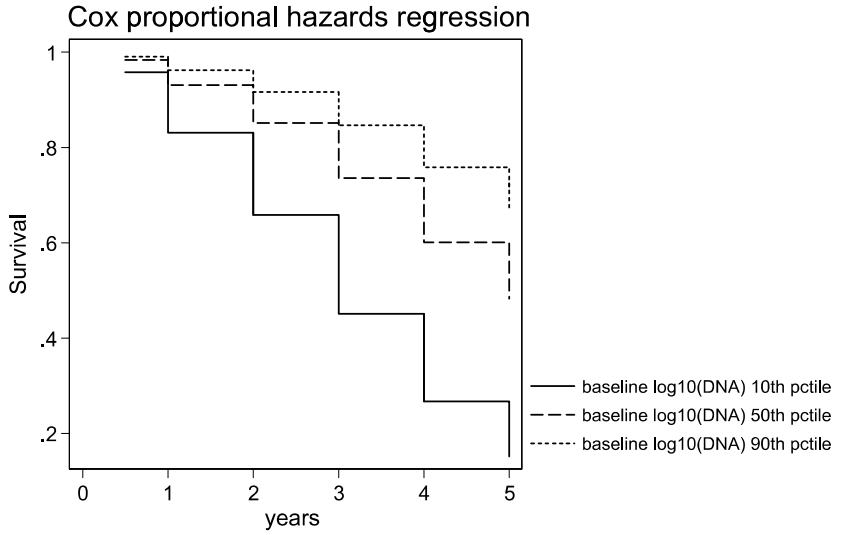


FIG 2 The proportion of patients who were exempt from the relevant event (final $\log_{10}$ cellular HIV DNA level reduced to ≤5 copies) was plotted as a function of time (years). The baseline $\log_{10}$ proviral HIV DNA levels are indicated as the 10th, 50th, and 90th percentiles, corresponding to 269, 1,838, and 6,284 copies, respectively.

Parisi et al. 2012 Journal of Clinical Microbiology p. 258–263

Low Total HIV DNA correlate with VIREMIA and CD4/8 RATIO

Low total HIV DNA loads correlated with:

- residual plasma viremia of <1 copy/ml
- high CD4/CD8 cell ratio

J Antimicrob Chemother 2014; **69**: 753–756
doi:10.1093/jac/dkt428 Advance Access publication 1 November 2013

Journal of Antimicrobial Chemotherapy

Factors associated with a low HIV reservoir in patients with prolonged suppressive antiretroviral therapy

Slim Fourati[1,2*], Philippe Flandre[1,3], Ruxandra Calin[4], Guislaine Carcelain[5,6], Cathia Soulie[1,2], Sidonie Lambert-Niclot[1,2], Almoustapha Maiga[7], Zaina Ait-Arkoub[2], Roland Tubiana[1,8], Marc-Antoine Valantin[1,8], Brigitte Autran[5,6,9], Christine Katlama[1,8,9], Vincent Calvez[1,2,9] and Anne-Genevieve Marcelin[1,2,9]

Table 2. Factors associated with obtaining a low total HIV-1 DNA in patients

	Univariate analysis		Multivariate analysis	
	OR (95% CI)	*P*	OR (95% CI)	*P*
Variable associated with a low HIV-1 DNA load (<100 copies/10^6 PBMCs)				
Age per 1 year increase	0.98 (0.96–1.02)	0.3864		
Sex (female versus male)	2.31 (1.23–4.32)	0.0086		
Nadir CD4 cell count per 100 cells/μL increase	1.18	0.0433		
Plasma HIV-1 RNA peak per 1 $\log_{10}$ copies/mL increase	0.40 (0.27–0.57)	<0.0001	0.42 (0.26–0.64)	0.0001
Time since HIV diagnosis per 1 year increase	1.00 (0.96–1.04)	0.9858		
Duration of antiretroviral treatment per 1 year increase	1.00 (0.94–1.06)	0.9923		
Duration of undetectable HIV-1 RNA (<50 copies/mL) per 1 year increase	1.09 (1.00–1.20)	0.0281		
At the time of HIV DNA measurements				
ultrasensitive plasma viral load (<1 copy/mL versus ≥1 copy/mL)	4.57 (2.19–11.05)	0.0003	2.97 (1.21–8.12)	0.0228
current CD4 cell count per 100 cells/μL increase	1.02 (0.92–1.13)	0.725		
% CD4 T cells per 1% increase	1.04 (1.01–1.08)	0.0086		
current CD8 cell count per 100 cells/μL increase	0.90 (0.82–0.98)	0.0256		
% CD8 T cells per 1% increase	0.96 (0.93–0.98)	0.0043		
current CD4/CD8 ratio	2.33 (1.36–4.09)	0.0024	2.31 (1.12–4.80)	0.0227

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[¶]

Visconti study:

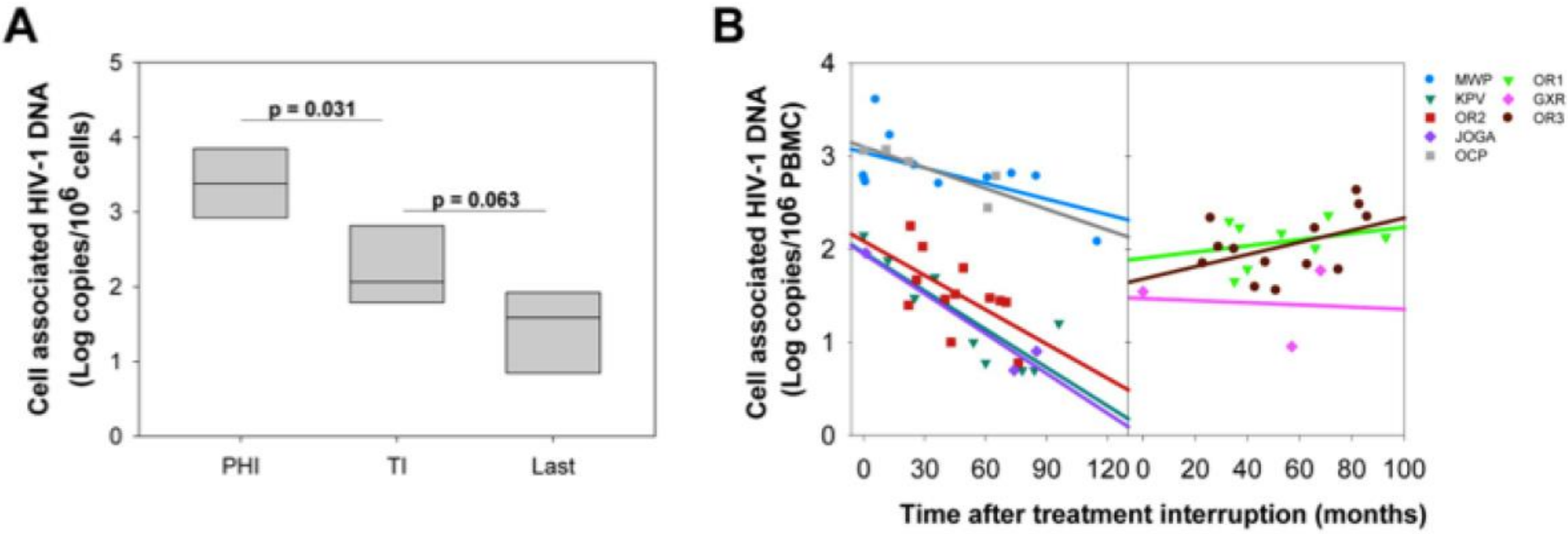
-HIV infected patients

-Treated during acute infection

-Haart interrupted

-HIV control

Post-treatment controllers have very low levels of cell-associated HIV DNA which keep decreasing after treatment interruption for some patients

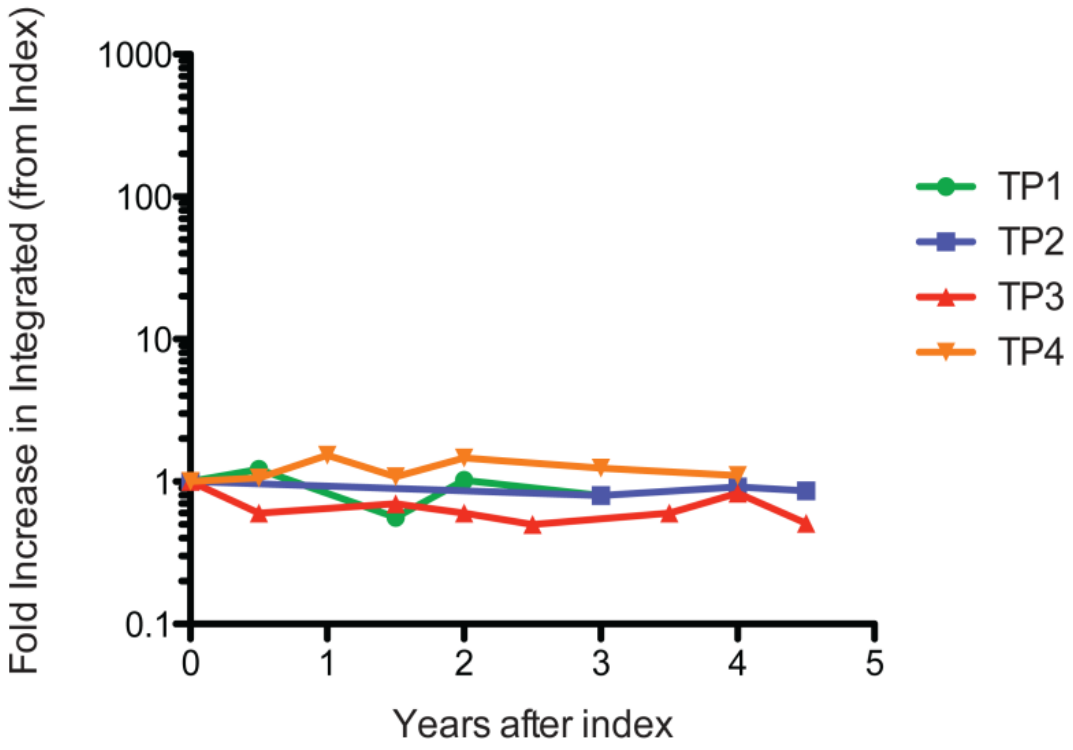


TOTAL HIV DNA IS A CLINICALLY RELEVANT MARKER

1) **Pretherapeutic Total HIV DNA Levels in PBMC is Predictive of:**
   - **Virologic**
   - **Immunologic**
   - **Clinical Responses to Antiretroviral Therapy**

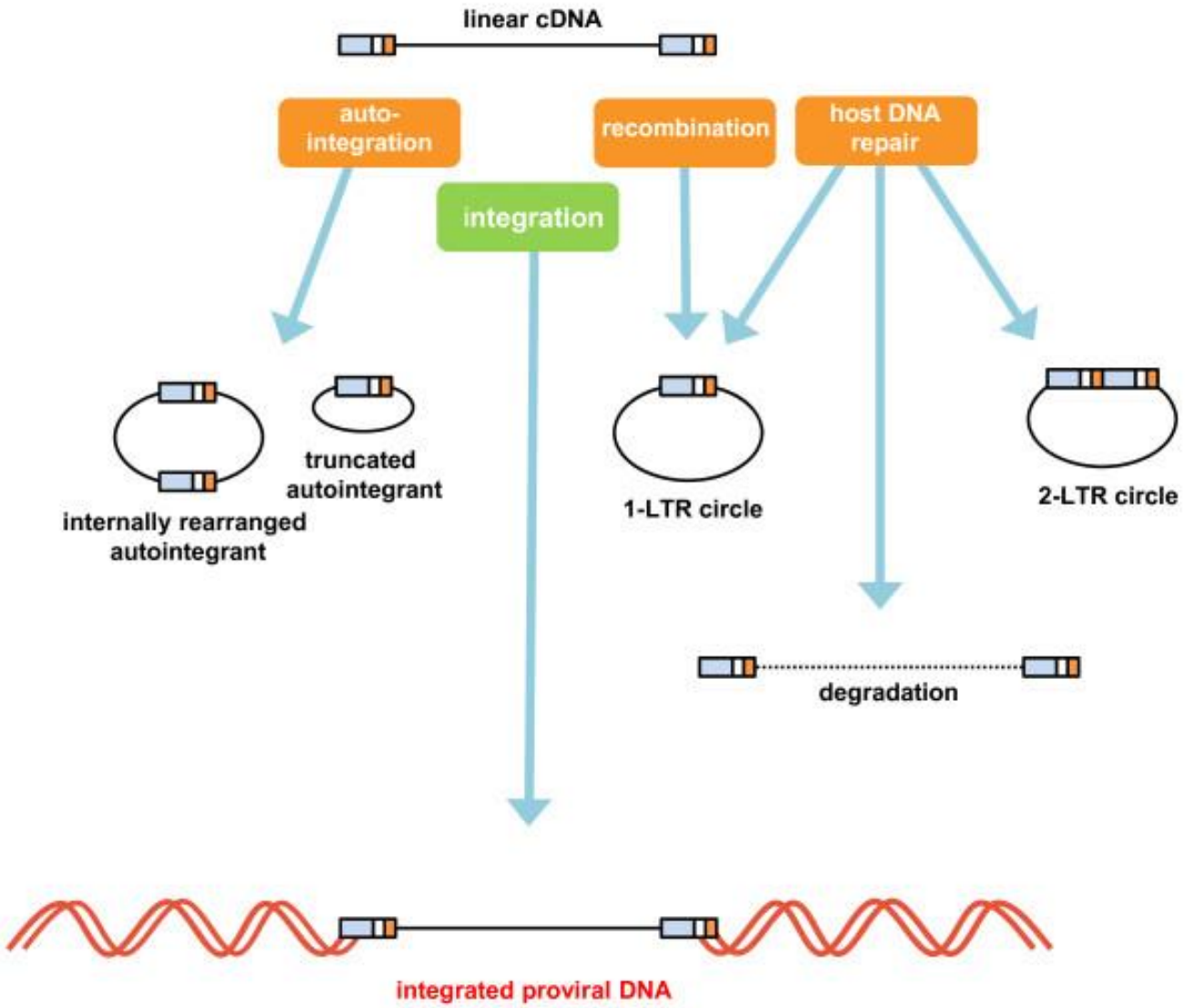
2) **HIV DNA declines but reaches a plateau**

Decay of integrated HIV DNA is minimal to none



Mexas 2012 AIDS
Besson 2014 CID

HIV DNA FORMS



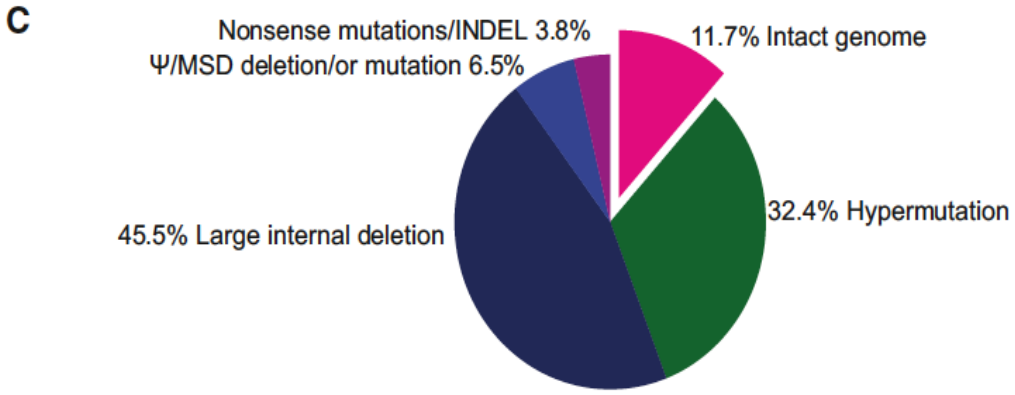
Replication-Competent Noninduced Proviruses in the Latent Reservoir Increase Barrier to HIV-1 Cure

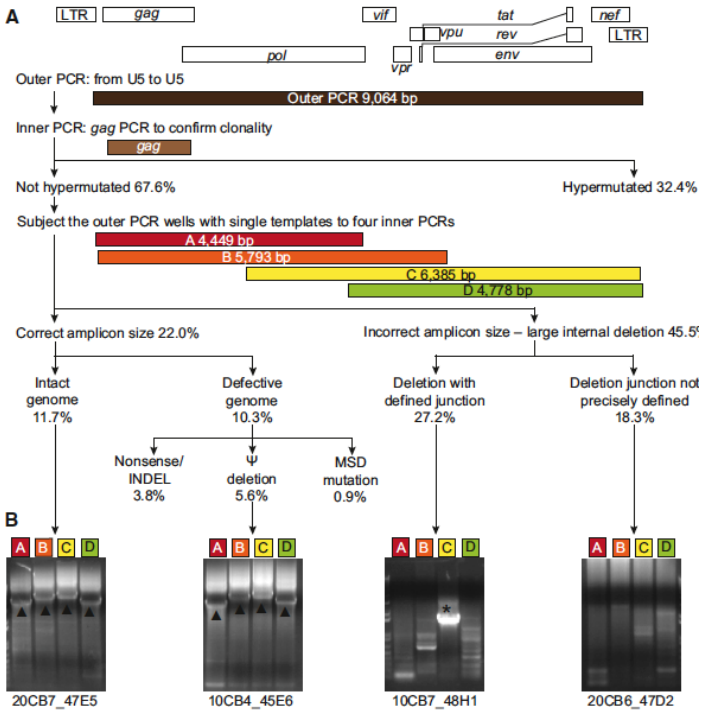
Cell

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,*]
[1]Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA
[2]Health Sciences Center, School of Medicine, Louisiana State University, New Orleans, LA 70112, USA
[3]Program for Evolutionary Dynamics, Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA 02138, USA
[4]Howard Hughes Medical Institute, Baltimore, MD 21205, USA
[5]Present address: Department of Immunobiology, School of Medicine, Yale University, New Haven, CT 06510, USA
*Correspondence: rsiliciano@jhmi.edu
http://dx.doi.org/10.1016/j.cell.2013.09.020

88.3% with identifiable defects
11.7% of intact genome





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,*]
[1]Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA
[2]Health Sciences Center, School of Medicine, Louisiana State University, New Orleans, LA 70112, USA
[3]Program for Evolutionary Dynamics, Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA 02138, USA
[4]Howard Hughes Medical Institute, Baltimore, MD 21205, USA
[5]Present address: Department of Immunobiology, School of Medicine, Yale University, New Haven, CT 06510, USA
*Correspondence: rsiliciano@jhmi.edu
http://dx.doi.org/10.1016/j.cell.2013.09.020

nature COMMUNICATIONS

ARTICLE

https://doi.org/10.1038/s41467-019-08431-7 OPEN

Longitudinal HIV sequencing reveals reservoir expression leading to decay which is obscured by clonal expansion

Marilia Rita Pinzone[1], D. Jake VanBelzen[1,2], Sam Weissman[1], Maria Paola Bertuccio[1], LaMont Cannon[1], Emmanuele Venanzi-Rullo[1,3], Stephen Migueles[4], R. Brad Jones[5], Talia Mota[5], Sarah B. Joseph[6], Kevin Groen[7], Alexander O. Pasternak[7], Wei-Ting Hwang[8], Brad Sherman[9], Anastasios Vourekas[1], Giuseppe Nunnari[3] & Una O'Doherty[1]

After initiating antiretroviral therapy (ART), a rapid decline in HIV viral load is followed by a long period of undetectable viremia. Viral outgrowth assay suggests the reservoir continues to decline slowly. Here, we use full-length sequencing to longitudinally study the proviral landscape of four subjects on ART to investigate the selective pressures influencing the dynamics of the treatment-resistant HIV reservoir. We find intact and defective proviruses that contain genetic elements favoring efficient protein expression decrease over time. Moreover, proviruses that lack these genetic elements, yet contain strong donor splice sequences, increase relatively to other defective proviruses, especially among clones. Our work suggests that HIV expression occurs to a significant extent during ART and results in HIV clearance, but this is obscured by the expansion of proviral clones. Paradoxically, clonal expansion may also be enhanced by HIV expression that leads to splicing between HIV donor splice sites and downstream human exons.

LETTER

https://doi.org/10.1038/s41586-019-0898-8

A quantitative approach for measuring the reservoir of latent HIV-1 proviruses

Katherine M. Bruner[1,8,10], Zheng Wang[1,10], Francesco R. Simonetti[1], Alexandra M. Bender[1], Kyungyoon J. Kwon[1], Srona Sengupta[1], Emily J. Fray[1], Subul A. Beg[1], Annukka A. R. Antar[1], Katharine M. Jenike[1], Lynn N. Bertagnolli[1], Adam A. Capoferri[1], Joshua T. Kufera[1], Andrew Timmons[1], Christopher Nobles[2], John Gregg[2], Nikolas Wada[3], Ya-Chi Ho[1,9], Hao Zhang[4], Joseph B. Margolick[4], Joel N. Blankson[1], Steven G. Deeks[5], Frederic D. Bushman[2], Janet D. Siliciano[1], Gregory M. Laird[6] & Robert F. Siliciano[1,7*]

A stable latent reservoir for HIV-1 in resting CD4$^+$ T cells is the principal barrier to a cure[1–3]. Curative strategies that target the reservoir are being tested[4,5] and require accurate, scalable reservoir assays. The reservoir was defined with quantitative viral outgrowth assays for cells that release infectious virus after one round of T cell activation[1]. However, these quantitative outgrowth assays and newer assays for cells that produce viral RNA after activation[6] may underestimate the reservoir size because one round of activation does not induce all proviruses[7]. Many studies rely on simple assays based on polymerase chain reaction to detect proviral DNA regardless of transcriptional status, but the clinical relevance of these assays is unclear, as the vast majority of proviruses are defective[7–9]. Here we describe a more accurate method of measuring the HIV-1 reservoir that separately quantifies intact and defective proviruses. We show that the dynamics of cells that carry intact and defective proviruses are different in vitro and in vivo. These findings have implications for targeting the intact proviruses that are a barrier to curing HIV infection.

Evaluation of cure strategies requires assays that detect infected cells and distinguish intact proviruses from the vast excess of defective proviruses. We define 'intact proviruses' as those that lack overt fatal defects such as large deletions and hypermutation[7,8], and recognize that some proviruses defined in this way may have minor defects that affect fitness. We analysed 431 near full genome HIV-1 sequences obtained by single genome analysis[8,9] from 28 HIV-1-infected adults. The near full genome sequencing (nFGS) methods used identify defects throughout the genome except the 5′ long terminal repeat (LTR). Consistent with previous reports[8,9], only 2.4% of proviruses were intact (Fig. 1a). The remaining 97.6% had fatal defects including deletions, encompassing on average 49.6% of the genome, and/or G→A hypermutation, which altered start codons and/or introduced stop codons in most open-reading frames (ORFs)[8]. Most defective proviruses had defects in most HIV-1 genes (Fig. 1b, Supplementary Table 1). Ninety-seven per cent of defective proviruses had defects that affect the transcriptional activator Tat (Fig. 1b, Supplementary Table 1) and might not be efficiently transcribed after latency reversal. Thus, cure interventions dependent on viral gene expression[4,5] may affect cells with intact and defective proviruses differently. Hence, separate quantification is essential.

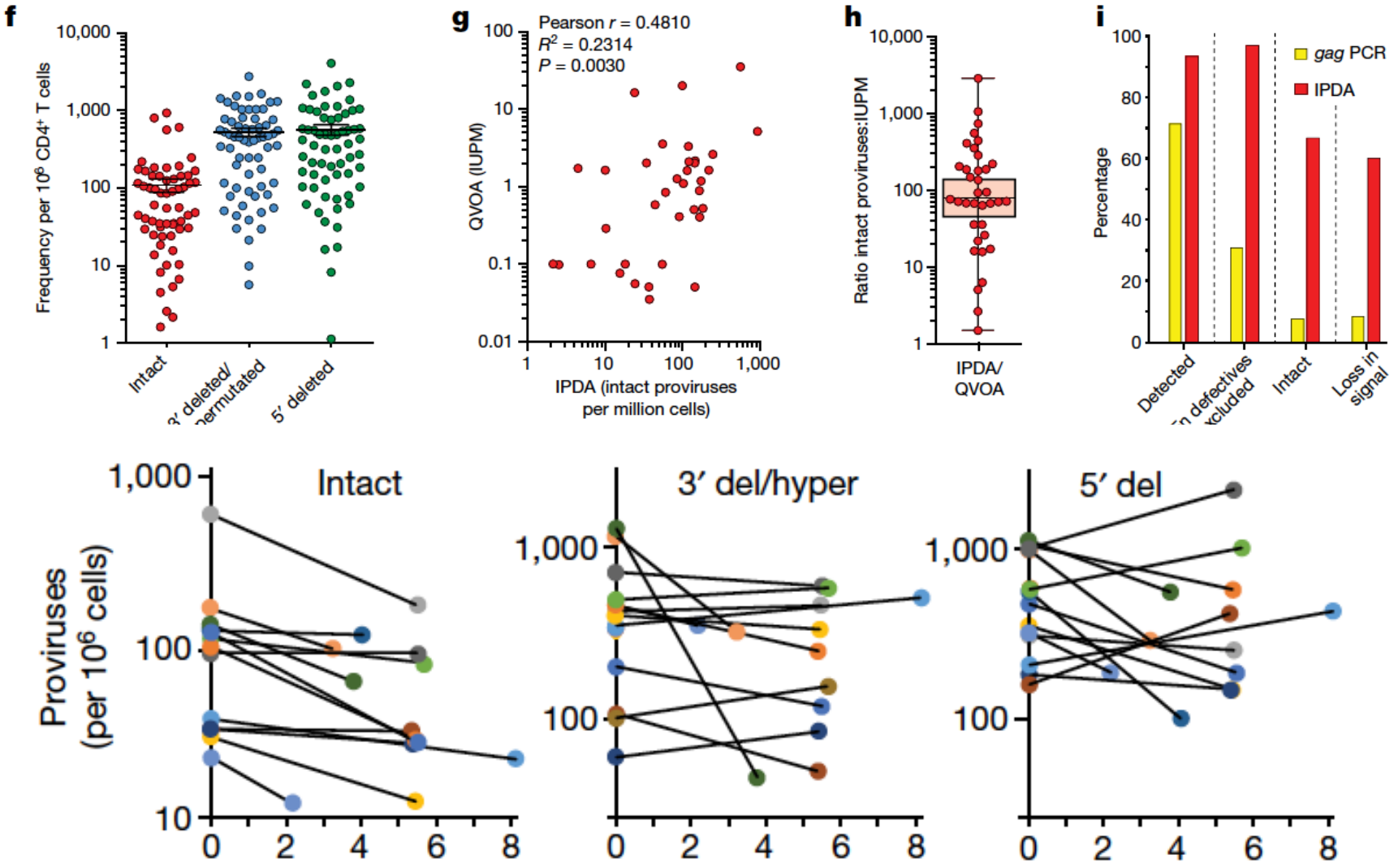
Standard PCR assays use short subgenomic amplicons in conserved regions (Fig. 1c, d) and do not distinguish intact and defective proviruses. Deletions occur throughout the genome (Fig. 1e), affecting not only the fraction of proviruses detected (Fig. 1f) but also the fraction of detected proviruses that are intact (Fig. 1g). For most standard PCR assays, less than 10% of detected sequences are intact (Fig. 1g). Thus, efficacy of a cure intervention causing a selective one log reduction in intact proviruses may be inapparent (Fig. 1h).

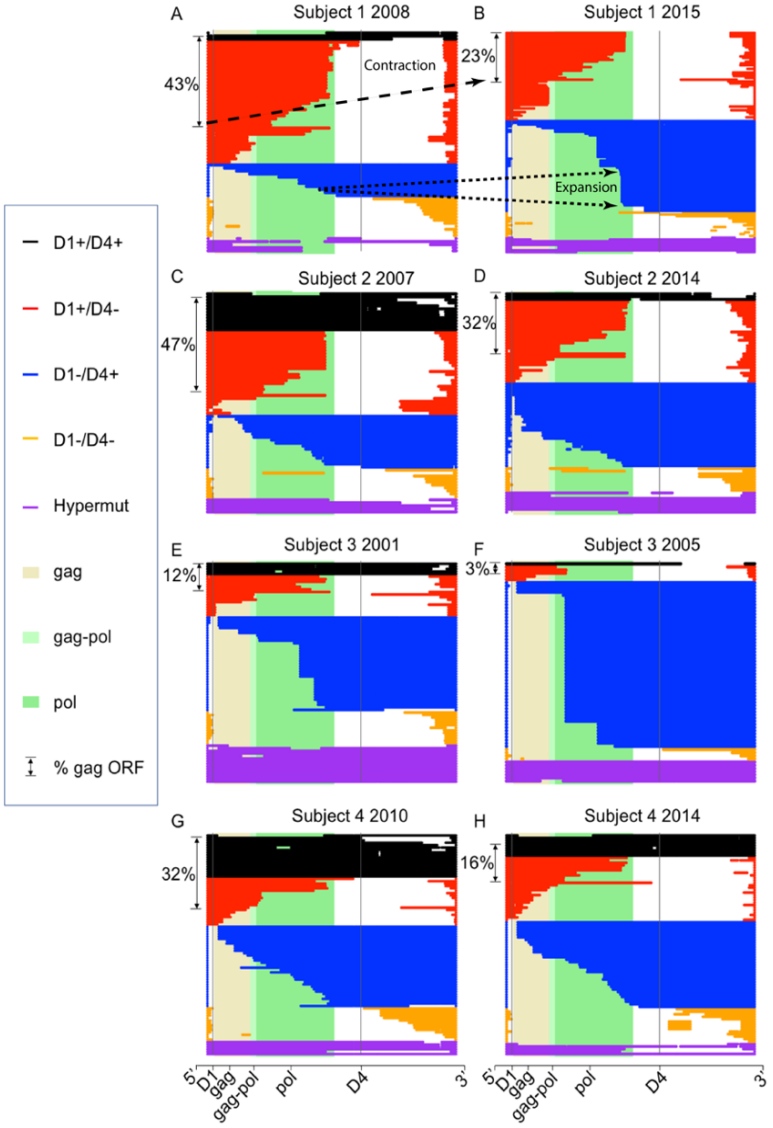
Interrogating individual proviruses simultaneously at several positions could differentiate intact from defective proviruses. Analysis of nFGS data revealed that strategically placed amplicons in the packaging signal (Ψ) and *env* regions could jointly identify more than 90% of deleted proviruses as defective (Fig. 2a). Hypermutated proviruses must also be identified (Fig. 1a). Seventy-three per cent of these have mutations in the GG→AG context (Extended Data Fig. 1a). Most also have GA→AA mutations. Only 27% of hypermutated proviruses had only GA→AA mutations, and most of these also had deletions (Extended Data Fig. 1a). Therefore, we focused on GG→AG hypermutation. We identified a conserved region in the Rev-response element (RRE) with adjacent consensus sites (TGGG) for the responsible enzyme, APOBEC3G[10] (Fig. 2b–e). Of the sequences with GG→AG hypermutation, 97% had one or more mutations in this region (Fig. 2c, e), with 13 distinct patterns (Fig. 2f). Using mutant plasmids carrying each pattern, we developed allelic discrimination probes (Extended Data Fig. 1b) that correctly identify 95% of hypermutated sequences as defective (Fig. 2f).

These analyses allowed design of a droplet digital PCR (ddPCR) method that distinguishes most deleted and/or hypermutated proviruses from intact proviruses using two amplicons and hypermutation discrimination probes (Fig. 3a, b). Genomic DNA (gDNA) is isolated using an optimized method to minimize DNA shearing between targeted regions (see below) and partitioned into nanodroplets such that individual droplets rarely contain more than one provirus ($P = 0.00416$). Proviruses within droplets are analysed simultaneously at the Ψ and *env* regions via multiplex PCR, with the *env* PCR also discriminating hypermutated proviruses. Intact proviruses give amplification at both regions (Fig. 3a, b). Intact proviruses per 10^6 cells are calculated using separate amplification of a cellular gene (*RPP30*) after correction for DNA shearing.

The ability of this intact proviral DNA assay (IPDA) to distinguish intact and defective proviruses was verified using plasmid controls that represent proviruses with different defects. These templates gave positive droplets in the expected quadrants: quadrant 1 (Q1) for 3′ deletion and/or hypermutation and Q4 for 5′ deletion (Extended Data Fig. 2). Cultured clonal populations of infected patient cells also give single quadrant patterns (see below). By contrast, uncultured polyclonal patient CD4$^+$ T cell populations gave droplets in all four quadrants, allowing separate quantification of intact and defective proviruses (Fig. 3c).

Validation of the IPDA is described in Extended Data Figs. 3 and 4. DNA shearing is essential for droplet formation but artificially reduces


f
Frequency per 10^6 CD4+ T cells
10,000
1,000
100
10
1
Intact
3′ deleted/ hypermutated
5′ deleted
g
Pearson r = 0.4810
R^2 = 0.2314
P = 0.0030
QVOA (IUPM)
100
10
1
0.1
0.01
1
10
100
1,000
IPDA (intact proviruses per million cells)
h
Ratio intact proviruses:IUPM
10,000
1,000
100
10
1
IPDA/ QVOA
i
Percentage
100
80
60
40
20
0
gag PCR
IPDA
Detected
Intact
Loss in signal
Proviruses (per 10^6 cells)
1,000
100
10
Intact
3′ del/hyper
5′ del
0
2
4
6
8

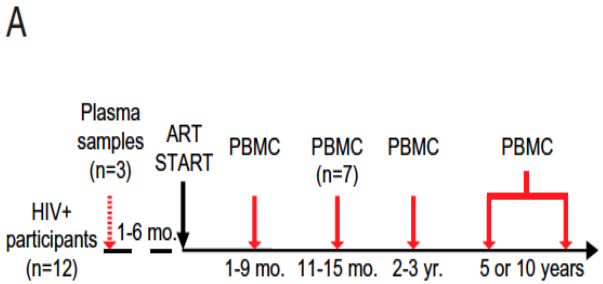


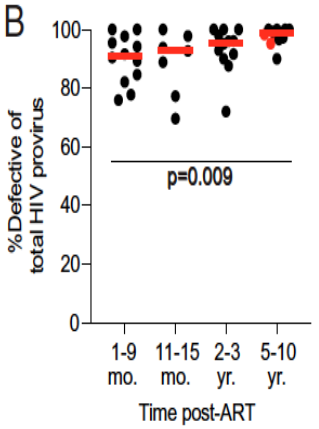
This pattern is present in 4 chronically infected individuals thus far analyzed!

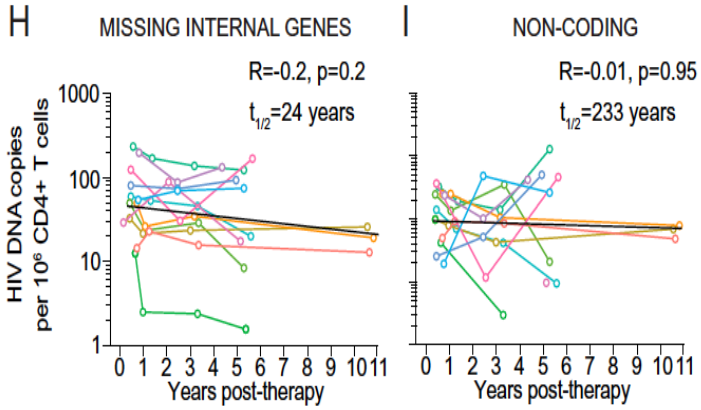
Longitudinal clonal dynamics of HIV-1 latent reservoirs measured by combination quadruplex polymerase chain reaction and sequencing

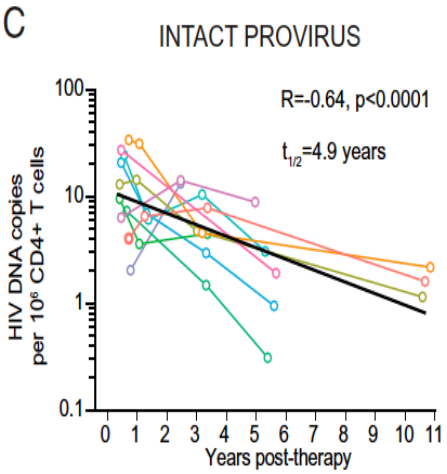
Alice Cho[a], Christian Gaebler[a], Thiago Olveira[a], Victor Ramos[a], Marwa Saad[a], Julio C. C. Lorenzi[a], Anna Gazumyan[a], Susan Moir[b], Marina Caskey[a], Tae-Wook Chun[b], and Michel C. Nussenzweig[a,c,1]

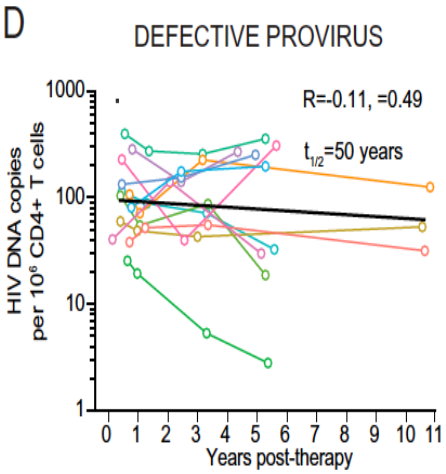
- **Intact and defective proviruses are under distinct selective pressure**
- **The intact proviral reservoir is progressively enriched in expanded clones of CD4+ T cells resulting in diminishing complexity over time**











GERIATRIC HIV CONFERENCE

CLINICAL RESEARCH AND CLINICAL PRACTICE FOR A HEALTHY AGEING IN HIV

INR: immunological profile
Higher HLA DR
Lower Naive cells
Lower Naive/Memory ratio

INR : Virological profile
Equal HIV DNA
HIGHER HIV RNA

The Journal of Infectious Diseases
BRIEF REPORT

Transcriptionally Active Defective HIV-1 Proviruses and Their Association With Immunological Nonresponse to Antiretroviral Therapy

Francesca Scrimieri,[1] Estella Bastian,[2] Mindy Smith,[2] Catherine A. Rehm,[2] Caryn Morse,[3] Janaki Kuruppu,[2] Mary McLaughlin,[2] Weizhong Chang,[1,a] Irini Sereti,[2] Joseph A. Kovacs,[3] H. Clifford Lane,[2] and Hiromi Imamichi[2,a]

[1]Applied and Developmental Research Directorate, Frederick National Laboratory for Cancer Research, Frederick, Maryland, USA; [2]National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, USA; and [3]Critical Care Medicine Department, National Institutes of Health Clinical Center, Bethesda, Maryland, USA

A subset of antiretroviral therapy-treated persons with human immunodeficiency virus (HIV), referred to as immunological nonresponders (INRs), fails to normalize $CD4^+$ T-cell numbers. In a case-control study involving 26 INRs ($CD4 < 250$ cells/μL) and 25 immunological responders (IRs; $CD4 \geq 250$ cells/μL), we evaluated the potential contribution of transcriptionally competent defective HIV-1 proviruses to poor $CD4^+$ T-cell recovery. Compared to the responders, the INRs had higher levels of cell-associated HIV RNA ($P = .034$) and higher percentages of HLA-DR^+ $CD4^+$ T cells ($P < .001$). While not encoding replication-competent viruses, the RNA transcripts frequently encoded HIV-1 Gag-p17 and Nef proteins. These transcripts and/or resulting proteins may activate pathway(s) leading to the immunological nonresponse phenotype.

Keywords. HIV immunological nonresponse; defective HIV-1 proviruses; transcription.

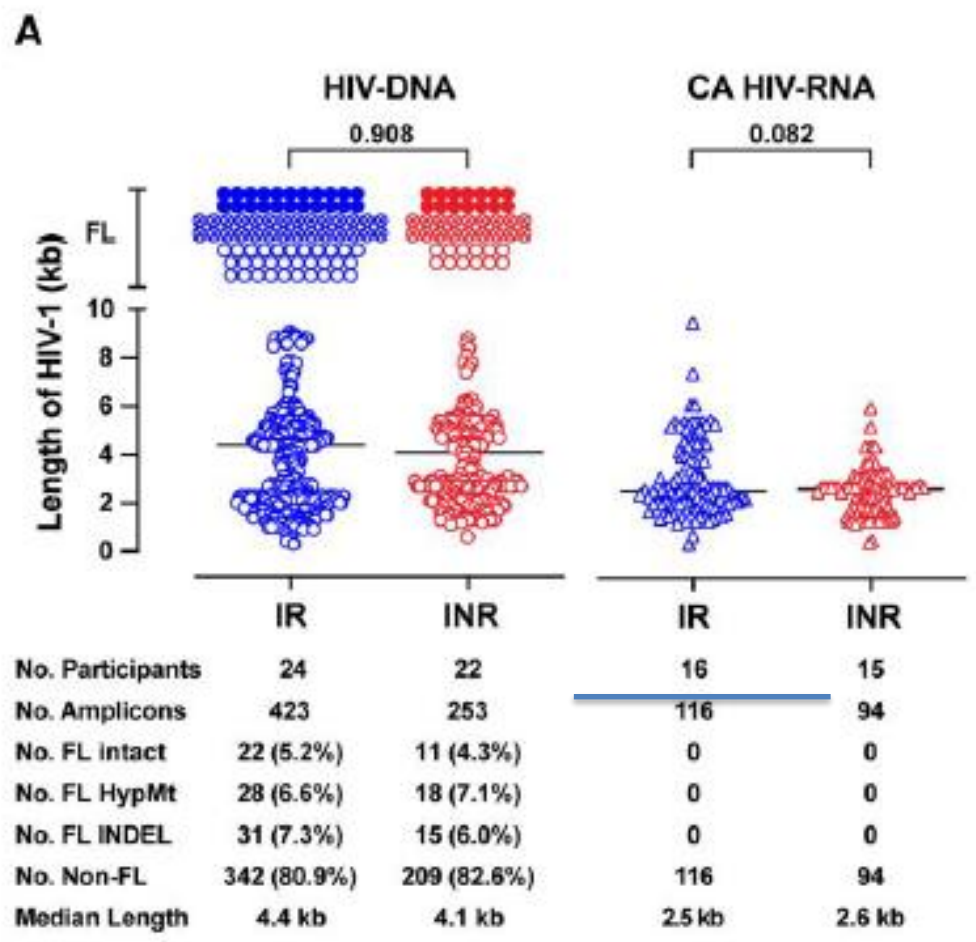
Table 1. T-Cell Characteristics and Levels of HIV DNA and CA HIV RNA of Study Participants

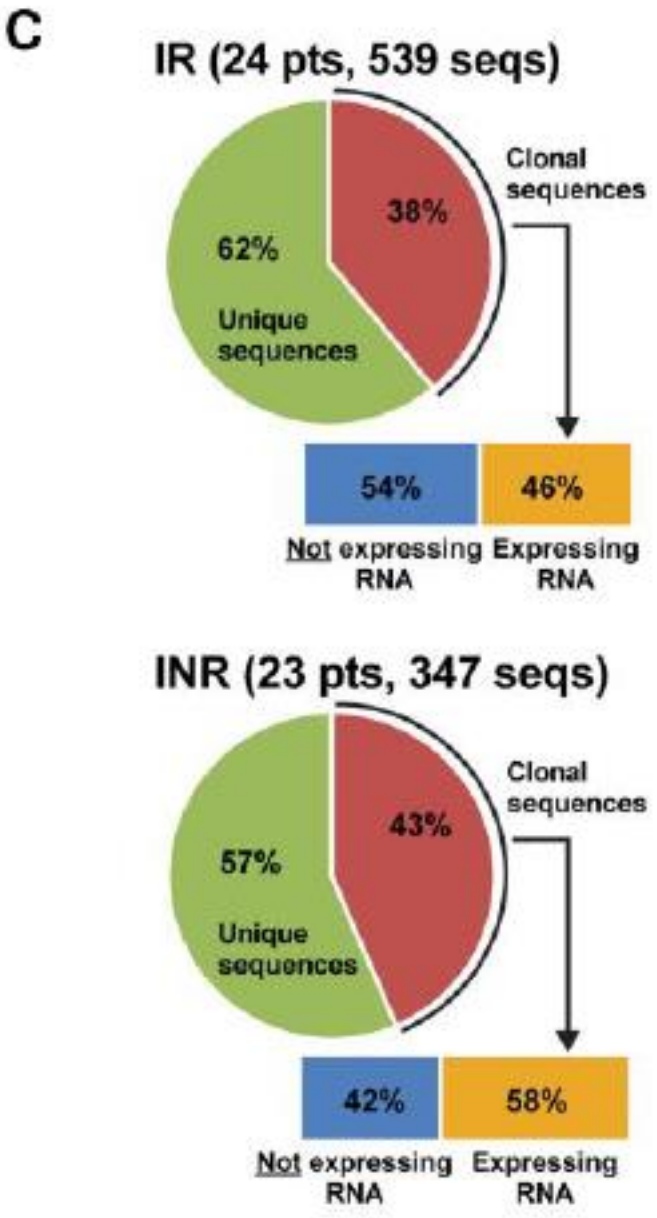
Characteristic	IR (n = 25)	INR (n = 26)	*P* Value, IR vs INR	HNC (n = 25)	*P* Value, IR vs HNC	*P* Value, INR vs HNC
$CD4^+$ count, cells/μL	605 (548–730)	187 (155–246)	<.001	784 (677–1030)	.002	<.001
$CD8^+$ count, cells/μL	940 (682–1050)	556 (381–744)	.001	471 (365–631)	<.001	.249
CD4/CD8 ratio	0.8 (0.5–0.9)	0.3 (0.2–0.5)	<.001	1.9 (1.2–2.3)	<.001	<.001
% $CD4^+$ HLA-DR^+	12.0 (10.0–17.0)	21.5 (16.0–29.0)	<.001	8.0 (7.0–10.0)	.003	<.001
% $CD8^+$ HLA-DR^+	31.0 (24.0–43.0)	41.5 (32.0–57.0)	.037	27.0 (15.0–36.0)	.013	<.001
% $CD4^+$ naive, $CD4^+$ $CD45RO^-$	25.0 (11.0–39.0)	16.5 (7.5–23.0)	.038	26.0 (19.0–41.0)	.634	.005
% $CD4^+$ memory, $CD4^+$ $CD45RO^+$	75.0 (61.0–88.0)	83.5 (76.3–93.0)	.034	74.0 (60.0–81.0)	.627	.005
% $CD8^+$ naive, $CD8^+$ $CD45RO^-$	23.0 (12.0–41.0)	14.0 (11.3–25.0)	.146	37.0 (22.0–49.0)	.087	<.001
% $CD8^+$ memory, $CD8^+$ $CD45RO^+$	77.0 (59.0–89.0)	85.0 (75.0–90.0)	.139	63.0 (51.0–79.0)	.073	<.001
$CD4^+$ naive/memory ratio	0.3 (0.1–0.6)	0.2 (0.1–0.3)	.035	0.4 (0.2–0.7)	.627	.005
$CD8^+$ naive/memory ratio	0.3 (0.1–0.7)	0.2 (0.1–0.3)	.152	0.6 (0.3–1.0)	.083	<.001
HIV DNA copies/10^6 $CD4^+$ cells[a]	1575 (540–7882)	1827 (642–5434)	.835	…		
CA HIV RNA copies/10^6 $CD4^+$ cells[a]	580 (40–1331)	1593 (450–3622)	.034	…		

Data are median (interquartile range). *P* values were calculated by Mann-Whitney test.

Abbreviations: CA, cell-associated; HNC, HIV negative control; INR, immunological nonresponder; IR, immunological responder.

[a]Data from 24 IRs and 25 INRs.





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BRIEF REPORT

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[1]Applied and Developmental Research Directorate, Frederick National Laboratory for Cancer Research, Frederick, Maryland, USA; [2]National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, USA; and [3]Critical Care Medicine Department, National Institutes of Health Clinical Center, Bethesda, Maryland, USA

A subset of antiretroviral therapy-treated persons with human immunodeficiency virus (HIV), referred to as immunological nonresponders (INRs), fails to normalize $CD4^+$ T-cell numbers. In a case-control study involving 26 INRs ($CD4 < 250$ cells/μL) and 25 immunological responders (IRs; $CD4 \geq 250$ cells/μL), we evaluated the potential contribution of transcriptionally competent defective HIV-1 proviruses to poor $CD4^+$ T-cell recovery. Compared to the responders, the INRs had higher levels of cell-associated HIV RNA ($P = .034$) and higher percentages of HLA-DR^+ $CD4^+$ T cells ($P < .001$). While not encoding replication-competent viruses, the RNA transcripts frequently encoded HIV-1 Gag-p17 and Nef proteins. These transcripts and/or resulting proteins may activate pathway(s) leading to the immunological nonresponse phenotype.

Keywords. HIV immunological nonresponse; defective HIV-1 proviruses; transcription.

HIGHER HIV RNA:

All defective viruses
No damage from virus production
DAMAGE from viral transcripts/proteins

Co-infections

High antigenic burden

In chronic infections, persistent exposure of T-cells to a high antigenic burden results in a T-cell dysfunctional state called exhaustion.

T-cell exhaustion leads to a suboptimal immune- mediated control of multiple viral infections including HIV (Fenwick et al., 2019).

INRs showed higher proportions of exhausted (PD-1+ and/or TIGIT+) and senescent (CD57+) CD4+ and CD8+ memory T-cells, as well as elevated markers of inflammation, e.g. IP-10 and IL-6

CMV replication & lower CD4/CD8 Ratio

Clinical Infectious Diseases

MAJOR ARTICLE

HIV/AIDS

Asymptomatic CMV Replication During Early Human Immunodeficiency Virus (HIV) Infection Is Associated With Lower CD4/CD8 Ratio During HIV Treatment

Davey M. Smith,[1,2] Masato Nakazawa,[1] Michael L. Freeman,[3] Christy M. Anderson,[1] Michelli F. Oliveira,[1] Susan J. Little,[1] and Sara Gianella[1]

[1]University of California, San Diego, La Jolla, and [2]Veterans Affairs San Diego Healthcare System, San Diego, California; and [3]Case Western Reserve University/University Hospitals Case Medical Center, Cleveland, Ohio

Background. A low CD4/CD8 ratio in human immunodeficiency virus (HIV)–infected individuals is associated with inflammation and higher risk of non-AIDS morbidity and mortality. In this study, we investigated the effect of subclinical cytomegalovirus (CMV) and Epstein-Barr virus (EBV) replication on $CD4^+$ and $CD8^+$ T-cell dynamics when antiretroviral therapy (ART) is started during early infection.

Methods. We investigated 604 peripheral blood mononuclear cell samples from 108 CMV- and EBV-seropositive HIV-infected men who have sex with men, who started ART within a median of 4 months from their estimated date of infection and were followed for a median of 29.1 months thereafter. Levels of CMV and EBV DNA were measured at each timepoint. Mixed-effects asymptotic regression models were applied to characterize $CD4^+$ and $CD8^+$ T-cell dynamics, and Bayesian hierarchical models were used to quantify individual differences in CMV and EBV DNA replication.

Results. Higher levels of subclinical CMV replication were associated with lower predicted maximum levels of CD4/CD8 ratio ($P < .05$), which was driven by higher levels of $CD8^+$ T-cell counts ($P < .05$), without affecting $CD4^+$ T-cell counts ($P > .1$). Age was negatively associated with CD4/CD8 levels ($P < .05$), and this effect was independent of the CMV association ($P < .05$ for both CMV and age in a multivariate model).

Conclusions. Subclinical CMV replication in blood cells during early HIV infection and younger age were associated with lower CD4/CD8 ratios during suppressive ART. These findings suggest that active CMV infection in the setting of treated HIV may represent an attractive potential target for therapeutic intervention.

Keywords. cytomegalovirus; Epstein-Barr virus; CD4/CD8 ratio; ART; HIV.

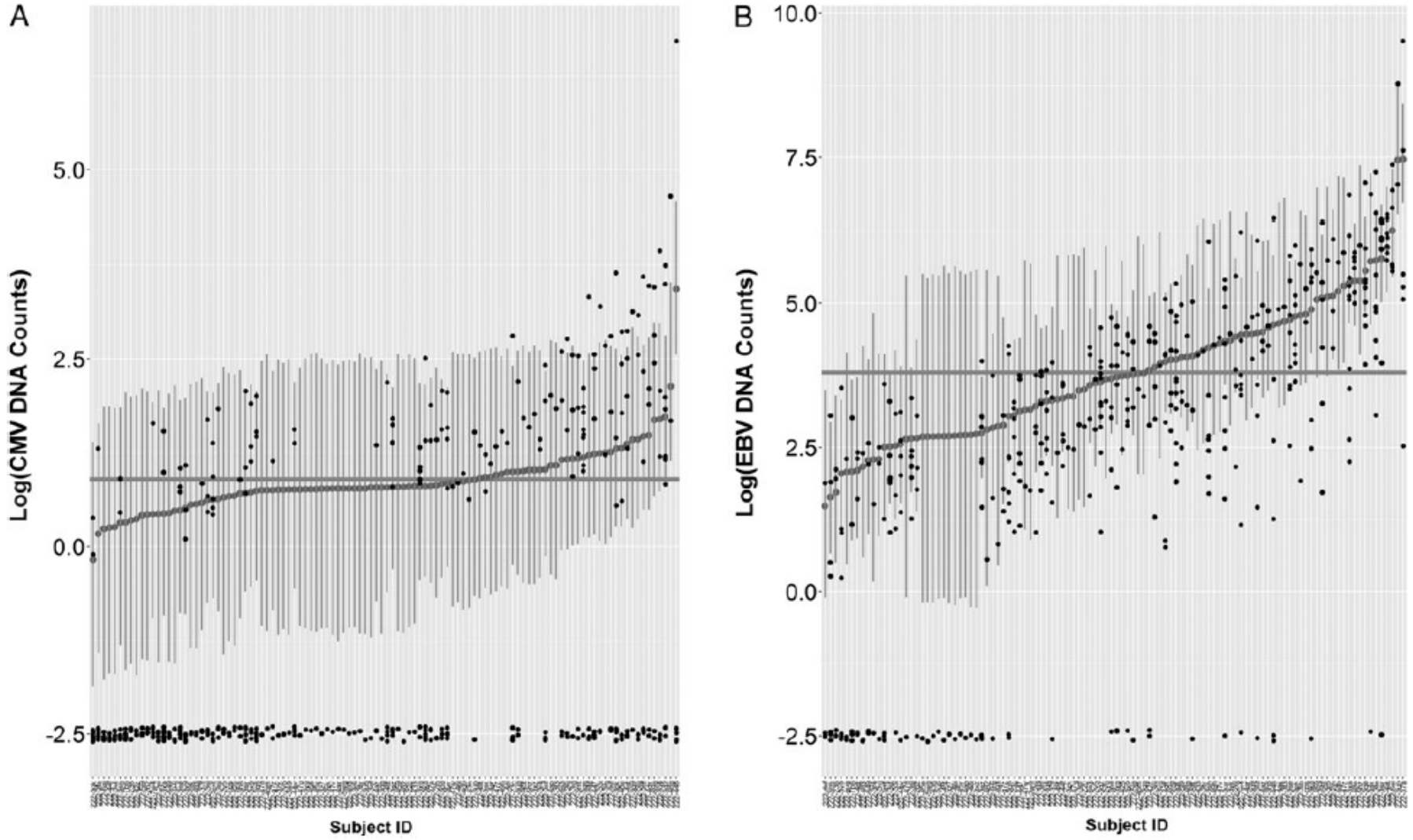


Figure 2. Posterior medians and 95% credible intervals (CIs) for cytomegalovirus (CMV; *A*) and Epstein-Barr virus (EBV; *B*) DNA counts, derived from Bayesian hierarchical regression models. Small black dots indicate observed data. Large gray dots, vertical gray lines, and the horizontal gray line indicate estimated random intercepts for participants, 95% CIs, and the global intercept, respectively. Observed values are jittered to avoid overlap.

Valganciclovir Reduded Activation

Valganciclovir Reduces T Cell Activation in HIV-infected Individuals With Incomplete $CD4^+$ T Cell Recovery on Antiretroviral Therapy

Peter W. Hunt,[1] Jeffrey N. Martin,[1] Elizabeth Sinclair,[1] Lorrie Epling,[1] Juli Teague,[1] Mark A. Jacobson,[1] Russell P. Tracy,[2] Lawrence Corey,[3] and Steven G. Deeks[1]

[1]Departments of Medicine, Epidemiology, and Biostatistics, University of California, San Francisco; [2]Departments of Pathology and Biochemisty, University of Vermont College of Medicine, Burlington; [3]Departments of Laboratory Medicine, Medicine and Microbiology, University of Washington, Fred Hutchison Cancer Research Center, Seattle

Background. Elevated immune activation persists during treated human immunodeficiency virus (HIV) infection and is associated with blunted CD4 recovery and premature mortality, but its causes remain incompletely characterized. We hypothesized that asymptomatic cytomegalovirus (CMV) replication might contribute to immune activation in this setting.

Methods. Thirty antiretroviral therapy–treated HIV-infected CMV-seropositive participants with CD4 counts <350 cells/mm^3 were randomized to receive valganciclovir 900 mg daily or placebo for 8 weeks, followed by an additional 4-week observation period. The primary outcome was the week 8 change in percentage of activated ($CD38^+$ HLA-DR^+) $CD8^+$ T cells.

Results. Fourteen participants were randomized to valganciclovir and 16 to placebo. Most participants (21 [70%] of 30) had plasma HIV RNA levels <75 copies/mL. The median CD4 count was 190 (IQR: 134–232) cells/mm^3, and 12 (40%) of 30 had detectable CMV DNA in saliva, plasma, or semen at baseline. CMV DNA continued to be detectable at weeks 4–12 in 7 (44%) of 16 placebo-treated participants, but in none of the valganciclovir-treated participants ($P = .007$). Valganciclovir-treated participants had significantly greater reductions in CD8 activation at weeks 8 ($P = .03$) and 12 ($P = .02$) than did placebo-treated participants. These trends were significant even among those with undetectable plasma HIV RNA levels.

Conclusions. CMV (and/or other herpesvirus) replication is a significant cause of immune activation in HIV-infected individuals with incomplete antiretroviral therapy–mediated $CD4^+$ T cell recovery.

Clinical Trials Registration. NCT00264290.

Lower inflammation
No CMV replication
No CD4 Increase

EBV
HHV-8
HHV-6

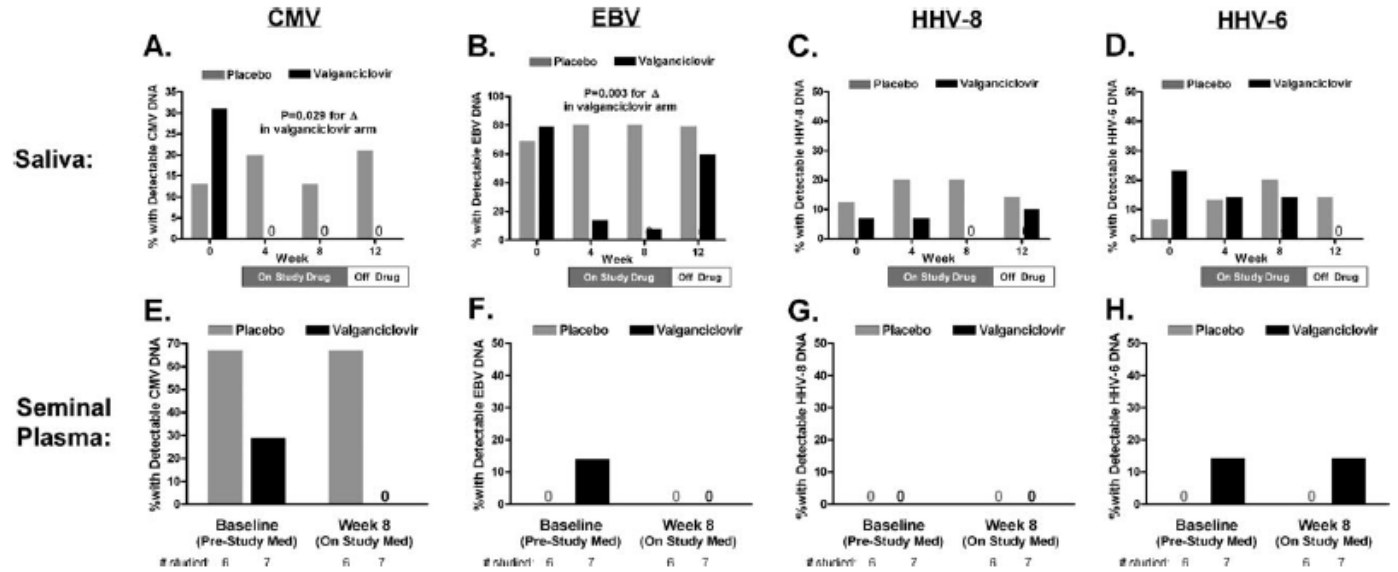
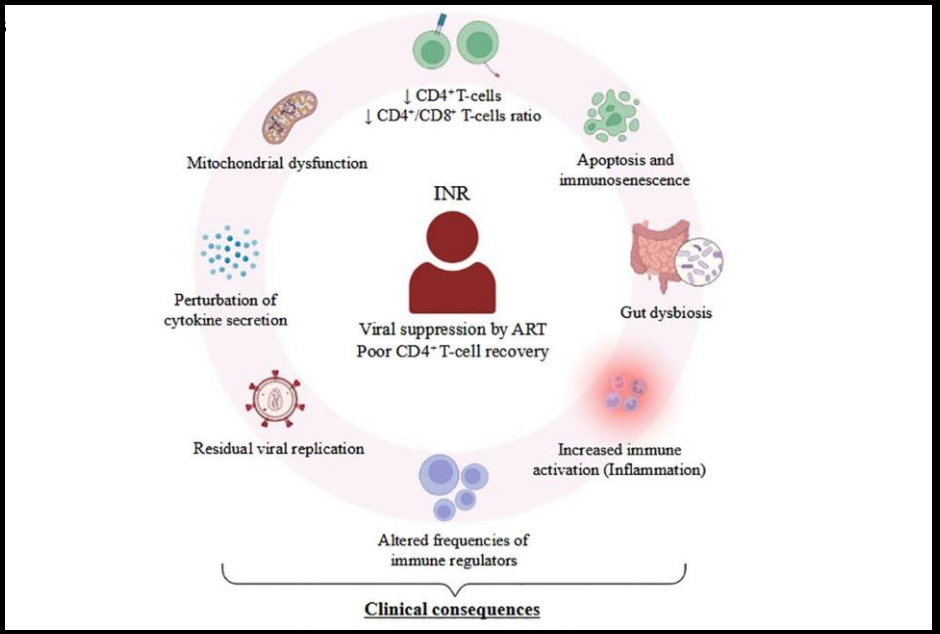


Figure 2. Changes in herpesvirus DNA levels in saliva and semen with valganciclovir therapy. Herpesvirus DNA levels were assessed by polymerase chain reaction (lower limit of detection, 150 copies/mL) on saliva (at weeks 0, 4, 8, and 12) and seminal plasma (at weeks 0 and 8 only). The proportion of participants with a positive herpesvirus DNA level result is plotted for both valganciclovir and placebo groups in saliva and seminal plasma for cytomegalovirus (CMV) (*A* and *E*), *Epstein-Barr virus (EBV)* (*B* and *F*), human herpesvirus (HHV) type 8 (*C* and *G*), and HHV type 6 (*D* and *H*). Plotted *P* values test whether the proportion of participants with a positive DNA level result changes over time within each treatment group (Cochran *Q* test). The proportion of participants with positive CMV and EBV DNA levels in saliva declined significantly with valganciclovir therapy but not with placebo. The proportion of participants with detectable salivary CMV DNA levels remained suppressed 4 weeks after study drug discontinuation, whereas the proportion with detectable EBV levels rebounded to pretreatment levels. Whereas 2 (29%) of 7 valganciclovir-treated participants had detectable CMV DNA plasma at baseline, none had it at week 8.

Chronic/Infectious Replicating Agents

- Herpes Virus
- HBV
- HCV
- HPV
- Other chronic Viruses
- TB
- Leishmania



Author	INR definition	Analytical technique	Interest factors	Conclusions related to INR	Reference
			GENOMICS		
El-Beeli et al.	$CD4^+$ T-cell count <500 and increase cell count by less than 50-150 cells/mm^3 per year.	PCR-SSP	*HLA-A68* *HLA-B15*	*HLA-A68* and *HLA-B15* alleles were associated with a poor immune response in HIV patients taking ART.	(28)
Pereira et al.	$CD4^+$ T-cell count <500 cells/μL.	PCR-SSO	*HLA-B13* *HLA-B35* *HLA-B39*	*HLA-B13*, *HLA-B35* and *HLA-B39* alleles are related to the poor immune response during cART.	(29)
Soria et al.	$CD4^+$ T-cell count <200/mm under suppressive cART	PCR-SSP	*KIR2DL2* *KIR2DL3* *KIR2DS2* *HLA-Cw*04:01*	Combination of *KIR2DS2*$^+$/*HLA-Cw*04:01*$^+$ multi-allelic complex was highly associated with poor recovery of $CD4^+$ T-cells.	(30)
Carvalho-Silva et al.	PLHIV that gained less than 200 $CD4^+$ T-cells/μL during the first year of ART	RFLP	*CCR5Δ32*	Heterozygosity for *CCR5Δ32* and lower $CD4^+$ T-cell count before treatment was associated with INR status.	(31)
García et al.	PLHIV starting cART with $CD4^+$ T-cells <200 cells/μL	Sequenom's MassARRAY	*IFN-γ rs2430561* *IL-19 rs2243191*	Polymorphisms in IFN-γ and IL-19 genes significantly impact the probability of not achieving an optimal immune recovery.	(32)
Yeregui et al.	pre-ART low $CD4^+$ T nadir <200 cells/μl and $CD4^+$ T-cell counts lower than 250 cells/μl at 48 months on ART.	DNA sequencing	*CXCL12 rs1801157* *CCR2 rs1799864_814*	*SDF-1/CXCL12* and *MCP-1/CCL2* prognostic markers of immune failure.	(33)
Restrepo et al.	pre-ART low nadir <200 cells/μl and $CD4^+$ T-cell count lower than 200 cells/μl at 48 months on ART	Sequenom's MassARRAY platform	*CCR2 rs1799864-AG* *CXCL12 rs1801157-TT*	*CCR2 rs1799864-AG* or *CXCL12 rs1801157-TT* genotypes influence the probability of poor $CD4^+$ T-cell recovery in the population of HIV patients.	(34)
Masson et al.	$CD4^+$ T-cell count of <500 cells/μL despite at least 3 years on cART	DNA sequencing	*SLC2A1 rs1385129* *AKT rs1130214*	The homozygous genotype (GG) associated with *SLC2A1 rs1385129* is linked to poor $CD4^+$ T-cell recovery and major frequency of GLUT1 in $CD4^+$ T-cells.	(35)

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TYPE Mini Review
PUBLISHED 15 August 2023
DOI 10.3389/fimmu.2023.1228795

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OPEN ACCESS

EDITED BY
Scott Sieg,
Case Western Reserve University,
United States

REVIEWED BY
Carey Shive,
United States Department of Veterans Affairs, United States
Joseph Mudd,
Tulane University, United States

*CORRESPONDENCE
Silvia Chafino
silvia.chafino@ciberinfec.es
Anna Rull

Multi-omics in HIV: searching insights to understand immunological non-response in PLHIV

Sonia Espineira[1,2,3†], Marina Flores-Piñas[1,2†], Silvia Chafino[2,4*], Consuelo Viladés[1,2,3,4], Eugenia Negredo[4,5,6,7], Salvador Fernández-Arroyo[8], Josep Mallolas[4,9], Beatriz Villar[1,2,3], Santiago Moreno[4,10,11], Francesc Vidal[1,2,3,4], Anna Rull[1,2,3,4*‡] and Joaquim Peraire[1,2,3,4‡]

Author	INR definition	Analytical technique	Interest factors	Conclusions related to INR	Reference
			PROTEOMICS		
Azzam et al.	$CD4^+$ T-cell count of less than 350 cells/μl at 2 years on ART.	LC-MS/MS	155 proteins	153 proteins associated with INR/IR. 99 proteins associated with INR/HC. 97 proteins overlapped between INR/IR and INR/HC.	(43)
Liu et al.	$CD4^+$ T-cell count less than 350 cells/μl at 96 weeks on ART.	LC-MS/MS	2500 proteins	2500 proteins TGF-β, NF-κB and CD35, were highly altered in INR patients compared to IR and HC.	(44)

HLA?

CD4/CD8 ratio is also considered a surrogatemarker of immune senescence in the general population.

CD4/CD8 ratio < 1.0 :

- HIV infection
- In the elderly
- 5% of the healthy population

Wikby A, et al. Changes in CD8 and CD4 lymphocyte subsets, T cell proliferation responses and non-survival in the very old: the Swedish longitudinal OCTO-immunestudy. Mech Ageing Dev. 1998;102:187-98

Amadori A, Zamarchi R, De Silvestro G, et al. Genetic control of the CD4/CD8 T-cell ratio in humans. Nat Med. 1995;1:1279-83.

The OCTO Immune Longitudinal Study Swedish octogenarians IMMUNE RISK PROFILE (IRP) ASSOCIATED WITH TWO-YEAR MORTALITY

- High percentages of CD8 +
- Low percentages of CD4 + (ferguson et al., 1995).

In follow-up studies of the octo

- Persistent cmv infection
- Cd4/cd8 ratio less than one
- The accumulation of lately differentiated cd8 + temra cells

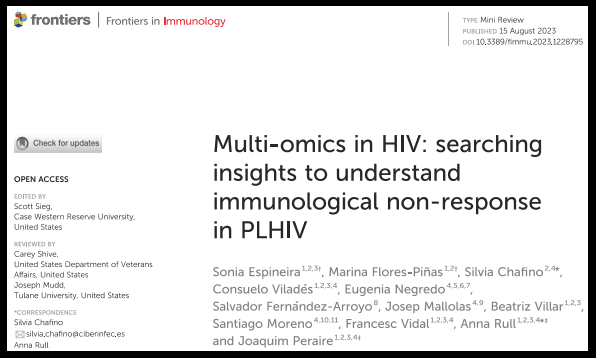
CD4/CD8 ratio < 1 is associated with lymphocyte subsets, CMV and gender in 71-year old individuals: 5-Year follow-up of the Swedish HEXA Immune Longitudinal Study
Jan Strindhall et al. **Experimental Gerontology** Volume 95, September 2017, Pages 82-87

In the Kristianstad Survey

Objective: to examine the proportion of individuals with a CD4/CD8 ratio less than one relative age and gender across the entire adult lifespan
population-based sample in the age range of 20–79 years

- 8% in the age range of 20–59 years
- 16% in the age range of 60–94 years
- higher in men compared with women

(Wikby et al., 2008).



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REVIEWED BY
Carey Shive,
United States Department of Veterans Affairs, United States
Joseph Mudd,
Tulane University, United States

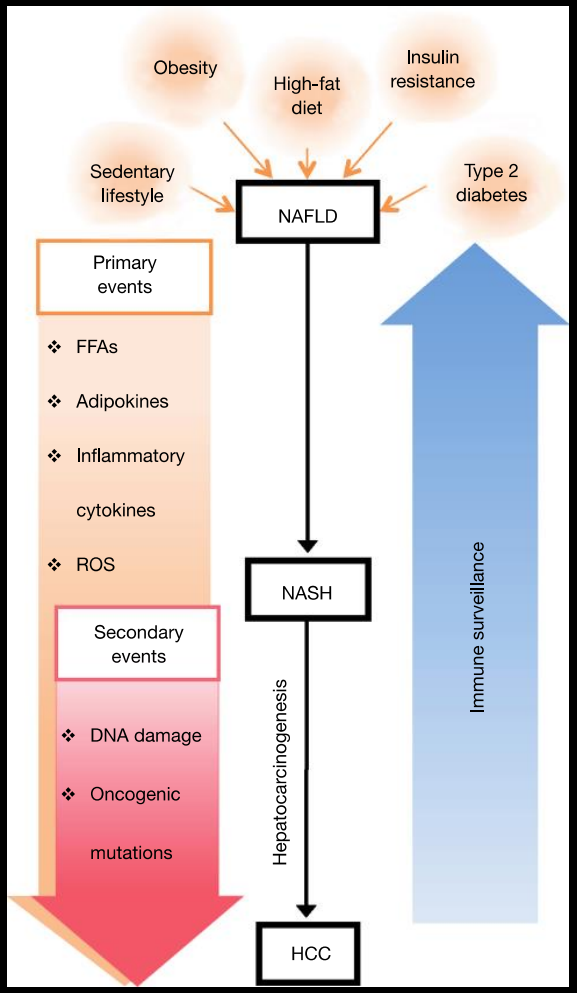
*CORRESPONDENCE
Silvia Chafino
silvia.chafino@ciberinfec.es
Anna Rull

Multi-omics in HIV: searching insights to understand immunological non-response in PLHIV

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Metabolomics

METABOLOMICS					
Scarpellini et al.	PLHIV with fail to increase $CD4^+$ T-cell count by at least 30%	Targeted MS/MS	40 acylcarnitines 19 proteinogenic amino acids, ornithine and citrulline, 19 biogenic amines, the sum of hexoses, 76 phosphatidylcholines, 14 lyso-phosphatidylcholines 15 sphingomyelins	↑ Ether lipids ↓ SGPP1 β-oxidation	(45)
Rodríguez-Gallego et. al	pre-ART low nadir (<200 cells/μl) and $CD4^+$ T-cell count lower than 250 cells/μl at 36 months on ART (baseline study).	NMR	HDL HDL-Cholesterol HDL- TGs VLDL VLDL-Cholesterol VLDL- TGs LDL/HDL	non-HDL lipoprotein particle ↑ VLDL particles ('medium' subclass) ↑ glucose	(46)
Masip et al.	pre-ART low nadir (<200 cells/μl) and $CD4^+$ T-cell count lower than 250 cells/μl at 36 months on ART (longitudinal study).	NMR	HDL HDL-Cholesterol HDL- TGs VLDL VLDL-Cholesterol VLDL- TGs LDL/HDL	↑ large HDL-P ↑ small HDL-P (increased from baseline levels, Rodríguez-Gallego et. al)	(47)
Qian et al.	$CD4^+$ T-cell count rise after 2 years of < 100 or > 300 cells/μl of ART.	PLC-MS/MS-ESI RP/UPLC-MS/MS-ESI+ RP/UPLC-MS/MS-ESI-	125 lipids 68 amino acids 7 peptides 14 carbohydrates 12 cofactors and vitamins 9 nucleotides 6 energy metabolites	↑ Acylcarnitines (MC, PC, OC, and SC) associated with INR	(48)
Ferrari et al.	$CD4^+$ T-cells <350/μL receiving ART for 2 or more years	UPLC-MS/MS	125 metabolites	↑ citrate, aconitate,linolenate ↓ nicotinamide, fumarate, malate and phospholipids ↓ amino acids (isoleucina, alalnina glycine…)	(49)
Nyström et al.	pre-ART low nadir (<200 cells/μl) and rise in $CD4^+$ T-cells <50 cells/year in the first 2 years following suppressive ART.	LC-MS technique	200 metabolites	↓ levels of cysteine could be associated with poor CD4+ T-cell recovery	(50)
Malo et al.	$CD4^+$ T-cell count rises after 2 years of < 100 or > 300 cells/μl of ART.	NMR	Plasma glycoprotein profiles	↑ levels of GlycA and GlycB associated with a worse immunological state. ↑ levels of baseline glycoprotein concentrations tend to respond less to ART.	(51)



New players in non-alcoholic fatty liver disease induced carcinogenesis: lipid dysregulation impairs liver immune surveillance

Ngoc Lan Tran, Giovanni Sitia

Division of Immunology, Transplantation and Infectious Diseases, IRCCS San Raffaele Scientific Institute, Milan, Italy

Nature. 2016 March 10; 531(7593): 253–257. doi:10.1038/nature16969.

NAFLD causes selective CD4+ T lymphocyte loss and promotes hepatocarcinogenesis

Chi Ma[1], **Aparna H. Kesarwala**[2], **Tobias Eggert**[1], **José Medina-Echeverz**[1], **David E. Kleiner**[3], **Ping Jin**[4], **David F. Stroncek**[4], **Masaki Terabe**[5], **Veena Kapoor**[6], **Mei ElGindi**[1], **Miaojun Han**[1], **Angela M. Thornton**[7], **Haibo Zhang**[8], **Michèle Egger**[9], **Ji Luo**[8], **Dean W. Felsher**[10], **Daniel W. McVicar**[11], **Achim Weber**[9], **Mathias Heikenwalder**[12,13], and **Tim F. Greten**[1,*]

Dysregulation of lipid metabolism causes a selective loss of intrahepatic $CD4_+$ but not $CD8_+$ T lymphocytes, leading to accelerated hepatocarcinogenesis

Microbial traslocation

Heliyon 9 (2023) e20790

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Severe intestinal barrier damage in HIV-infected immunological non-responders

Xiao-Yan Guo [a,b,1], Yun-Tian Guo [b,c,1], Ze-Rui Wang [d], Yan-Mei Jiao [b,*], Yue Hu [e], Li-Na Fan [e], Rui-Qin Cheng [f], Meng-Meng Qu [b], Chao Zhang [b], Jin-Wen Song [b], Ruo-Nan Xu [b], Xing Fan [b], Wen Xu [b], Ji-Yuan Zhang [b], Bing-Ke Bai [b], En-Qiang Linghu [d], Yao-Kai Chen [g,**], Ping Ma [e,***], Fu-Sheng Wang [a,b,c,2,****]

[a] Beijing Ditan Hospital, Capital Medical University, Beijing, China
[b] Senior Department of Infectious Diseases, the Fifth Medical Centre of Chinese PLA General Hospital, National Clinical Research Center for Infectious Diseases, Beijing, China
[c] The Second School of Clinical Medicine, Southern Medical University, Guangzhou, China
[d] Department of Gastroenterology, First Medical Center of Chinese PLA General Hospital, Beijing, China
[e] Department of Infectious Diseases, Tianjin Second People's Hospital, Tianjin, China
[f] Tianjin Bright Eye Hospital, Tianjin, China
[g] Department of Infectious Disease, Chongqing Public Health Medical Center, Chongqing, China

Lu et al. BMC Infect Dis (2021) 21:742
https://doi.org/10.1186/s12879-021-06491-z

BMC Infectious Diseases

RESEARCH Open Access

Association between CD4$^+$ T cell counts and gut microbiota and serum cytokines levels in HIV-infected immunological non-responders

Danfeng Lu[1,2], Jian-Bo Zhang[4], Yue-Xin Wang[1,3], Shi-Tao Geng[1,3], Zunyue Zhang[1,2], Yu Xu[1,3], Shao-You Li[1,2], Kun-Hua Wang[1,3*] and Yi-Qun Kuang[1,2*]

Targets of Immune Restoration

- CD8 elevati ed immunoattivati
  - HIV e HIV Proteins
  - CMV-EBV-altri patogeni
  - Espansione clonale
- CD4
  - Elevata morte cellulare
  - Killing of uninfected cells
  - Scarse riserve midollari o timiche

Progenitori midollari e Timo

- Perche le riserve midollari/toimiche sono ridotte ma sufficienti a produrre CD8?
- Timo: induzione a selezionare CD8 e non CD4?
- Possiamo indirizzare i precursori timici verso i CD4 e non i CD8?
- Trials con IL2 IL7 IL15 IL6: quali risultati???

Immunological/Virological Tayloring

- New anti HIV drugs (gp120, p24, maturation, etc)
- Test and potentially treat other infections
- Immune therapy: IL7?
- Reduce Inflammation
- Microbial translocation

