

Virology of Eradication

Carlo Federico Perno



**10th Residential Course
on Clinical Pharmacology of Antiretrovirals**

Starhotels Majestic, corso Vittorio Emanuele II 54, Torino

21-23 January 2015

Is HIV ERADICABLE???

From real lives to research: lessons from HIV
cure cases

FAILURE TO ERADICATE HIV DESPITE FULLY SUCCESSFUL HAART INITIATED IN THE FIRST DAYS OF LIFE

ALESSANDRA VIGANÒ, MD, DARIA TRABATTONI, PhD, LAURA SCHNEIDER, MD, FRANCESCO OTTAVIANI, MD, ANTONIA ALIFFI, MD, ERIKA LONGHI, PhD, STEFANO RUSCONI, MD, AND MARIO CLERICI, MD

Highly active antiretroviral therapy (HAART) started shortly after birth resulted in reversion of human immunodeficiency virus (HIV) plasma viremia, proviral DNA in PBMC, viral culture, and serum HIV antibodies to negative. Discontinuation of HAART 2 years after apparent HIV eradication, however, was followed by virus replication, CD4 decline, and destruction of HIV-specific lymphocytes, epitomizing the impossibility of HIV eradication. (*J Pediatr* 2006;148:389-91)

Table. Virologic and immunologic test results from birth to discontinuation of antiretroviral therapy at 24 months of age

	Birth	1 Month	6 Months	12 Months	18 Months	24 Months
CD4 count (cell/mm ³)	NT ¹	3531	5872	3240	3176	2461
CD4:CD8 ratio	NT	2.40	2.50	3.00	1.08	2.05
HIV RNA (copies/mL)	15300	<50	<50	<50	<50	<50
Serum HIV-specific Ab	+	N.T.	+	+	—	—

¹NT = Not tested.



No cure of HIV infection in a child despite early treatment and apparent viral clearance

Vania Giacomel, Daria Trabattoni, Nadia Zanchetta, Mara Biasin, Mariarita Gismondo, Mario Clerici, Gianvincenzo Zuccotti

A woman at 41 weeks' gestation presented to our hospital in December, 2009, in labour. She had a history of intravenous drug use, and was unaware of her HIV status. Rapid HIV-1 testing was positive and she had high viral load. A small-for-gestational-age boy was delivered vaginally, in good condition. 12 h after his birth virological analyses, comprising HIV 1-2 antibody western blot (gag, pol, env) and HIV-1 antigen p24, were positive, and his HIV-RNA viral load was 152 560 copies per mL. We started

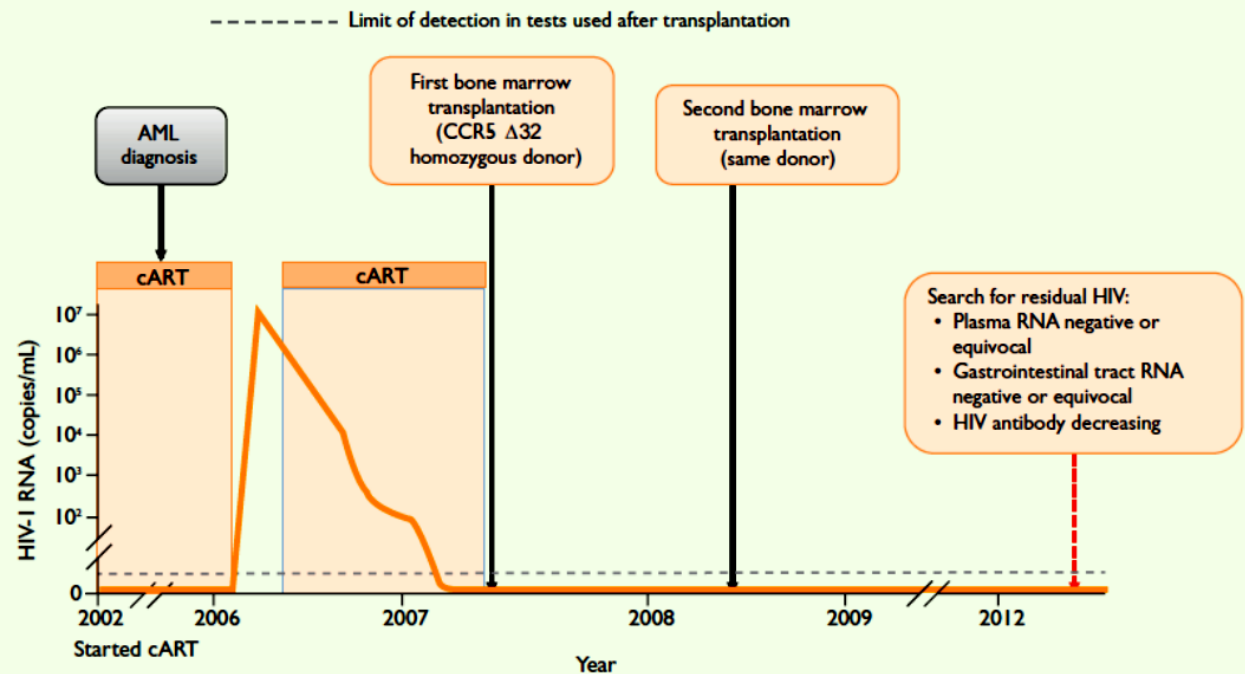
prophylaxis with zidovudine and nevirapine within 12 h of birth, but at day 4 his viral load was 13 530 copies per mL. After checking for possible viral resistance we started triple antiretroviral therapy with ritonavir boosted lopinavir, zidovudine, and lamivudine. Plasma viral load was 3971 copies per mL at day 15, 49 copies per mL after 3 months, and below assay detection at 6 months. Viral load remained undetectable and CD4 T cells were higher than normal range (appendix).

AT 3 YEARS OF AGE, TESTS FOR HIV ANTIBODIES, DNA, P24, RNA, AND VIRAL CULTURES WERE NEGATIVE. IN VIEW OF THIS, WE STOPPED TREATMENT.

1 WEEK AFTER STOPPING TREATMENT, THE PATIENT'S VIRAL LOAD WAS UNDETECTABLE AND ANTIGEN AND ANTIBODY TESTS WERE NEGATIVE; HOWEVER, VIRAL LOAD REBOUNDED (36,840 copies/ml) WITHIN 2 WEEKS. TESTS FOR HIV DNA, ANTIGENS AND ANTIBODIES, WESTERN BLOT BECAME POSITIVE. VIRAEMIA WAS SUPPRESSED BELOW DETECTABILITY BY MONTH 3 OF THERAPY

2007: 'Berlin patient' receives first bone marrow transplant^{1,2}

Figure 1. Timeline for the 'Berlin patient'. First reported case of HIV cure, following haematopoietic stem cell transplant (HSCT) for acute myelogenous leukaemia (AML). After 5 years, there has been no recurrence of HIV replication and HIV-specific immune responses continue to wane^{1,2}



2008 and 2010: 'Boston patients' receive bone marrow transplants³

- **Patient A:** male, perinatally acquired HIV infection, diagnosed with stage IV nodular sclerosing Hodgkin lymphoma in 2006, received HSCT in 2008
- **Patient B:** male, sexually acquired HIV-1 diagnosed in the mid-1980s, diagnosed with diffuse large B-cell lymphoma in 2003, received matched, sibling-donor allogeneic HSCT in 2010
- Both remained on cART
- HIV-1 DNA and RNA undetectable in peripheral blood mononucleated cells (PBMCs), CD4+ T cells, and plasma up to 21 and 42 months after transplant

2011: 'Australian patients' receive bone marrow transplants⁴

- **Patient 1:** aged 53 years, diagnosed with HIV in 2003, received transplant for AML in 2011
- **Patient 2:** aged 47 years, diagnosed with HIV in 1987, received transplant for non-Hodgkin lymphoma in 2010
- Both remained on cART throughout transplant and after
- At 3 years post-transplant, neither had detectable HIV-1 RNA in plasma or any HIV-1 DNA in CD4+ T cells or PBMCs measured using real-time polymerase chain reaction (PCR) assays
- Neither had CD4+ T-cell response to HIV antigen

2013: 'Mississippi baby' found to have undetectable HIV RNA⁵

- Infant born prematurely in a Mississippi clinic in 2010, to an HIV-infected mother not diagnosed until delivery and therefore did not receive cART during pregnancy
- Because of the high risk of exposure, at 30 h of age the infant was started on three-drug cART
- Testing confirmed that the infant had been infected with HIV, so cART was continued after discharge from hospital; however, mother and infant were lost to follow-up at 18 months and treatment was discontinued
- At 23 months mother and infant returned to clinic, but no HIV-1 plasma RNA and no HIV-specific antibodies were detectable in the infant

2013: VISCONTI cohort of post-treatment controllers reported⁶

- Most post-treatment controllers (PTCs) lacked the protective human leukocyte antigen (HLA) B alleles that are overrepresented in spontaneous HIV controllers (HICs); instead, they carried risk-associated HLA alleles that were largely absent among the HICs

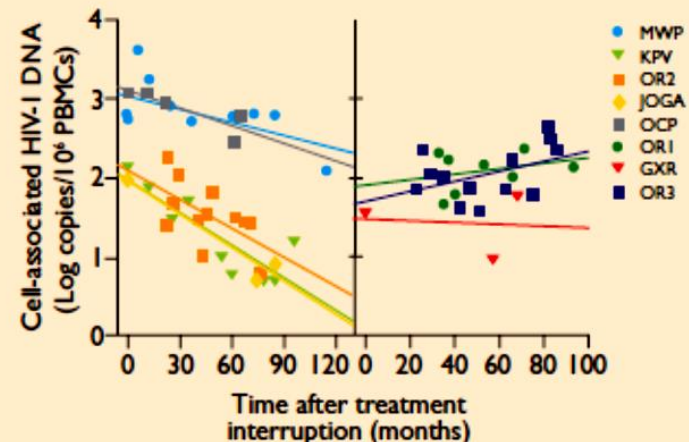


Figure 2. Evolution of cell-associated HIV-1 DNA after treatment interruption in PBMCs from eight PTCs.⁶ **Left:** five PTCs experienced a decline in their cell-associated HIV-1 DNA levels. **Right:** two PTCs maintained stable HIV-1 DNA levels and a positive slope was calculated for OR3

2014: HIV rebound in the 'Boston patients'⁷

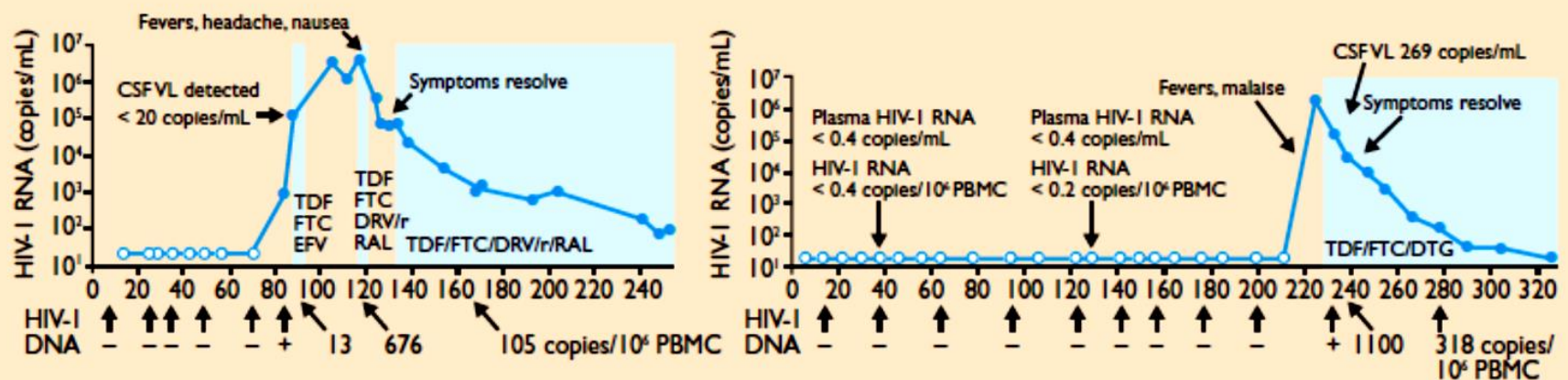


Figure 3. HIV rebound in the 'Boston patients' following analytic treatment interruptions⁷

CSF, cerebrospinal fluid; DRV, darunavir; DTG, dolutegravir; EFV, efavirenz; FTC, emtricitabine; r, ritonavir; RAL, raltegravir; TDF, tenofovir; VL, viral load

2014: HIV rebound in the 'Mississippi baby'⁸

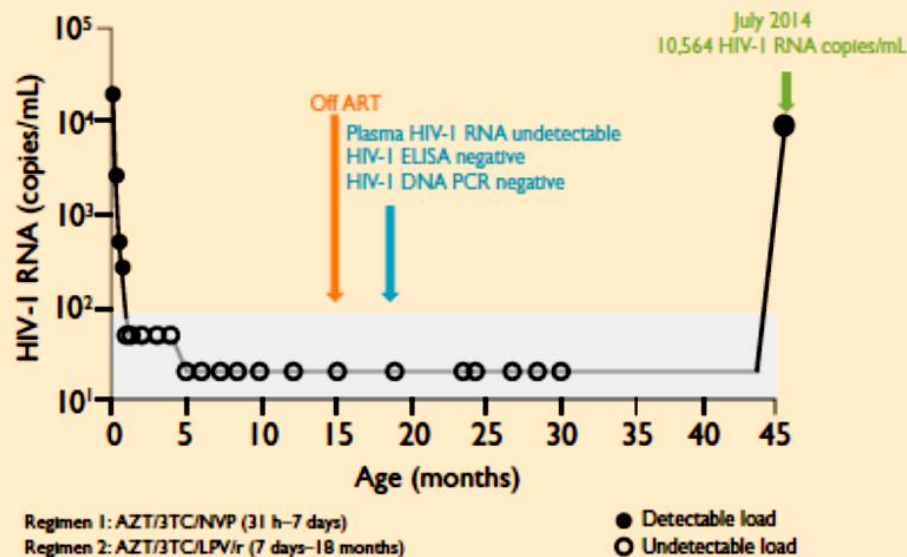


Figure 4. HIV rebound in the 'Mississippi baby', reported July 2014^{5,8}
3TC, lamivudine; AZT, zidovudine; ELISA, enzyme-linked immunosorbent assay;
LPV, lopinavir; NVP, nevirapine

2014: Argentine woman – functional cure during chronic infection?⁹

- A 51-year-old woman diagnosed with HIV after hospital admittance in 1996
- In 1997, with a CD4⁺ T-cell count of 490, began indinavir plus stavudine and lamivudine
- cART stopped in 2007 due to lipodystrophy and abnormal lipids but viral load has remained undetectable ever since
- Antibodies against HIV-1 and HIV-2 cannot be detected and PCR confirms absence of the CCR5 Δ 32 deletion linked to protection against HIV

What have we learned from cure cases?

- The 'Berlin patient' continues to provide a proof-of-concept that HIV cure is possible (*Yukl SA, et al. PLoS Pathog 2013;9:e1003347*)
- Although haematopoietic stem cell transplant (HSCT) may lead to significant and sustained reductions in HIV reservoirs, infected tissue or cell-bound virus persists in many cases (*Henrich TJ, et al. CROI 2014. Abstract 144LB. Available from: <http://www.croiwebcasts.org/console/player/22281?mediaType=audio&> [Accessed August 2014]*)
- cART during primary infection may provide significant benefits in terms of reduction in viral reservoirs, preservation of immune responses and protection from chronic immune activation, but is unlikely to lead to cure even in infants (*Persaud D, et al. N Engl J Med 2013;369:1828–35; National Institute of Allergy and Infectious Diseases. NIH News. Available from: <http://www.niaid.nih.gov/news/newsreleases/2014/Pages/MississippiBabyHIV.aspx> [Accessed August 2014]*)
- The time to HIV re-emergence after reservoir reduction is highly variable
- Data from cure cases has been used to develop a stochastic model of infection dynamics to estimate the efficacy of latency-reversing agents (LRAs) needed to prevent viral rebound after cART interruption (*Hill AL, et al. Proc Nat Acad Sci 2014;37:13475–80.*)

Model suggests ~2000-fold reductions are required to permit a majority of patients to interrupt cART for 1 year without rebound

Hopes of HIV cure in 'Boston patients' dashed

Announcement reveals hurdles to virus detection in patients.

Erika Check Hayden

06 December 2013

“It is now clear viral rebounds can happen at any time, even months after stopping therapy. **People will have to be followed very carefully for more prolonged periods than in the past**”

Steven Deeks

- HIV naturally hides in different body compartments
- During latency it cannot be hit directly
 - All available antiviral agents are virustatic, not virucidal!!
- Continuous cell-to-cell transmission cycles, less sensitive to antivirals, cannot be excluded also when virus replication is apparently controlled

**UNDER THESE CONDITIONS, NEITHER
NATURAL-, NOR THERAPY DRIVEN,
HIV-ELIMINATION (BIOLOGICAL CURE)
IS POSSIBLE!!**

HIV reservoirs: pathogenesis and obstacles to viral eradication and cure

Tae-Wook Chun and Anthony S. Fauci

AIDS 2012, **26**:1261–1268

AIDS 2012, Vol 26 No 10

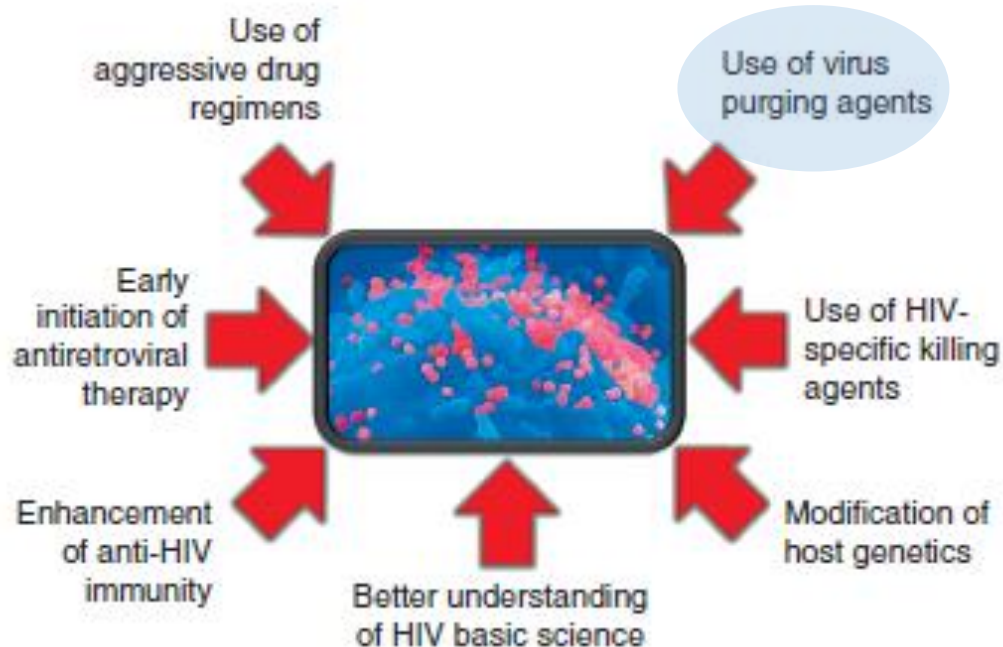


Fig. 2. Potential strategies for eradicating HIV in infected individuals receiving antiretroviral therapy.

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Nat Rev Immunol. ; 12(8): 607–614. doi:10.1038/nri3262.

Towards an HIV cure: a global scientific strategy

The International AIDS Society Scientific Working Group on HIV Cure

Abstract

Given the limitations of antiretroviral therapy and recent advances in our understanding of HIV persistence during effective treatment, there is a growing recognition that a cure for HIV infection is both needed and feasible. The International AIDS Society convened a group of international experts to develop a scientific strategy for research towards an HIV cure. Several priorities for basic, translational and clinical research were identified. This Opinion article summarizes the group's recommended key goals for the international community.

Box 2

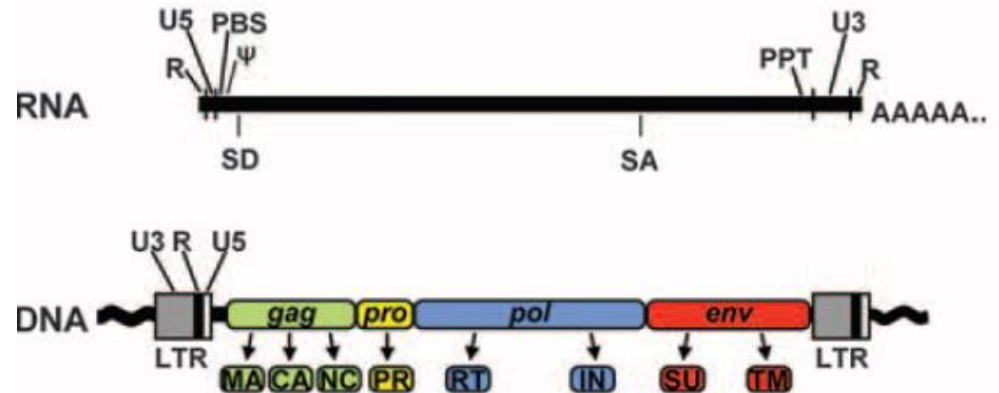
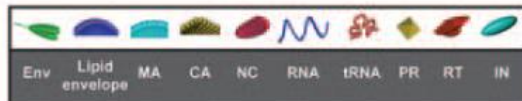
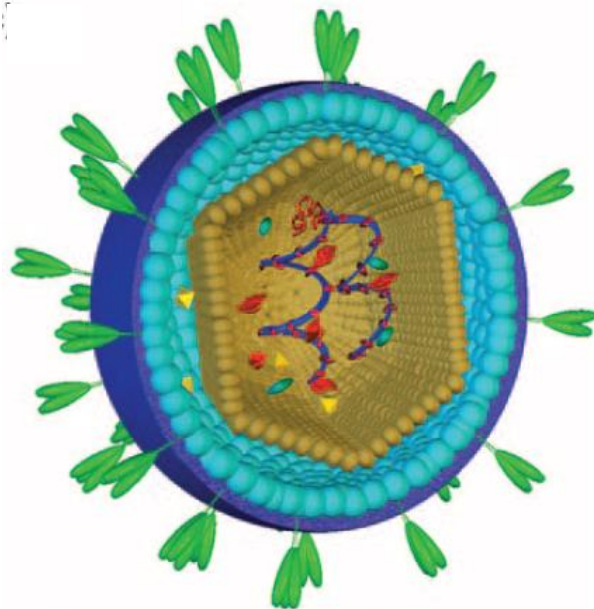
Seven key scientific priorities for HIV cure research

- Determine the cellular and viral mechanisms that maintain HIV persistence during prolonged antiretroviral therapy and in rare natural controllers. This includes defining the role of mechanisms that contribute to the establishment and maintenance of latent infection, as well as defining the role of ongoing viral replication and/or homeostatic proliferation.
- Determine the tissue and cellular sources of persistent simian immunodeficiency virus (SIV) or HIV in animal models and in individuals on long-term antiretroviral therapy.
- Determine the origins of immune activation and inflammation in the presence of antiretroviral therapy and their consequences for HIV or SIV persistence.
- Determine host mechanisms that control HIV replication in the absence of therapy.
- Study, compare and validate assays to measure persistent HIV infection and to detect latently infected cells.
- Develop and test therapeutic agents or immunological strategies to safely eliminate latent infection in animal models and in individuals on antiretroviral therapy. This includes strategies aimed at reversing latency, as well as strategies aimed at clearing latently infected cells.
- Develop and test strategies to enhance the capacity of the host immune response to control active viral replication.

Is HIV behaviour characteristic only of this virus?

RETROVIRUSES

Retroviruses are RNA viruses defined by their expression of reverse transcriptase and **are characterized by an extraordinary ability to persist indefinitely in their natural hosts**

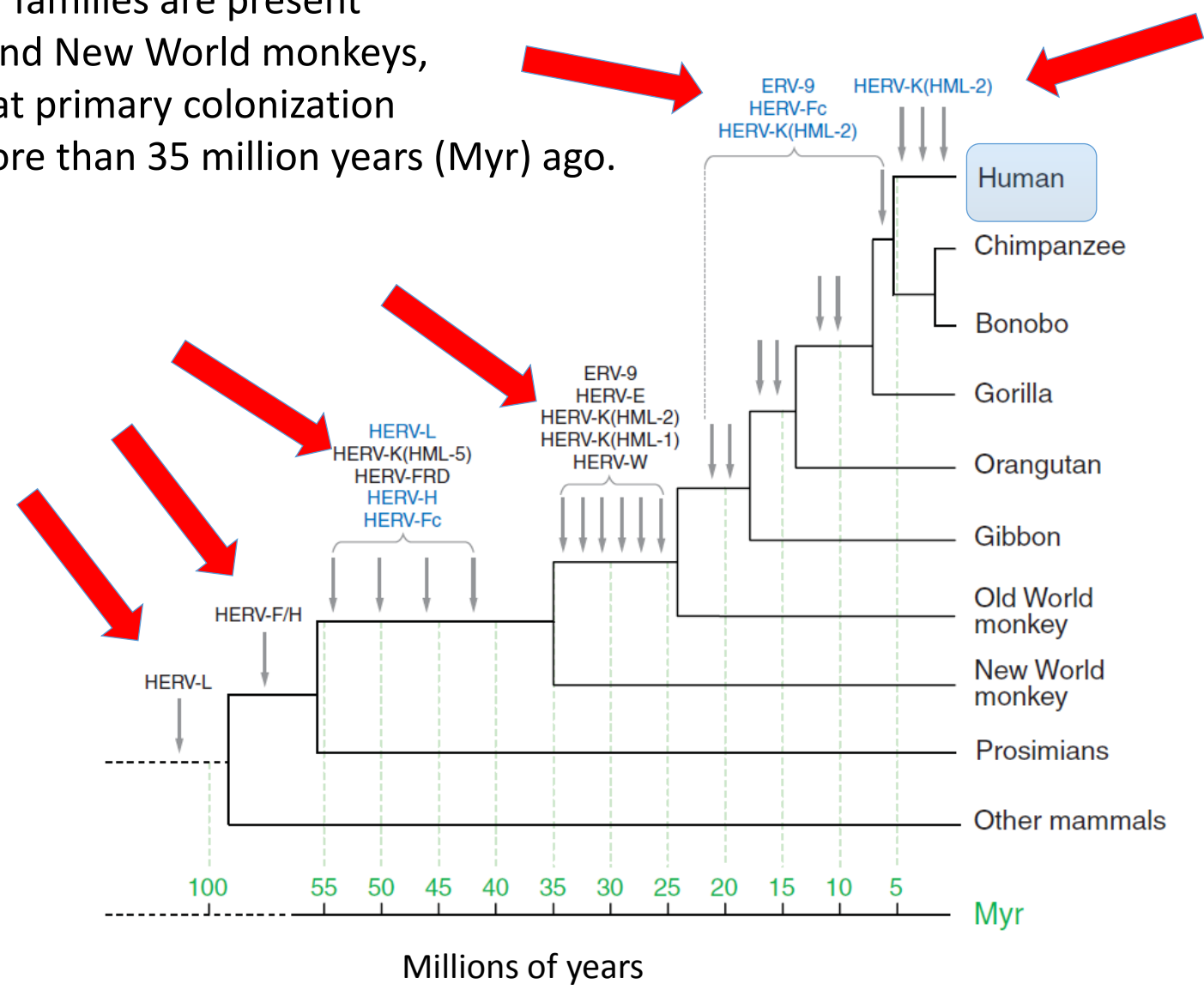


Human Endogenous Retroviruses (HERVs)

- **HERVs** are genetic elements that reside as **proviruses** in host's genome
- **HERVs** represent footprints of previous retroviral infection and have been termed “**fossil viruses**”
- Constitute about 7%–8% of the human genome

Most endogenous retrovirus families in humans appear to be ancient.

Several HERV families are present in both Old and New World monkeys, indicating that primary colonization occurred more than 35 million years (Myr) ago.



Phylogeny of primate species with approximate primary colonization times of selected HERV families. Important subsequent amplification periods of some families are indicated in blue.

HERVs: disease and health

- HERVs have been associated with a range of disease processes including **neoplasia**, **auto-immunity** and **fetal malformations**.
- HERVs have also been found to be expressed in **healthy normal tissues**, such as **placenta** where they are assumed to exert **beneficial effects**.



RESEARCH

Open Access

The distribution of insertionally polymorphic endogenous retroviruses in breast cancer patients and cancer-free controls

Julia H Wildschutte^{1,4}, Daniel Ram², Ravi Subramanian¹, Victoria L Stevens³ and John M Coffin^{1*}

Abstract

Background: Integration of retroviral DNA into a germ cell can result in a provirus that is transmitted vertically to the host's offspring. In humans, such endogenous retroviruses (HERVs) comprise >8% of the genome. The HERV-K (HML-2) proviruses consist of ~90 elements related to mouse mammary tumor virus, which causes breast cancer in mice. A subset of HERV-K(HML-2) proviruses has some or all genes intact, and even encodes functional proteins, though a replication competent copy has yet to be observed. More than 10% of HML-2 proviruses are human-specific, having integrated subsequent to the *Homo-Pan* divergence, and, of these, 11 are currently known to be polymorphic in integration site with variable frequencies among individuals. Increased expression of the most recent HML-2 proviruses has been observed in tissues and cell lines from several types of cancer, including breast cancer, for which expression may provide a meaningful marker of the disease.

Results: In this study, we performed a case-control analysis to investigate the possible relationship between the genome-wide presence of individual polymorphic HML-2 proviruses with the occurrence of breast cancer. For this purpose, we screened 50 genomic DNA samples from individuals diagnosed with breast cancer or without history of the disease ($n = 25$ per group) utilizing a combination of locus-specific PCR screening, *in silico* analysis of HML-2 content within the reference human genome sequence, and high-resolution genomic hybridization in semi-dried agarose. By implementing this strategy, we were able to analyze the distribution of both annotated and previously undescribed polymorphic HML-2 proviruses within our sample set, and to assess their possible association with disease outcome.

Conclusions: In a case-control analysis of 50 humans with regard to breast cancer diagnosis, we found no significant difference in the prevalence of proviruses between groups, suggesting common polymorphic HML-2 proviruses are not associated with breast cancer. Our findings indicate a higher level of putatively novel HML-2 sites within the population, providing support for additional recent insertion events, implying ongoing, yet rare, activities. These findings do not rule out either the possibility of involvement of such proviruses in a subset of breast cancers, or their possible utility as tissue-specific markers of disease.

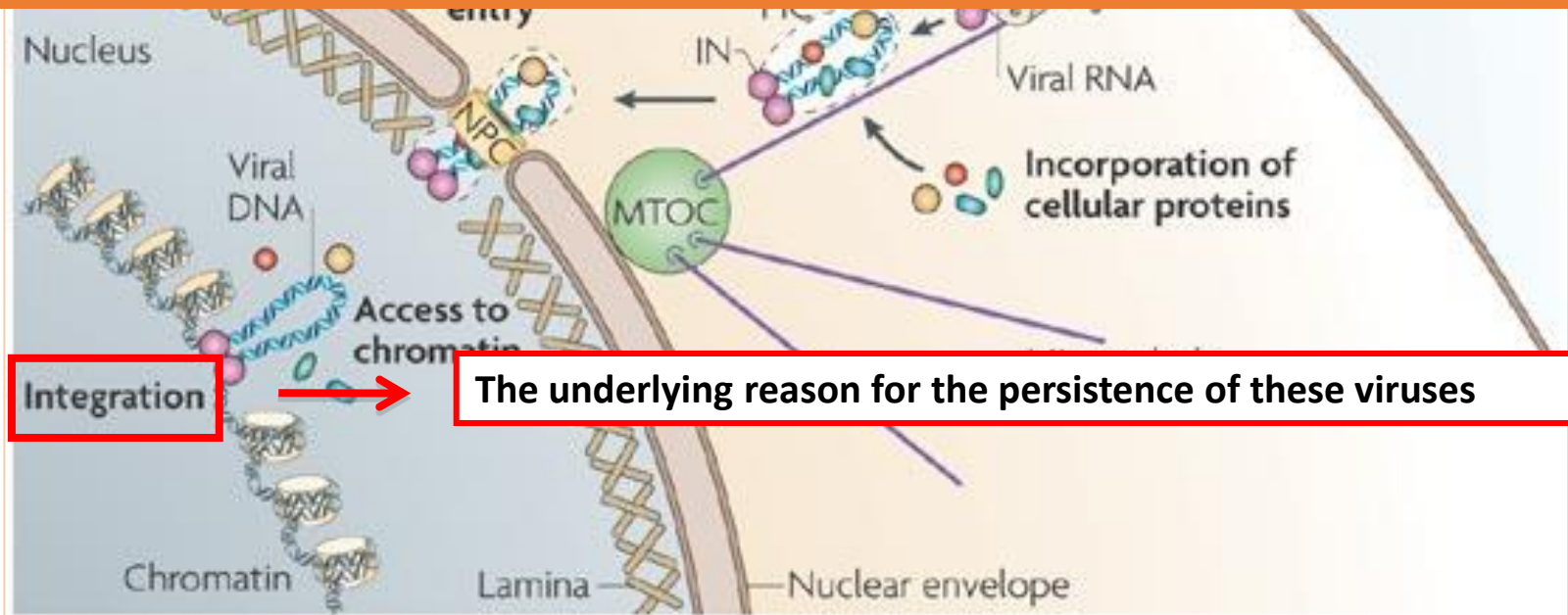
Keywords: Endogenous retrovirus, Provirus, HERV-K, Breast cancer, Betaretroviridae, MMTV, JSRV

So.....

- Retroviruses commonly interact with eucaryotic genomes far before humans appeared on the face of Earth
 - Retroviruses that interacted with human ancestors are still present (and active!!) in human genome
- Their integration is definitive
 - Once viral DNA gets integrated within human genome (so called, provirus), it cannot be naturally eliminated
- If integrated in active parts of human genome, they can be metabolically active
 - Their metabolites can harm the host or, due to adaptation and symbiosis, become important for body homeostasis, or even essential for life

What about the viral life cycle makes retroviruses so difficult to eradicate?

- **Integration** of retroviral DNA into the host cell's genome is an **essential and mandatory step in the viral replication cycle**.
- Normally, HIV does not show post-integration latency, and is metabolically active.
- Therefore, clinical latency is an uncommon phenomenon (= disease progression)
- Biological latency is restricted only to cells in quiescent phase, such as CD4-resting lymphocytes (= reservoirs that make eradication impossible)



Integration

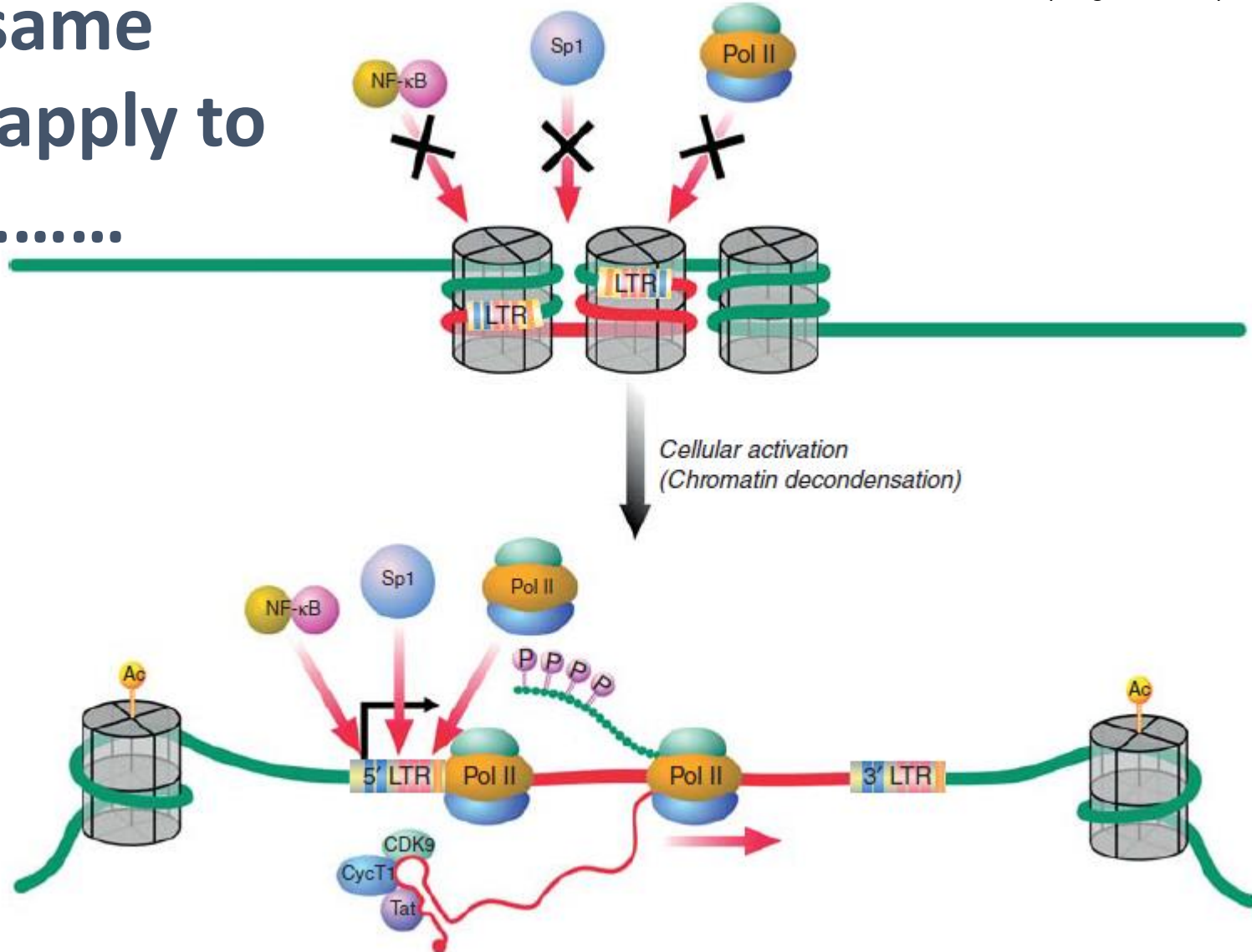
The underlying reason for the persistence of these viruses

Latent virus infections

Virus	Site of latency
Herpesviruses	
THESE VIRUSES CAN BECOME NATURALLY LATENT FOR DECADES, OR EVEN FOR THE WHOLE LIFE!!!	
Papovavirus	
Papillomavirus	Epithelium
Retroviruses	
Endogenous oncornaviruses	Somatic and germ cells
Lentiviruses	
Human immunodeficiency viruses	T lymphocytes, macrophages, brain cells

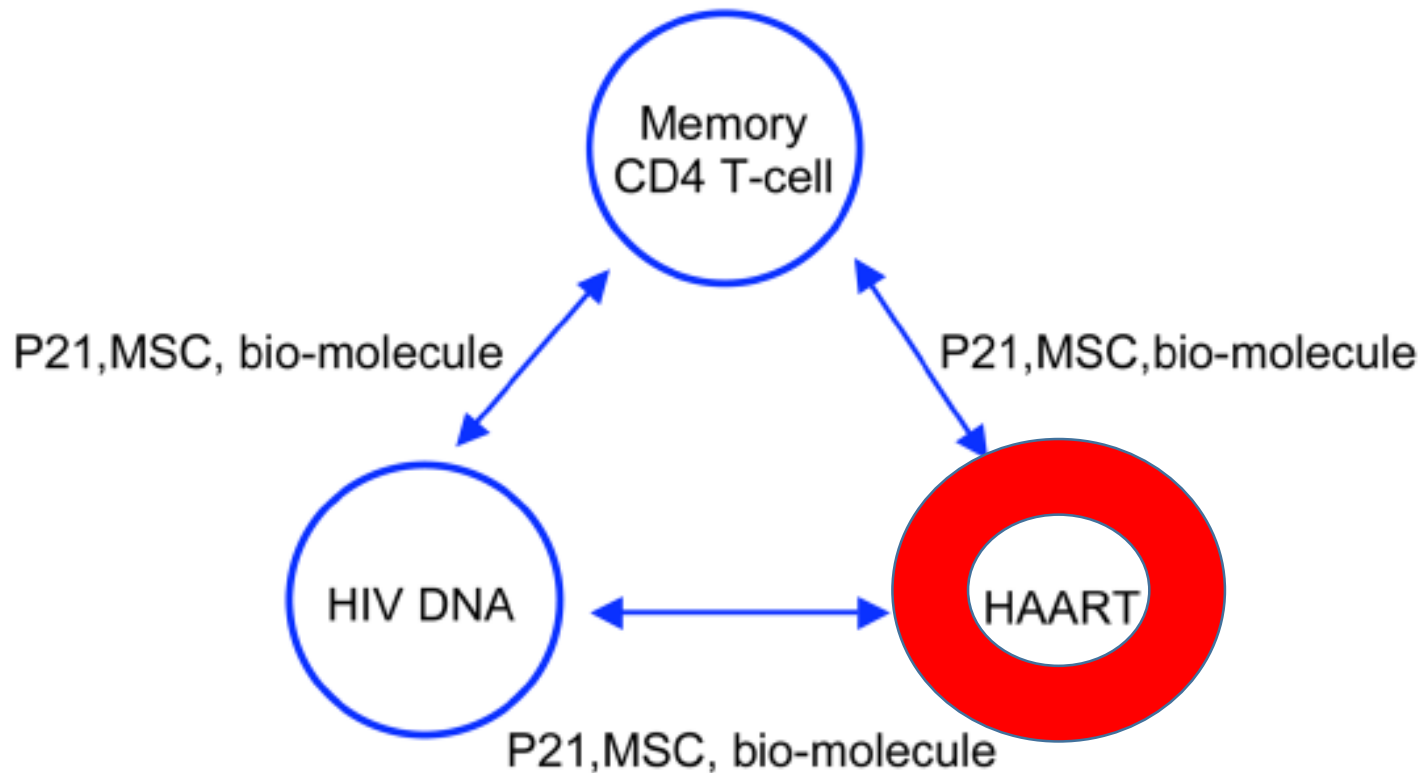
- For herpesviruses and HBV, the clinically relevant achievement is not the eradication (biological cure, nearly impossible), but the stable control of the virus, kept in a continuous (lifelong?) status of biological and clinical latency (**FUNCTIONAL CURE**)
- Functional cure is naturally achievable for these viruses, without therapeutic interventions
- WHAT ABOUT HIV?

The same
may apply to
HIV.....



Compaction of chromatin around integrated HIV-1 proviruses may restrict access of key transcription factors to the 50 long terminal repeat. However, this condensed chromatin state is reversed when cells are activated. Activation leads to transcription factor access and effective RNA Pol II elongation, giving rise to high-level virus production when HIV-1 Tat is produced.

A trinity of HIV eradication and AIDS cure



Strategies and approaches stem from the three achievements in the field of studying HIV/AIDS. Each alone, however, cannot achieve the goal toward an AIDS-free world. HAART decreases the HIV DNA thus increases the memory CD4 T-cell function. Increased memory T-cell function in turn controls the activity of viral DNA therefore increases the efficacy of HAART. Approaches with impacts on the triad, such as modulating memory CD4 T-cell turnover, eliminating HIV reservoir, and silencing HIV DNA, ultimately lead to reprogram memory CD4 T-cell repertoire and patient anti-HIV immunity. An AIDS-free world will be built on patient immunity against HIV, regulated by memory CD4 T-cells endowed with long living, self-renewing, and differentiating into antigen specific effector cells to control HIV provirus activation.

Maximal Suppression of HIV Replication

- Effective, highly bioavailable ARVs that have robust lymphoid tissue penetration

Fletcher, CROI 2012

- Initiation of ARVs as early as possible during acute infection

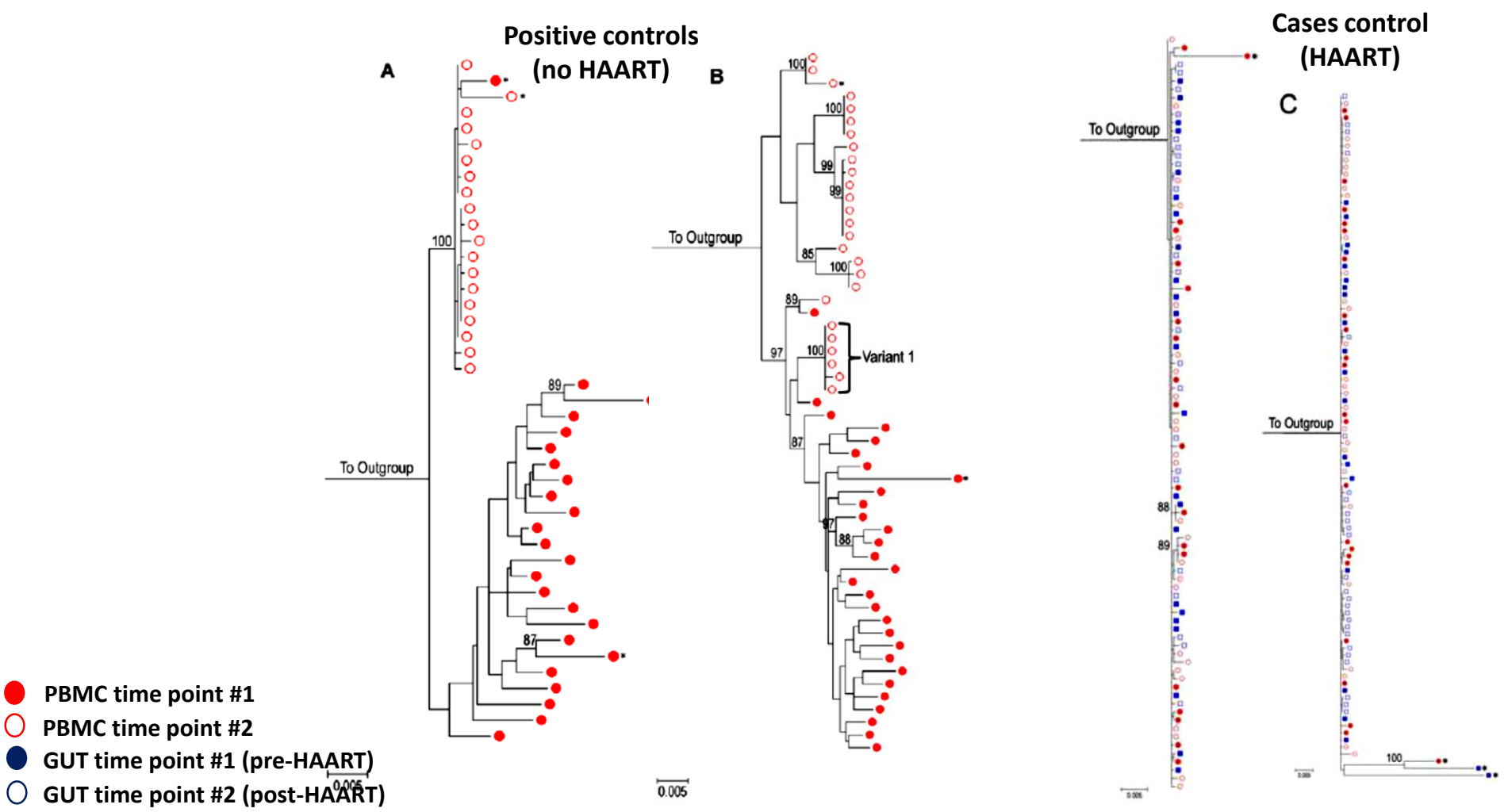
Jain, JID 2013; Saez-Cirion, PLoS Path 2013; Ananworanich, CROI 2013; Persaud, NEJM 2013

Absence of HIV-1 Evolution in the Gut-Associated Lymphoid Tissue from Patients on Combination Antiviral Therapy Initiated during Primary Infection

Teresa H. Evering¹, Saurabh Mehandru¹✉, Paul Racz², Klara Tenner-Racz², Michael A. Poles³, Amir Figueroa¹, Hiroshi Mohri¹, Martin Markowitz^{1*}

Mucosal mononuclear (MMC) CCR5+CD4+ T cells of the gastrointestinal (GI) tract are selectively infected and depleted during acute HIV-1 infection. Despite early initiation of combination antiretroviral therapy (cART), gut-associated lymphoid tissue (GALT) CD4+ T cell depletion and activation persist in the majority of HIV-1 positive individuals studied. This may result from ongoing HIV-1 replication and T-cell activation despite effective cART. We hypothesized that ongoing viral replication in the GI tract during cART would result in measurable viral evolution, with divergent populations emerging over time. Subjects treated during early HIV-1 infection underwent phlebotomy and flexible sigmoidoscopy with biopsies prior to and 15–24 months post initiation of cART. At the 2nd biopsy, three GALT phenotypes were noted, characterized by high, intermediate and low levels of immune activation. A representative case from each phenotype was analyzed. Each subject had plasma HIV-1 RNA levels <50 copies/ml at 2nd GI biopsy and CD4+ T cell reconstitution in the peripheral blood. Single genome amplification of full-length HIV-1 envelope was performed for each subject pre- and post-initiation of cART in GALT and PBMC. A total of 280 confirmed single genome sequences (SGS) were analyzed for experimental cases. For each subject, maximum likelihood phylogenetic trees derived from molecular sequence data showed no evidence of evolved forms in the GALT over the study period. **During treatment, HIV-1 envelope diversity in GALT-derived SGS did not increase and post-treatment GALT-derived SGS showed no substantial genetic divergence from pre-treatment sequences within transmitted groups.** Similar results were obtained from PBMC-derived SGS. **Our results reveal that initiation of cART during acute/early HIV-1 infection can result in the interruption of measurable viral evolution in the GALT, suggesting the absence of de-novo rounds of HIV-1 replication in this compartment during suppressive cART.**

HIV-1 env phylogenies do not suggest measurable evolution on suppressive cART



Although intensification does not affect plasma viremia, it does alter episomal DNA levels (2-LTR circles), suggesting replication is occurring at low levels

**nature
medicine**



HIV-1 replication and immune dynamics are affected by raltegravir intensification of HAART-suppressed subjects

Maria J Buzón^{1,9}, Marta Massanella^{1,9}, Josep M Llibre², Anna Esteve³, Viktor Dahl⁴, Maria C Puertas¹, Josep M Gatell⁵, Pere Domingo⁶, Roger Paredes^{1,2}, Mark Sharkey⁷, Sarah Palmer⁴, Mario Stevenson⁷, Bonaventura Clotet^{1,2}, Julià Blanco¹ & Javier Martinez-Picado^{1,8}

Effect of raltegravir-containing intensification on HIV burden and T-cell activation in multiple gut sites of HIV-positive adults on suppressive antiretroviral therapy

Steven A. Yukl^a, Amandeep K. Shergill^a, Kenneth McQuaid^a, Sara Gianella^b, Harry Lampiris^a, C. Bradley Hare^c, Mark Pandori^d, Elizabeth Sinclair^c, Huldrych F. Günthard^b, Marek Fischer^b, Joseph K. Wong^a and Diane V. Havlir^c

Increase in 2-Long Terminal Repeat Circles and Decrease in D-dimer After Raltegravir Intensification in Patients With Treated HIV Infection: A Randomized, Placebo-Controlled Trial

Hiroyu Hatano,¹ Matthew C. Strain,^{4,5} Rebecca Scherzer,^{1,3} Peter Bacchetti,² Deborah Wentworth,⁵ Rebecca Hoh,¹ Jeffrey N. Martin,² Joseph M. McCune,¹ James D. Neaton,⁶ Russell P. Tracy,⁷ Priscilla Y. Hsue,¹ Douglas D. Richman,^{4,5} and Steven G. Deeks¹

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 Vandergeeten⁷, Irini Sereti⁸, Mark de Souza^{5,6}, Rungsun Rerknimitr⁴, Robin Dewar⁹, Mary Marovich⁶, Frits van Griensven¹⁰, Rafick Sekaly⁷, Suteeraporn Pinyakorn^{1,2,3}, Nittaya Phanuphak^{1,2}, Rapee Trichavaroj⁵, Wiriya Rutvisuttinunt⁵, Nitiya Chomchey^{1,2}, Robert Paris^{5,6}, Sheila Peel⁶, Victor Valcour^{1,11}, Frank Maldarelli¹², Nicolas Chomont⁷, Nelson Michael⁶, Praphan Phanuphak^{1,2,3,4}, Jerome H. Kim^{1,5,6}, on behalf of the RV254/SEARCH 010 Study Group

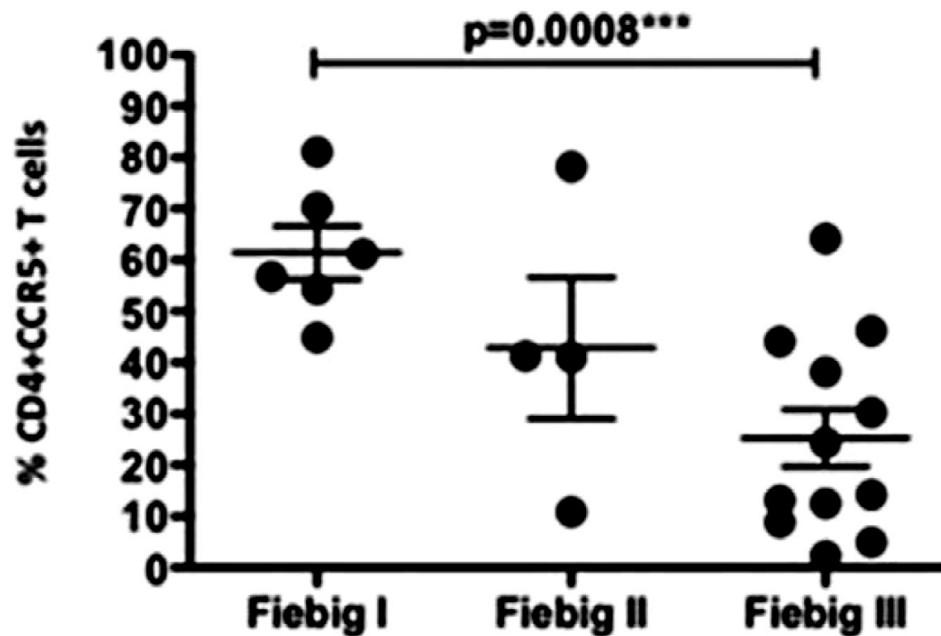
Abstract

Background: Limited knowledge exists on early HIV events that may inform preventive and therapeutic strategies. This study aims to characterize the earliest immunologic and virologic HIV events following infection and investigates the usage of a novel therapeutic strategy.

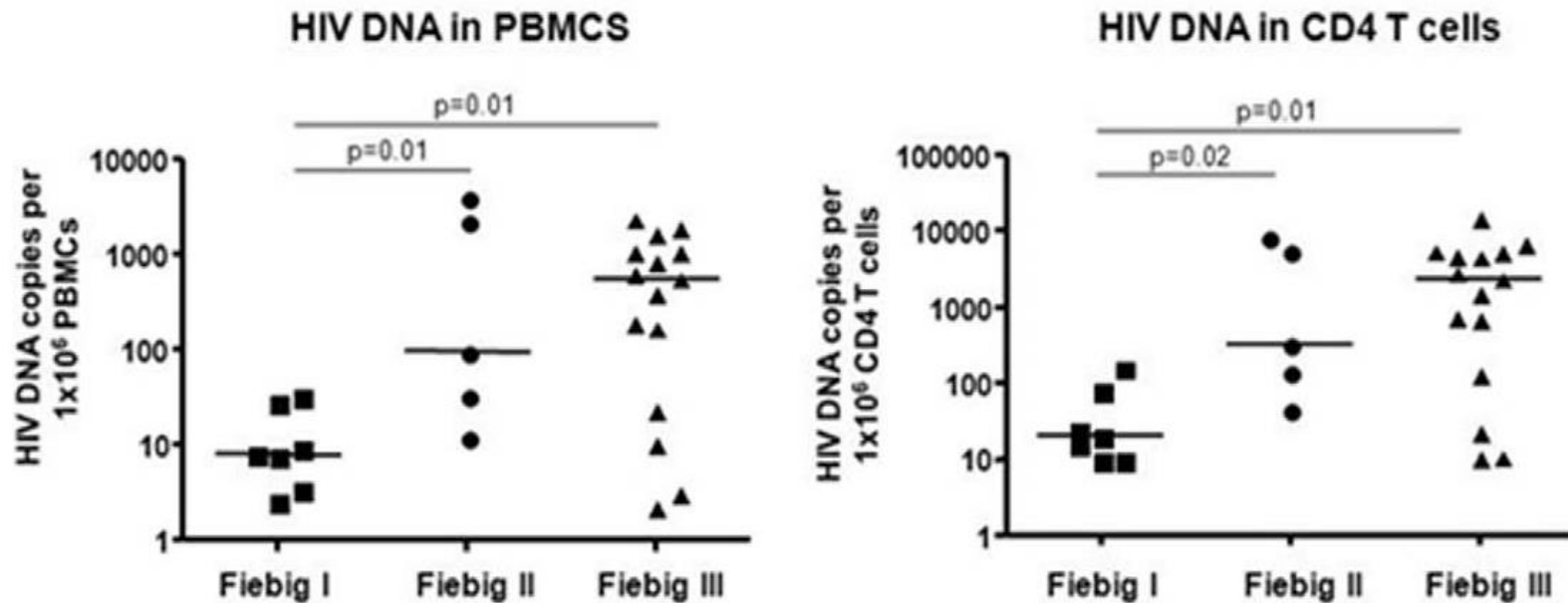
Methods and Findings: We prospectively screened 24,430 subjects in Bangkok and identified 40 AHI individuals. Thirty Thais were enrolled (8 Fiebig I, 5 Fiebig II, 15 Fiebig III, 2 Fiebig IV) of whom 15 completed 24 weeks of megaHAART (tenofovir/emtricitabine/efavirenz/raltegravir/maraviroc). Sigmoid biopsies were completed in 24/30 at baseline and 13/15 at week 24. At baseline, the median age was 29 years and 83% were MSM. Most were symptomatic (87%), and were infected with R5-tropic (77%) CRF01_AE (70%). Median CD4 was 406 cells/mm³. HIV RNA was 5.5 log₁₀ copies/ml. Median total blood HIV DNA was higher in Fiebig III (550 copy/10⁶ PBMC) vs. Fiebig I (8 copy/10⁶ PBMC) ($p = 0.01$) while the median %CD4+CCR5+ gut T cells was lower in Fiebig III (19%) vs. Fiebig I (59%) ($p = 0.0008$). After 24 weeks of megaHAART, HIV RNA levels of <50 copies were achieved in 14/15 in blood and 13/13 in gut. Total blood HIV DNA at week 0 predicted reservoir size at week 24 ($p < 0.001$). Total HIV DNA declined significantly and was undetectable in 3 of 15 in blood and 3 of 7 in gut. Frequency of CD4+CCR5+ gut T cells increased from 41% at baseline to 64% at week 24 ($p > 0.050$); subjects with less than 40% at baseline had a significant increase in CD4+CCR5+ T cells from baseline to week 24 (14% vs. 71%, $p = 0.02$).

Conclusions: Gut T cell depletion and HIV reservoir seeding increases with progression of AHI. MegaHAART was associated with immune restoration and reduced reservoir size. Our findings could inform research on strategies to achieve HIV drug-free remission.

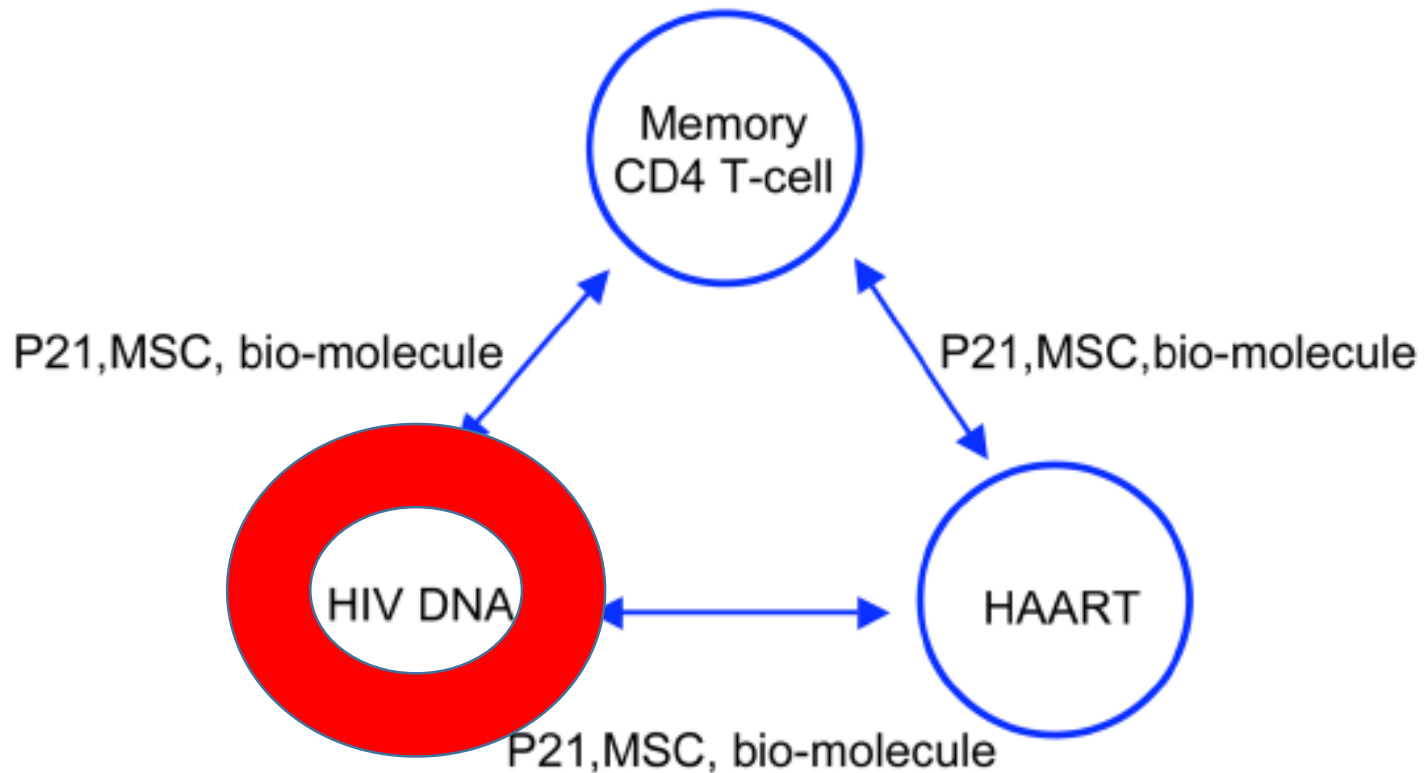
In gut, for patients with an available sigmoid biopsy at baseline , a significant decrease in the median frequency of CD4+CCR5+ T cells was observed with increasing of Fiebig stages (53% at Fiebig I and 19.3% at Fiebig III)



The total HIV DNA in PBMCs and CD4 T cells was significantly higher in subjects at Fiebig III and Fiebig II, compared to those at Fiebig I



A trinity of HIV eradication and AIDS cure



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- **Very early treatment decreases HIV-DNA in reservoir**
- **This may imbalance virus/host relationship (to the advantage of the host), thus favouring the conditions of host-driven virus-control also in the absence of therapy**

HIV-1 DNA Decay Dynamics in Blood During More Than a Decade of Suppressive Antiretroviral Therapy

CID 2014

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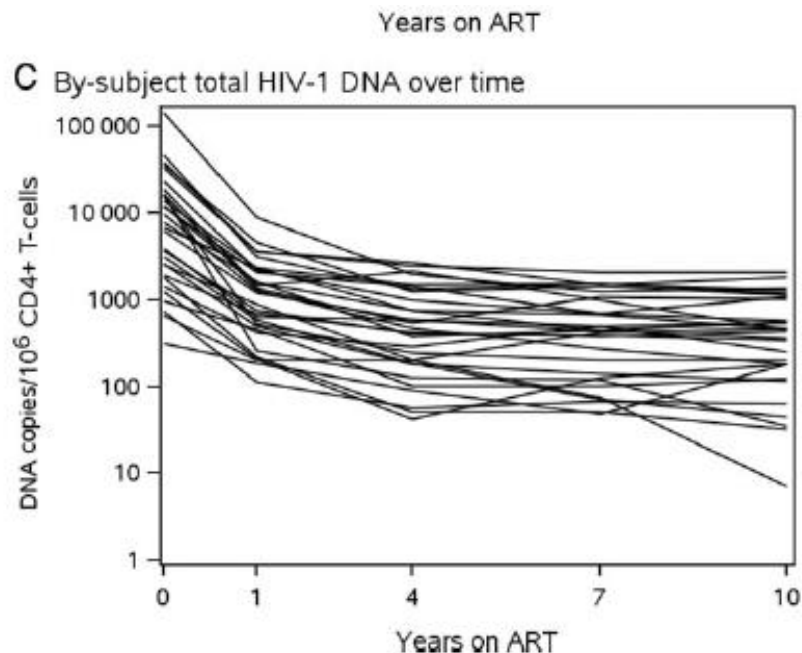
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Background. Human immunodeficiency virus type 1 (HIV-1) DNA dynamics during long-term antiretroviral therapy (ART) are not defined.

Methods. Blood mononuclear cells obtained during 7–12 years of effective ART were assayed for total HIV-1 DNA and 2-long terminal repeat (LTR) circles by quantitative polymerase chain reaction (qPCR). Slopes of HIV-1 DNA were estimated by participant-specific linear regressions. Plasma was assayed for residual viremia (HIV-1 RNA) by qPCR.

Results. Thirty participants were studied. HIV-1 DNA decreased significantly from years 0–1 and 1–4 of ART with median decay slopes of -0.86 (interquartile range, -1.05 , -0.59) and -0.11 (-0.17 , -0.06) $\log_{10}(\text{copies}/10^6 \text{ CD4}^+ \text{ T-cells})/\text{year}$, respectively ($P < .001$). Decay was not significant for years 4–7 (-0.02 [-0.06 , 0.02]; $P = .09$) or after year 7 of ART (-0.006 [-0.030 , 0.015]; $P = .17$). All participants had detectable HIV-1 DNA after 10 years (median $439 \text{ copies}/10^6 \text{ CD4}^+ \text{ T-cells}$; range: 7–2074). Pre-ART HIV-1 DNA levels were positively associated with pre-ART HIV-1 RNA levels (Spearman $= 0.71$, $P < .001$) and with HIV-1 DNA at years 4, 7, and 10 on ART (Spearman ≥ 0.75 , $P < .001$). No associations were found ($P \geq .25$) between HIV-1 DNA slopes or levels and % activated CD8⁺ T-cells (average during years 1–4) or residual viremia ($n = 18$). 2-LTR circles were detected pre-ART in 20/29 and in 8/30 participants at last follow-up.

Conclusions. Decay of HIV-1 DNA in blood is rapid in the first year after ART initiation (86% decline), slows during years 1–4 (23% decline/year), and subsequently plateaus. HIV-1 DNA decay is not associated with the levels of CD8⁺ T-cell activation or persistent viremia. The determinants of stable HIV-1 DNA persistence require further elucidation.



HIV-1 DNA decreased significantly from years 0–1 and 1–4 of ART. Decay was not significant for years 4–7 or after year 7 of ART. All participants had detectable HIV-1 DNA after 10 years.

Participant-specific decay patterns of total HIV-1 DNA per million CD4+ T-cells.

Table 3. Summary of Total Human Immunodeficiency Virus Type 1 DNA Slopes Over Time

log ₁₀ (Total HIV-1 DNA cps/10 ⁶ CD4+ T-cells) Slope/Year	Pre-ART to Year 1 on ART ^a	Year 1 to Year 4 on ART ^a	Year 4 to Year 7 on ART ^a	Year 7 Through Follow-up on ART ^{a,b}
N	30	30	29	29
Median (Q1, Q3)	−0.856 (−1.048, −0.590)	−0.111 (−0.169, −0.062)	−0.017 (−0.061, 0.020)	−0.006 (−0.030, 0.015)
Min, Max	−1.965, −0.246	−0.241, 0.057	−0.195, 0.166	−0.415, 0.252
Negative slope				
Yes	30 (100%)	29 (97%)	20 (69%)	19 (66%)
No		1 (3%)	9 (31%)	10 (34%)

Abbreviations: ART, antiretroviral therapy; HIV-1, human immunodeficiency virus type 1.

^a Wilcoxon signed rank $P = <.001$, $<.001$, .09, and .17, respectively.

^b Last year of ART follow-up with available total HIV-1 DNA: median 10 (range, 7–12; interquartile range, 10–12).

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,*}

SUMMARY

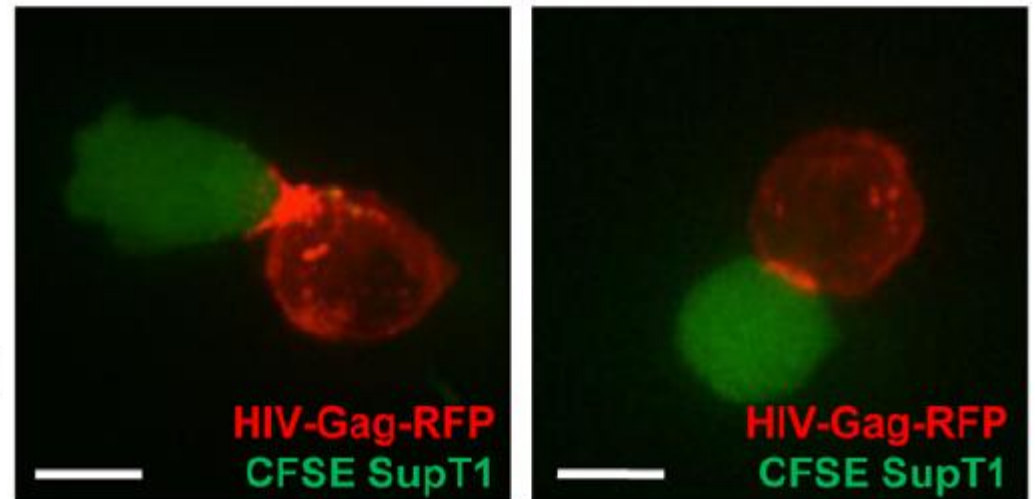
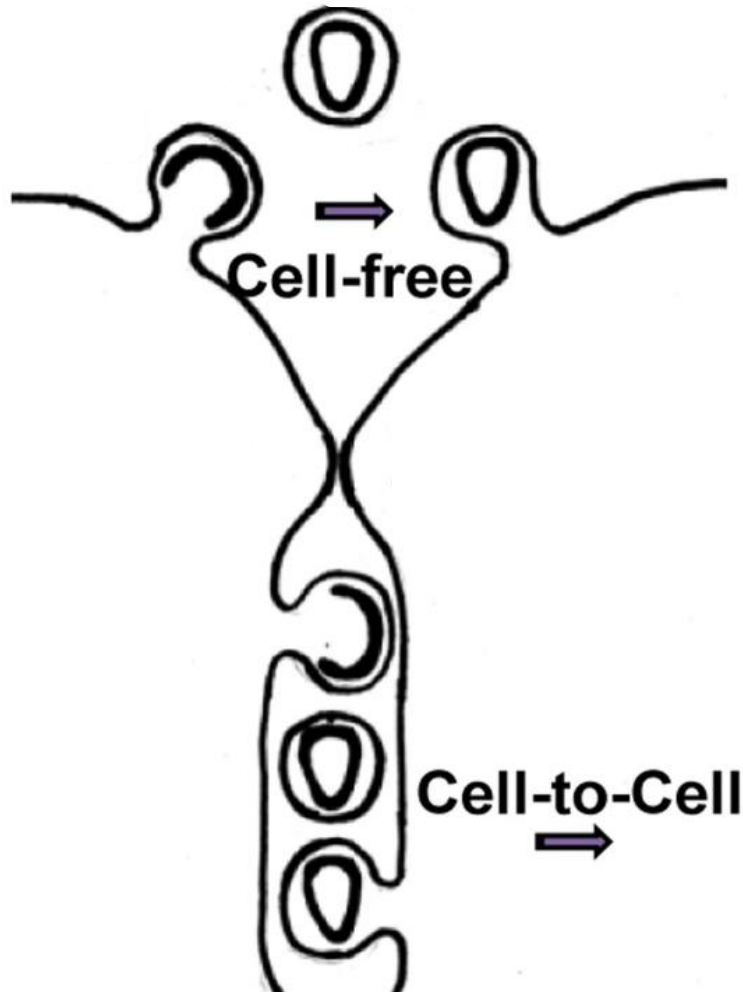
Antiretroviral therapy fails to cure HIV-1 infection because latent proviruses persist in resting CD4⁺ T cells. T cell activation reverses latency, but <1% of proviruses are induced to release infectious virus after maximum in vitro activation. The noninduced proviruses are generally considered defective but have not been characterized. Analysis of 213 noninduced proviral clones from treated patients showed 88.3% with identifiable defects but 11.7% with intact genomes and normal long terminal repeat (LTR) function. Using direct sequencing and genome synthesis, we reconstructed full-length intact noninduced proviral clones and demonstrated growth kinetics comparable to reconstructed induced proviruses from the same patients. Noninduced proviruses have unmethylated promoters and are integrated into active transcription units. Thus, it cannot be excluded that they may become activated in vivo. 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.

Barriers to cure

- Residual viral replication
- Latently infected T-cells
- Anatomical reservoirs

- **There is the evidence that cell-to-cell transmission can contribute to ongoing viral replication during suppressive HAART.....**

Cell-to-cell HIV transmission occurs at the interface two cells. It is **a highly efficient mode of HIV transmission** since it **allows multiple viral particles to infect a single cell**, and...



Zhong et al., Plos One 2013

- Cell-to-cell HIV transmission is so efficient that **cannot be totally controlled** by the current antiretroviral drugs

● Cell-to-cell spread of HIV permits ongoing replication despite antiretroviral therapy

Alex Sigal¹, Jocelyn T. Kim^{1,2}, Alejandro B. Balazs¹, Erez Dekel³, Avi Mayo³, Ron Milo⁴ & David Baltimore¹

Nature 2011

This gives rise to a cryptic ongoing replication representing:

- another source of residual viremia
- an obstacle against HIV cure

Barriers to cure

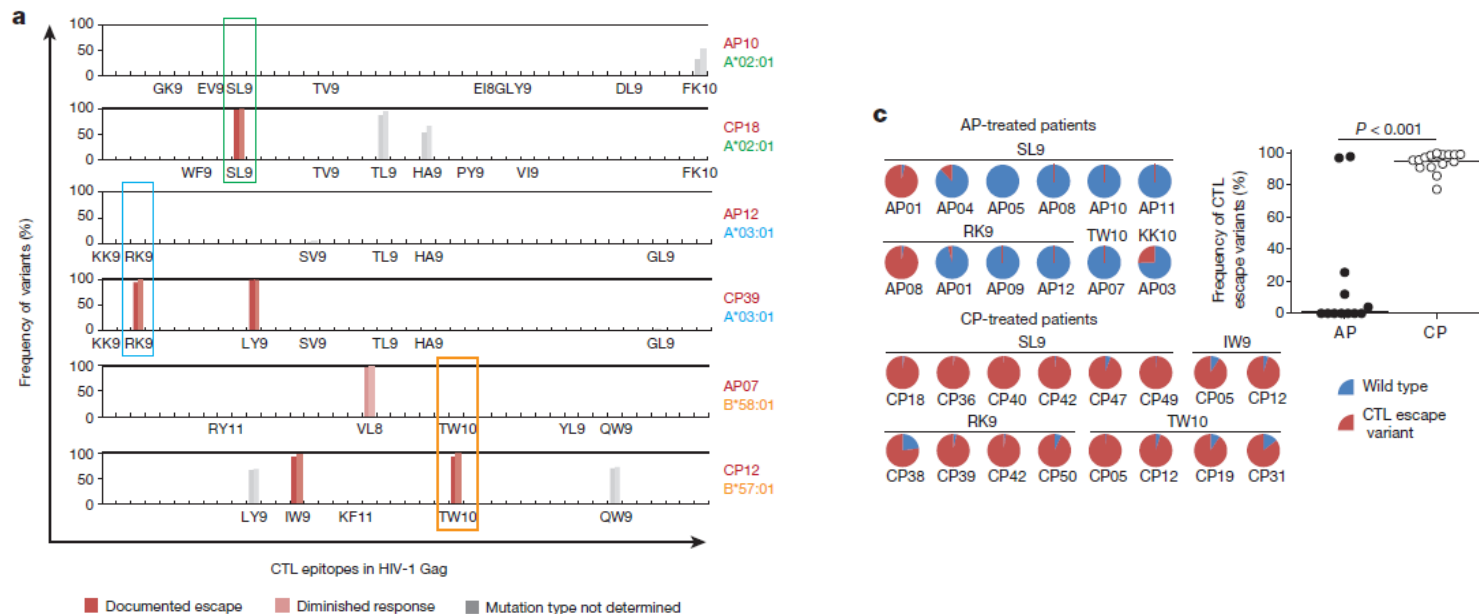
- Residual viral replication
- Latently infected T-cells
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The problem: 1 in a million cells is
latently infected



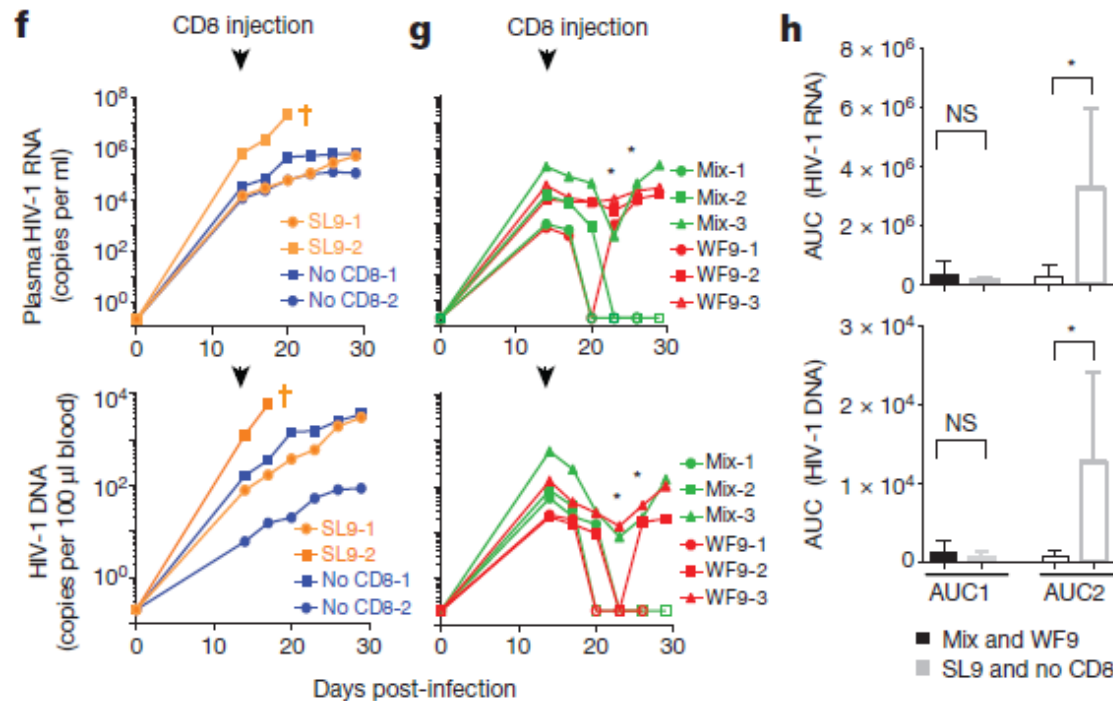
Broad CTL response is required to clear latent HIV-1 due to dominance of escape mutations

Kai Deng¹, Mihaela Perte^{1,2}, Anthony Rongvaux³, Leyao Wang⁴, Christine M. Durand¹, Gabriel Ghiaur⁵, Jun Lai¹, Holly L. McHugh¹, Haiping Hao⁶, Hao Zhang⁷, Joseph B. Margolick⁷, Cagan Gurer⁸, Andrew J. Murphy⁸, David M. Valenzuela⁸, George D. Yancopoulos⁸, Steven G. Deeks⁹, Till Strowig³, Priti Kumar¹⁰, Janet D. Siliciano¹, Steven L. Salzberg^{2,11}, Richard A. Flavell^{3,12}, Liang Shan³ & Robert F. Siliciano^{1,13}



Patients treated during Acute infection (AP) differed from patients treated during Chronic infection (CP) by the dominance in latent reservoir of variants resistant to dominant CTL responses.

Latent HIV-1 can be eliminated in chronically infected patients despite the overwhelming presence of CTL escape variants only after receiving CD81 T cells pre-stimulated with the mixture of Gag peptides including dominant and subdominant epitopes.



Indeed, dramatic decreases in plasma HIV-1 RNA of 100- to 1,000-fold were observed in all three mice that received CD81 T cells pre-stimulated

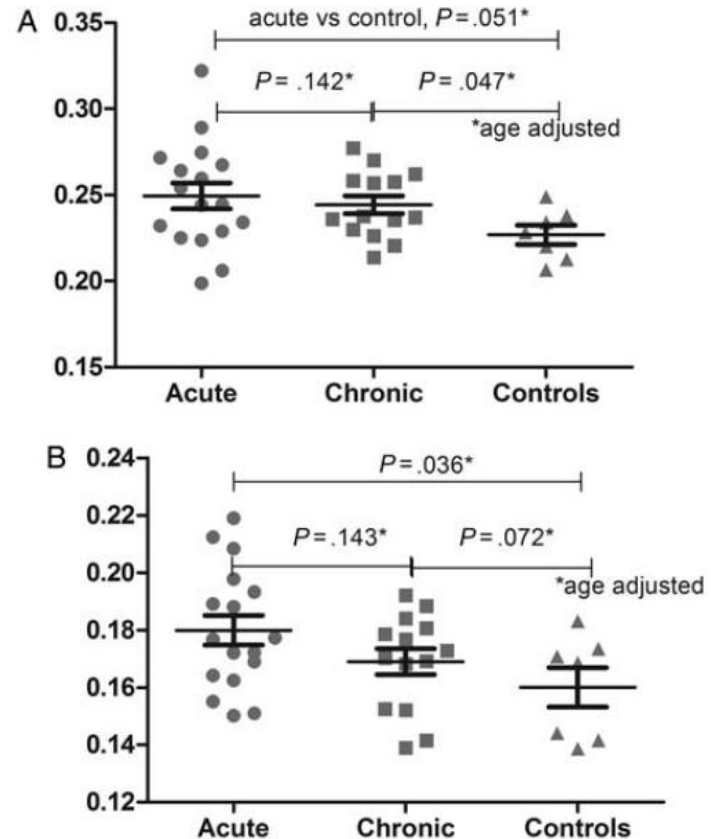
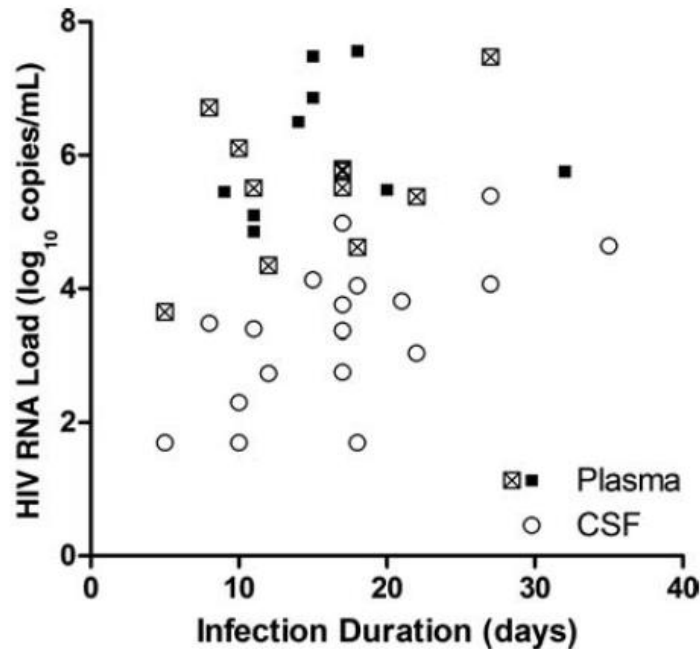
Barriers to cure

- Residual viral replication
- Latently infected T-cells
- Anatomical reservoirs

Central Nervous System Viral Invasion and Inflammation During Acute HIV Infection

Victor Valcour,¹ Thep Chalermchai,² Napapon Sailasuta,³ Mary Marovich,⁴ Sukalaya Lerdlum,^{5,6} Duanghathai Suttichom,² Nijasri C. Suwanwela,^{5,6} Linda Jagodzinski,⁴ Nelson Michael,⁴ Serena Spudich,⁷ Frits van Griensven,^{8,9} Mark de Souza,¹⁰ Jerome Kim,^{5,6} and Jintanat Ananworanich,^{2,5,11,12}
on behalf of the RV254/SEARCH 010 Study Group

Conclusions. CSF HIV RNA was detectable in humans as early as 8 days after exposure. CNS inflammation was apparent by CSF analysis and MRS in some individuals during acute HIV infection.



**Clinical Case: ID 15757 Patient
infected with HIV-1 F1 subtype**

**Age:
46**

**Sex:
M**

**First Seropositivity:
Sept-2014 with acute infection**

GRT from Plasma:

VL: 6,890,459 cps/ml

CD4: 944 cells/ul

PR: L10V K20R M36I L63T L89M

RT: None

IN: None

**GP-120(V3): E25D T2I S11G I14L R18Q
A19T T22A Q32K**

**Tropism*: Prevalence of a R5 tropic
virus (FPR*: 91.4%)**

** Geno2pheno algorithm*

Other *pol* mutations

**PR: I13V K14R E35D R41K R57K E65D K70R
I72T**

**RT: V35T K43N V60I S68G D123E I135T
S162H K173D Q174K D177E I178L T200A
Q207A R211K V245Q E248N A272P T286A
E291D V292I I293V P294T E297T A304E
K311R D324E**

**IN: S17N R20KR S57GS I60V I84L L101I T112I
T124S K136Q V201I A205S T218I L234V
D256E S283G D286N**

GRT from CSF:

VL: 164,686 cps/ml

CD4: 944 cells/ul

PR: L10V K20R M36I L63T L89M

RT: None

IN: None

**GP-120(V3): E25D T2I S11G I14L R18Q
A19T T22A Q32K**

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D256E S283G D286N**

Young patient infected with HIV : Clinical Case ID 13698

GRT from plasma 3 July 2012

HIV RNA: 1,519,237 cps/ml

Subtype : B

CD4: 234 cells/mm³ (14%)

Resistance mutations

•PR: L63P V77I

•RT: V179E

•Int: none

•GP41: none

Other mutations

•PR: N37S I64V

•RT: S162C Q207E A272P T286A I293V E297T
Q334G G335D

•Int: E11D S17T M50I I72V Y99F L101I T112I S119P
T122I T124A I135V I182V V201I K219N N222K
D253E D256E

•GP41: L54M S129G N140I T268A

Tropism: R5 virus (FPR: 54.4%)

GP120/V3: N5G H13P T22A E25D Q32K

GRT from CSF 5 July 2012

HIV-RNA : 2,997,146 cps/ml

Subtype: B

Resistance mutations

•PR: L63P V77I

•RT: V179E

•Int: none

• GP41: none

Other mutations

•PR: N37S I64V

•RT: S162C Q207E A272P T286A I293V E297T
Q334G G335D

• Int: E11D S17T M50I I72V Y99F L101I T112I
S119P T122I T124A I135V I182V V201I K219N
N222K D253E D256E

•GP41: L54M S129G N140I T268A

Tropism: R5 virus (FPR: 54.4%)

GP120/V3: N5G H13P T22A E25D Q32K

CONCLUSIONS

- There are many obstacles, beyond the natural characteristics of retroviruses, and HIV in particular, that make very difficult (impossible??) the eradication of the virus from the body (biological cure)
 - Latency, natural reservoirs, and residual replication, are the most relevant ones
- The seeding the HIV throughout the body occurs in the first hours/days after entry. All intervention beyond this time point has limited (or even no) chances to be successful in eradicating the virus
- A handful of proof-of-concept situations whereby the virus is stably under control without antiviral therapy, demonstrates the possibility of achieving a functional cure, without the need of eradicating the virus.
- This latter condition represents an achievable target based upon new and innovative therapeutic approaches
 - Investments in this specific area may bring to the discovery of new therapeutic tools aiming at promising clinical results