The virology of two-drug regimens and the tale of the reservoir

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HIV Cure: Definition and Potential Outcomes

Eradication	Functional Cure
Elimination of the HIV reservoir	Partial elimination and control of cells containing replication competent virus
Berlin Patient London Patient	Elite controllers Post cART controllers

Hutter et al. NEJM 2009, Gupta et al, Nature 2019; Saez-Cirion et al, Plos Pathogens 2013

The Berlin Patient – The First but not Unique Person Cured of HIV Infection

BRIEF REPORT

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

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

N Engl J Med. 2009

The New York Times

Health

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

Heidi Schumann for The New York Times

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

By ANDREW POLLACK

Published: November 28, 2011

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

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

Yukl SA, et al. PLoS Pathog. 2013

The London Patient

nature Accelerated Article Preview

LETTER

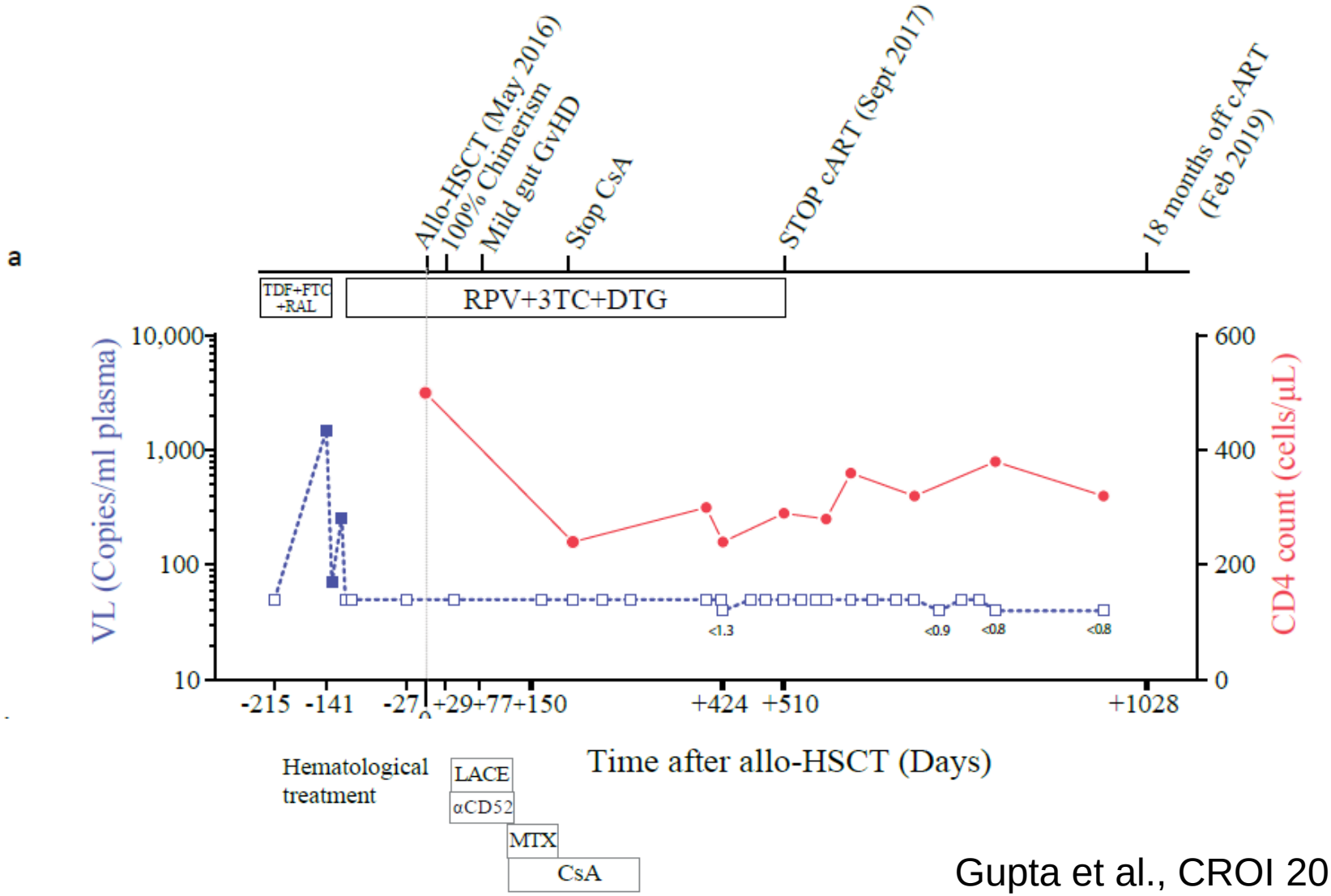
doi:10.1038/s41586-019-1027-4

HIV-1 remission following CCR5Δ32/Δ32 haematopoietic stem-cell transplantation

Ravindra K Gupta, Sultan Abdul-jawad, Laura E McCoy, Hoi Ping Mok, Dimitra Peppa, Maria Salgado, Javier Martinez-Picado, Monique Nijhuis, Annemarie M.J. Wensing, Helen Lee, Paul Grant, Eleni Nastouli, Jonathan Lambert, Matthew Pace, Fanny Salasc, Christopher Monit, Andrew Innes, Luke Muir, Laura Waters, John Frater, Andrew ML Lever, SG Edwards, Ian H Gabriel & Eduardo Olavarria

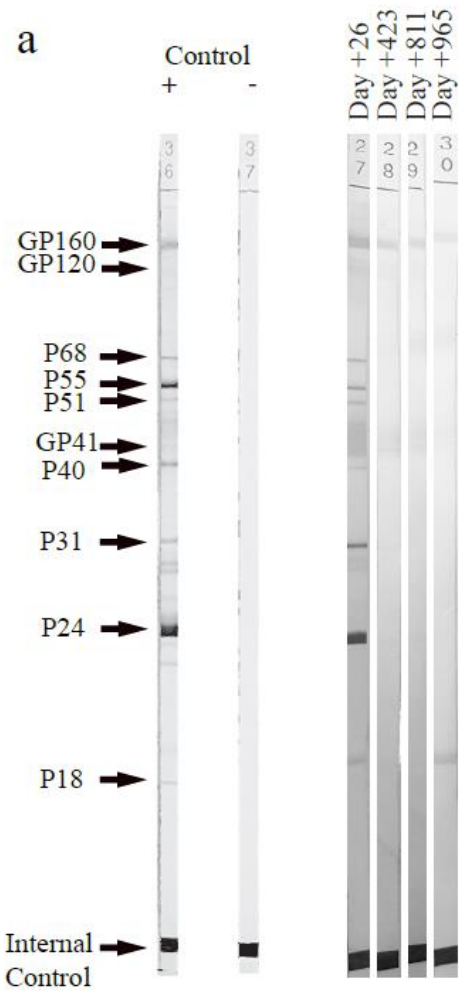
HIV-1 cure remains elusive with only one reported case a decade ago[1,2]. Termed the 'Berlin patient', the individual underwent two allogeneic haematopoietic stem-cell transplantation (allo-HSCT) procedures using a donor with a homozygous mutation in the HIV coreceptor CCR5 (CCR5Δ32/Δ32) to treat his acute myeloid leukaemia. Total body irradiation was given with each HSCT. Critically, it is unclear which treatment or patient parameters contributed to this only documented case of long-term HIV remission. Here we show that HIV-1 remission may be possible with a less aggressive and toxic approach. An HIV-1-infected adult underwent allo-HSCT for Hodgkin's lymphoma using cells from a CCR5Δ32/Δ32 donor. He experienced mild gut graft versus host disease. Antiretroviral therapy was interrupted 16 months after transplantation. HIV-1 remission has been maintained through a further 18 months. Plasma HIV-1 RNA has been undetectable at less than 1 copy per millilitre along with undetectable HIV-1 DNA in peripheral CD4 T lymphocytes. Quantitative viral outgrowth assay from peripheral CD4 T lymphocytes shows no reactivatable virus using a total of 24 million resting CD4 T cells. CCR5-tropic, but not CXCR4-tropic viruses were identified in HIV-1 DNA from CD4 T cells of the patient prior to transplant. CD4 T cells isolated from peripheral blood post-transplant did not express CCR5 and were only susceptible to CXCR4-tropic virus ex vivo. HIV-1 Gag-specific CD4 and CD8 T cell responses were lost after transplantation, whereas cytomegalovirus (CMV)-specific responses were detectable. Likewise, HIV-1-specific antibodies and avidities fell to levels comparable to those in the Berlin patient following transplantation. Although at 18 months post-treatment interruption it is premature to conclude that this patient has been cured, these data suggest that single allo-HSCT with homozygous CCR5Δ32 donor cells may be sufficient to achieve HIV-1 remission with reduced intensity conditioning and no irradiation, and the findings further support the development of HIV remission strategies based on preventing CCR5 expression.

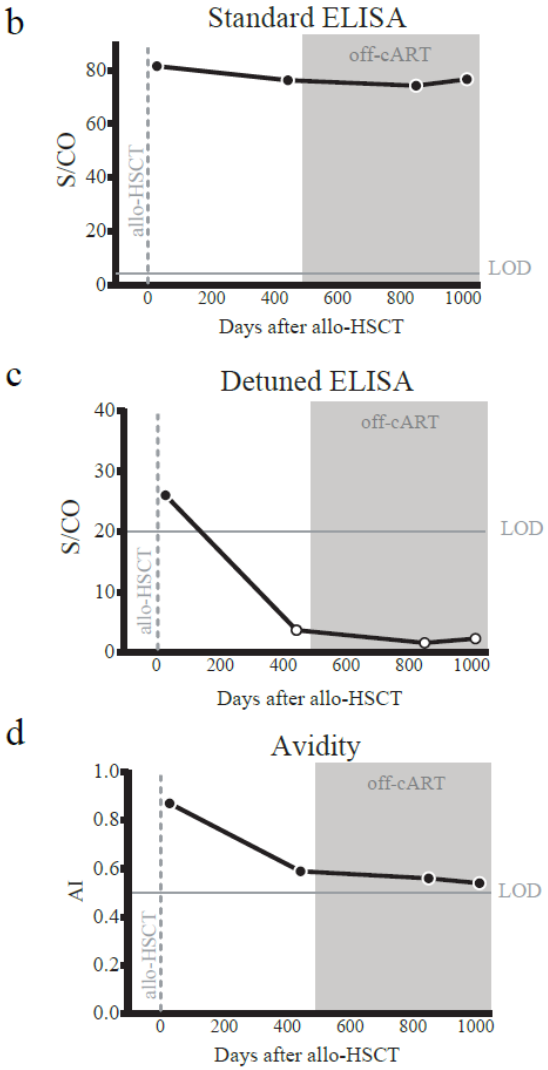
The London Patient: HIV-RNA 18 months of follow-up



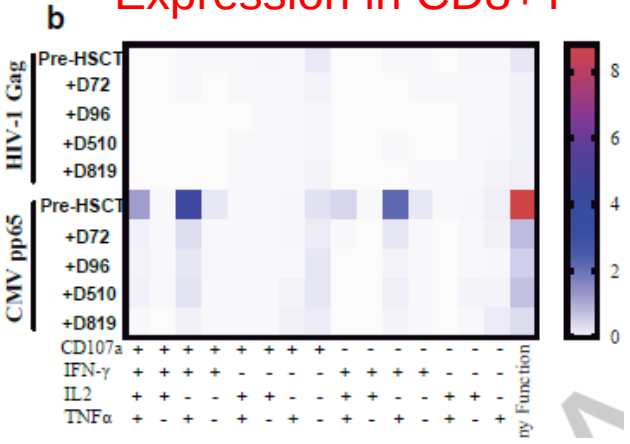
Gupta et al., CROI 2019

The London Patient: Loss of antibody and specific T-cell responses to HIV-1

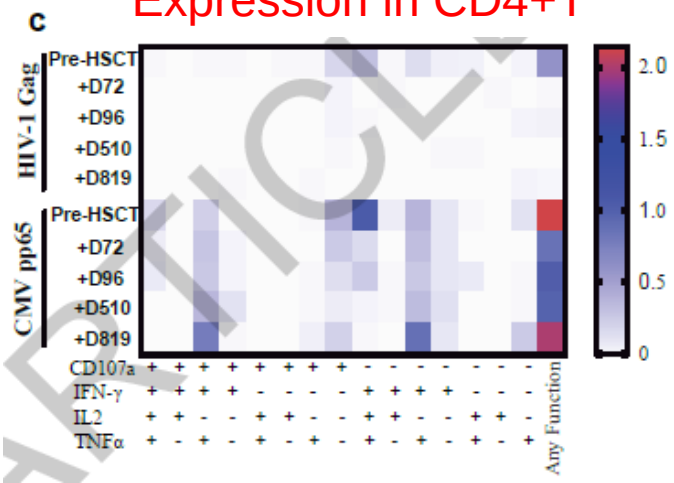




Expression in CD8+T



Expression in CD4+T



Gupta et al., CROI 2019

- Although it is not a viable large-scale strategy for a cure … these new findings reaffirm our belief that there exists a proof of concept that HIV is curable.

  Anton Pozniak

- The breakthrough suggests first case was not a one-off and could pave way for future treatments, based on preventing the expression of the wild-type CCR5 co-receptor.

- Gene-editing in infected patients might be a justifiable goal

  Ravi Gupta

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

Multiplex Genome Engineering Using CRISPR/Cas Systems

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

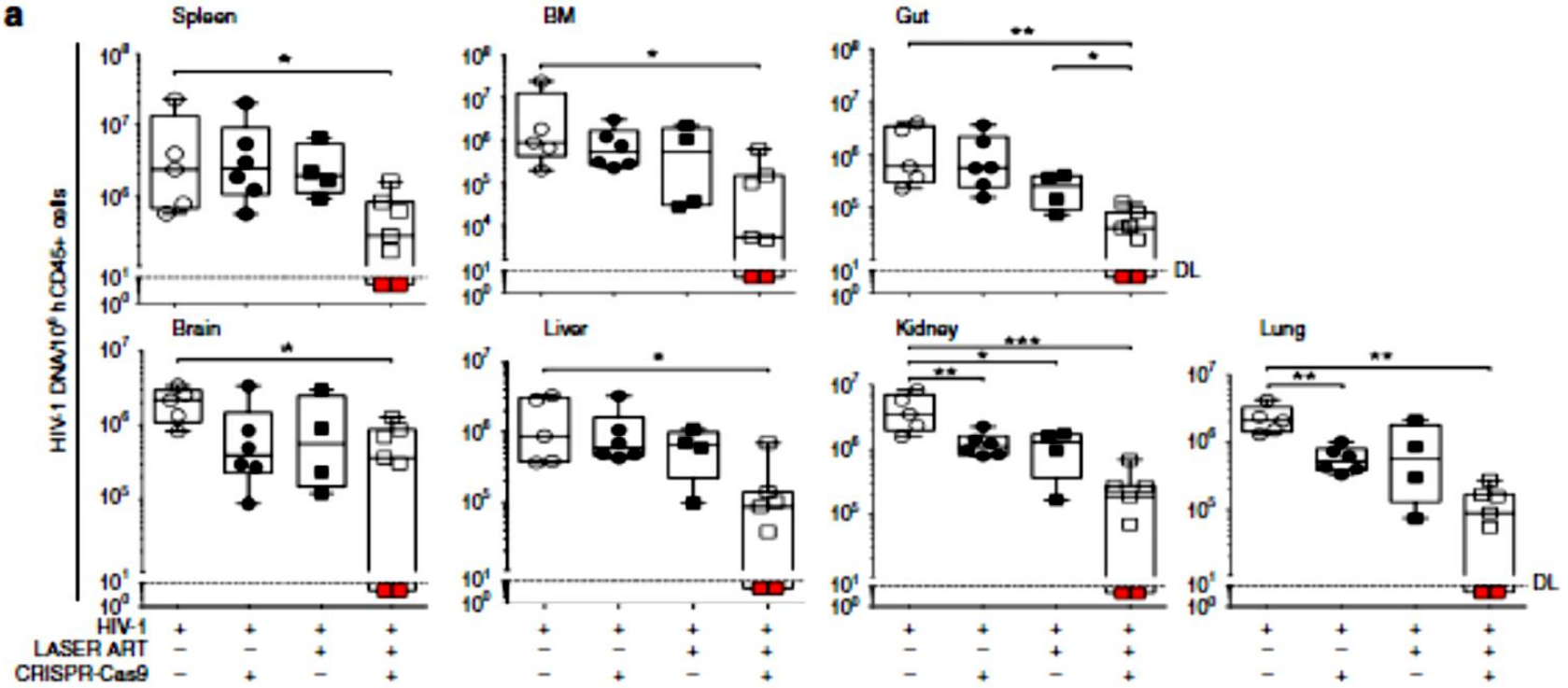
Science 2013

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

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

Sequential LASER ART and CRISPR Treatments Eliminate HIV-1 in a Subset of Infected Humanized Mice

Sequential long-acting slow-effective release antiviral therapy (LASER ART) and CRISPR-Cas9 demonstrate viral clearance in latent infectious reservoirs in HIV-1 infected humanized mice. HIV-1 subgenomic DNA fragments, spanning the long terminal repeats and the Gag gene, are excised in vivo, resulting in elimination of integrated proviral DNA; virus is not detected in blood, lymphoid tissue, bone marrow and brain by nested and ddPCR as well as RNAscope tests.



Dash et a., Nature Comm 2019

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

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

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

Tracking HIV-1 recombination to resolve its contribution to HIV-1 evolution in natural infection

Hongshuo Song[1,14], Elena E. Giorgi[2], Vitaly V. Ganusov[3], Fangping Cai[1], Gayathri Athreya[4], Hyejin Yoon[2], Oana Carja[5], Bhavna Hora[1], Peter Hraber[2], Ethan Romero-Severson[2], Chunlai Jiang[1,6], Xiaojun Li[1], Shuyi Wang[7], Hui Li[7], Jesus F. Salazar-Gonzalez[8,15], Maria G. Salazar[8], Nilu Goonetilleke[9], Brandon F. Keele[10], David C. Montefiori[1], Myron S. Cohen[9], George M. Shaw[7,11], Beatrice H. Hahn[7,11], Andrew J. McMichael[12], Barton F. Haynes[1], Bette Korber[2], Tanmoy Bhattacharya[2,13] & Feng Gao[1,6]

Recombination in HIV-1 is well documented, but its importance in the low-diversity setting of within-host diversification is less understood. Here we develop a novel computational tool (RAPR (Recombination Analysis PRogram)) to enable a detailed view of in vivo viral recombination during early infection, and we apply it to near-full-length HIV-1 genome sequences from longitudinal samples. Recombinant genomes rapidly replace transmitted/founder (T/F) lineages, with a median half-time of 27 days, increasing the genetic complexity of the viral population. We identify recombination hot and cold spots that differ from those observed in inter-subtype recombinants. Furthermore, RAPR analysis of longitudinal samples from an individual with well-characterized neutralizing antibody responses shows that recombination helps carry forward resistance-conferring mutations in the diversifying quasispecies. These findings provide insight into molecular mechanisms by which viral recombination contributes to HIV-1 persistence and immunopathogenesis and have implications for studies of HIV transmission and evolution in vivo.

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

- Selection of resistance
- Presence of mutant viral strains showing poor or no-cleavage at all
- Delay but no complete elimination of viral replication
- Need of a safe and effective mechanisms of delivery
- No platform for gRNA candidate evaluation
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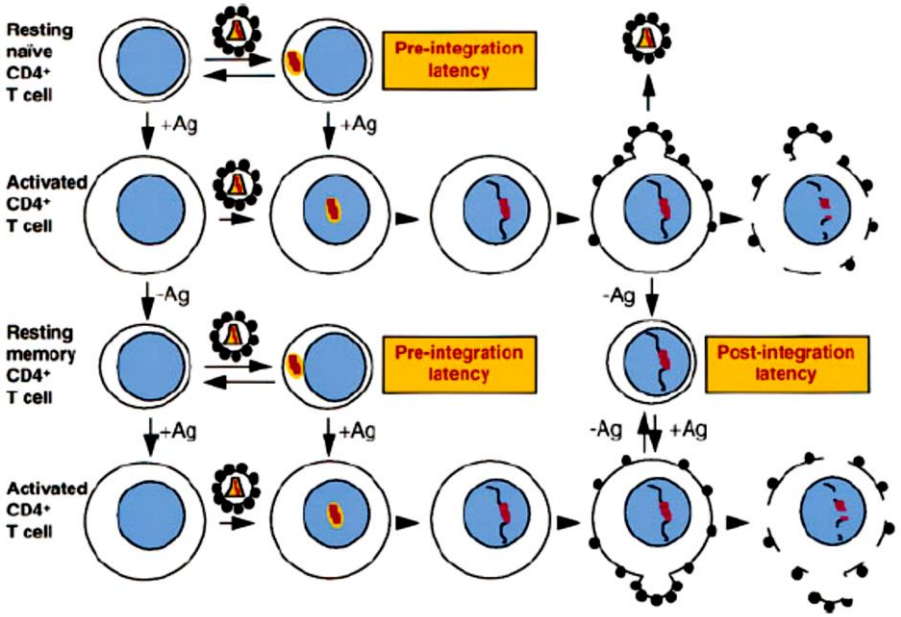
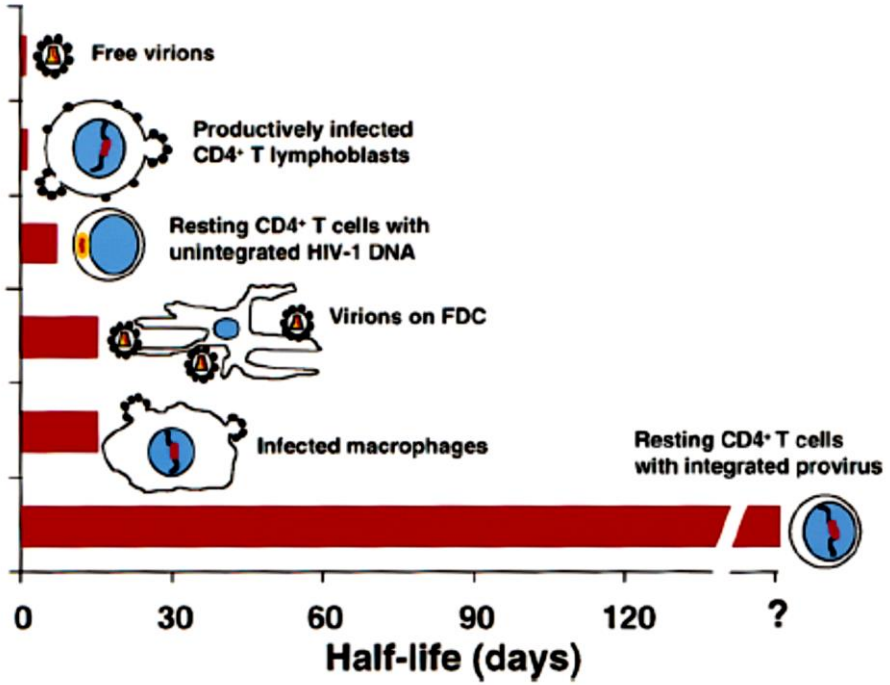
From Soriano V. in Gene Therapy with CRISPR/Cas9 Coming to Age for HIV Cure AIDS Rev 2017

..., thus much more is needed

- Definition of latent reservoir
- Definition of replication competent virus and its origin
- Definition of residual viremia origin
- New treatment strategies
- **We need to know that there is no harm at the reservoir level while proposing a maintenance therapy “lighter” than the previous one**

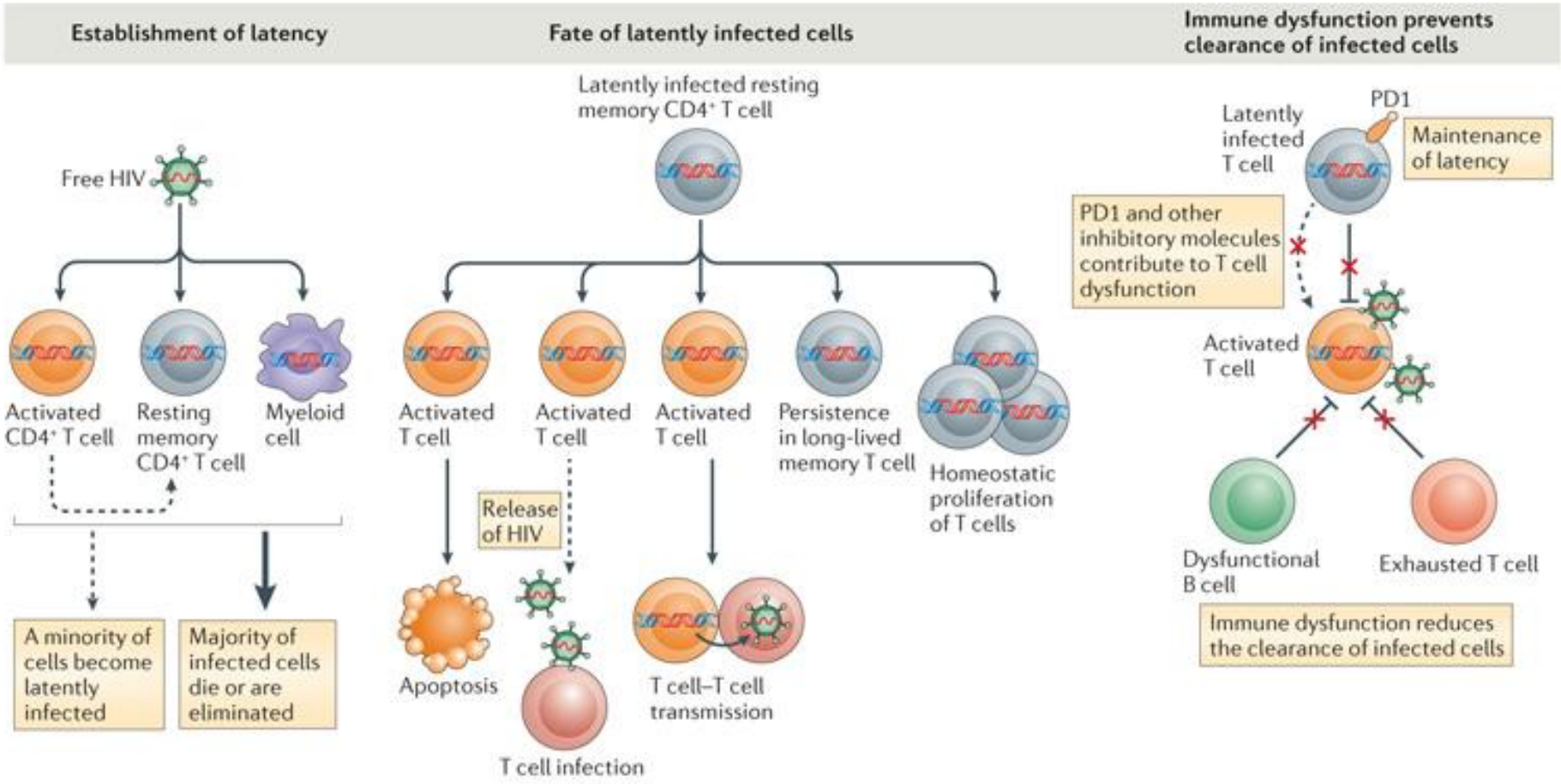
HIV-1 infected CD4 T cells: different subsets and half-lifes





Finzi, Siliciano Cell 1998

Establishment of latency



The Int Soc for HIV cure; Nature Imm 2012

Ongoing replication vs Homeostatic proliferation: unresolved question

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

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

Ramon Lorenzo-Redondo[#],

- Ongoing HIV Replication During ART Reconsidered

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

Open Forum Infectious Diseases

PERSPECTIVES

2017

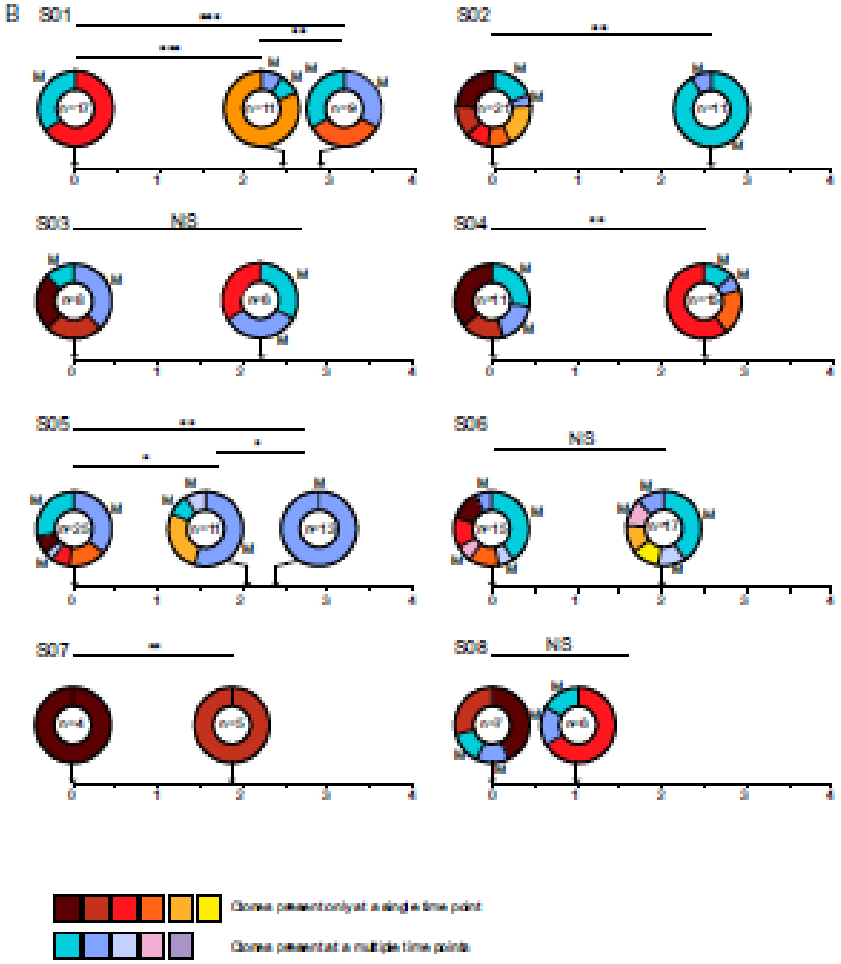
Expanded cellular clones carrying replication-competent HIV-1 persist, wax, and wane

2018

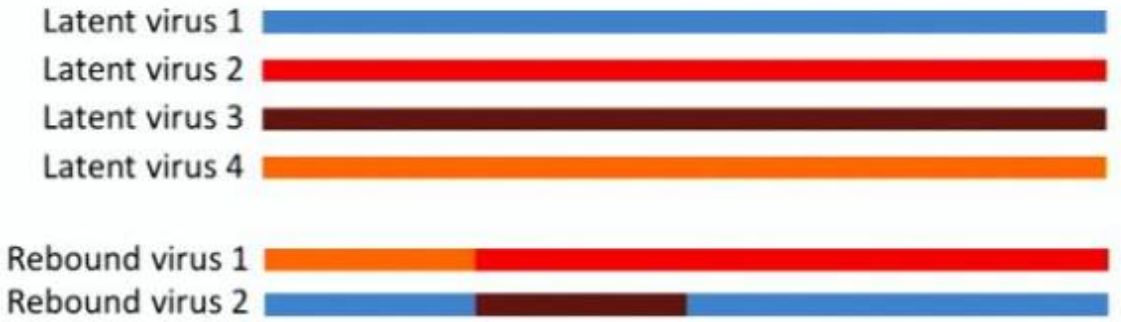
Zheng Wang[a], Evelyn E. Gurule[a], Timothy P. Brennan[a], Jeffrey M. Gerold[b], Kyungyoon J. Kwon[a], Nina N. Hosmane[a], Mithra R. Kumar[a], Subul A. Beg[a], Adam A. Capoferri[a], Stuart C. Ray[a], Ya-Chi Ho[c], Alison L. Hill[b], Janet D. Siliciano[a], and Robert F. Siliciano[a,d,1]

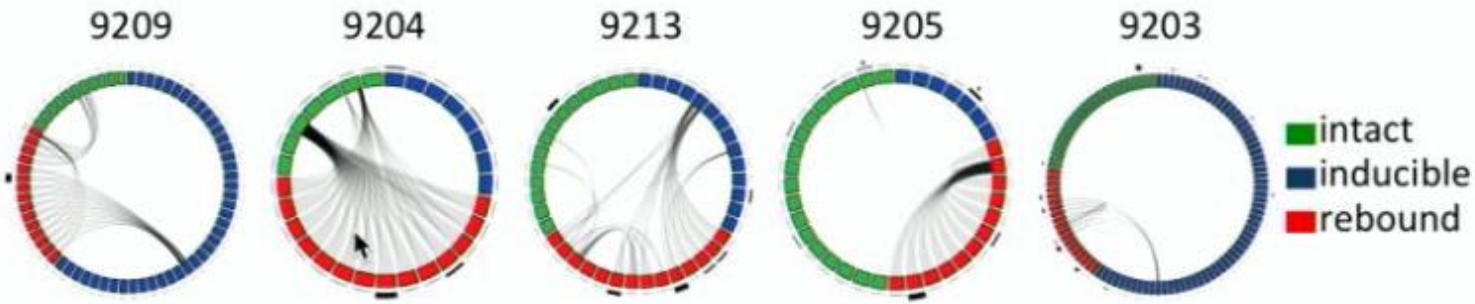
Contributed by Robert F. Siliciano, January 24, 2018 (sent for review December 4, 2017; reviewed by Douglas F. Nixon and Rafick Sekaly)

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



Recombinants were found in rebound viruses





- Many of the rebound viruses in the plasma appeared to be recombinants from the circulating latent reservoir

Ching-Lan, CROI 2019

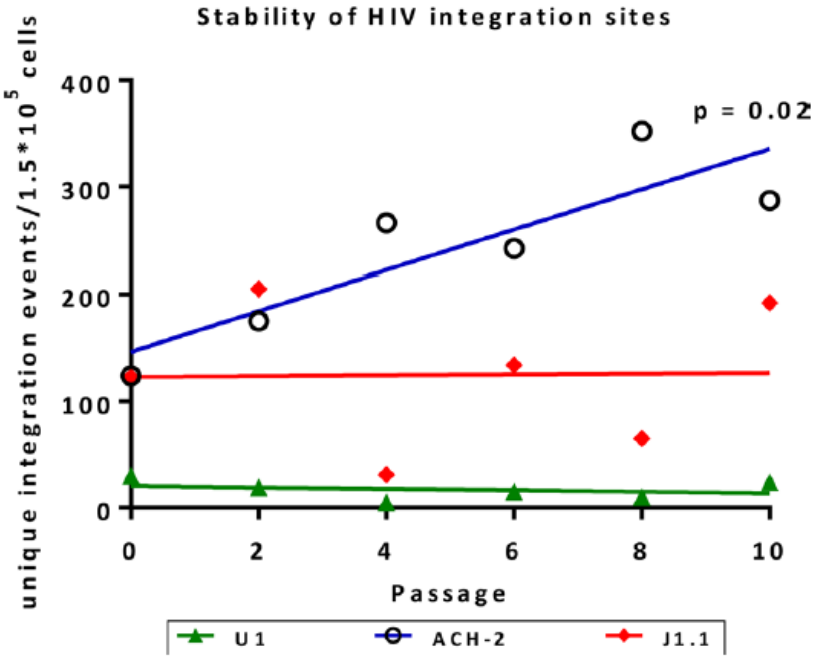
Symons *et al. Retrovirology (2017) 14:2*
DOI 10.1186/s12977-016-0325-2

Retrovirology

RESEARCH Open Access

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

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

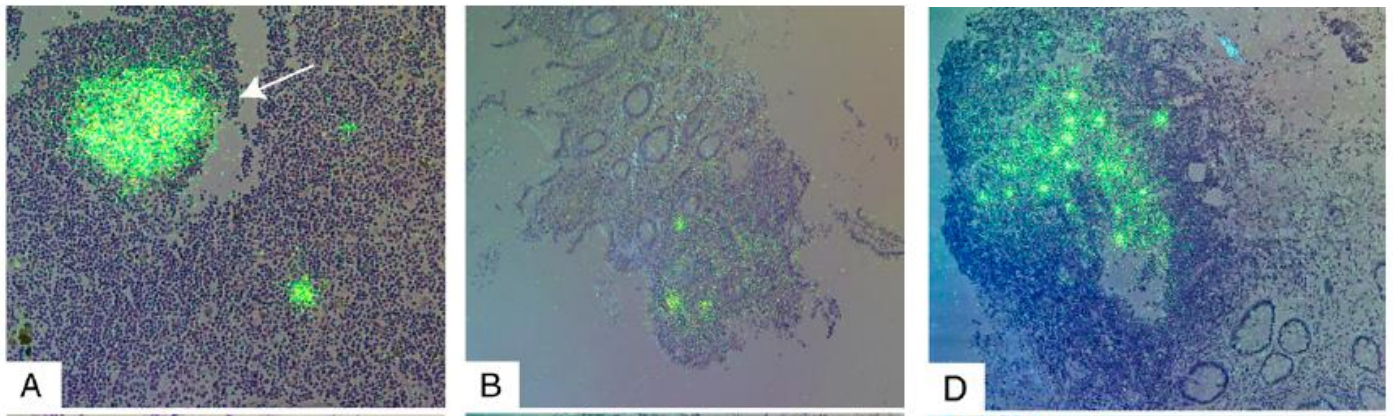


Conclusion: Cell lines latently infected with intact HIV demonstrated multiple unique HIV integration sites indicating that these cell lines are not clonal and in the ACH-2 cell line there was evidence of low level virus replication. These findings have implications for the use of latently infected cell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

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

- **Lymphoid organs are the primary anatomical compartments for HIV replication and spreading**

- **HIV recrudescence occurs from multiple anatomical sites on treatment interruption**



LN Ileum Rectum

Pantaleo, Nature 1993; Rothenberger Pnas 2015

The Journal of Clinical Investigation

HIV-1 latent reservoir size and diversity are stable following brief treatment interruption

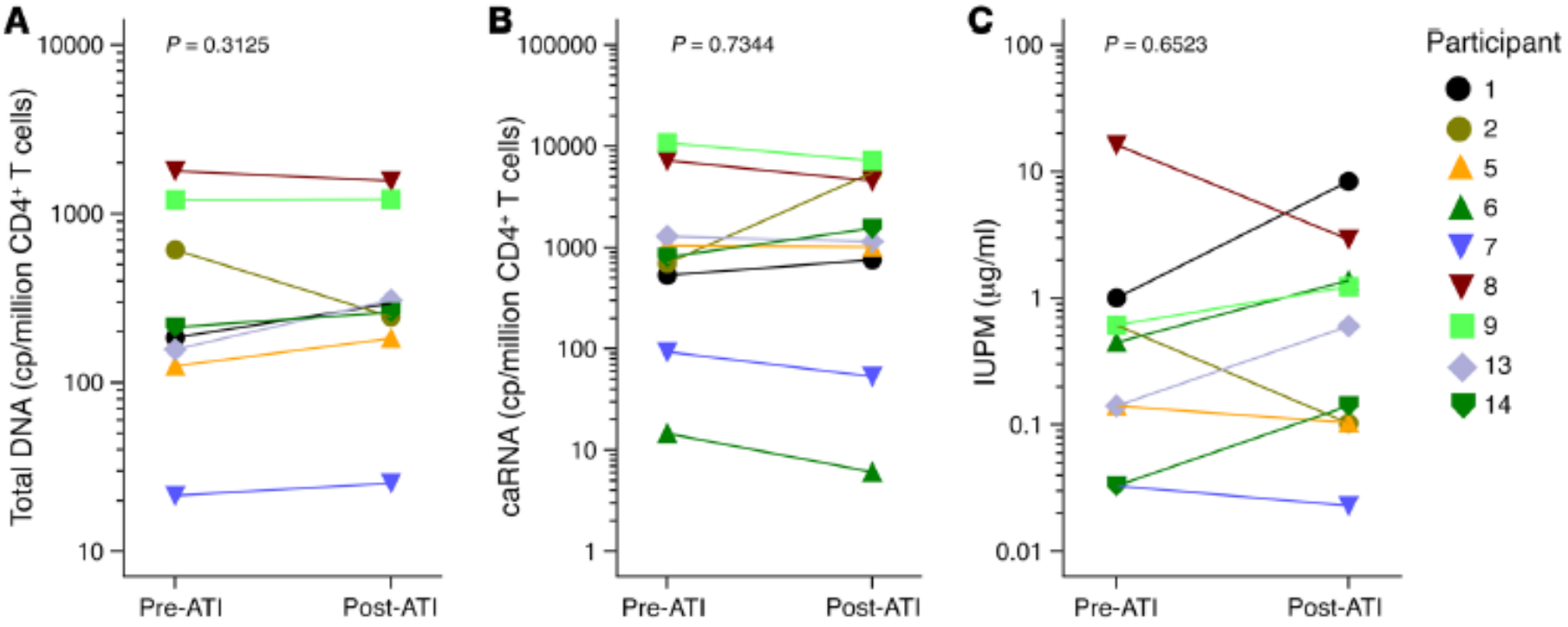
D. Brenda Salantes,[1] Yu Zheng,[2] Felicity Mampe,[1] Tuhina Srivastava,[1] Subul Beg,[3] Jun Lai,[3] Jonathan Z. Li,[2] Randall L. Tressler,[4] Richard A. Koup,[5] James Hoxie,[1] Mohamed Abdel-Mohsen,[6] Scott Sherrill-Mix,[1] Kevin McCormick,[1] E. Turner Overton,[7] Frederic D. Bushman,[1] Gerald H. Learn,[1] Robert F. Siliciano,[3,8] Janet M. Siliciano,[3] Pablo Tebas,[1] and Katharine J. Bar[1]

RESULTS. Measures of total HIV-1 DNA, cell-associated RNA, and infectious units per million cells (IUPM) (measured by quantitative viral outgrowth assay [QVOA]) were not statistically different before or after ATI. Phylogenetic analyses of HIV-1 *env* sequences from QVOA and proviral DNA demonstrated little change in the composition of the virus populations comprising the pre- and post-ATI reservoir. Expanded clones were common in both QVOA and proviral DNA sequences. The frequency of clonal populations differed significantly between QVOA viruses, proviral DNA sequences, and the viruses that reactivated in vivo.

CONCLUSIONS. The results indicate that transient viremia from ATI does not substantially alter measures of the latent reservoir, that clonal expansion is prevalent within the latent reservoir, and that characterization of latent viruses that can reactivate in vivo remains challenging.

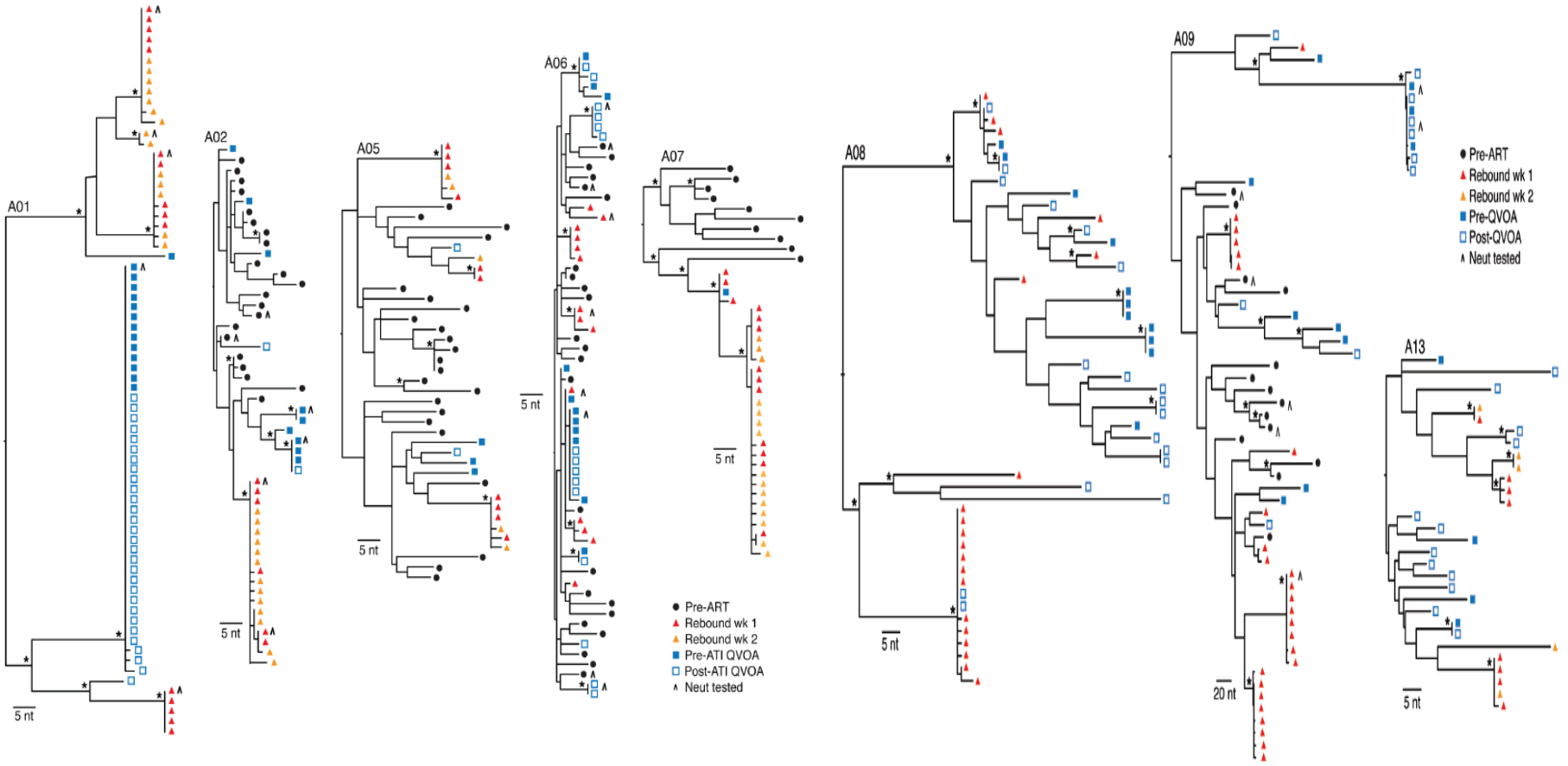
Brenda Salates, JCI 2019

- Analytical treatment interruptions with transient viremia did not substantially alter the size or diversity of the peripheral reservoir



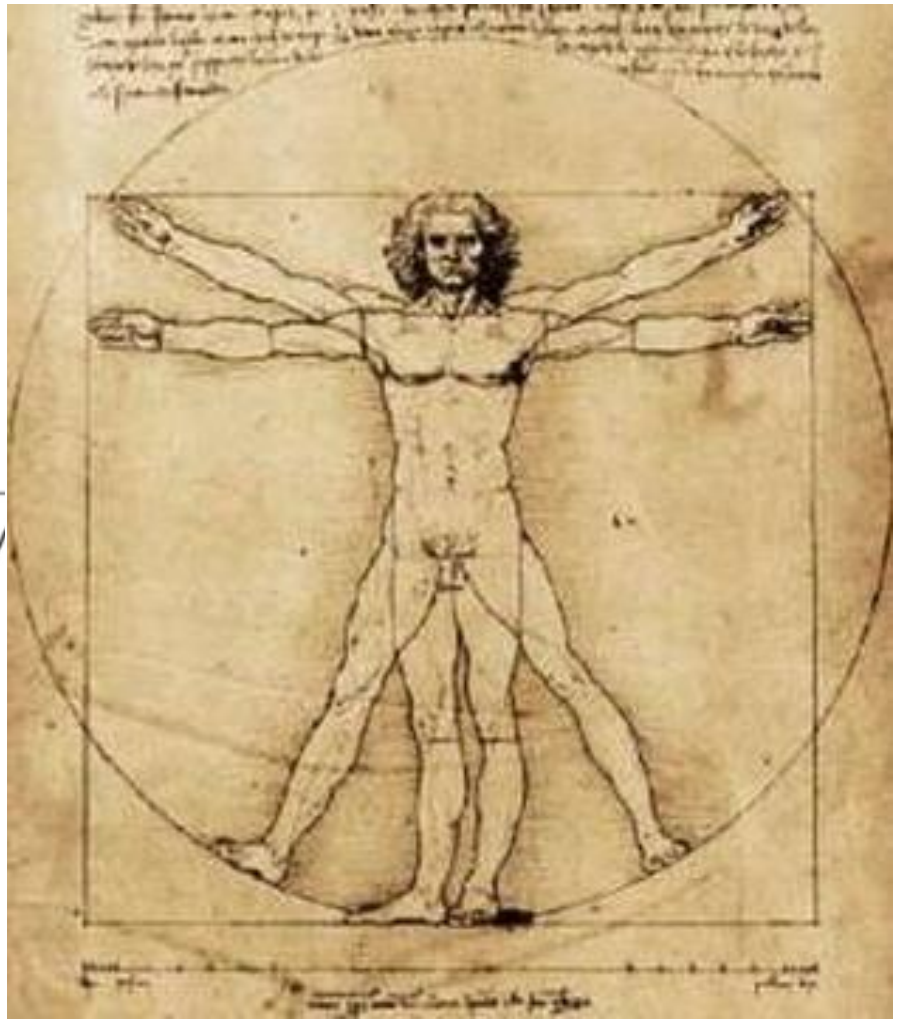
Brenda Salates, JCI 2019; Huting, CROI 2019

- Rebound and replication competent virus at ATI has different origin and potentially arises from different sanctuaries sites



Brenda Salates, JCI 2019; Huting, CROI 2019

The HIV hiding places



BONE MARROW - Lymphocytes, hematopoietic stem cells

GALT – chromaffin cells Lymphocytes

(1). GENITAL TRACT -Exposure to HIV at mucosal surface (sex). The virus is collected by DC SIGN+ dendritic cells

BRAIN – macrophages, glial cells

THYMUS - lymphocytes

LUNGS – alveolar macrophages

SPLEEN – Lymphocytes

(2). LYMPH NODES – Virus carried to lymph nodes by dendritic cells, exposes CD4+ cells (replication in CD4 cells)

(3). BLOOD – infected cells released into blood followed by dissemination to other organs.

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

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

2018

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

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

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

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

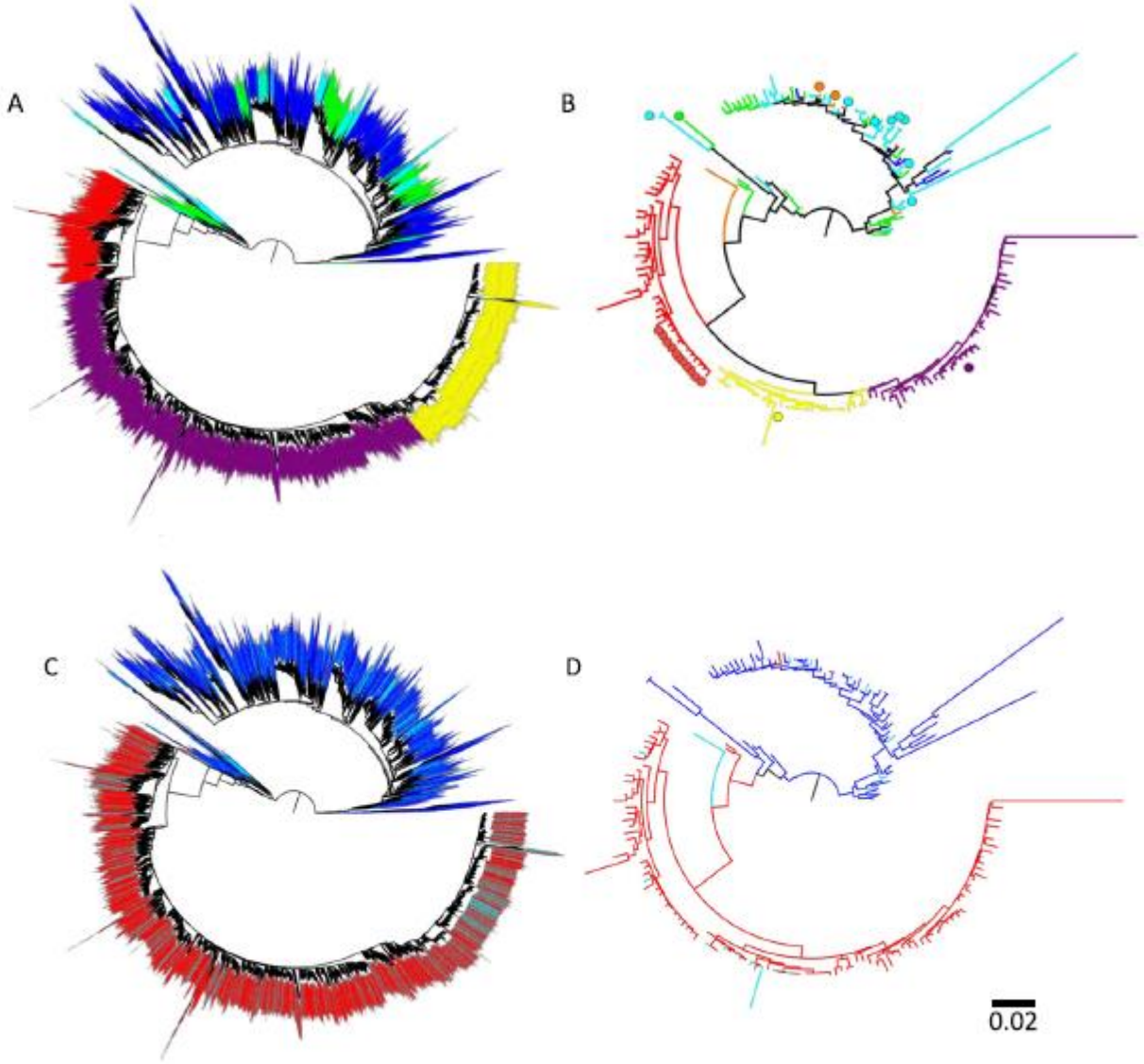
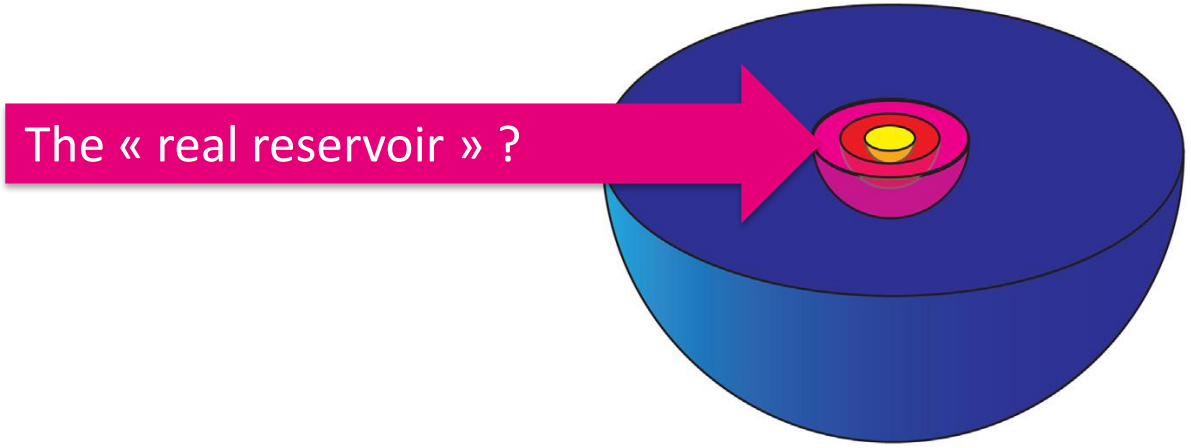
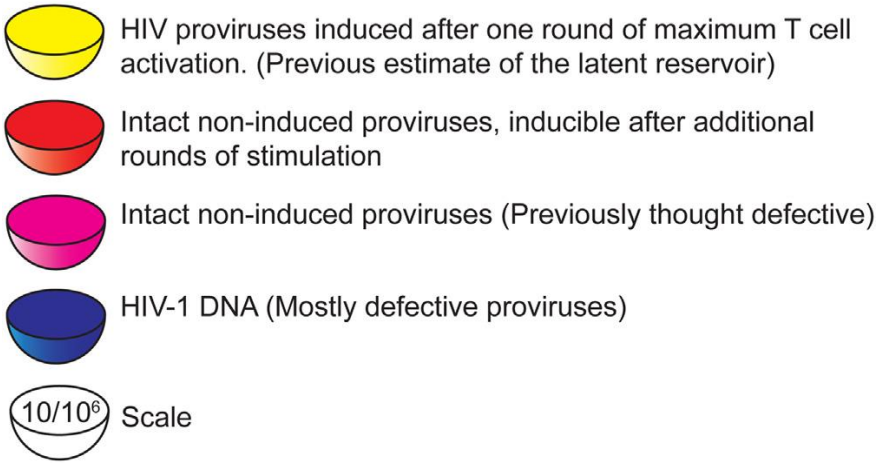


Fig. 1 Maximum-likelihood tree using the HQCS (**a**, **c**) and HQCS10 (**b**) datasets. Branches are scaled in substitutions/site according to the bar at the bottom of each tree. **a**, **b** Branches are colored to indicate the tissue of origin as follows: dark blue = blood, orange = colon, aqua =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

« Size » of the HIV reservoir



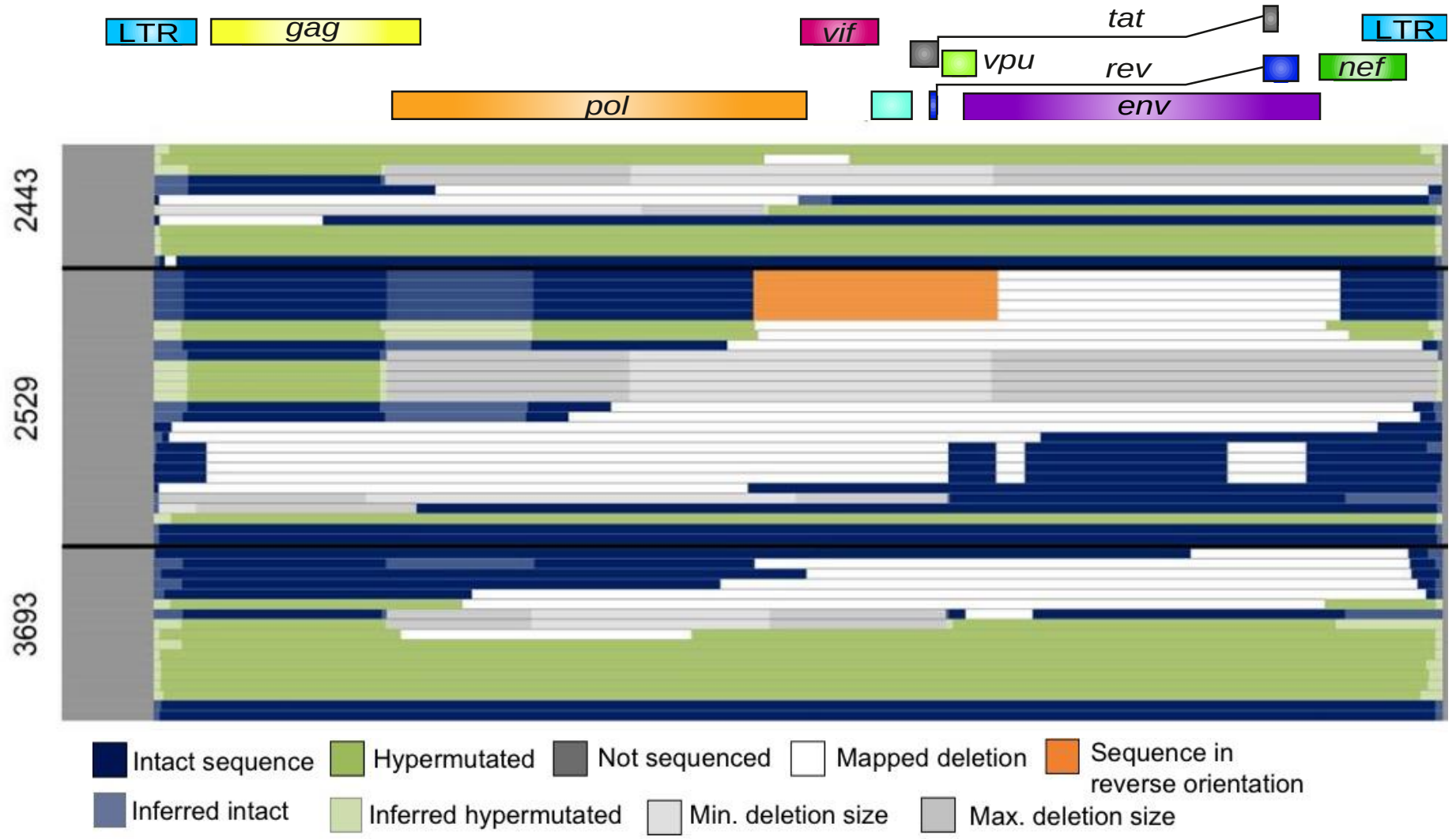
- The vast majority of proviruses that persist on ART are defective.



Ho et al. Cell 2013

Rapid accumulation of defective proviruses…

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

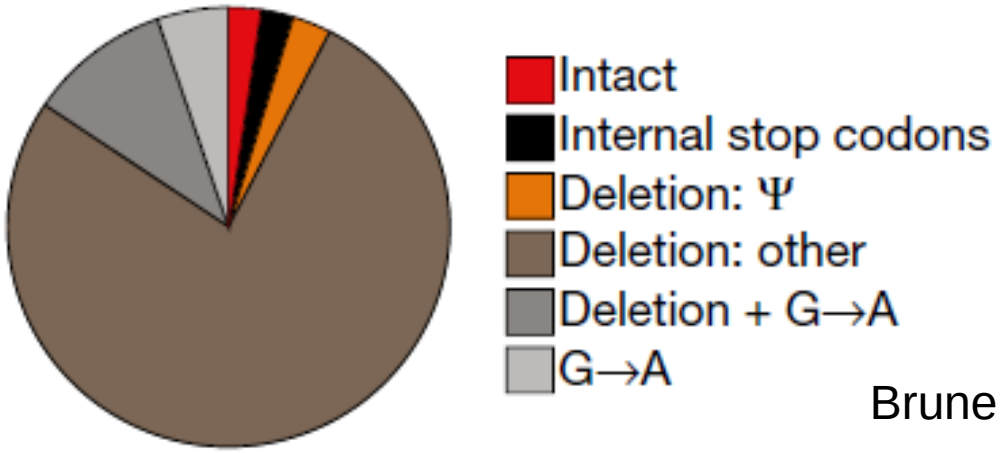


Bruner et al, Nat Med 2016

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

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

- The majority of defective proviruses contains a large internal deletion



Bruner et al., 2019

The Remarkable Frequency of Human Immunodeficiency Virus Type 1 Genetic Recombination

A Onafuwa-Nuga and A Telesnitsky
Microbiology and Molecular Biology Reviews - 2009

The vast majority of acute transforming retroviruses are replication defective, with the oncogene-containing genome being transmissible only during mixed infection with a replication-competent virus.

A defective retrovirus that relies on complementing functions can, in some instances, become replication competent by recombining with its replication-competent "helper."

In fact, there is some evidence that Rous sarcoma virus, possibly the only naturally arising replication-competent retrovirus containing a host oncogene, was replication defective initially

JOURNAL OF VIROLOGY, Mar. 1989, p. 1455–1459
0022-538X/89/031455-05$02.00/0

Vol. 63, No. 3

Genetic Recombination of Human Immunodeficiency Virus

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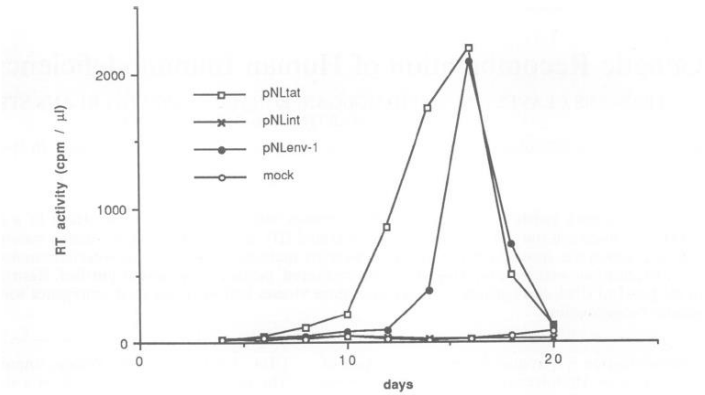
Received 7 September 1988/Accepted 11 November 1988

We investigated genetic recombination of the human immunodeficiency virus (HIV) in a tissue culture system. A clonal cell line expressing a single integrated HIV provirus with a termination codon affecting *pol* gene expression was transfected with different defective mutants derived from an infectious molecular clone of HIV. Replication-competent viral particles were recovered, passaged, and plaque purified. Restriction analyses of the proviral DNA corresponding to several of these viruses indicated that their emergence was the result of genetic recombination.



Infectivity of recombinant viruses generated following transfection of 8E5 cells with defective molecular clones of HIV.

As we observed for 8E5 in this study, genetic recombination could generate replication-competent viruses from such a collection of defective proviral sequences.

Tracking HIV-1 recombination to resolve its contribution to HIV-1 evolution in natural infection

Hongshuo Song[1,14], Elena E. Giorgi[2], Vitaly V. Ganusov[3], Fangping Cai[1], Gayathri Athreya[4], Hyejin Yoon[2], Oana Carja[5], Bhavna Hora[1], Peter Hraber[2], Ethan Romero-Severson[2], Chunlai Jiang[1,6], Xiaojun Li[1], Shuyi Wang[7], Hui Li[7], Jesus F. Salazar-Gonzalez[8,15], Maria G. Salazar[8], Nilu Goonetilleke[9], Brandon F. Keele[10], David C. Montefiori[1], Myron S. Cohen[9], George M. Shaw[7,11], Beatrice H. Hahn[7,11], Andrew J. McMichael[12], Barton F. Haynes[1], Bette Korber[2], Tanmoy Bhattacharya[2,13] & Feng Gao[1,6]

Recombination in HIV-1 is well documented, but its importance in the low-diversity setting of within-host diversification is less understood. Here we develop a novel computational tool (RAPR (Recombination Analysis PRogram)) to enable a detailed view of in vivo viral recombination during early infection, and we apply it to near-full-length HIV-1 genome sequences from longitudinal samples. Recombinant genomes rapidly replace transmitted/founder (T/F) lineages, with a median half-time of 27 days, increasing the genetic complexity of the viral population. We identify recombination hot and cold spots that differ from those observed in inter-subtype recombinants. Furthermore, RAPR analysis of longitudinal samples from an individual with well-characterized neutralizing antibody responses shows that recombination helps carry forward resistance-conferring mutations in the diversifying quasispecies. These findings provide insight into molecular mechanisms by which viral recombination contributes to HIV-1 persistence and immunopathogenesis and have implications for studies of HIV transmission and evolution in vivo.

nature COMMUNICATIONS

Determinants of HIV-1 reservoir size and long-term dynamics during suppressive ART

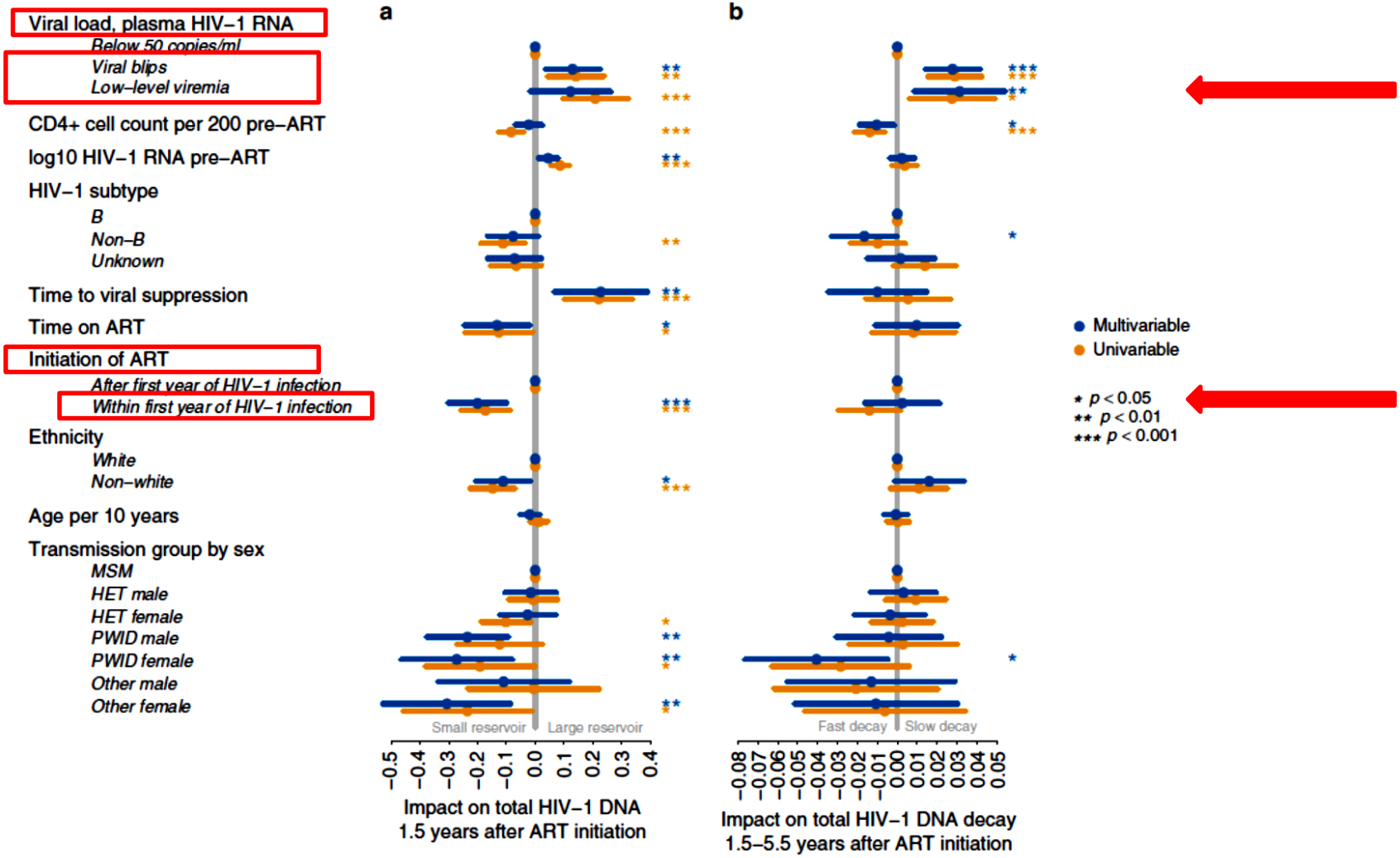
The HIV-1 reservoir is the major hurdle to a cure. We here evaluate viral and host characteristics associated with reservoir size and long-term dynamics in 1,057 individuals on suppressive antiretroviral therapy for a median of 5.4 years. At the population level, the reservoir decreases with diminishing differences over time, but increases in 26.6% of individuals. Viral blips and low-level viremia are significantly associated with slower reservoir decay. Initiation of ART within the first year of infection, pretreatment viral load, and ethnicity affect reservoir size, but less so long-term dynamics. Viral blips and low-level viremia are thus relevant for reservoir and cure studies.

Bachmann, Nat Comm 2019

ARTICLE

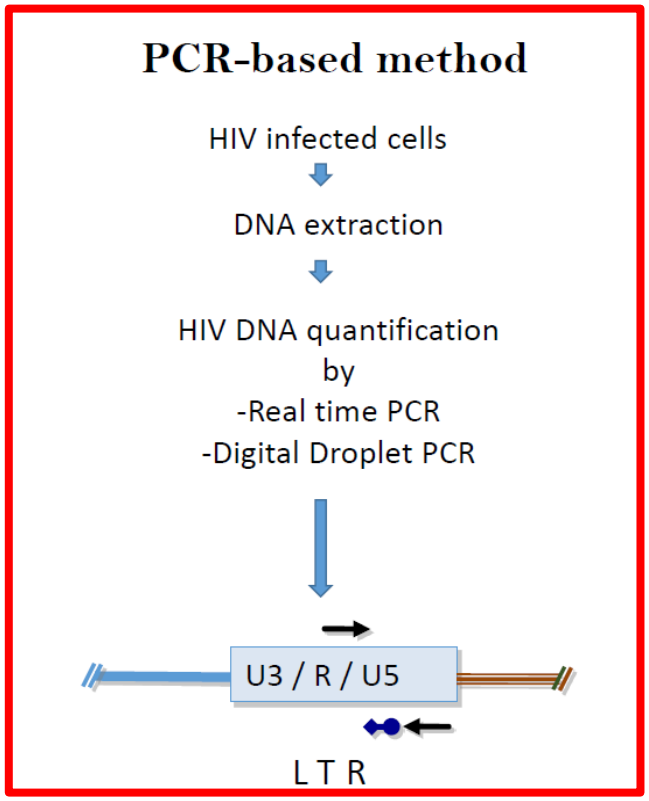
https://doi.org/10.1038/s41467-019-10884-9 OPEN

Determinants of HIV-1 reservoir size and long-term dynamics during suppressive ART



Bachmann, Nat Comm 2019

Quantification of cell-associated HIV DNA



•**When to use it?**
-Quantification of cells that are infected (i.e total reservoir size)

•**Sample:** few cells (>0,1*10^6) -> PBMC, CD4 T or Tissue cells

•**Advantages**
-High Sensitivity (1 infected cell/million cells)
-High Accuracy
-Cheap
-Rapid

•**Disadvantages**
-Overestimate the size of the reservoir
-Measure intact and defective provirus
(no exclusively replication competent)

Conclusions

- ✓ **From the Berlin Patient to 2 functionally cured HIV-1 infected patients (what about biological cure?)**

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

However…

- **The composition of the HIV reservoir is highly complex** because of the distinct cell lineages, with various phenotypes, transcriptional status and anatomical distributions

- The identification of **cells carrying replication competent or/and trascriptionally active viruses** is still far to be defined

- **Recombination events** are frequent and might regenerate infectious virus particles from replication-incompetent sequences

Conclusions

- ✓ **Based upon the current knowledge and information available, there is no evidence that rational and well-targeted therapy simplifications, including dual therapy (particularly if dolutegravir-based), have a negative effect upon the reservoir and harm the homeostasis of the patient**