6th National Conference on HIV/AIDS
Hangzhou
China

HIV reservoirs: a major obstacle for HIV cure

Prof Olivier Lambotte

Department of Internal Medicine and Clinical Immunology

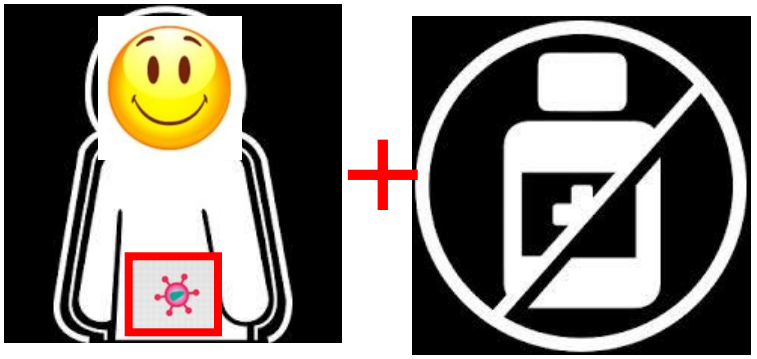
Hôpitaux Universitaires Paris Sud - Bicêtre

Research center UMR 1184 ImVA Paris Sud

Inserm CEA

HIV cure

- Eradication or remission ?



Eradication

Remission

HIV cure: eradication of HIV= myth or reality?

- The Berlin patient = cure with HIV eradication
- A second patient in Great Britain? (Gupta et al. Nature 2019)

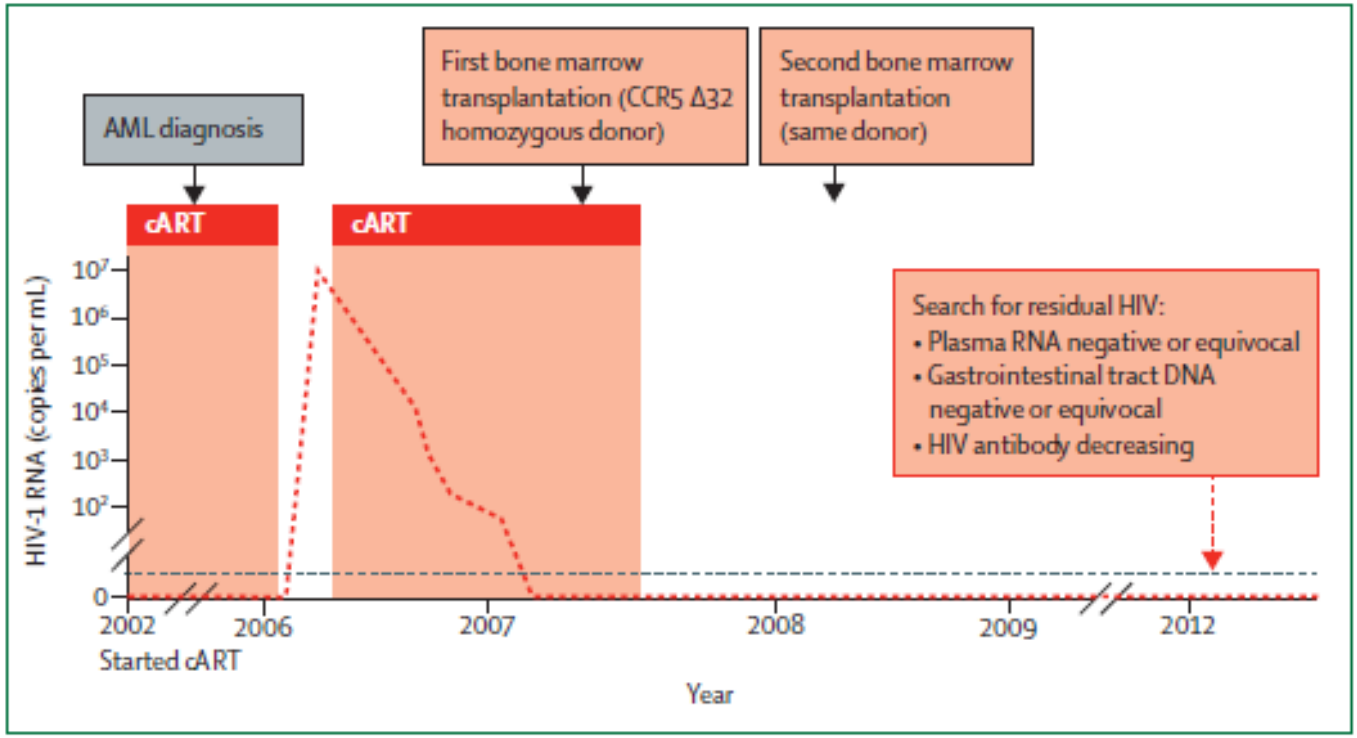


Figure 1: Timeline for treatment of the Berlin patient

The challenge: stay alive after two bone marrow transplants…
Risk of GVHD…

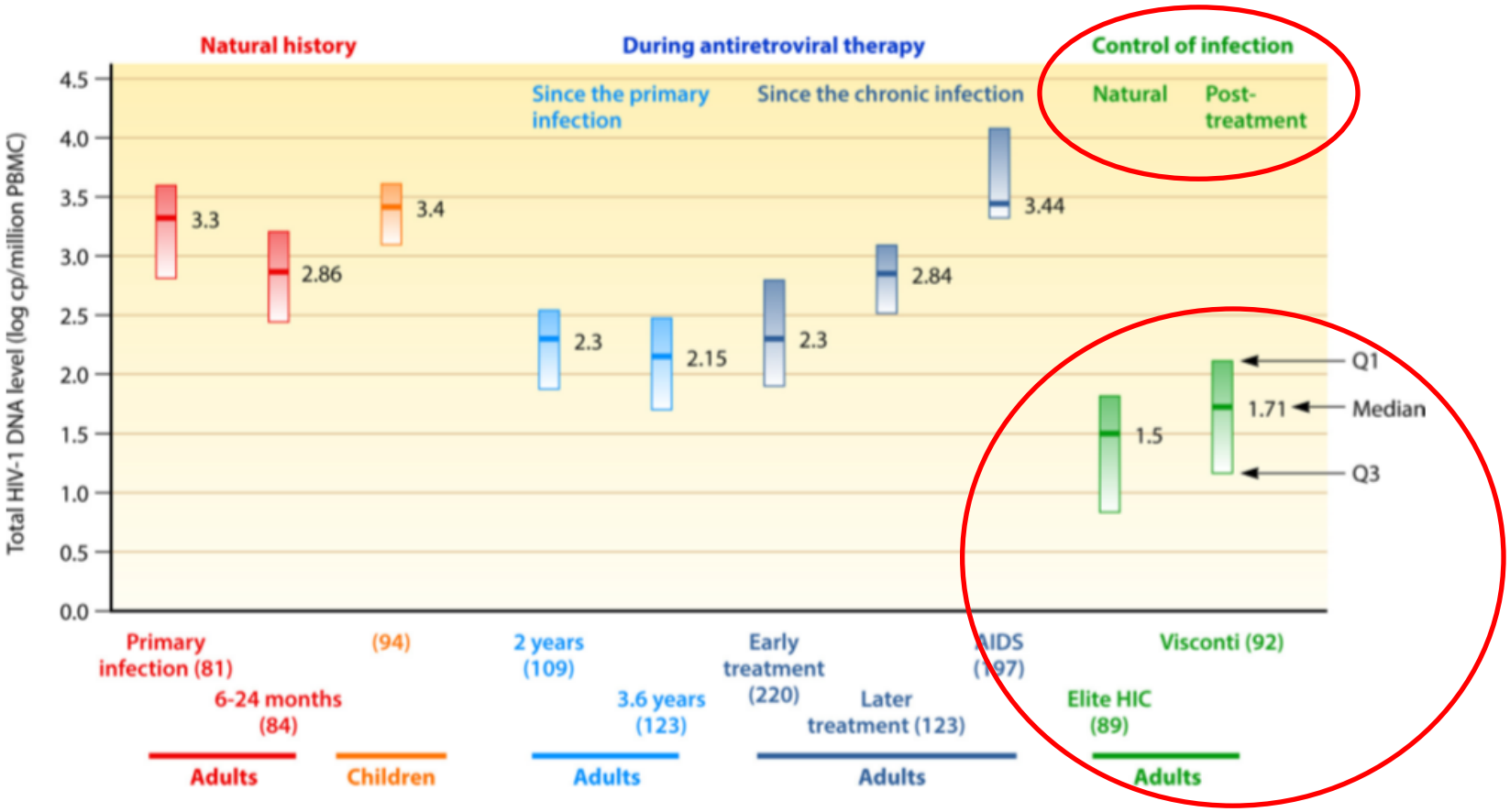
Kent et al. Lancet Infect Dis 2013

HIV cure, remission: myth or reality ?

HIV controllers: a functional cure / remission ?

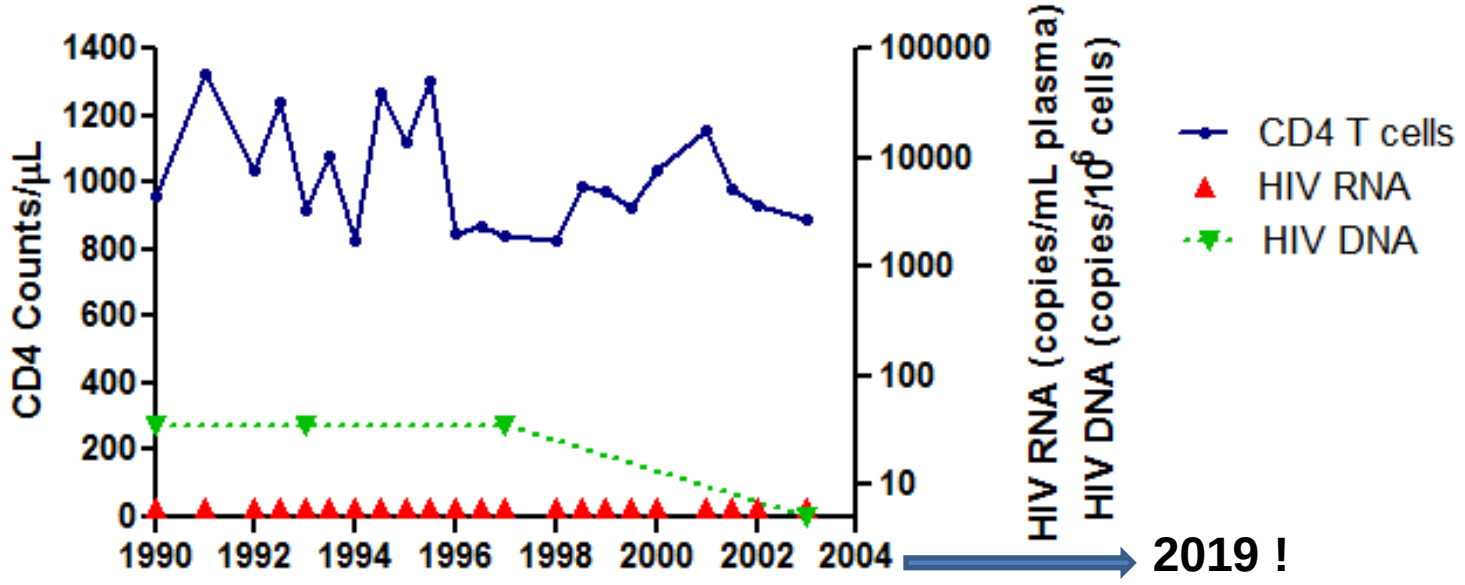
- « spontaneous » HIV Controllers
- « Post treatment » HIV controllers (Etude Visconti)

= extremely low HIV DNA level in PBMCs = small HIV reservoir in blood



Avettand et al. Clin Microbiol. Rev 2016

HIV controllers
A model of successful remission ?



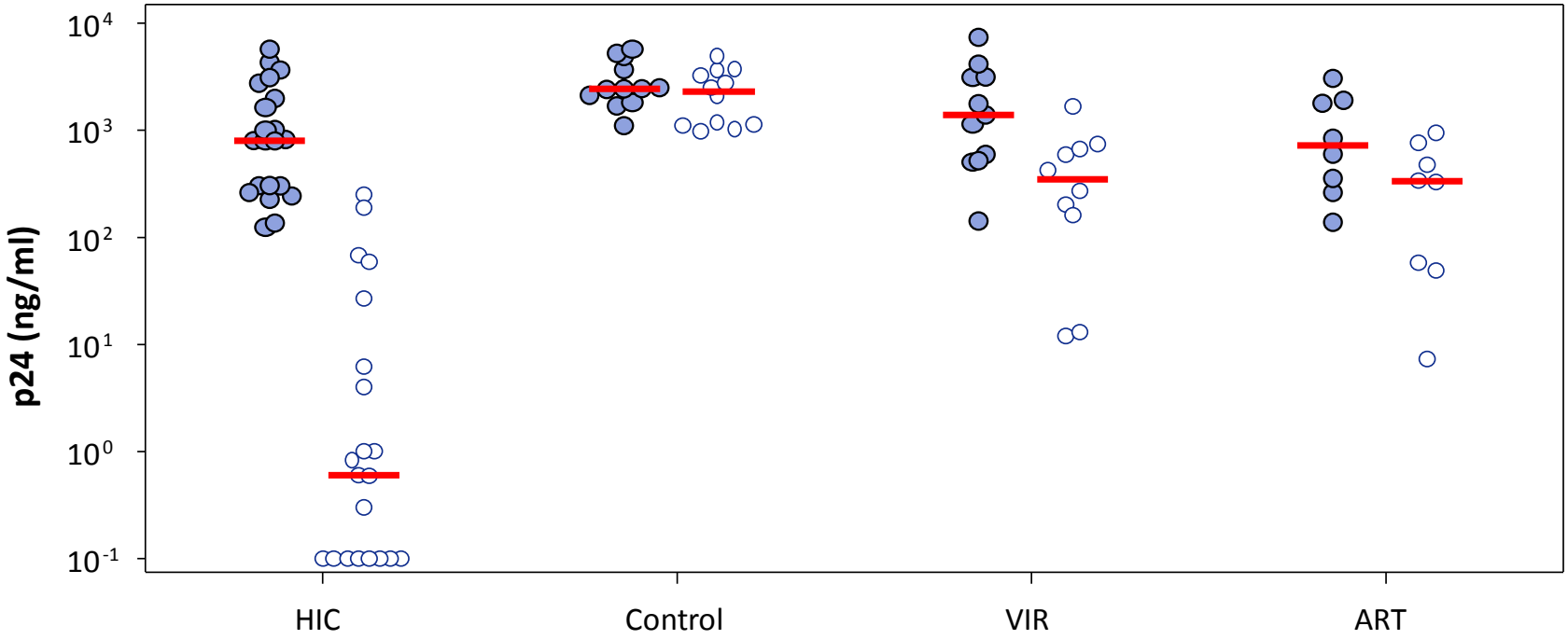
HIV CONTROLLER

- infection HIV > 10 years
- > 90% RNA viral loads < 400 copies/ml
- Never ART

<1% of HIV infected patients Lambotte et al. CID 2005

What do they learn?

1/ Unique characteristic of elite controllers CD8 T cells: without prior stimulation, they can block HIV replication, killing HIV-infected cells



Sáez-Cirión et al, PNAS. 2007 and JI 2009

Key role of HIV specific CD8 T cells in ECs

1. **HIV-specific CD8 T cells kill HIV-infected CD4 T cells by strong and fast production of perforine and granzyme** (Saez-Cirion et al 2007, 2009, Migueles et al 2008, Hersperger et al 2010)
2. **Polyfunctionality** (Betts 2006, Ndhlovu et al. Blood 2012, Lecuroux et al J Virol 2014)
3. **CD8 Tcm cells are able to use both glucose and fatty acid oxydation** (Angin et al. Nature Metabol. 2019 in press)
4. **HIV-specific CD8 are largely present in tissues** (Ferre et al. 2010)
5. **Strong CD4 help is provided to CD8 T cells by polyfunctional HIV-specific CD4 T cells with strong TCR avidity for HIV peptides** (*Potter et al.J Virol. 2007, Vingert et al.PLoS Pathog. 2010, Benati et al. JCI 2016)*

To get functional HIV-specific T cell responses is mandatory

To get HIV remission is achievable

What do we need?

- A small HIV reservoir = A better knowledge of the mechanisms of HIV persistence
- Be able to counteract these mechanisms with a favorable risk-reward ratio
- In a large number of patients
- Acceptable cost for the countries

1997 : identification of the first HIV reservoir

Latently infected resting CD4 T cells

Infectious virus as a form of silent integrated HIV DNA

Finzi et coll., Science 1997, Wong et coll., Science 1997, Chun et coll., PNAS 1997

Latently infected resting CD4 T cells are the HIV reservoir the « best » characterized since 1997

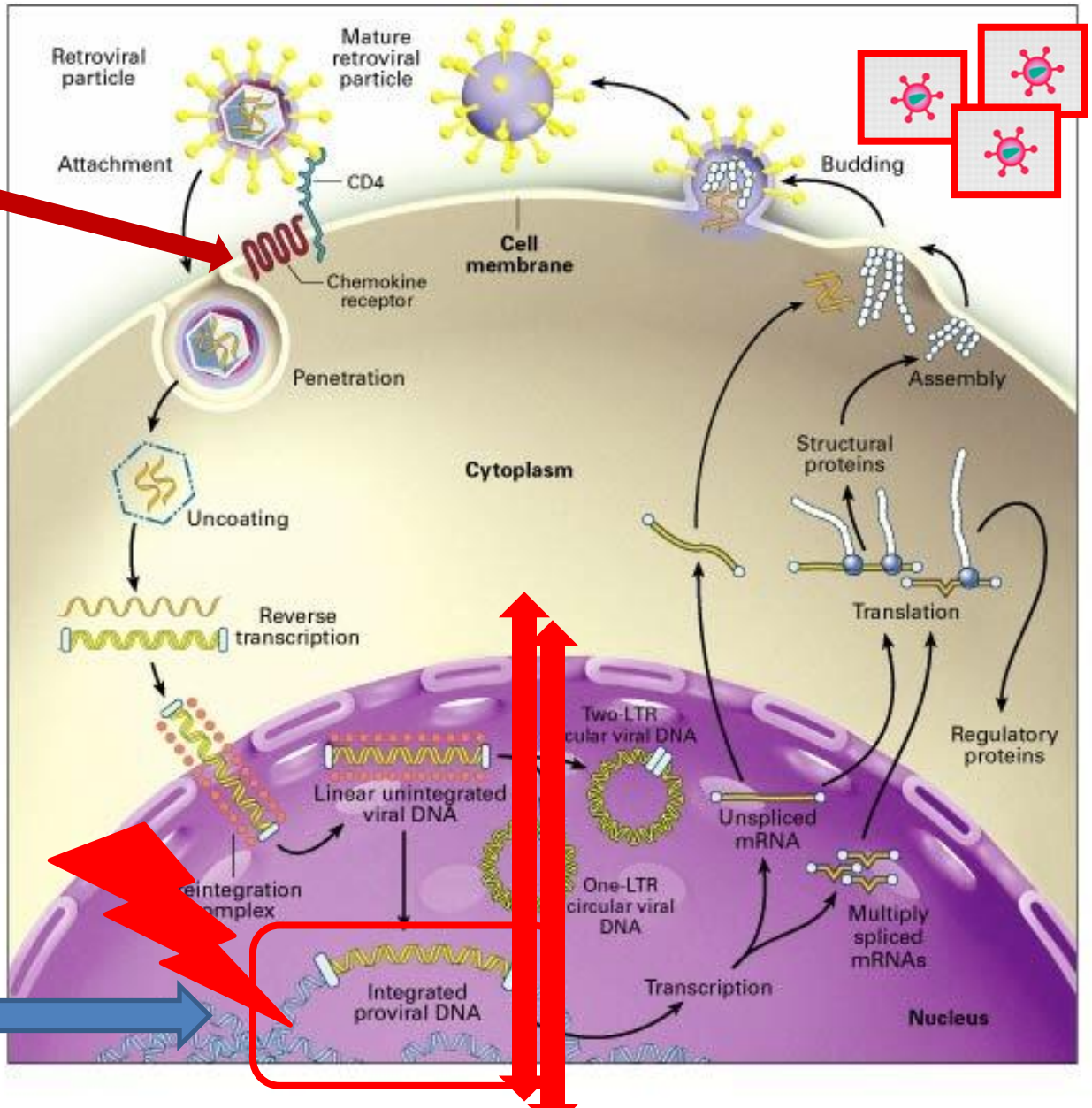
- Set up since the primary-infection (Chun et al. PNAS 1998, Whitney et al. Nature 2014)
- Latent infection in the different subsets of CD4 T cells (T_{CM} > T_{TM} > T_{EM} > T_{naive} > Tscm)

(Lambotte et al, AIDS 2002, Chomont et al. Nat Med 2009, Buzon et al Nat Med 2014)

- Long half-life of some cell subsets and homeostatic proliferation are major mechanisms of persistance of these reservoir cells
- To target that reservoir is mandatory

Several strategies can target CD4 T cell reservoirs of HIV

3/ Target CCR5 (analogy wit Berlin patient)



2/ Keep « frozen » HIV latency: Silencing

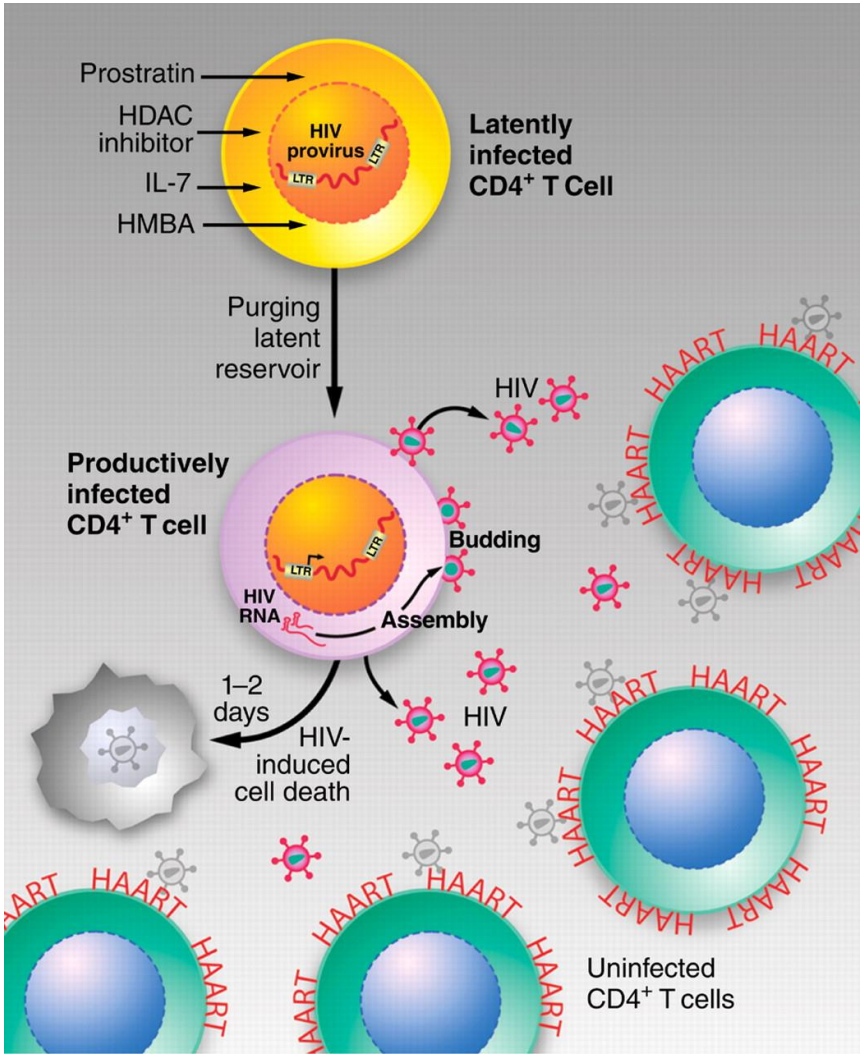
1/ Target post-integration latency = reactivation = <u>shock and kill</u>

(Furtado MR et al. NEJM, 1999)

The HIV reservoir CD4 T cells = target n°1 for cure strategies

- « shock and kill »: the most studied strategy

- To know the mechanisms of HIV latency
- To have an optimized ART
- To have a functional immune system (Shan et al Immunity 2013)



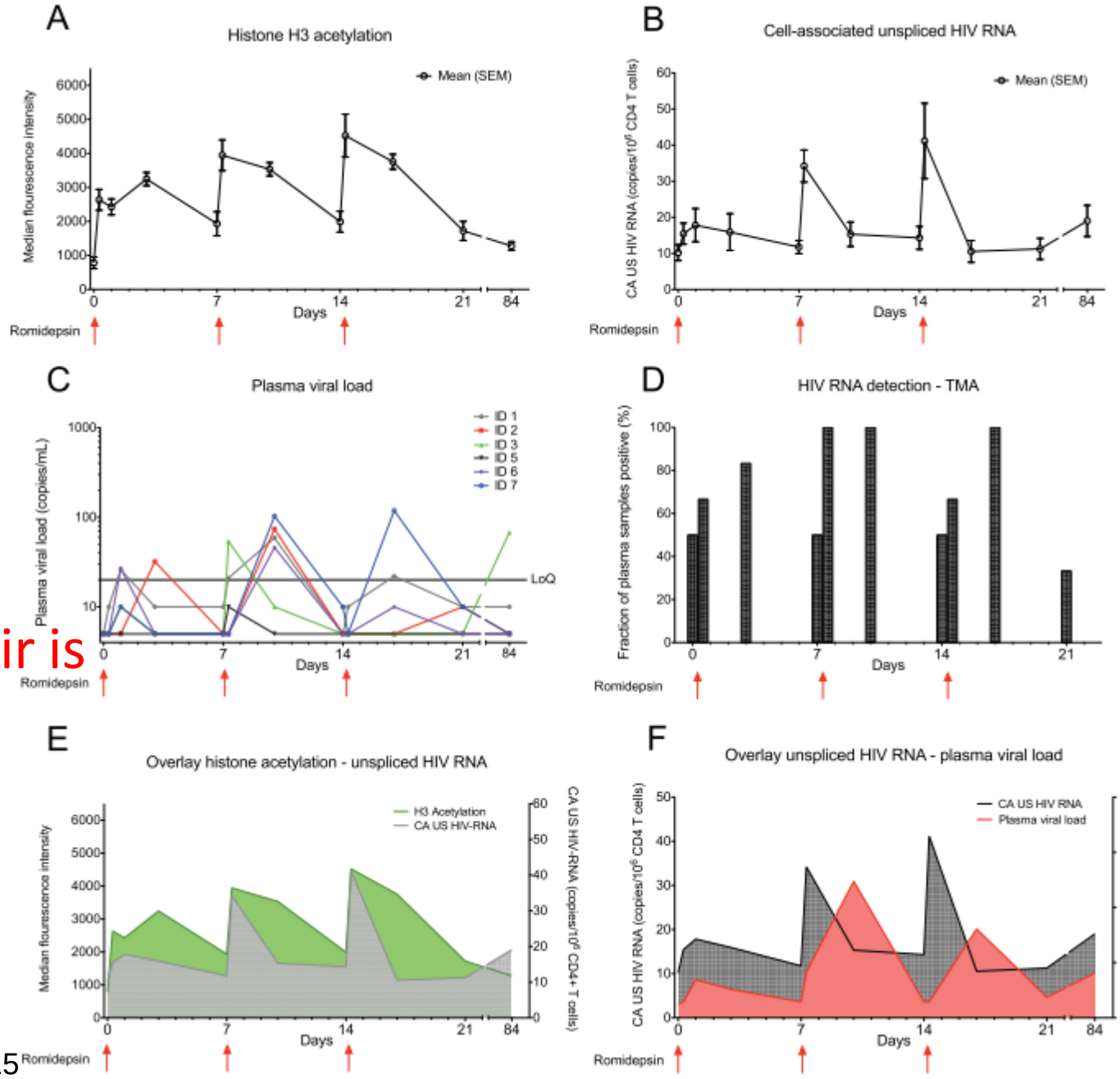
Do latency reversing agents (LRAs) work?

Numerous LRAs studied, an example, romidepsin (HDACi)…

6 patients on ART

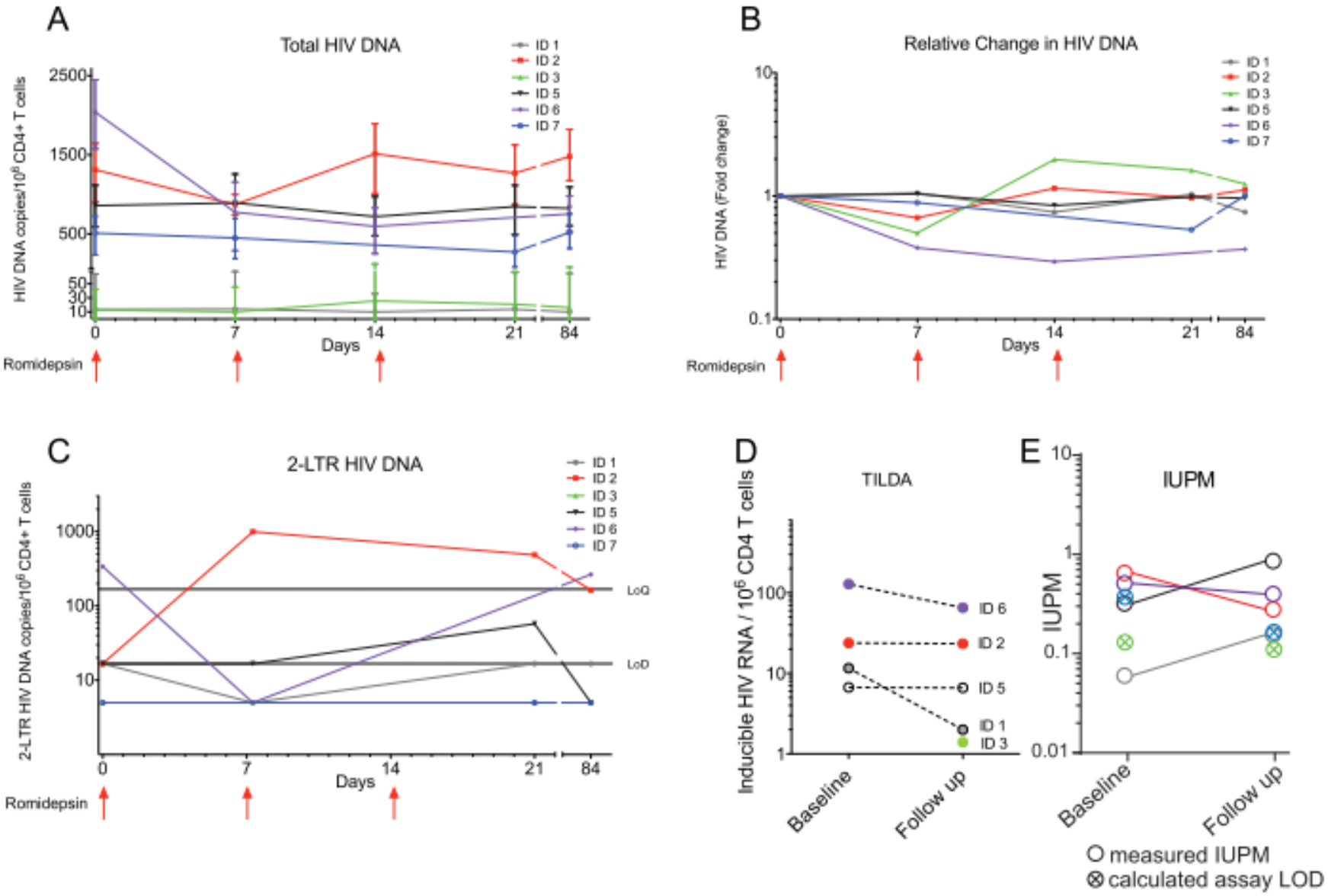
No AE > grade 1

The HIV reservoir is mobilized…



Sogaard et al. Plos Path 2015

BUT NO REDUCTION of the size of HIV reservoir



Sogaard et al. Plos Path 2015

First conclusion

- LRAs are able to act on HIV reservoir CD4 T cells but cannot decrease its size
- Interest of LRAs: efficacy of these drugs in oncology
  - Parallel of HIV cure and cancer cure
  - Doses can be lower thus safer
  - Major interest to combine different molecules (Reuse et al. Plos One 2009)

Research Article

TRANSPARENT PROCESS OPEN ACCESS

EMBO Molecular Medicine

Sequential treatment with 5-aza-2′-deoxycytidine and deacetylase inhibitors reactivates HIV-1

Sophie Bouchat[1], Nadège Delacourt[1], Anna Kula[1], Gilles Darcis[1,2], Benoit Van Driessche[1], Francis Corazza[3], Jean-Stéphane Gatot[1,†], Adeline Melard[4], Caroline Vanhulle[1], Kabamba Kabeya[5], Marion Pardons[1], Véronique Avettand-Fenoel[4], Nathan Clumeck[5], Stéphane De Wit[5], Olivier Rohr[6,7], Christine Rouzioux[4] & Carine Van Lint[1,*]

The model is too simple…!!

A more complex model of HIV persistence

- 4 obstacles !

1. The intrinsic stability of the CD4 T lymphocyte reservoir
2. Other reservoirs – tissues
3. A residual viral replication on ART : insufficient ART tissue diffusion ?
4. The lack of an effective HIV-specific immune response

HIV reservoirs are not only blood CD4 T cells….

1. **The tissues ARE the main problem !**

- HIV target cells are present in nearly all tissues
  - Tissular resident CD4 T cells (Tfh…)
  - Macrophages++
  - Follicular dendritic cells in lymph nodes

- Anatomic reservoirs (CNS, genital tract…)

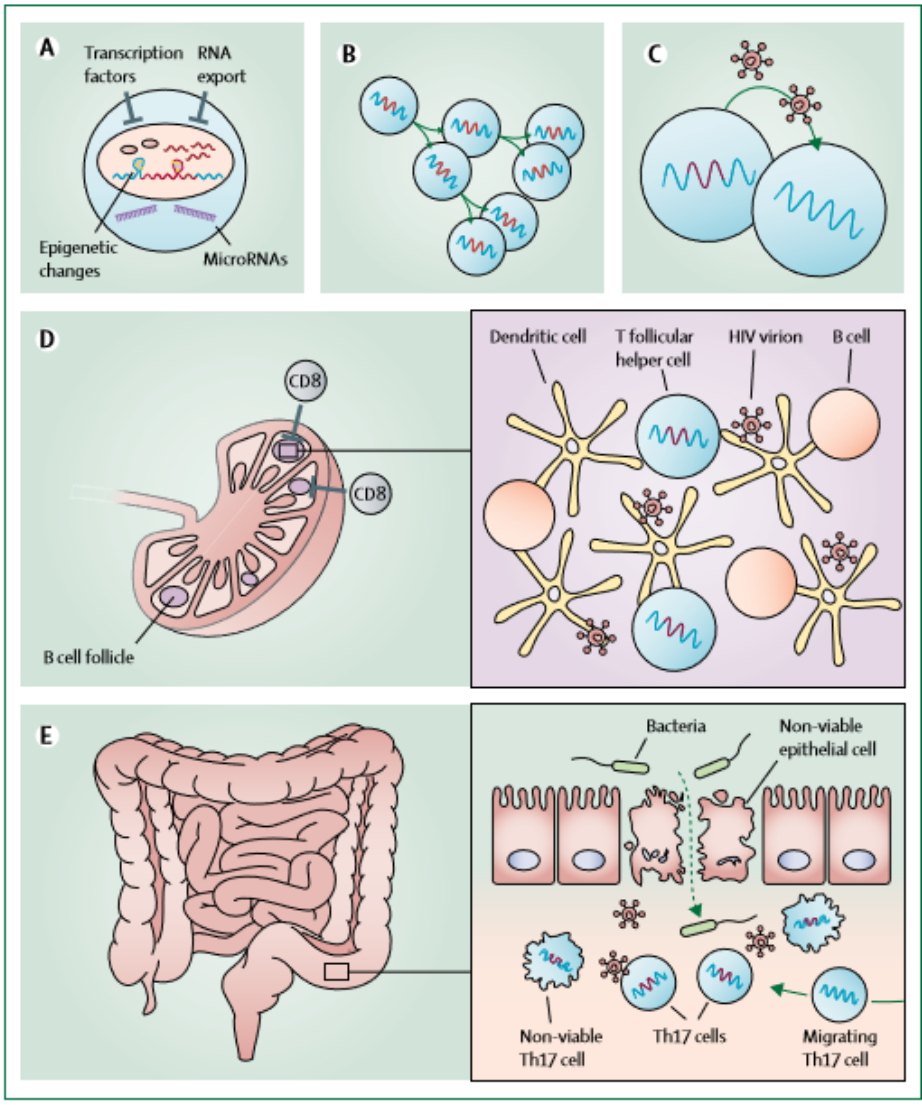


Figure 1: **Mechanisms of HIV persistence in cells and tissue** Pitman et al. Lancet 2018

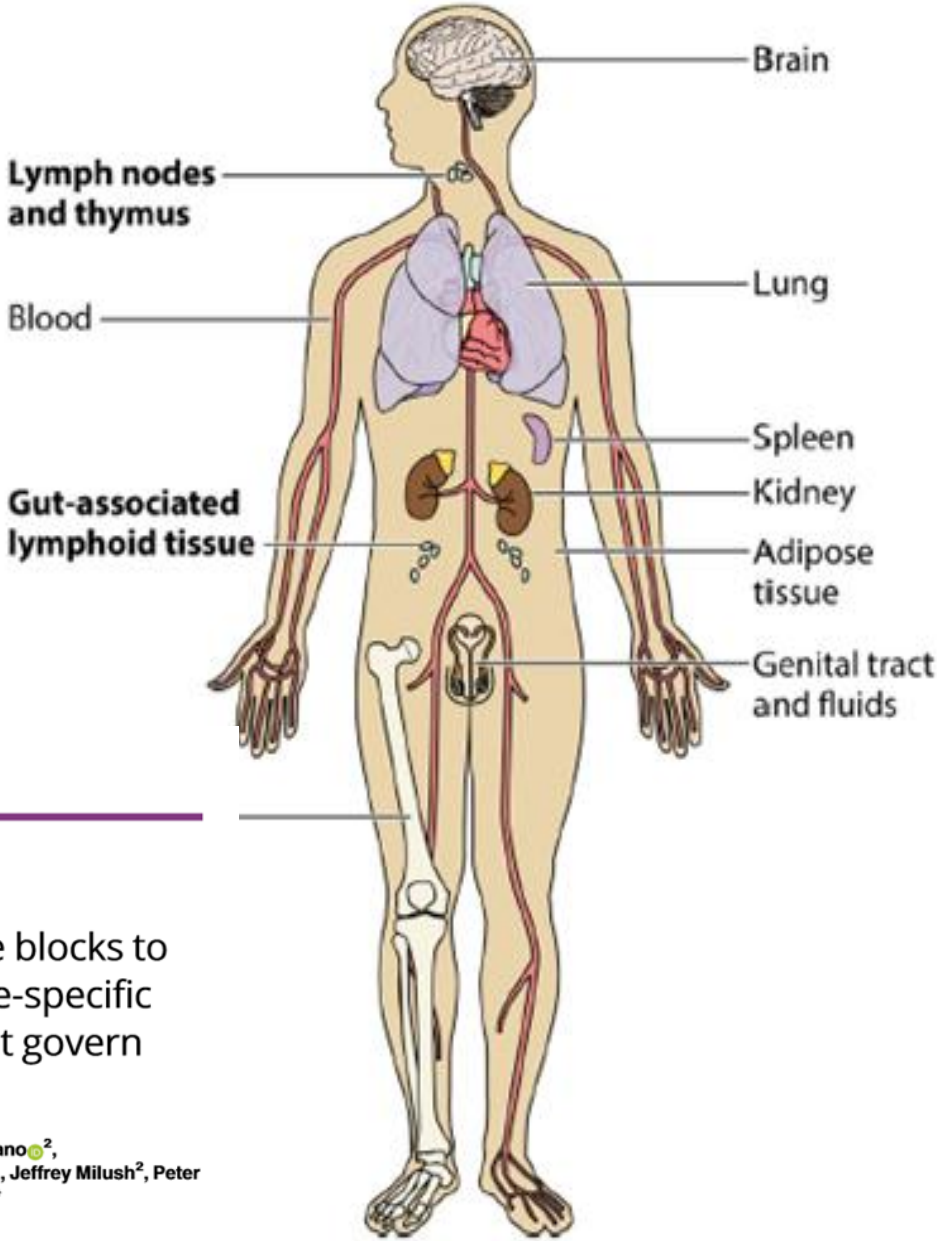
Chun et al JID 2008, Erikson Plos Path 2013, Yukl et al. JID 2013, Yukl et al. AIDS 2014

Tissular reservoirs are a maior problem…

- Numerous
- Hard to study
- Associated with insufficient biodiffusion of some antiretroviral drugs

(Fletcher C et al. PNAS 2014…)

- Intrinsic properties favoring HIV persistence



PLOS | PATHOGENS

RESEARCH ARTICLE

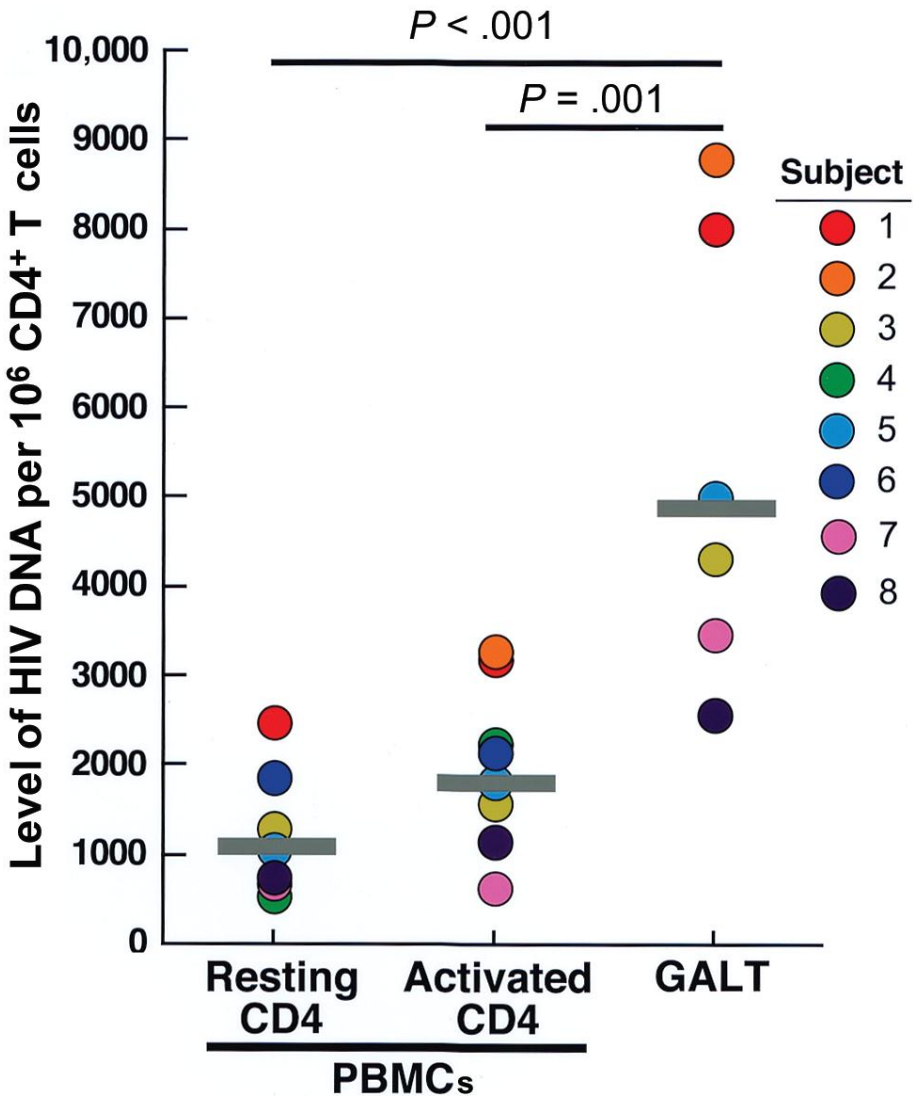
Gut and blood differ in constitutive blocks to HIV transcription, suggesting tissue-specific differences in the mechanisms that govern HIV latency

Sushama Telwatte[1‡], Sulggi Lee[2‡], Ma Somsouk[2], Hiroyu Hatano[2], Christopher Baker[2], Philipp Kaiser[1], Peggy Kim[1], Tsui-Hua Chen[1], Jeffrey Milush[2], Peter W. Hunt[2], Steven G. Deeks[2], Joseph K. Wong[1], Steven A. Yukl[1*]

Tissues data are rare in patients on HAART

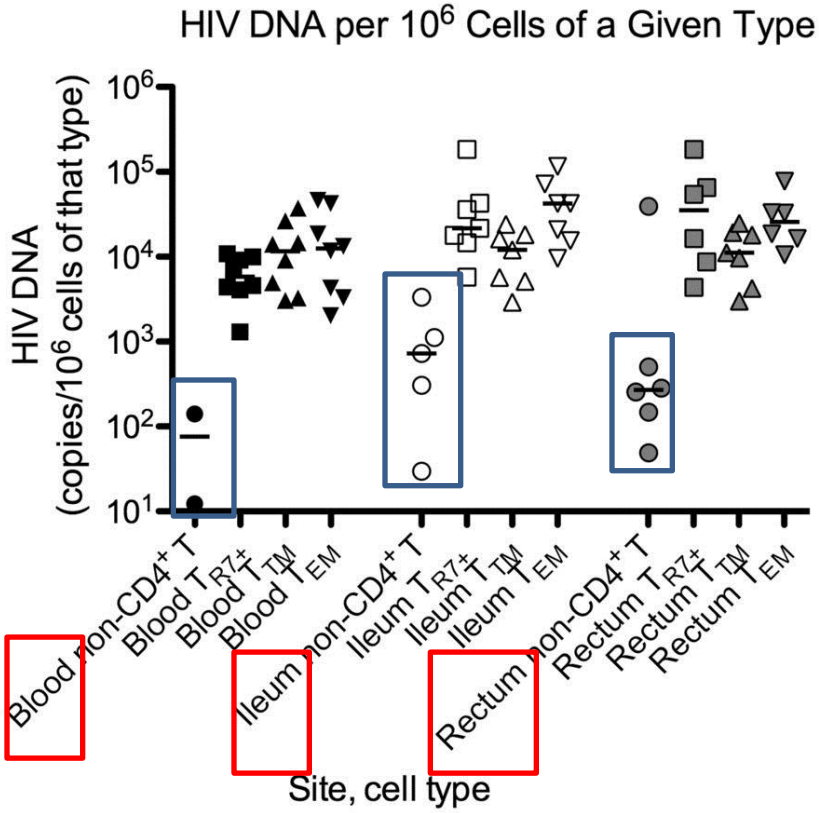
- HIV infection in GALT is more important than infection of PBMCs in patients on ART

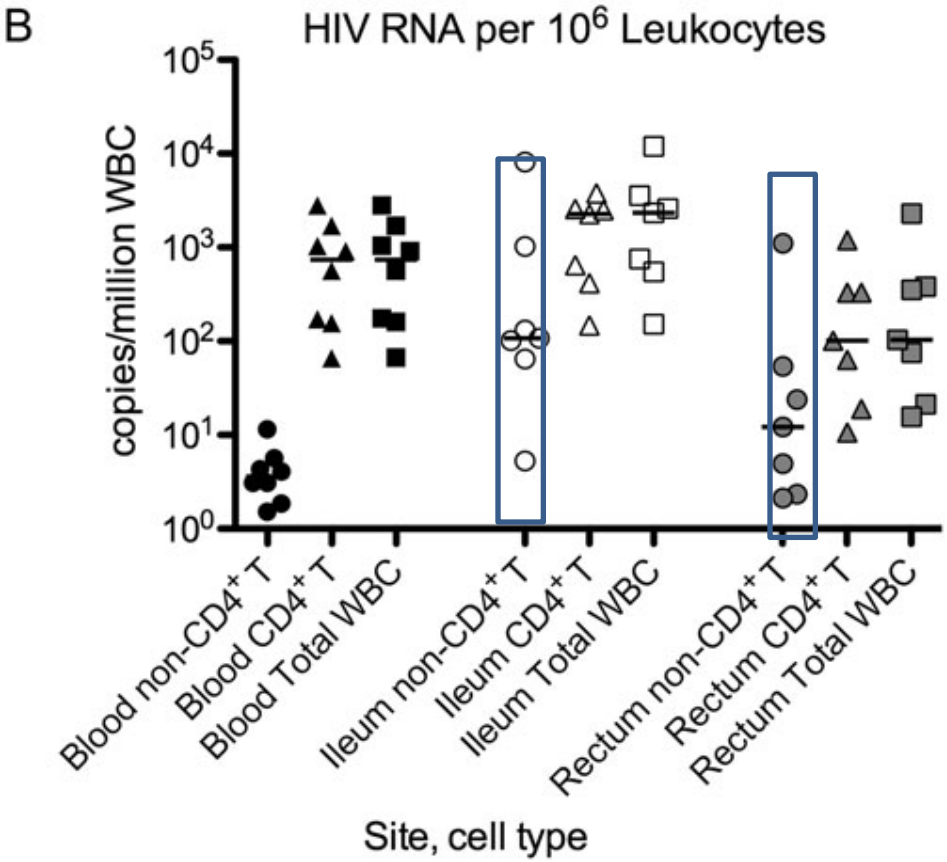
(Chun et al JID 2008)



Tissues and HIV persistence

- HIV persists on ART in CD4 T cells and macrophages in the GALT





(Chun et al JID 2008, Yukl et al. JID 2013, Yukl et al. AIDS 2014, Zalar et al. Antivir Res 2010)

Lymph nodes contain HIV-infected cells in patients on ART

- Persistent viral replication in lymphoid tissues in patients on ART

Zhang et al. NEJM 1999

Parallel with the persistence of infected CD4Tfh in germinal centers with few contact with CD8 T cells

(Banga et al. Nat Med 2016)

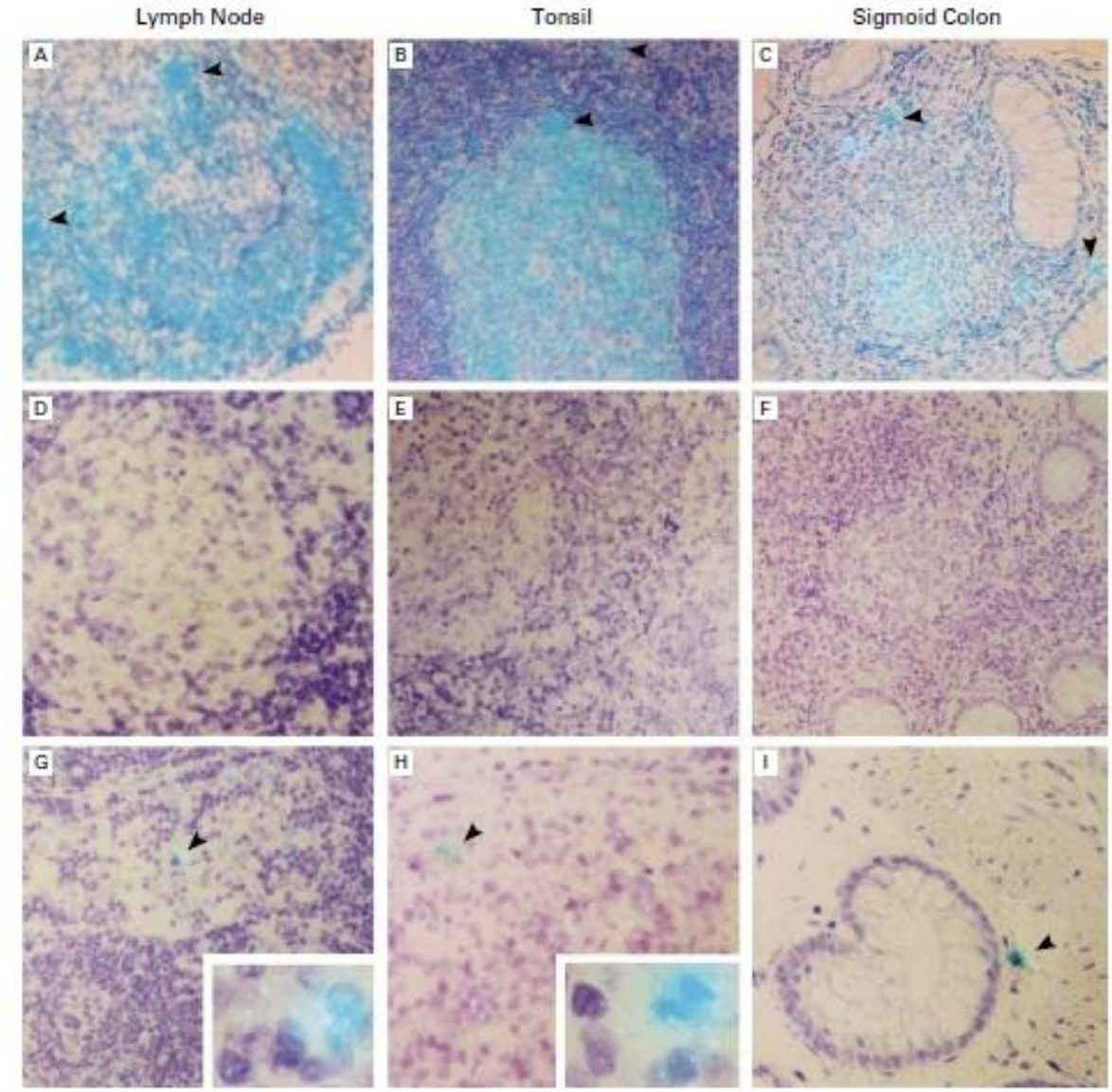
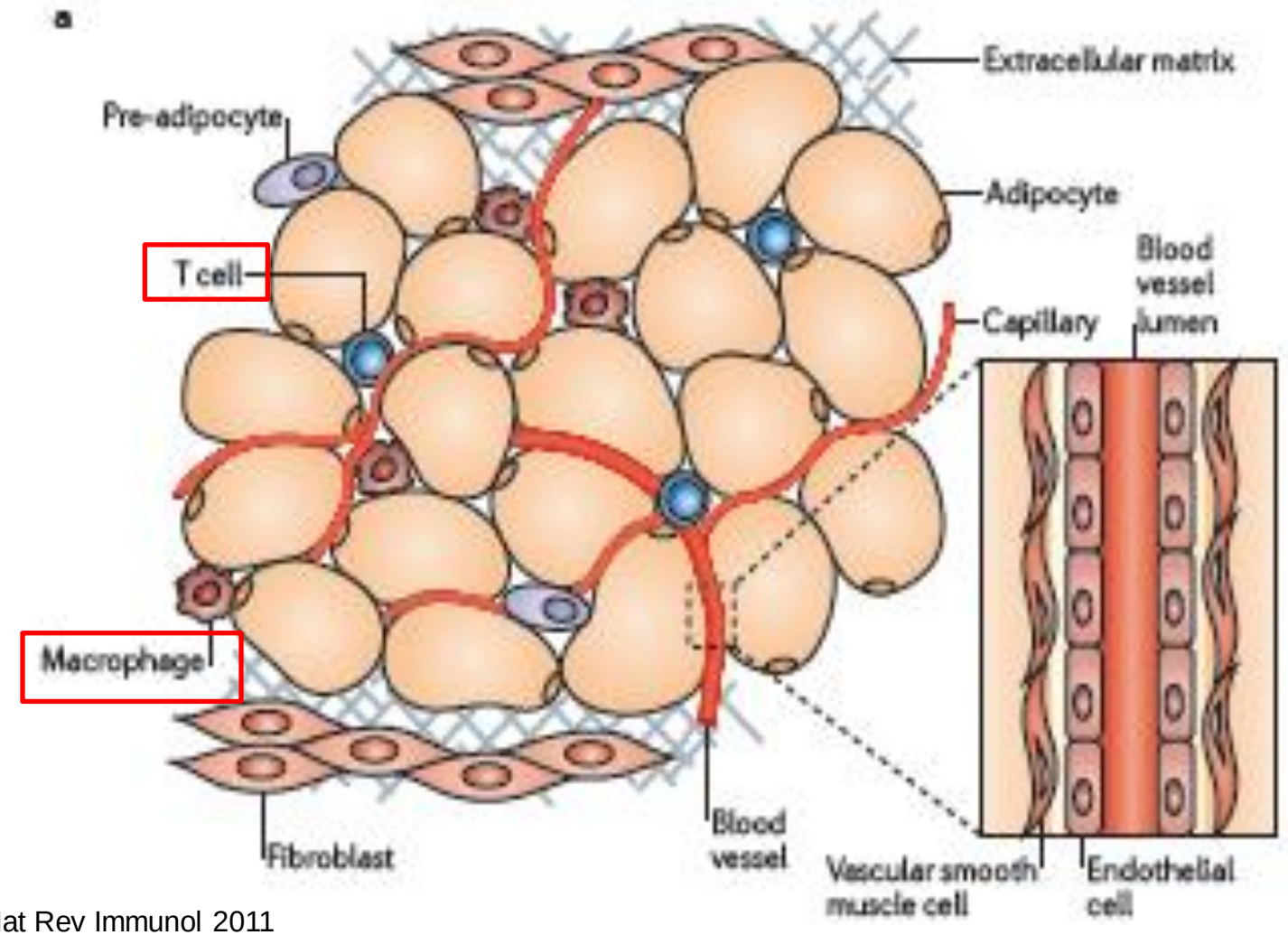


Figure 3. In Situ Hybridization Studies of Tissue from an Untreated Patient with HIV Infection (Panels A, B, and C) and Patient 5 (Panels D through I).

A neglected HIV reservoir: the adipose tissue

it contains HIV target cells (CD4 T cells and macrophages)



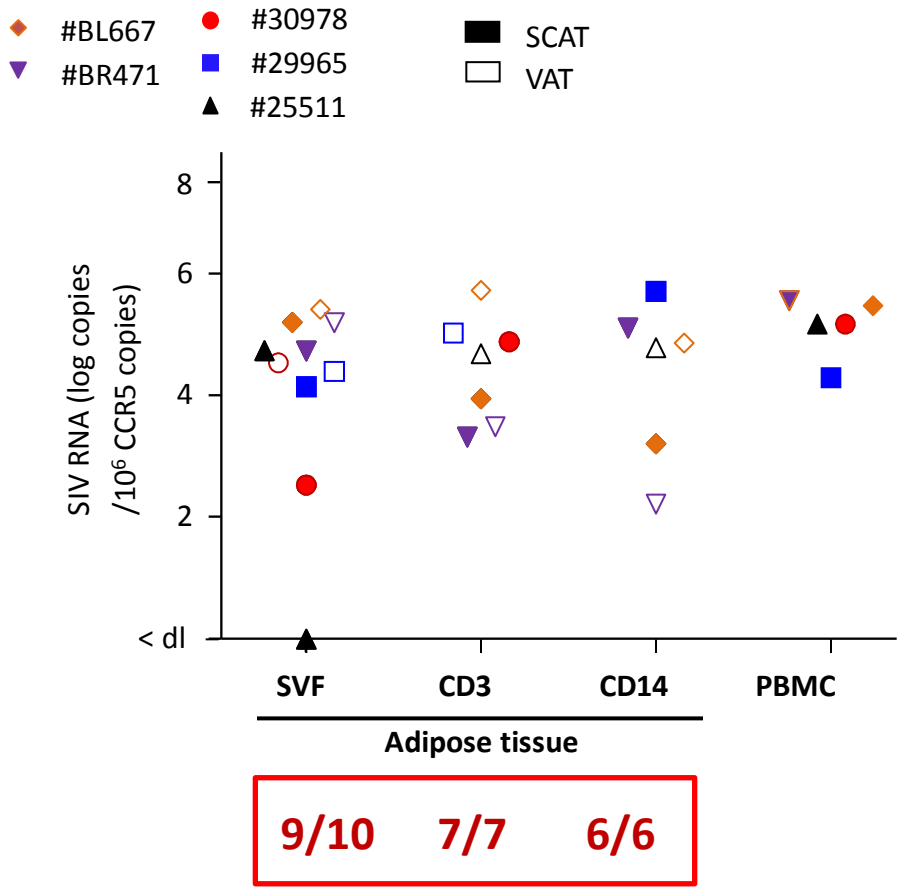
Ouchi et al. Nat Rev Immunol 2011

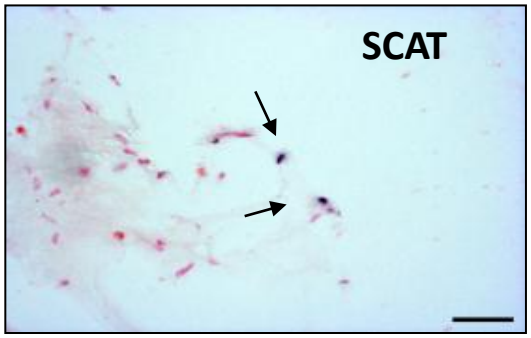
Study of CD4 T cells and macrophages in adipose tissue (AT)

- Use of the cynomolgus macaque model infected by SIV
- Access to perioperative samples during surgical procedures in HIV-infected patients on ART

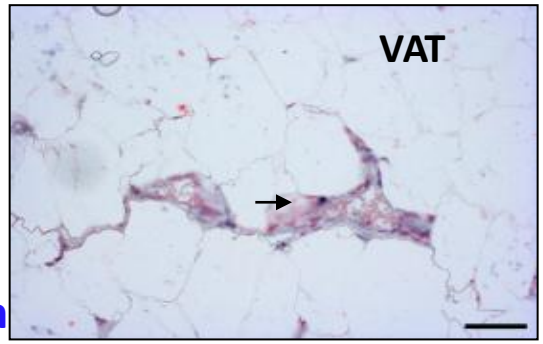
Adipose tissue includes SIV infected cells

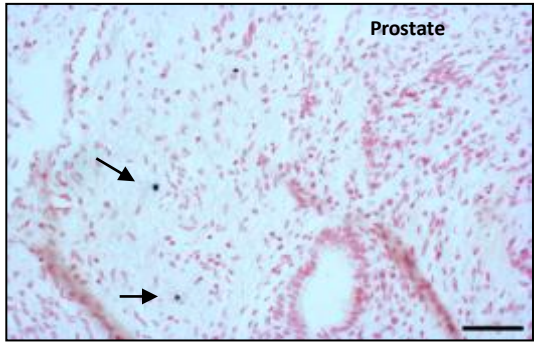
SIV RNA quantification in SVF and in sorted cells





***In situ* SIV RNA hybridization**



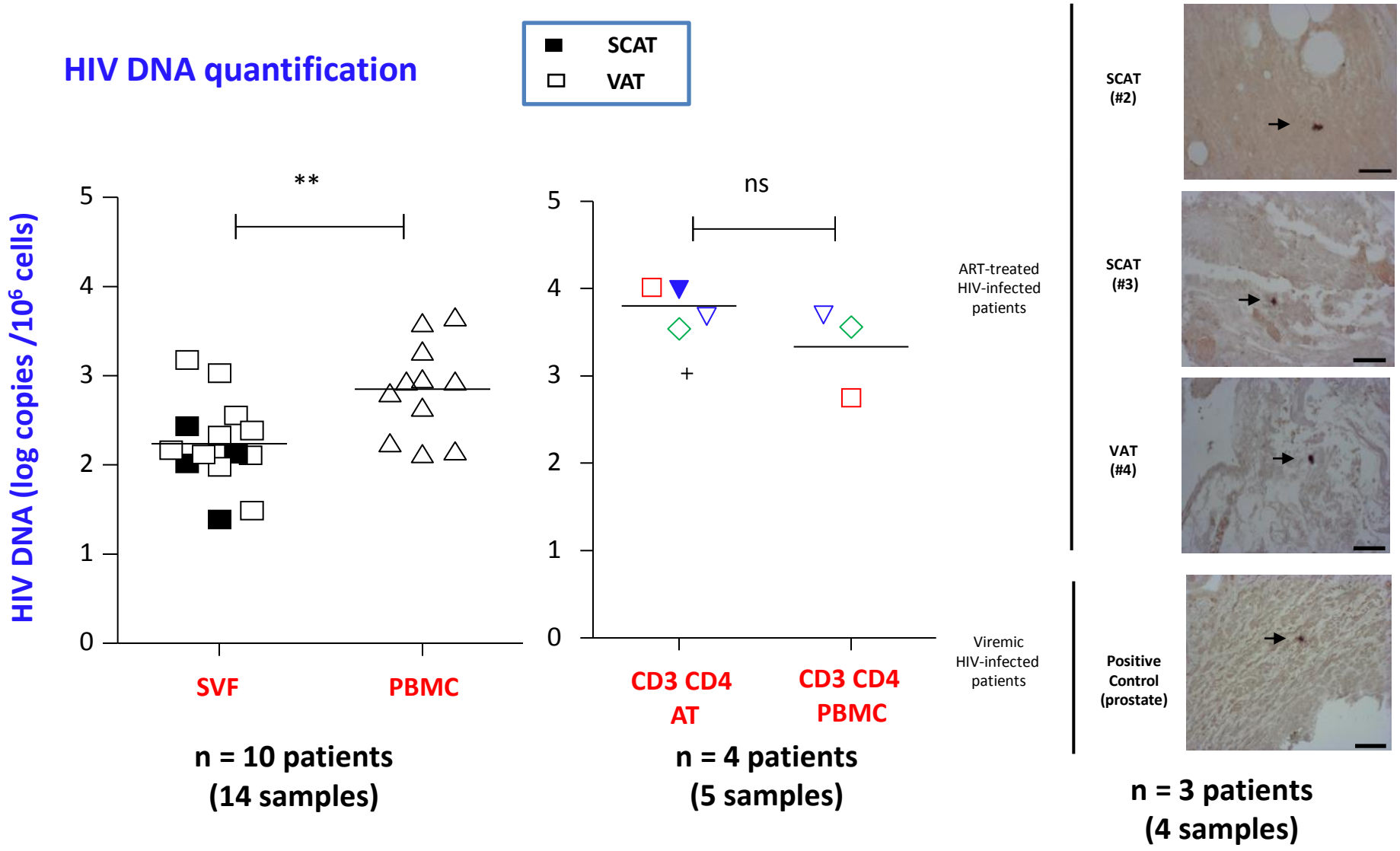


In 5 chronically SIV+ untreated macaques,

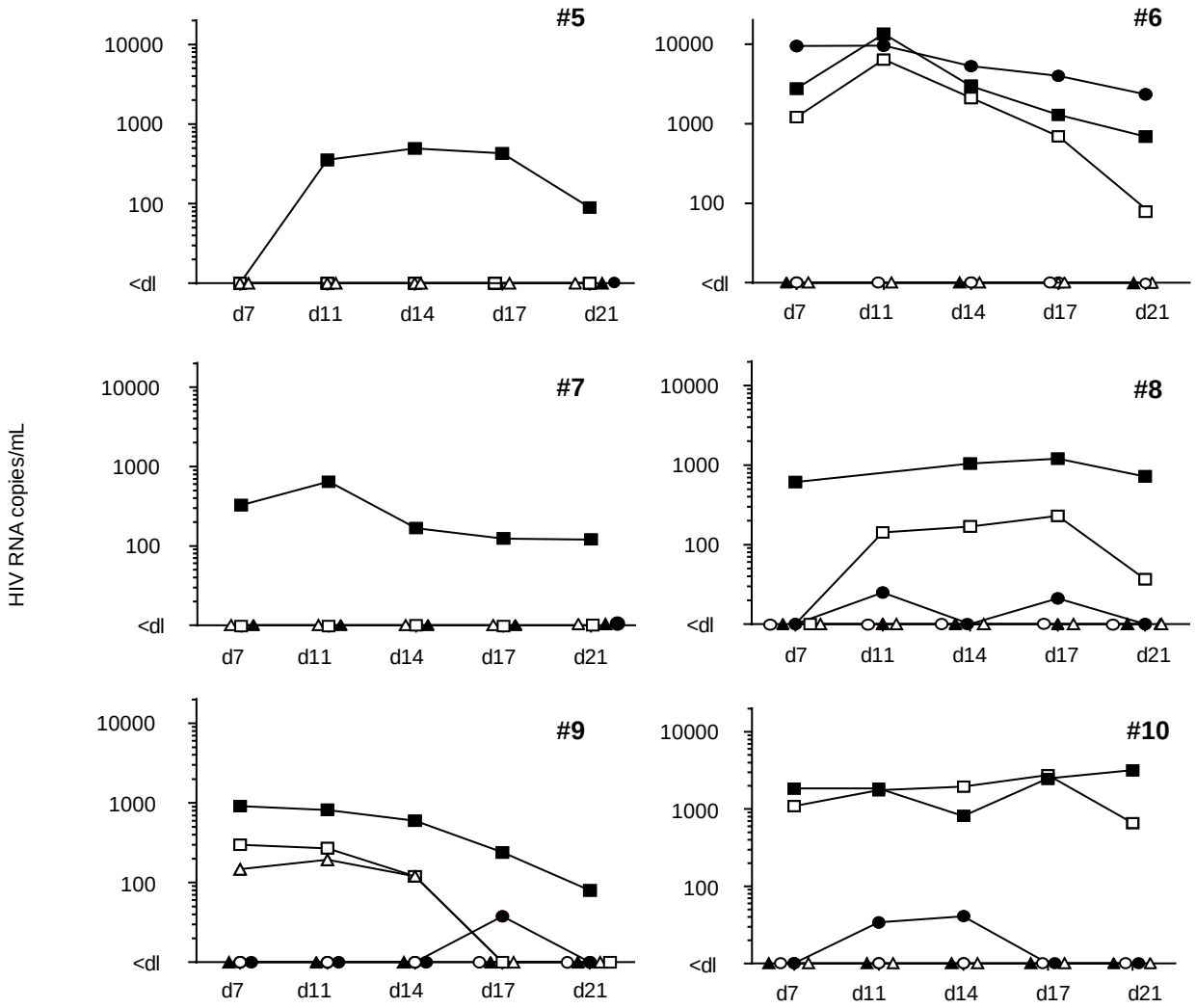
- SIV RNA detected in SVF collected from 9/10 samples (5 animals)
- SIV RNA detected in all CD3+ sorted fraction and CD14+ sorted fraction

Damouche et al. Plos Path 2015

Adipose tissue includes HIV infected cells



In ART-treated HIV-infected patients, HIV was detected in SVF and adipose tissue CD4 T cells (DNA and RNA by HIS)



Presence of CD4 T cells infected with replication competent virus in adipose tissue in patients on ART

Higher frequency than in PBMCs ?

Stim SVF
Stim CD4 SVF
Stim CD14 CD206 SVF
Non Stim SVF
Stim CD4 PBMC
Stim CD14 PBMC

Stimulated CD4 T cells Adipose Tissue

Stimulated CD4 T cells PBMCs

Damouche et al. Plos Path 2015

Tissue intrinsic properties can help HIV persistence in adipose tissue

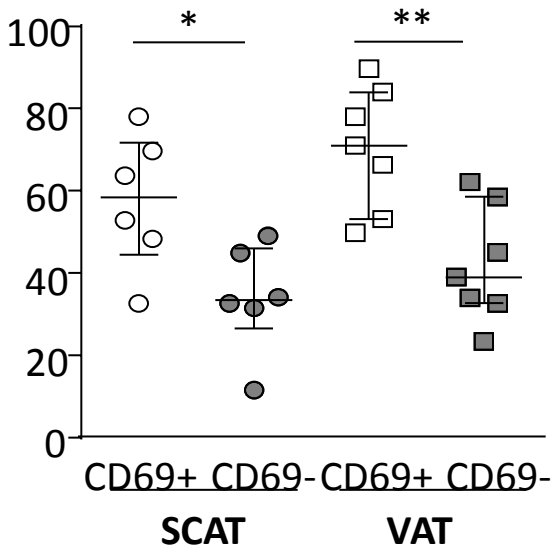
HIV+
HIV-

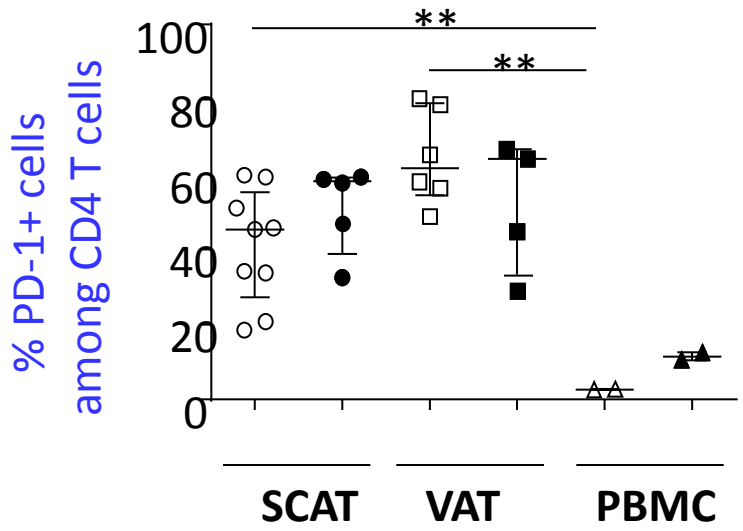
European Journal of Immunology
Immunity to infection

Research Article

High proportion of PD-1-expressing CD4+ T cells in adipose tissue constitutes an immunomodulatory microenvironment that may support HIV persistence

Abderaouf Damouche[1,2], Guillaume Pourcher[3], Valérie Pourcher[4], Stéphane Benoist[5], Elodie Busson[6], Jean-Jacques Lataillade[6], Mélanie Le Van[1,2], Thierry Lazure[7], Julien Adam[8], Benoit Favier[1,2], Bruno Vaslin[1,2], Michaela Müller-Trutwin[9], Olivier Lambotte[1,2,10] and Christine Bourgeois*[1,2]*





High proportion of PD-1+ CD4T cells in T resident cells

Why does HIV persist easily in tissues?

Tissues specificities favouring HIV persistence = **access to the target cells**

...

The structure of the germinal center in lymph nodes

Published in final edited form as:

Nat Med. 2015 February ; 21(2): 132–139. doi:10.1038/nm.3781.

A B cell follicle sanctuary permits persistent productive SIV infection in elite controllers

Yoshinori Fukazawa[1], **Richard Lum**[1], **Afam A. Okoye**[1], **Haesun Park**[1], **Kenta Matsuda**[2], **Jin Young Bae**[1], **Shoko I. Hagen**[1], **Rebecca Shoemaker**[3], **Claire Deleage**[3], **Carissa Lucero**[3], **David Morcock**[3], **Tonya Swanson**[1], **Alfred W. Legasse**[1], **Michael K. Axthelm**[1], **Joseph Hesselgesser**[4], **Romas Geleziunas**[4], **Vanessa M. Hirsch**[2], **Paul T. Edlefsen**[5], **Michael Piatak Jr.**[3], **Jacob D. Estes**[3], **Jeffrey D. Lifson**[3], and **Louis J. Picker**[1,*]

Tissues specificities favouring HIV persistence = **limited drug biodiffusion**

Fibrosis : an underestimated question ?

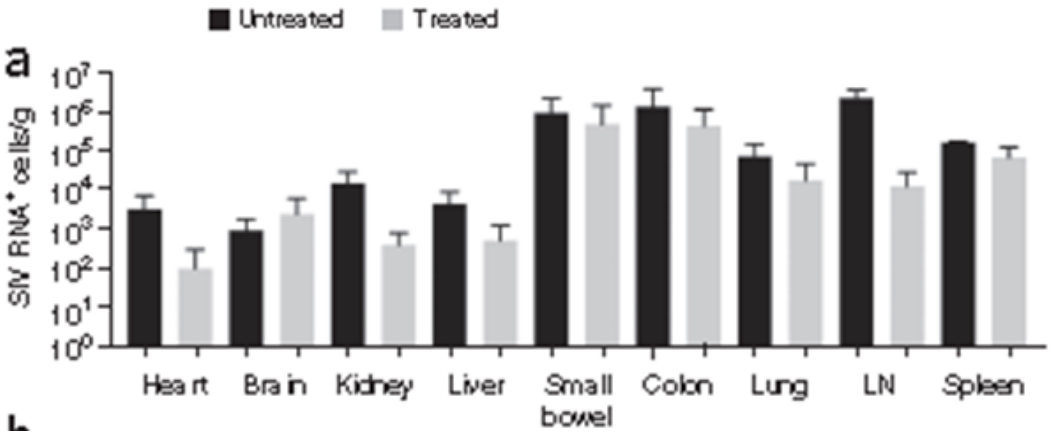
Impact of HIV/simian immunodeficiency virus infection and viral proteins on adipose tissue fibrosis and adipogenesis

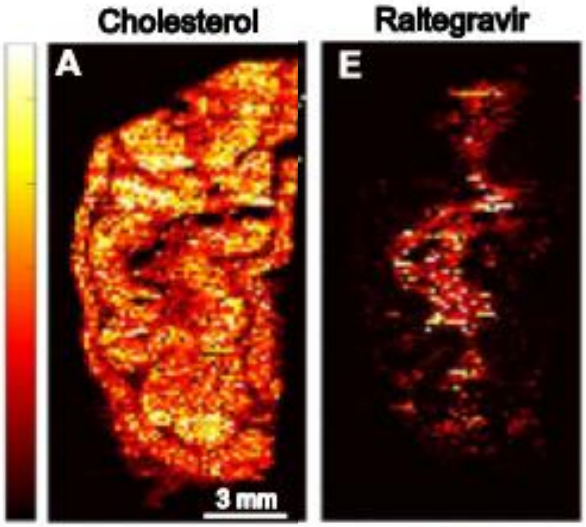
Gorwood, Jennifer[a]; Bourgeois, Christine[b]; Mantecon, Matthieu[a]; Atlan, Michael[a,c]; Pourcher, Valérie[d]; Pourcher, Guillaume[e]; Le Grand, Roger[b]; Desjardins, Delphine[b]; Fève, Bruno[a,f]; Lambotte, Olivier[b,g]; Capeau, Jacqueline[a]; Béréziat, Véronique[a,*]; Lagathu, Claire[a,*]

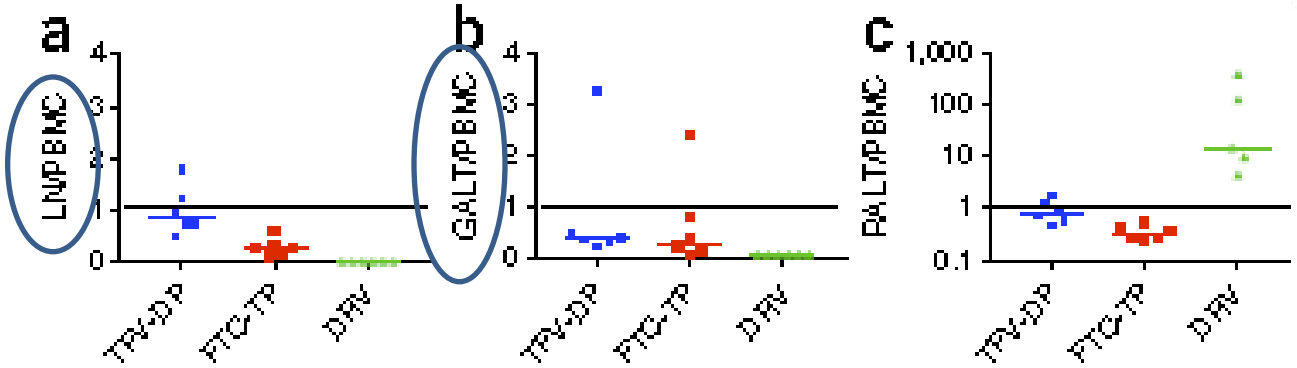
AIDS: May 1, 2019 - Volume 33 - Issue 6 - p 953–964

doi: 10.1097/QAD.0000000000002168

Insufficient drug diffusion could explain a limited impact on the tissular viral replication = under estimation of true viral reservoirs







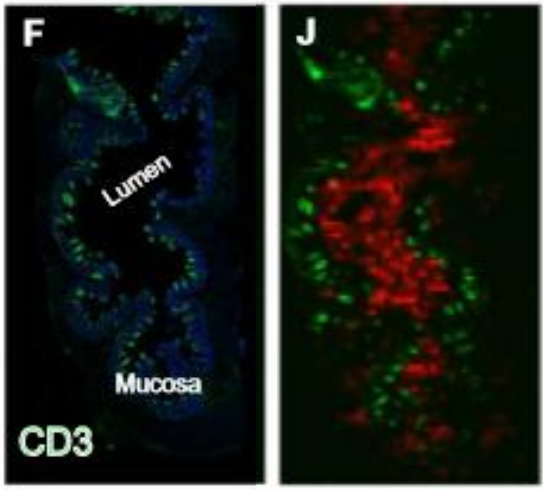
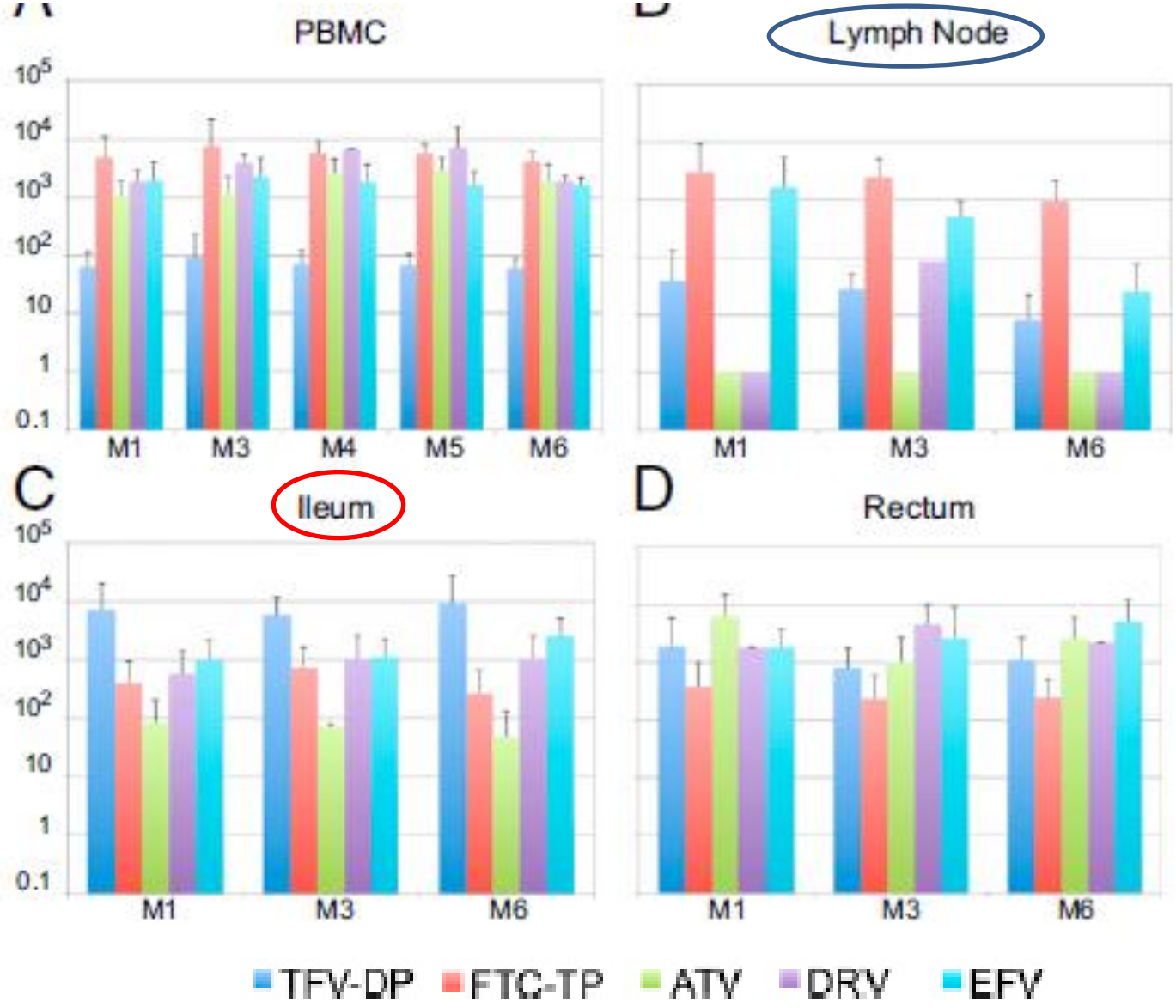


Figure 4 Intracellular concentrations of TFV-DP, FTC-TP, and DRV in LNs gut, and rectum, as compared with simultaneous measurements in PBMC

Estes et al. Nat Med 2017

Thompson al. Science Transl Med 2019

Insufficient ART diffusion in lymph nodes and gut in humans



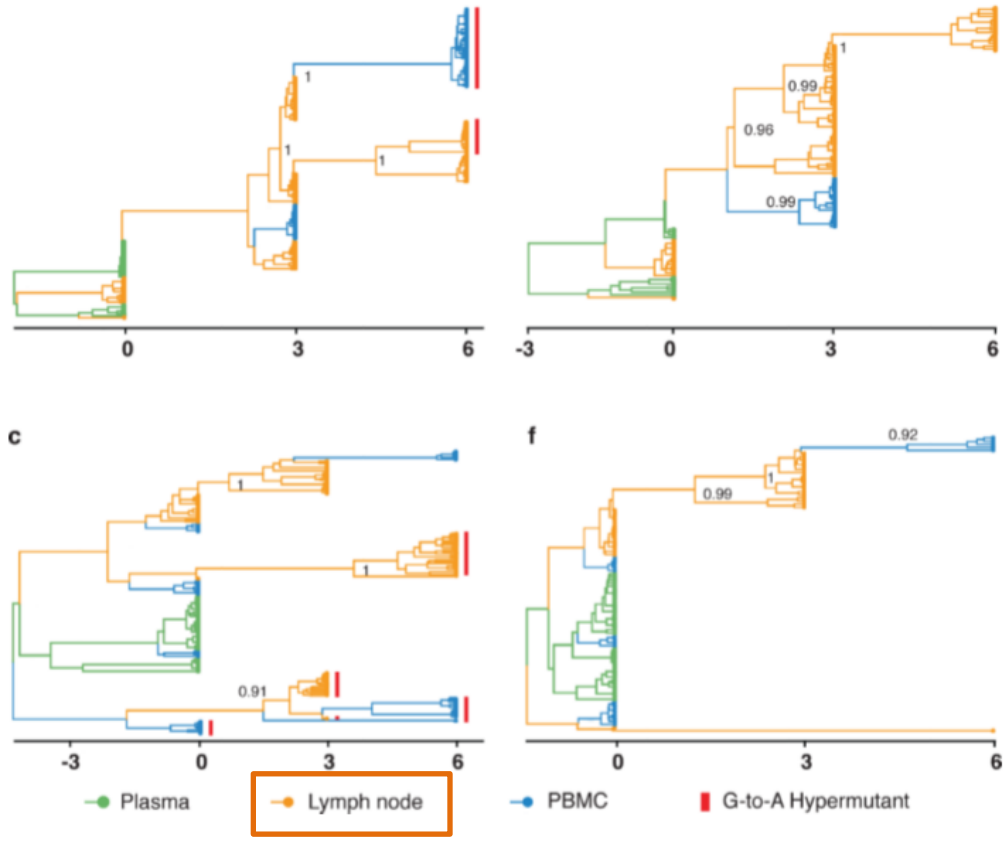
Fletcher C et al.
PNAS 2014

The persistence of HIV replication leads to viral strains evolution inside the tissues

Nature. 2016 Feb 4;530(7588):51-56. doi: 10.1038/nature16933. Epub 2016 Jan 27.

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

Lorenzo-Redondo R[#1], Fryer HR[#2], Bedford T[3], Kim EY[1], Archer J[4], Pond SLK[5], Chung YS[6], Penugonda S[1], Chipman J[7], Fletcher CV[8], Schacker TW[9], Malim MH[10], Rambaut A[11], Haase AT[12], McLean AR[2], Wolinsky SM[1].



. Time-structured phyloanatomic history of haplotypes in lymph nodes and blood

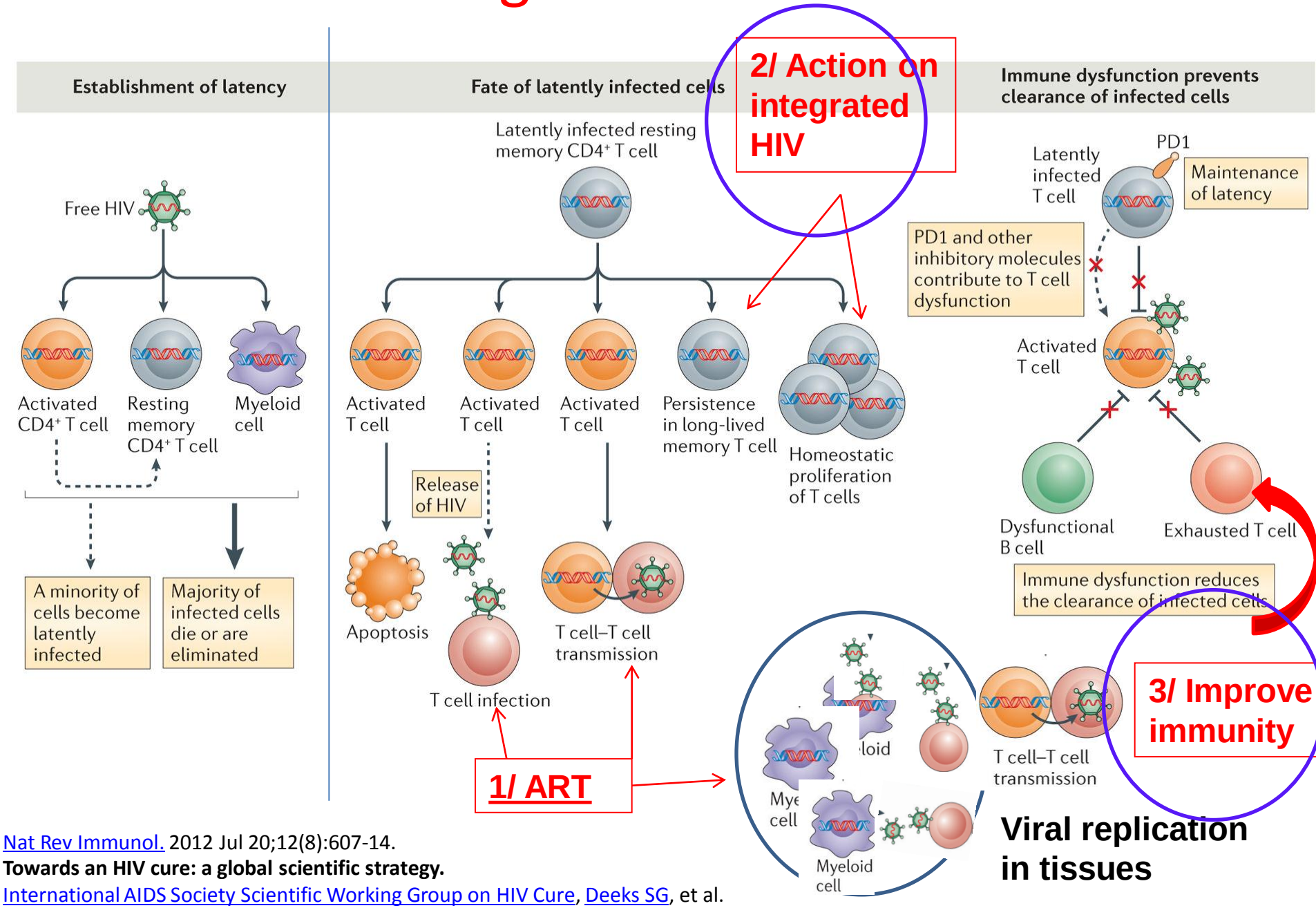
High drug concentrations = no possible viral replication

Low drug concentrations

Equilibrium ?

Emergency to study tissues!

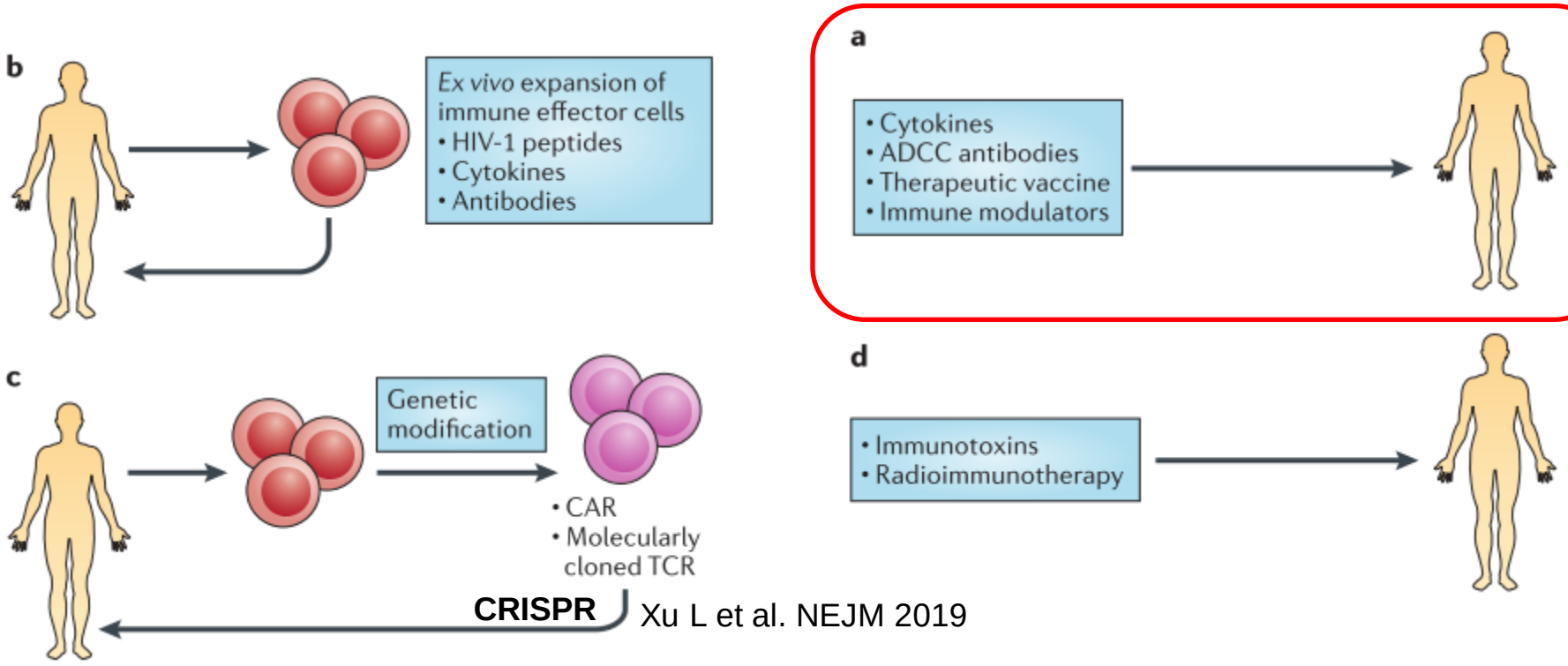
How to get HIV remission ?



Nat Rev Immunol. 2012 Jul 20;12(8):607-14.
Towards an HIV cure: a global scientific strategy.
International AIDS Society Scientific Working Group on HIV Cure, Deeks SG, et al.

Improve HIV-specific immune response… a challenge

- How to make a patient, elite controller



Archin et al. Nat Rev Microbiol 2014

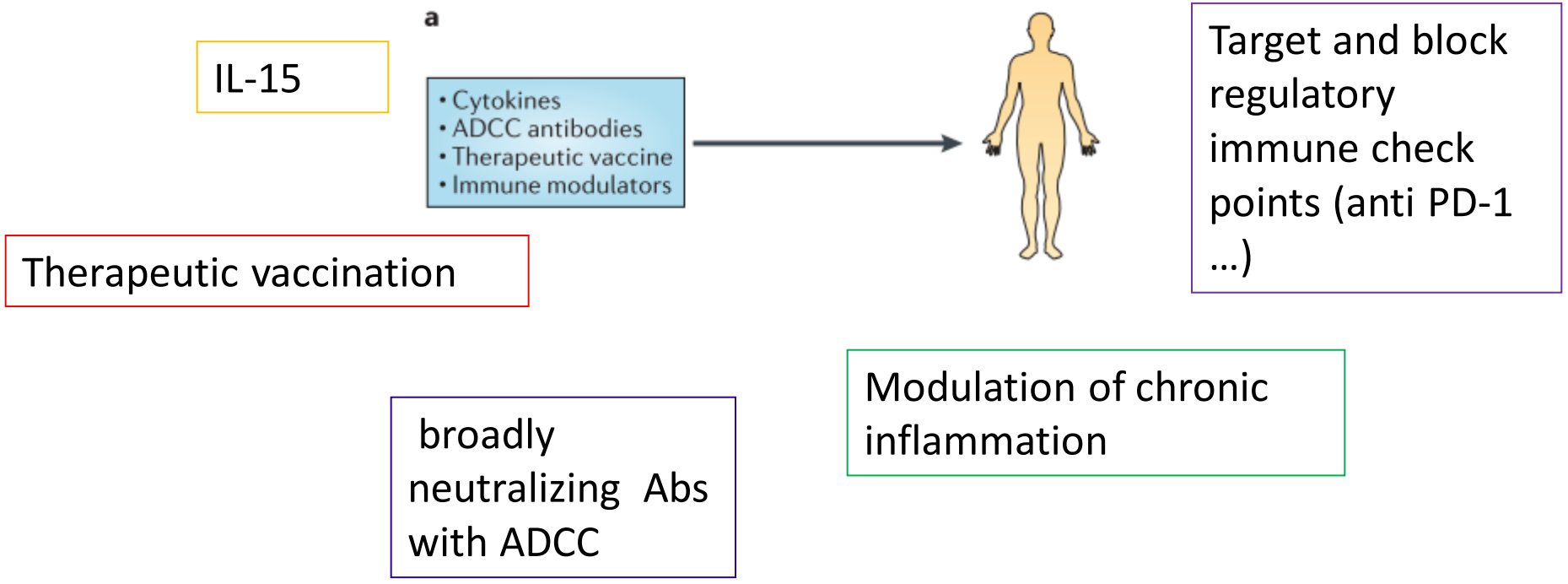
Xu L et al. NEJM 2019

Ex vivo manipulations of the immune system …

In vivo modulation of the immune system

Improve HIV-specific immune response… a challenge

- How to make a patient, elite controller = immunotherapy



Conclusion

- ART have to be optimized
- We have to focus on tissues (Human and NHP models)
  - Technical tools like imagery
  - Take into account tissues in clinical trials
- Importance of immunotherapy which can act into the tissues (like in cancer)
- Cure “eradication”…. ☹
- Cure “remission”……(a long time)…… ☺?

A long way to HIV remission…

- We know many things
- Some are more important than others… which ones ?
- We still lack some important information to win… tissues…

We have to work together within the HIV community and with other fields of interest (oncology, metabolism…)

Acknowledgements: the EP 36 study group / ANRS Cohort CODEX

- INSERM UMR 1184 Bicêtre / FAR
  - Dr Bruno Vaslin
  - Dr Benoit Favier
  - Dr Camille Lecuroux
  - Dr Nicolas Noel
  - Dr Christine Bourgeois
  - Dr Roger Legrand
- INSERM U 1018 Bicêtre
  - Pr Laurence Meyer
  - Dr Faroudy Boufassa
  - Dr Azeb Tadesse

Unit of Retroviruses, Pasteur Institute, Paris
  - Dr Gianfranco Pancino
  - Dr Asier Sáez-Cirión
  - Pr F Barré-Sinoussi
- Department of Molecular Medicine, Pasteur Institute, Paris
  - Dr Lisa Chakrabarti
- Virology Laboratory at Necker Hospital (Paris)
  - Dr Véronique Avettand Fenoel
  - Pr Christine Rouzioux
- INSERM U 778 (Strasbourg)
  - Dr Christiane Moog

Immunology Laboratory at Pitié Hospital (Paris)

Pr Brigitte Autran

CNRS UMR 8147 at Necker Hospital (Paris)

Dr Jean Philippe Herbeuval
Dr Lucie Barblu

- The clinicians and nurses
- **The patients !**

- Collaboration PRIMO cohort
  - Pr C Goujard
- Collaboration CASCADE
  - Dr Yoann Madec / Pr Laurence Meyer
- Collaborations USA
  - Duke University
    - Pr Barton Haynes
    - Dr Guido Ferari
    - Dr Georgia Tomaras

- Adipose tissue study
  - Dr Nathalie Dejucq (Univ Rennes)
  - Pr Jacqueline Capeau (Univ P6)
  - Dr Claire Lagathu (Univ P6)

anRS

Grants from ANRS, Sidaction, University Paris Sud, INSERM, CEA

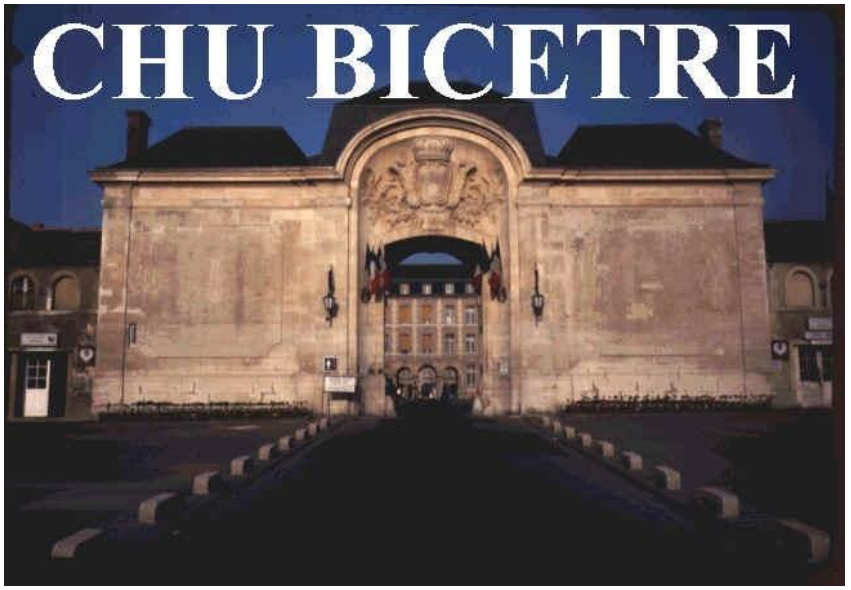
anRS

Agence nationale de recherches sur le sida et les hépatites virales
French national agency for research on AIDS and viral hepatitis

Inserm
Institut national de la santé et de la recherche médicale

cea

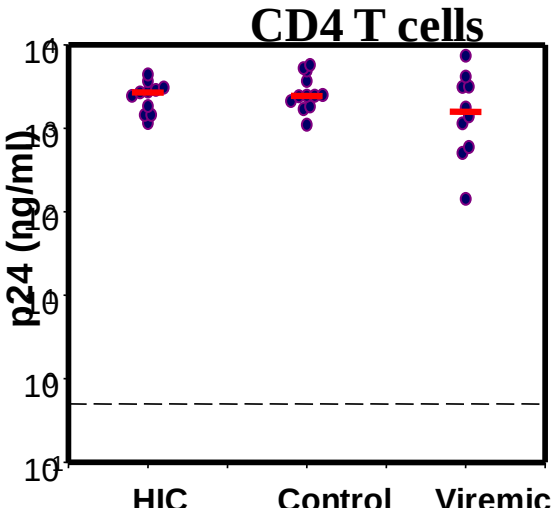
Sidaction
Ensemble Contre le Sida



The end…

Presence of saturable HIV restriction factors

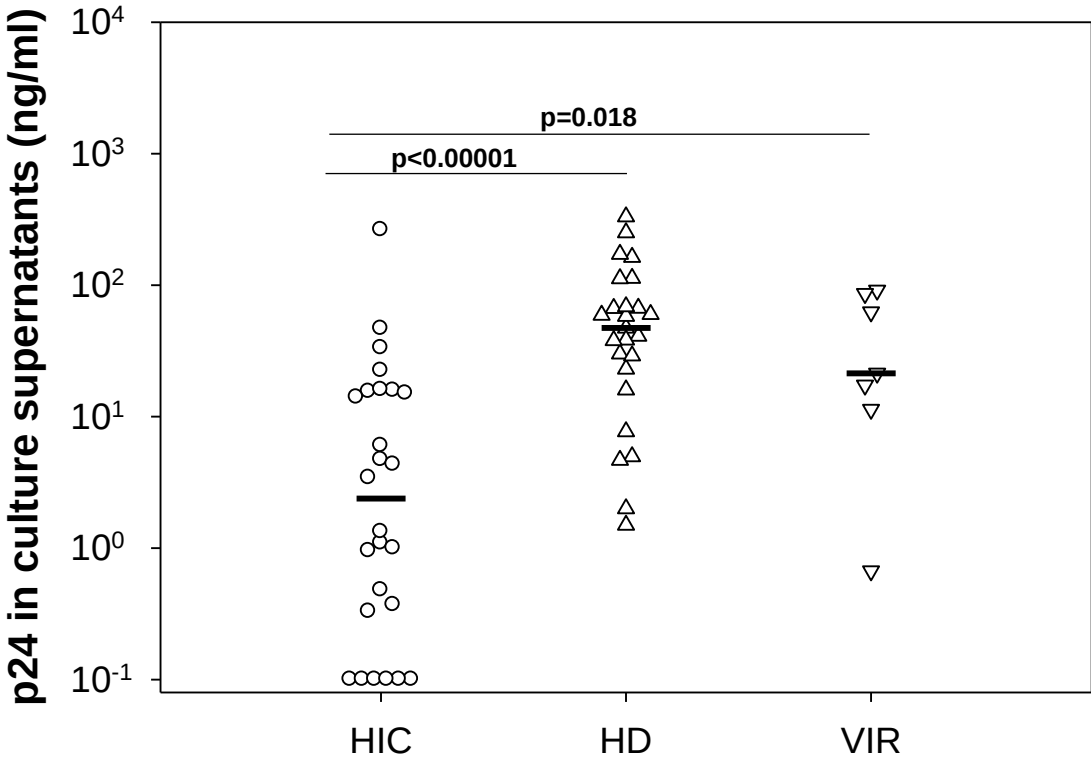
2006 : EC CD4 T cells can be infected by HIV



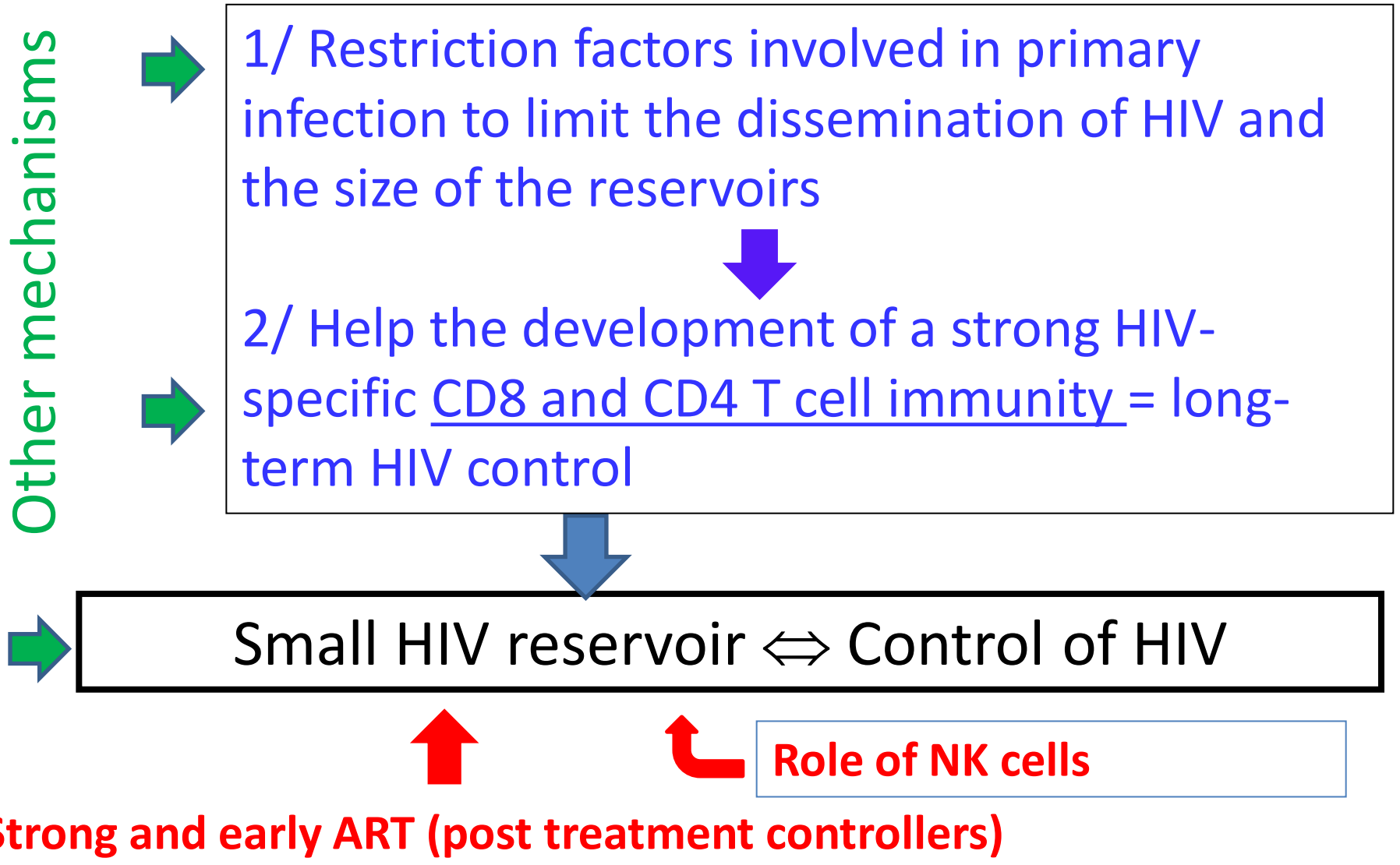
2011 : in suboptimal conditions of infection:

- **CD4⁺ T cells from HICs have low susceptibility to HIV-1 infection but the restriction mechanism is saturable**

(Saez-Cirion et al Blood 2011)



Proposal for a “remission” model



CD4 T cells and CAR-T

JCI The Journal of Clinical Investigation

About Editors Consulting Editors For authors Alerts Advertise Subscribe Contact

Current Issue Past Issues By specialty Videos Reviews Collections Clinical Medicine JCI This Month

Public T cell receptors confer high-avidity CD4 responses to HIV controllers

Daniela Benati,[1] Moran Galperin,[1] Olivier Lambotte,[2,3,4,5] Stéphanie Gras,[6,7] Annick Lim,[8] Madhura Mukhopadhyay,[1] Alexandre Nouël,[1] Kristy-Anne Campbell,[6] Brigitte Lemercier,[8] Mathieu Claireaux,[1] Samia Hendou,[9] Pierre Lechat,[10] Pierre de Truchis,[11] Faroudy Boufassa,[9] Jamie Rossjohn,[6,7,12] Jean-François Delfraissy,[2,3,4] Fernando Arenzana-Seisdedos,[1,13] and Lisa A. Chakrabarti[1,13]

First published April 25, 2016 - More info

Transmission of the properties of CD4 T cells EC TCR to allogenic CD8 T cells … ***CAR-T cells ?***

Persistence of a residual viral replication on HAART

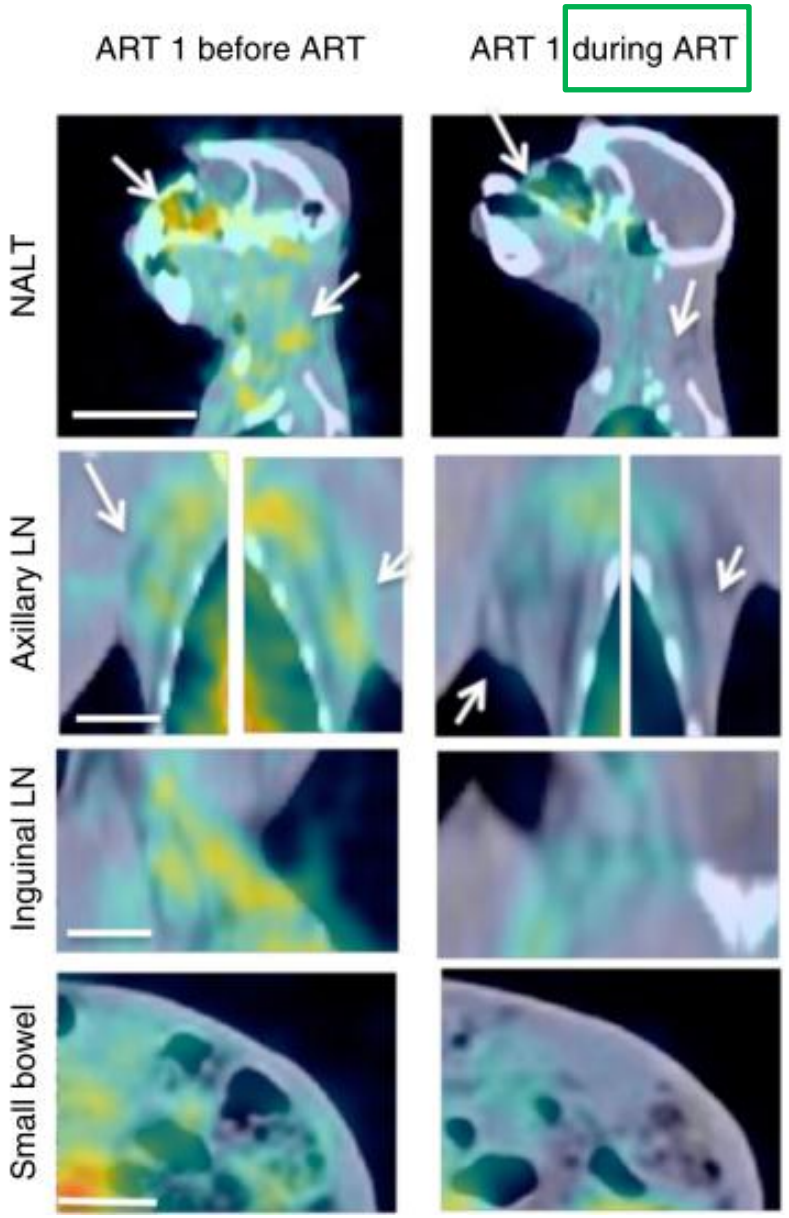
- Extremely low levels of plasma HIV RNA detectable on HAART (Dornadula et al. JAMA 1999, Palmer et al. PNAS 2008, Hatano et al. AIDS 2010)
- Persistance of HIV mRNA in PBMCs and tissues (Furtado et al NEJM 1999, Hockett et al. J Exp Med 1999)
- No relevant? Because no evidence of viral evolution neither development of drug resistance…
- But intensification with raltegravir decreased the us Viral load [usVL] (Buzon et al. Nat Med 2010)
- Large debate on the origins of the usVL: question of possible *de novo* cell infection despite HAART
  - Passive release of HIV RNA (Bailey 2006, Kieffer 2004, Nettles 2005, Persaud 2004)
  - Active cryptic viral replication in tissues (Bailey 2006, Sahu 2009)

Tissues and HIV persistence

- HIV could be present in <u>neglected</u> reservoirs

= the SIV model

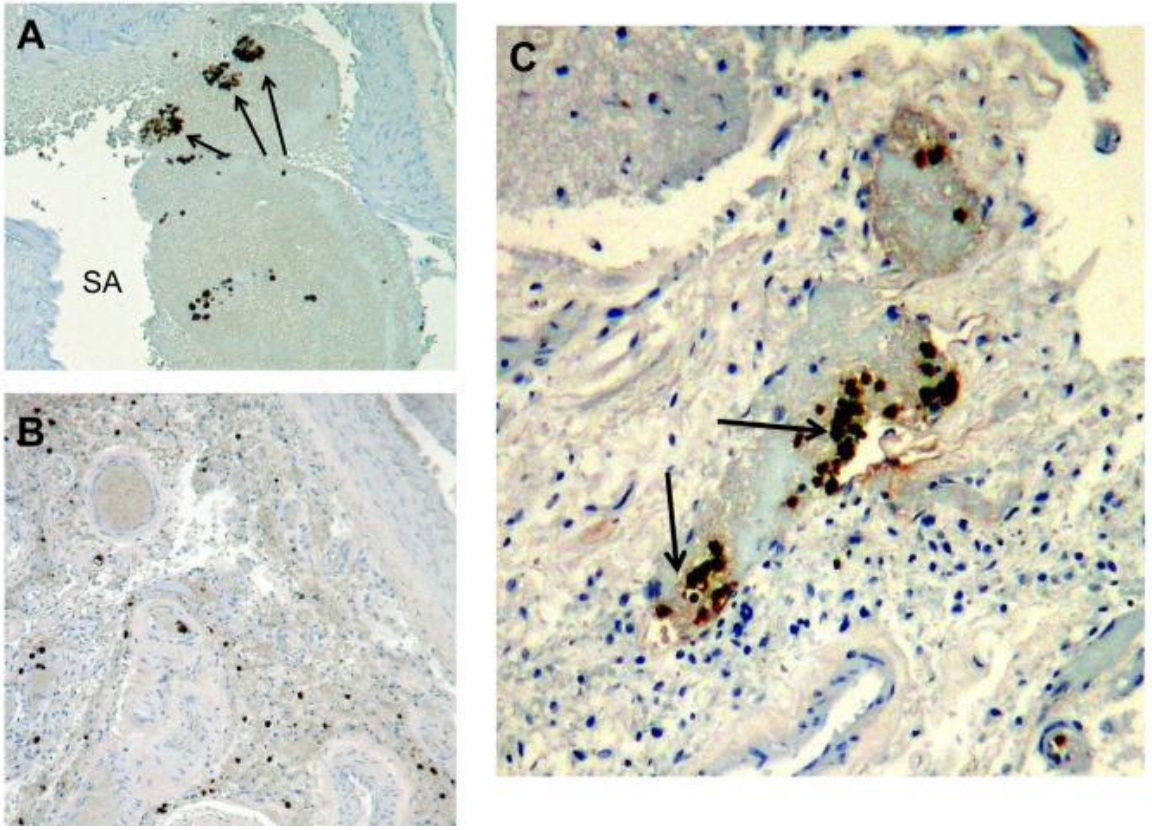
  - Colon and small bowel
  - select lymph nodes
  - the genital tract
  - <u>nasal turbinates</u>
  - <u>lungs</u>



Santangelo P & Villinger F., Nat Meth. 2015

Tissues and HIV persistence in patients on ART

- HIV is present in patients on ART
  - In the brain, in macrophages



HIV p24 stain of meninges: localization of perivascular and parenchyma macrophages (Lamers et al. Infect Genet Evol 2011)