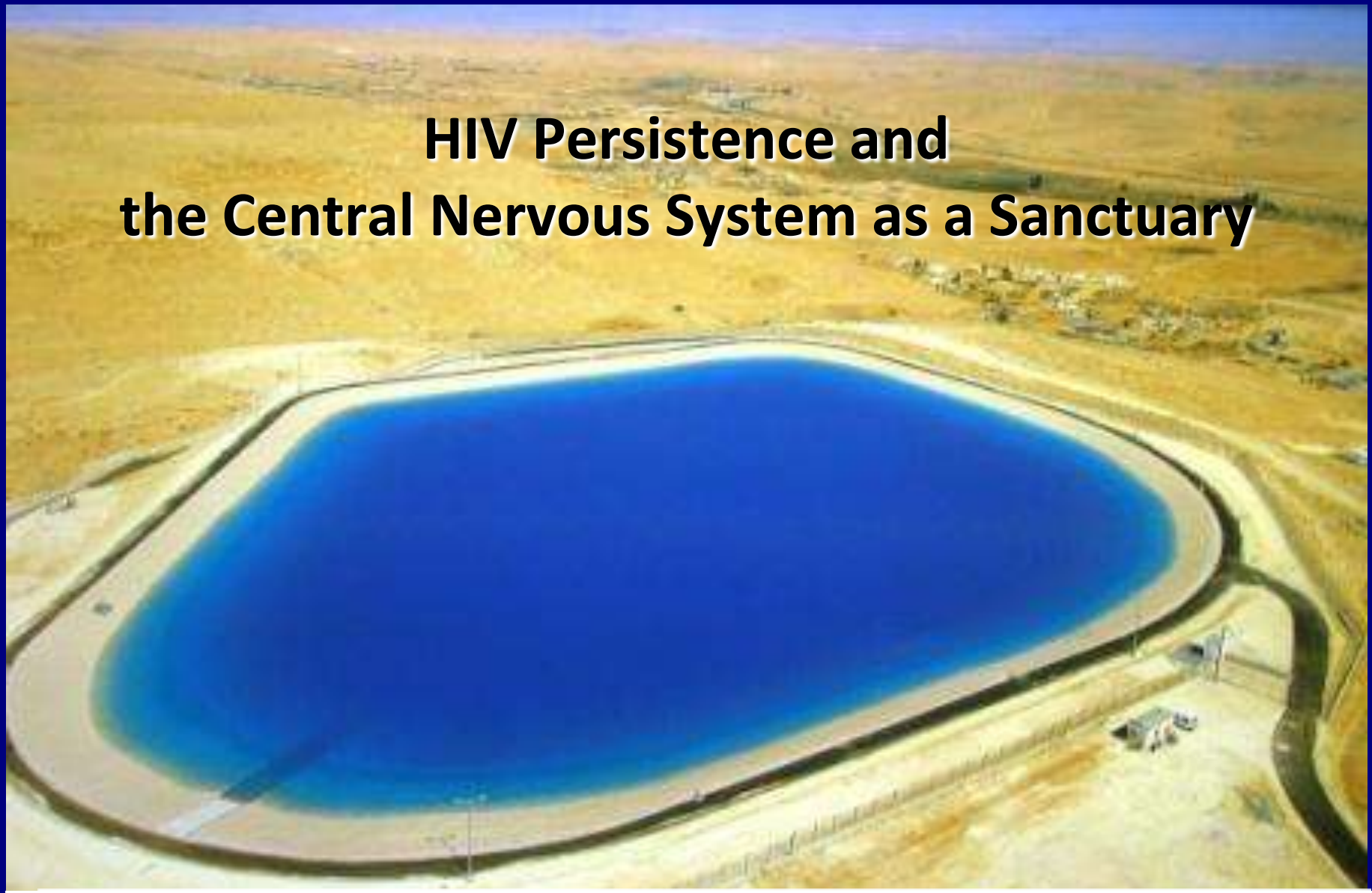
HIV Persistence and the Central Nervous System as a Sanctuary



THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

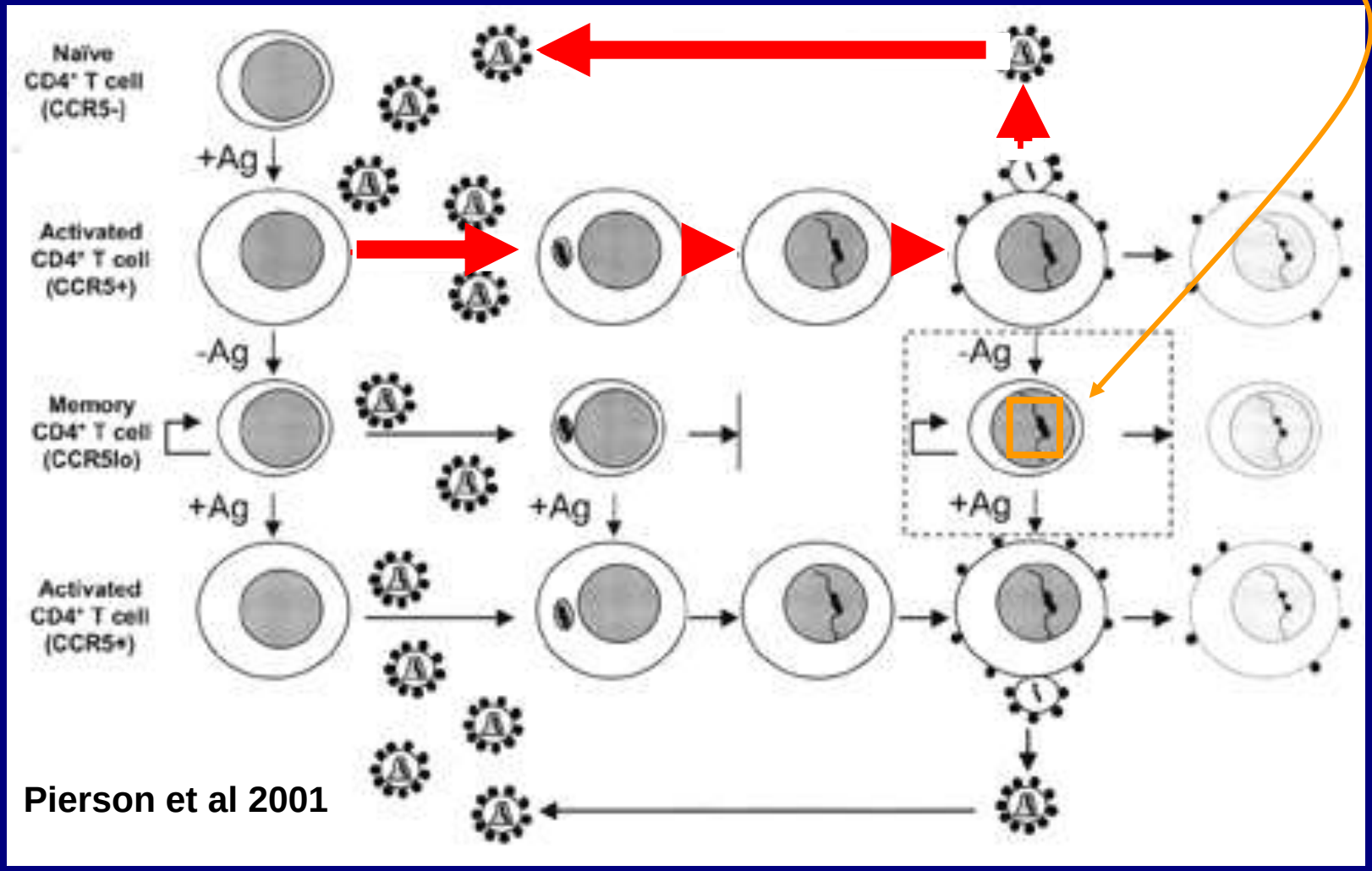
David M. Margolis, MD

Professor of Medicine, Microbiology & Immunology, Epidemiology

Persistent HIV Infection despite ART:

Is the CNS different?

Rare, persistent proviral genomes



Pierson et al 2001

Residual viral expression (without failure of ART)

a) **Pharmacologic and anatomic barriers to drug distribution and activity**
   - e.g. CNS penetration
   - Incomplete suppression in the tissues (eg. GALT)

b) **Persistent expression in long-lived cells**
   - Macrophages, brain microglia

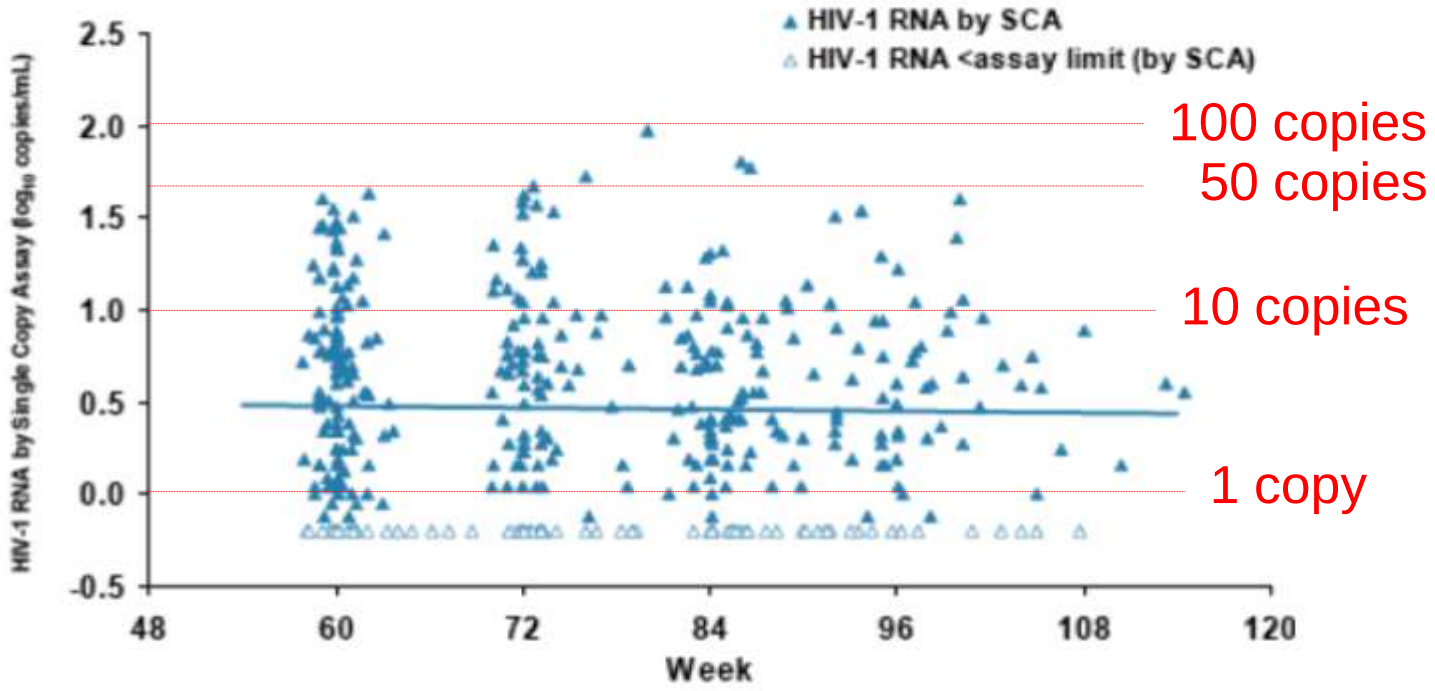
c) **Intermittent activation and release of virus from latently infected cells**
   - CD4+ cells
   - Recent renewal of the claim that marrow stem cells may be a long-term stable viral reservoir

The clinical detection of this event: **Low-level viremia**

- 1-copy assay Palmer et al. J Clin Micro 2003
- detected in ca. 75% ART-suppressed patients
- Level correlates with peak viremia, hypothesized to be “slow leak” from resting CD4+ cells by some, or from non-CD4 cells by others

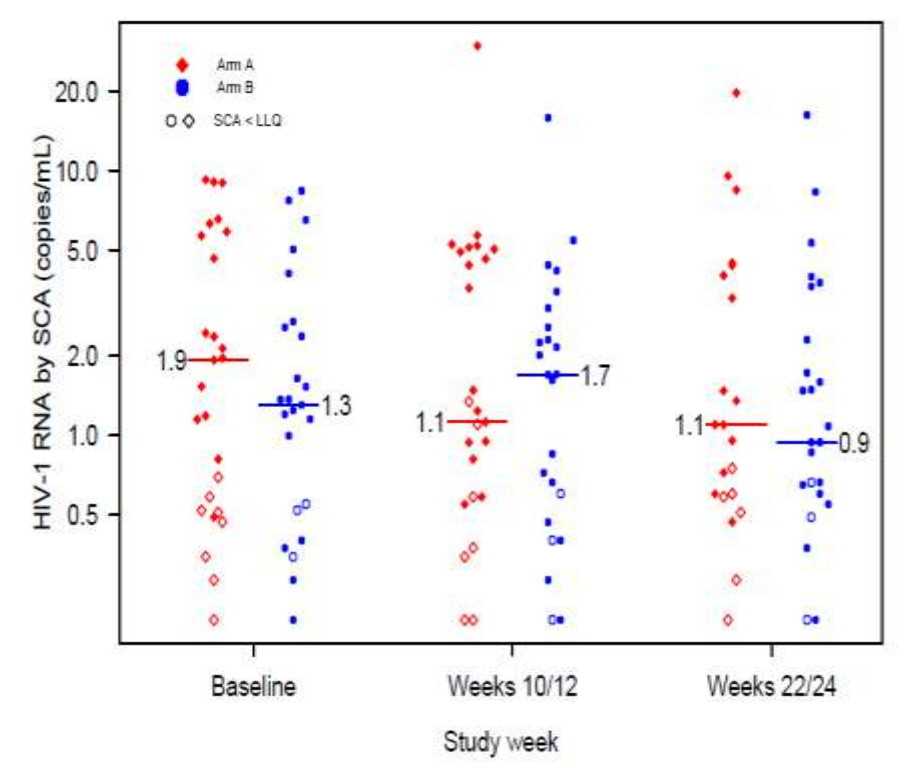
Persistent HIV *Production* despite ART

Figure 1. No decline in viremia setpoint over 60-110 weeks of therapy



Maldarelli et al. PLoS Path 2007

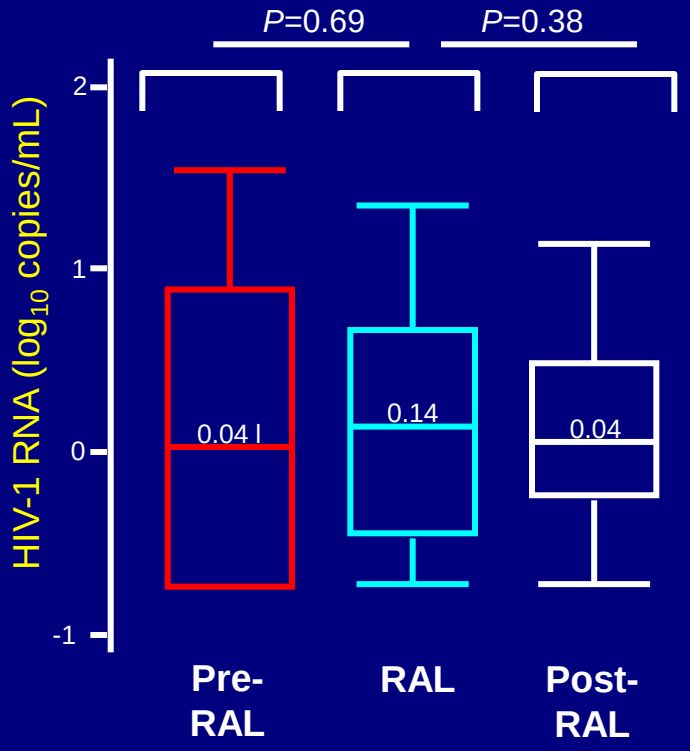
Low-level viremia is unaffected by intensification with PI, NNRTI, RAL, Enf



♦ **Arm A: RAL first, then placebo**

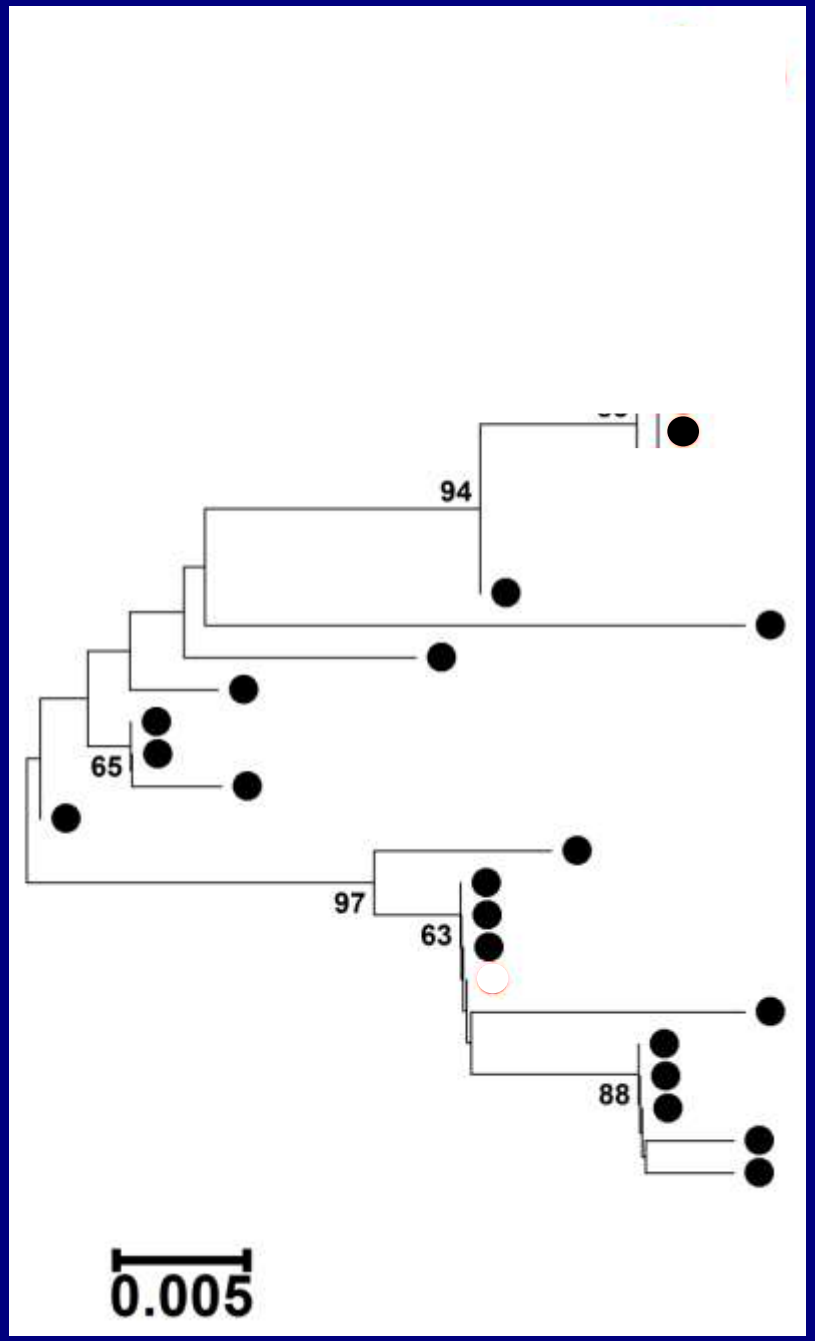
● **Arm B: Placebo first, then RAL**

Ghandi PLoS Med 2010



Raltegravir intensification does not reduce residual HIV-1 viremia in patients on highly active antiretroviral therapy Dinoso PNAS 2009

Single Genome sequences from resting CD4 cell outgrowth viruses ●



Anderson J Virology 2011

HIV Neuroimaging Consortium
Navia et al CROI 2011

- Prospective study of ~300 HIV-infected subjects across 7 centers
  - Brain bank centers at UCLA, UCSD and Harbor-UCLA
  - ACTG and primary HIV clinics Colorado, Pittsburgh, Harbor, Stanford
- Inclusion criteria:
  - Nadir CD4 count <200 cells/mL
  - ART for at least 12 weeks
- Exclusion criteria:
  - Confounding neurological, psychiatric and medical disorders (hepatic, renal, diabetes)
  - Active drug use

Conclusions

- Nadir CD4 prior to the initiation of treatment is significantly associated with persistent injury and NCI.

- Progressive brain injury likely due to the effects or several host and disease-related factors (or their interactions)
  - HIV RNA, immune activation, duration of treatment, duration of infection, and to a lesser extent, aging
  - Co-morbid disorders (hep C, cardiovascular risk factors) may also contribute.

Ongoing viral expression in the CNS
(as there is in the periphery)

- Some evidence that HIV-associated brain injury can continue to unfold in the setting of stable HIV disease and treatment.
- Ongoing evidence of markers of inflammation or injury in the CSF in some studies

- Is that different from the periphery?
- Is it ameliorated by earlier therapy?
- If patients are not treated until later disease, will adjunctive therapy (other than ART) be needed?

CNS Penetration-Effectiveness Ranks 2010

	4	3	2	1
NRTIs	**Zidovudine**	**Abacavir** Emtricitabine	Didanosine **Lamivudine** Stavudine	Tenofovir Zalcitabine
NNRTIs	Nevirapine	Delavirdine Efavirenz	Etravirine	
PIs	Indinavir/RTV	Darunavir/RTV Fosamprenavir/RTV Indinavir **Lopinavir/RTV**	Atazanavir Atazanavir/RTV Fosamprenavir	**Nelfinavir** Ritonavir Saquinavir Saquinavir/RTV Tipranavir/RTV
Entry/Fusion Inhibitors		Maraviroc		Enfuvirtide
Integrase Inhibitors		Raltegravir		

Letendre S, et al. CROI 2010. Abstract 172

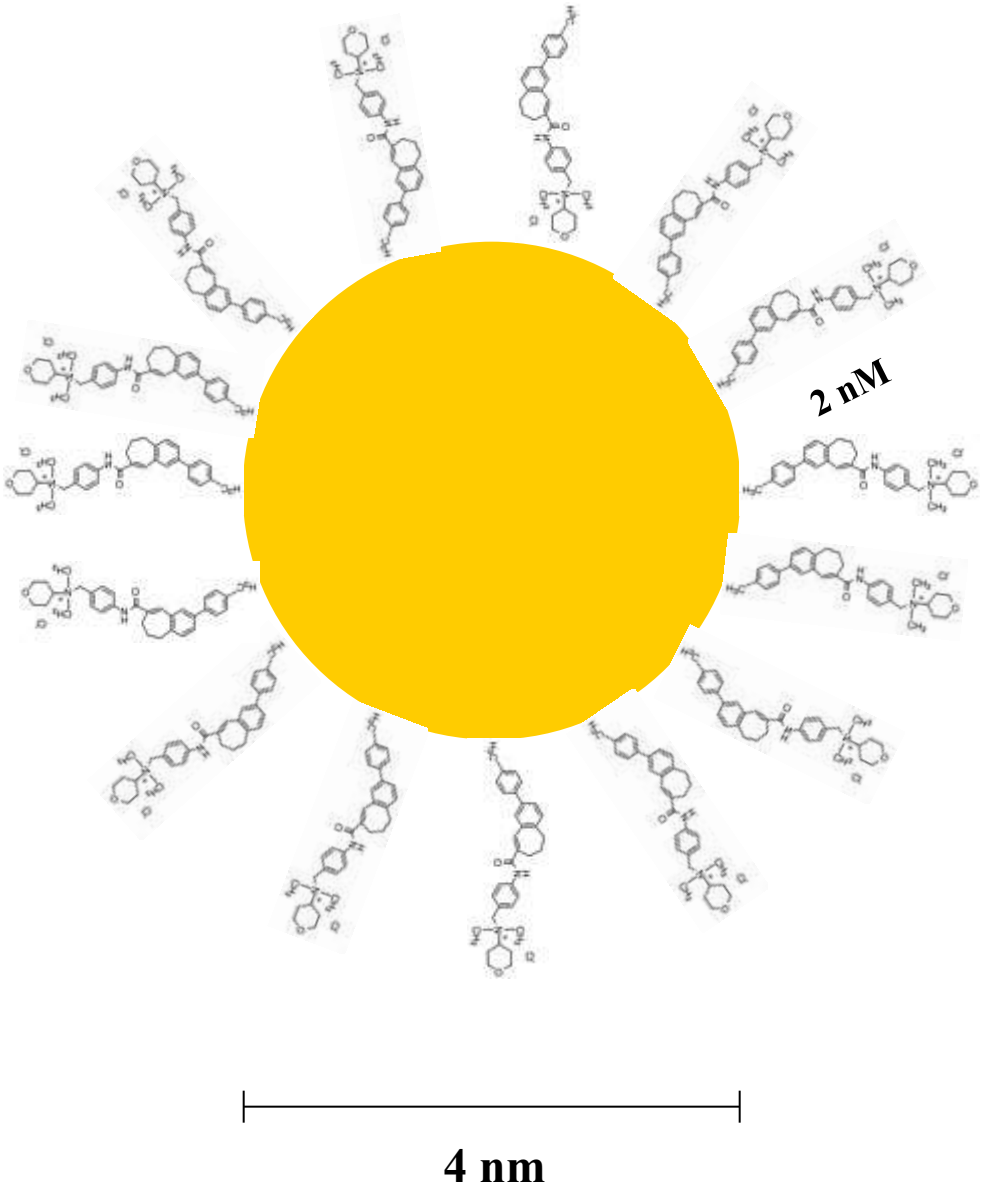
Improved Delivery of Standard ART

Macrophage Delivery of Nanoformulated Antiretroviral Drug to the Brain in a Murine Model of NeuroAIDS[1]

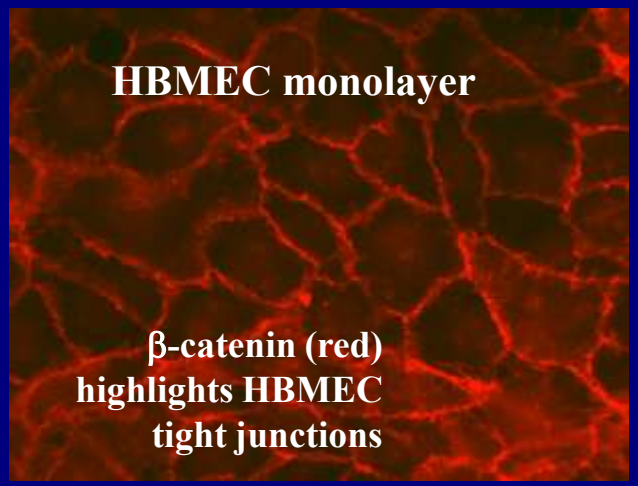
Huanyu Dou,* Cassi B. Grotepas,* JoEllyn M. McMillan,* Christopher J. Destache,[‡] Mahesh Chaubal,[§] Jane Werling,[§] James Kipp,[§] Barrett Rabinow,[§] and Howard E. Gendelman[2]*[†]

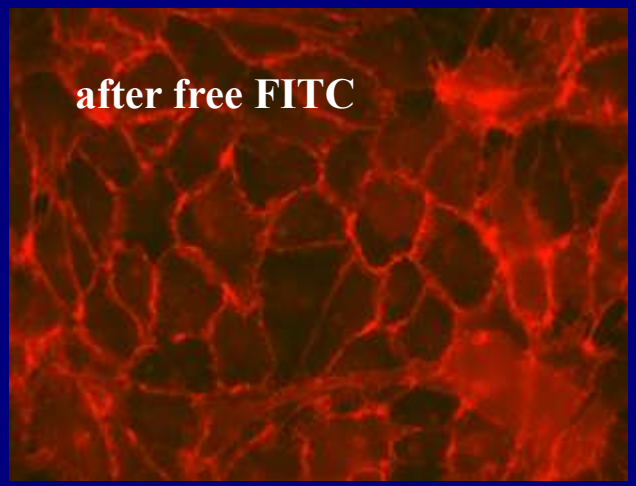
The Journal of Immunology, 2009, 183, 661-669

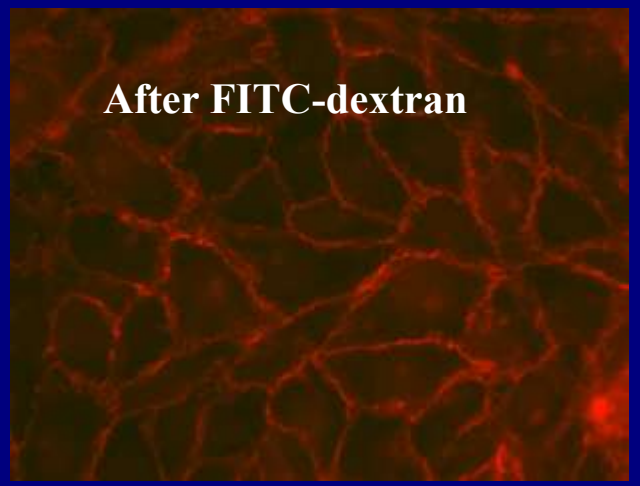
- **Gold nanoparticle preparations with 1 to 170 inhibitor moieties**
- **Covalently linked to 4 nm gold particle by thiol linker**

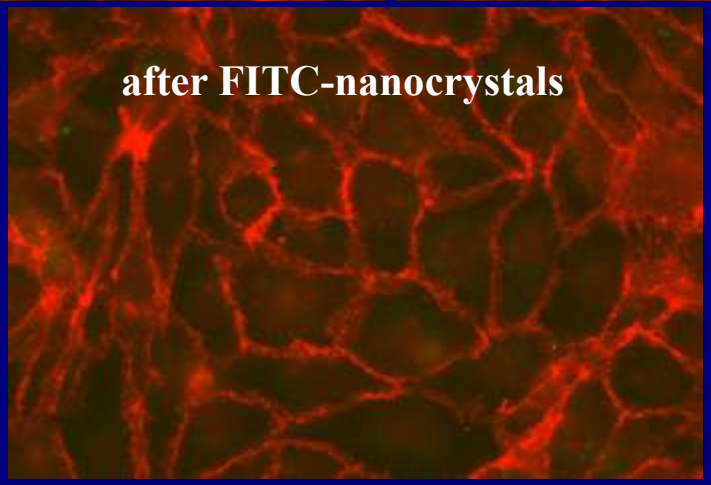


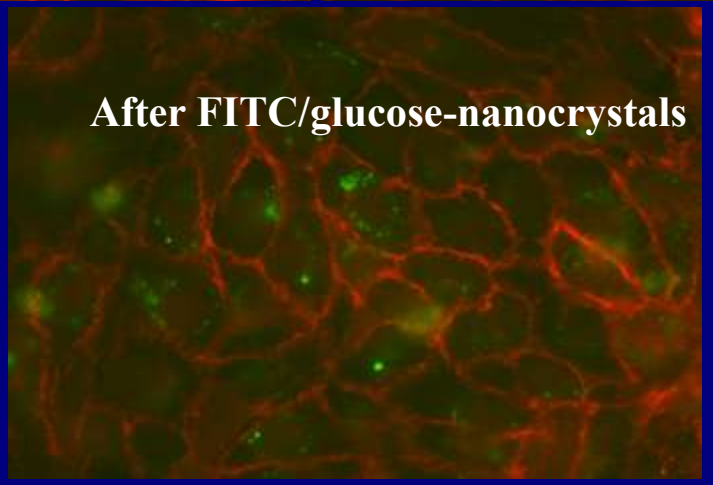
Bowman JACS 2008



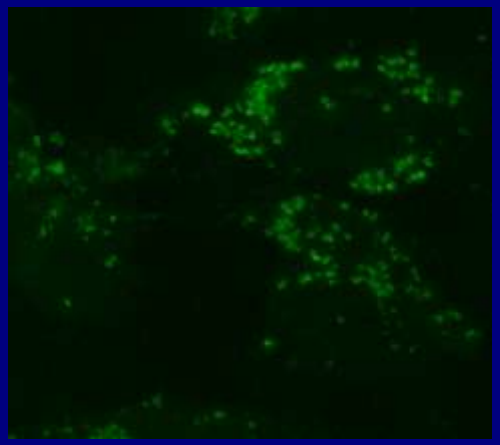


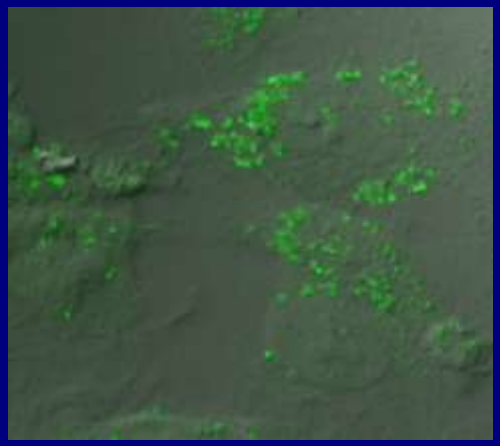






confocal image across central cell axis, illustrating intracellular entry of NC

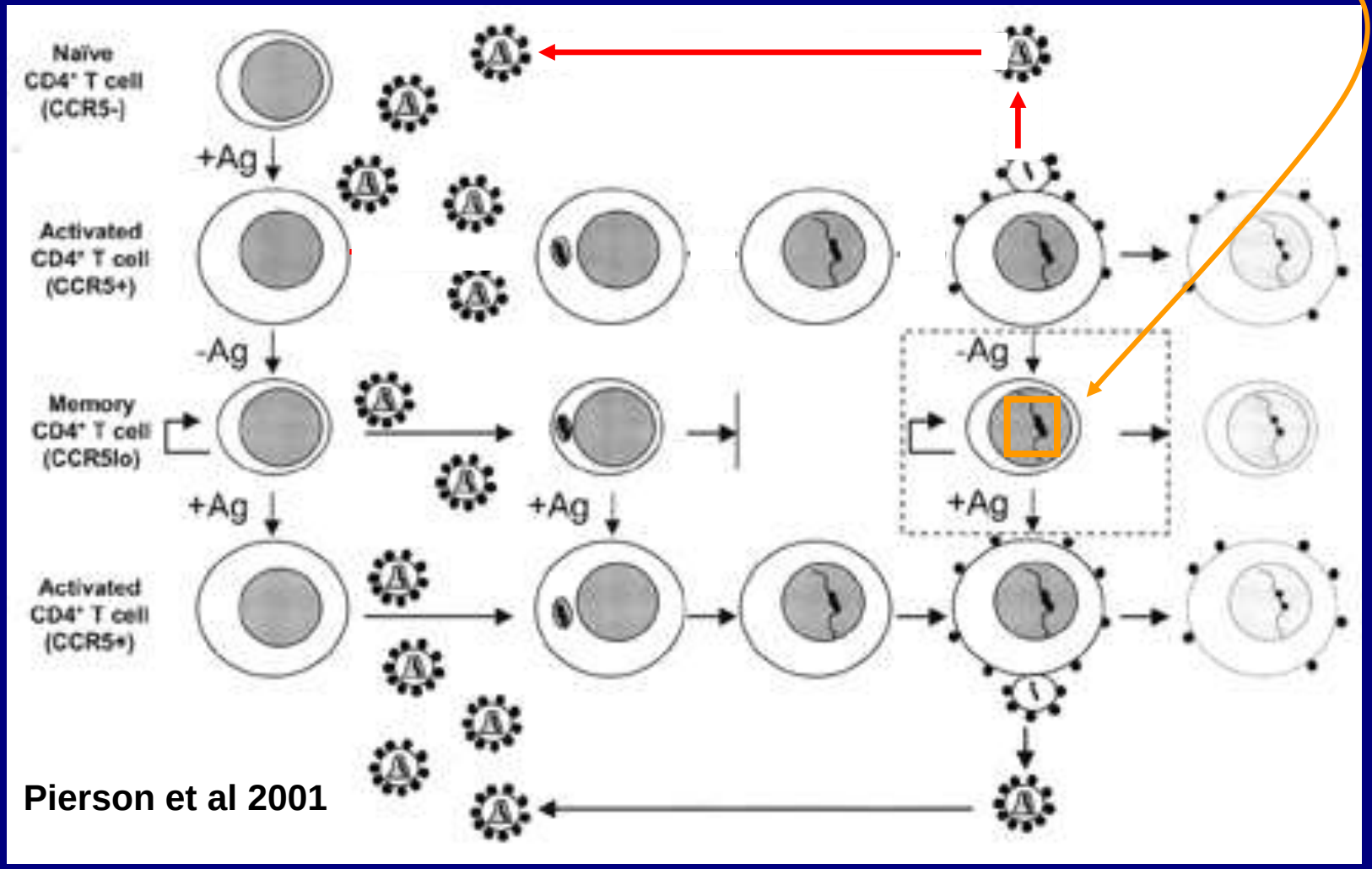




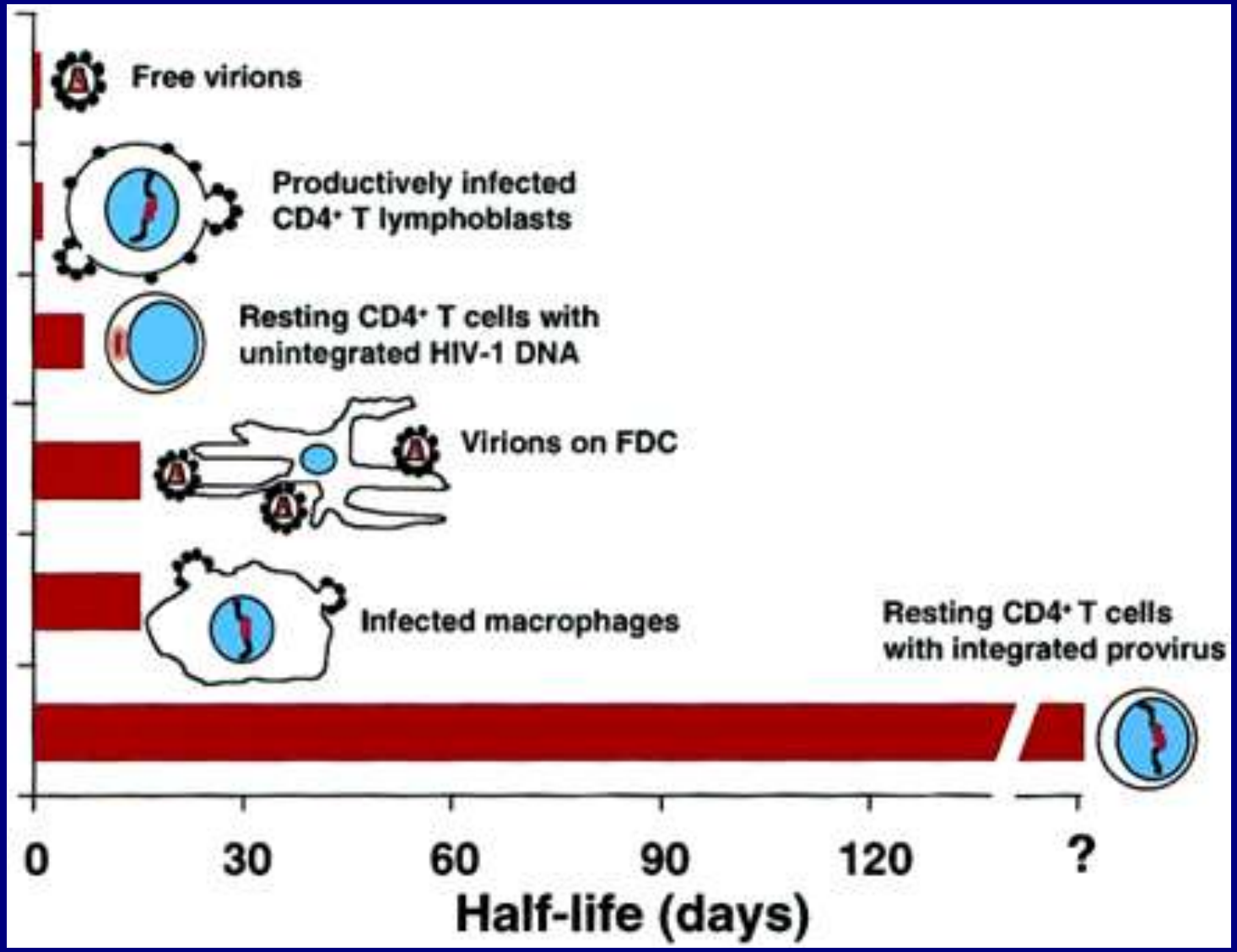
confocal image overlaid with phase contrast

Persistent HIV Infection despite ART

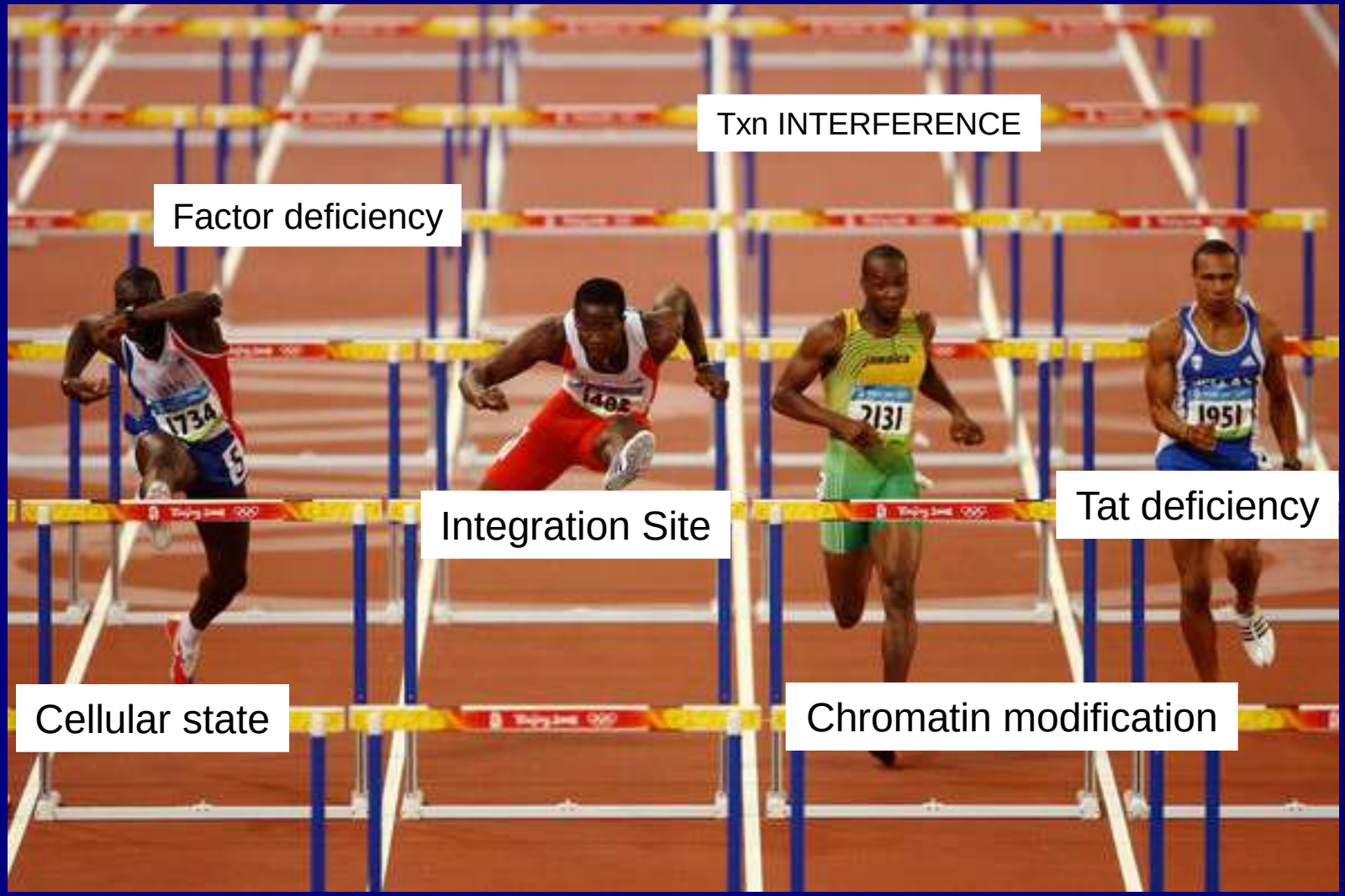
Rare, persistent proviral genomes



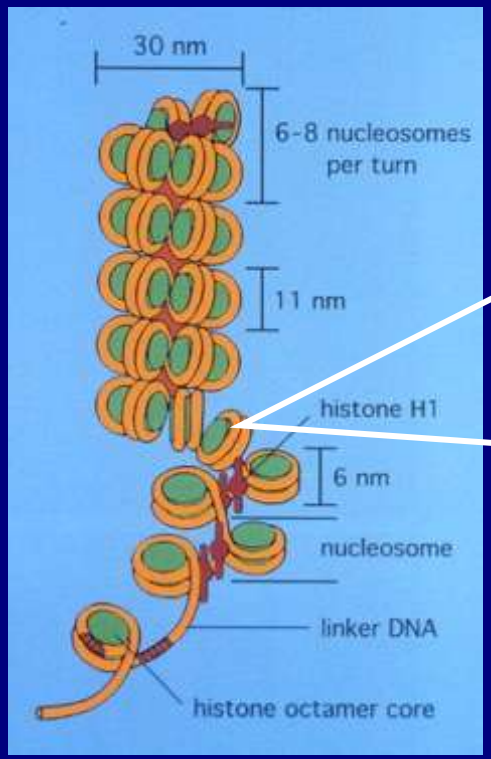
Pierson et al 2001


Free virions
Productively infected
CD4+ T lymphoblasts
Resting CD4+ T cells with
unintegrated HIV-1 DNA
Virions on FDC
Infected macrophages
Resting CD4+ T cells
with integrated provirus
0
30
60
90
120
?
Half-life (days)

MULTIPLE HURDLES TO VIRAL ESCAPE FROM LATENCY

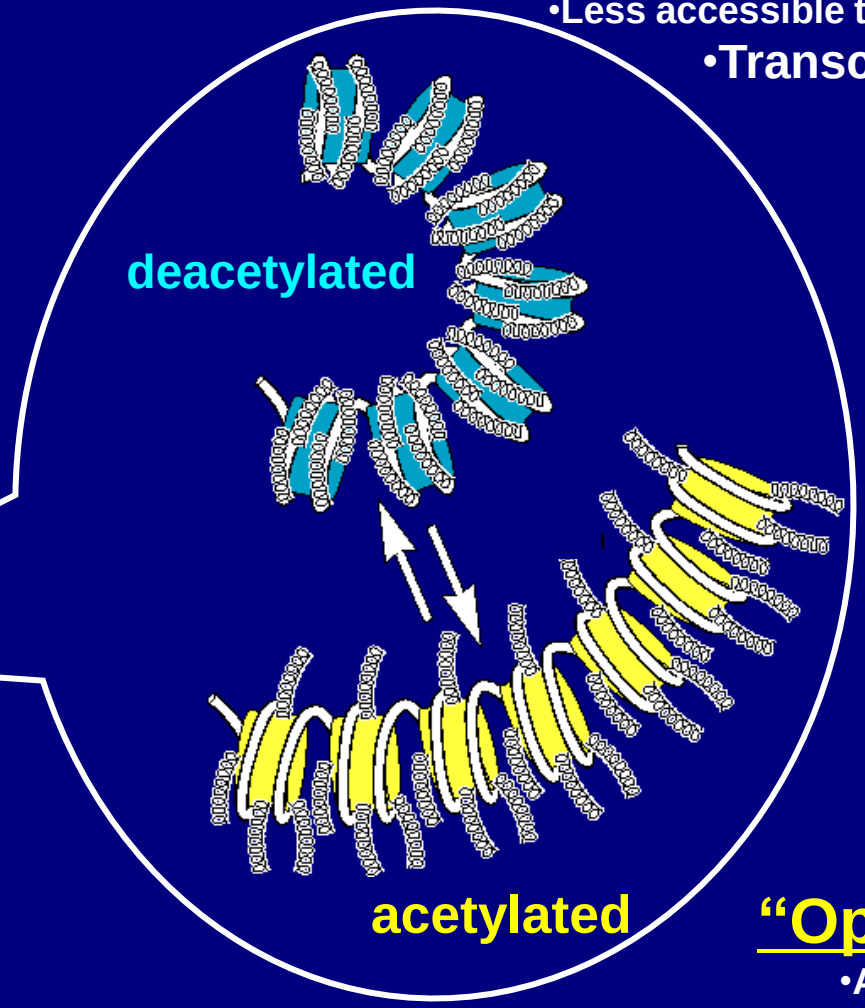


HIV lives within chromatin



"Closed" Nucleosome

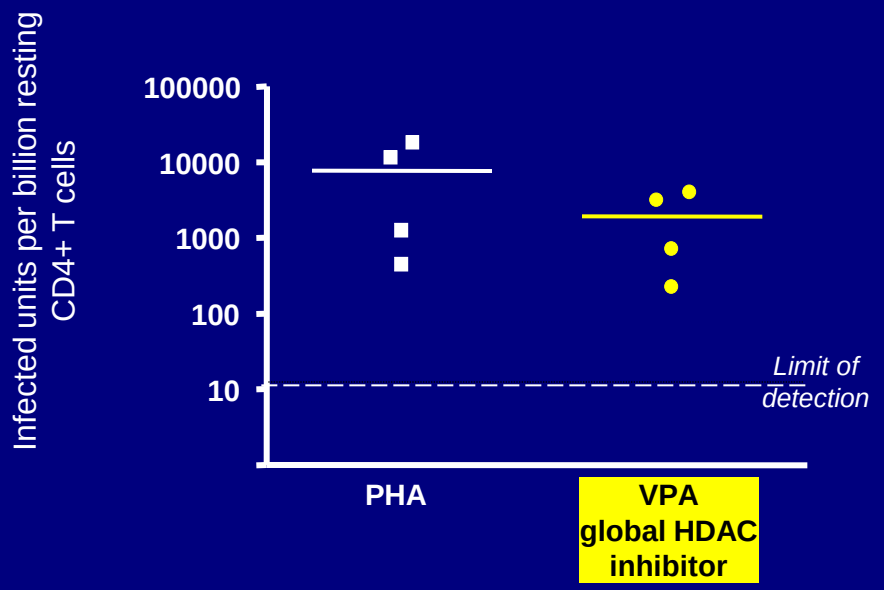
- Hypo-Acetylated Histone tails
- Stable, Compact Chromatin
- Less accessible to Transcription Factors
- Transcription Repressed

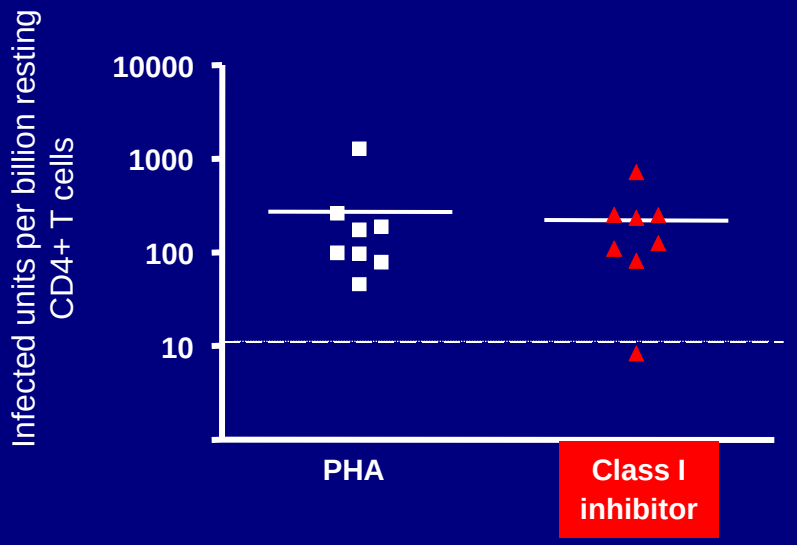


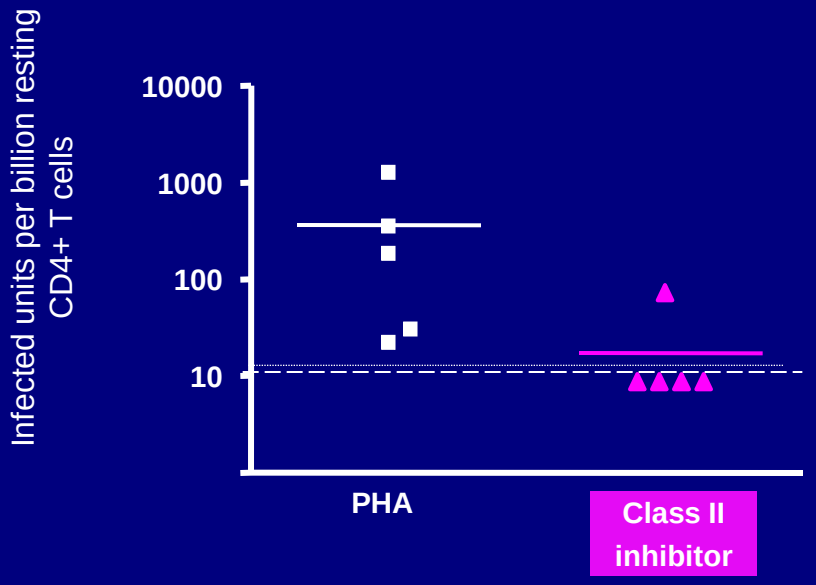
"Open" Histones

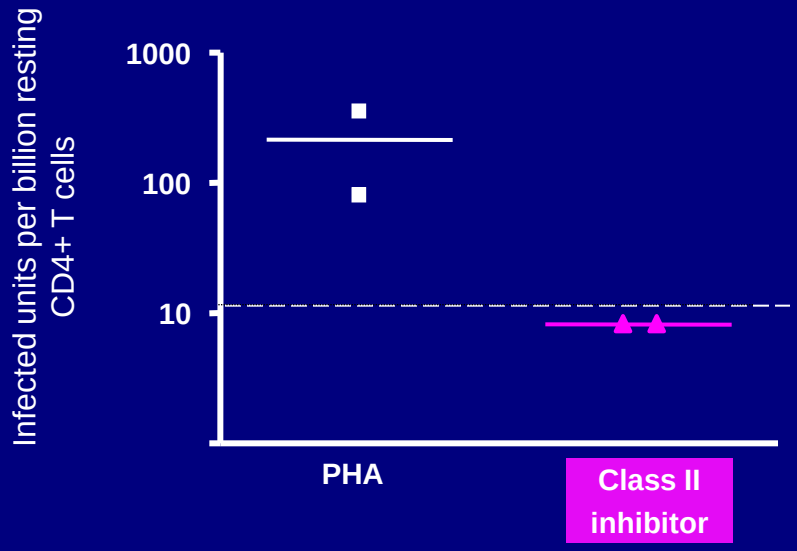
- Acetylated Histone tails
- Reduced Higher Order Structure
- Access to Transcription Factors
- Transcription Active

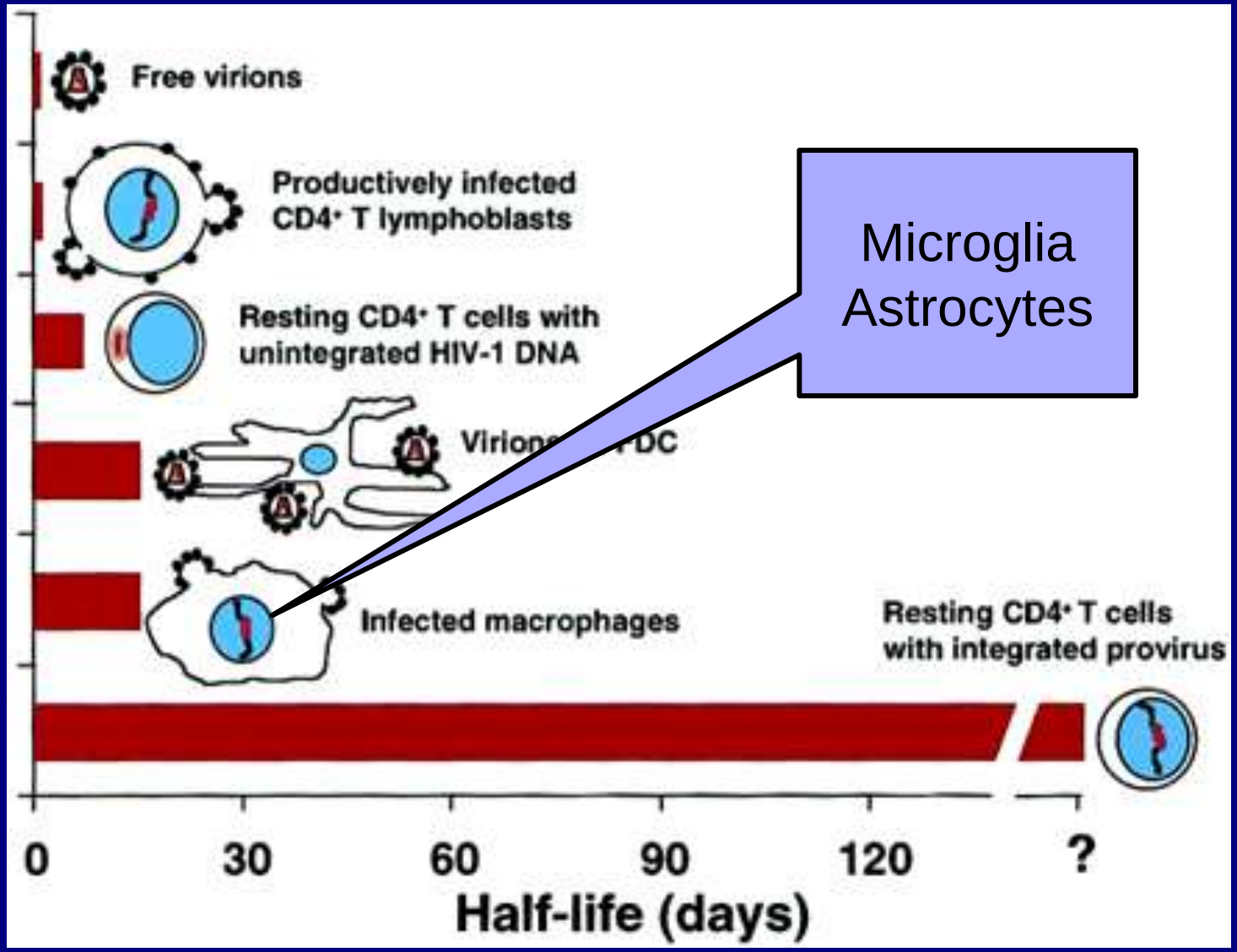
Archin *AIDS 2009*

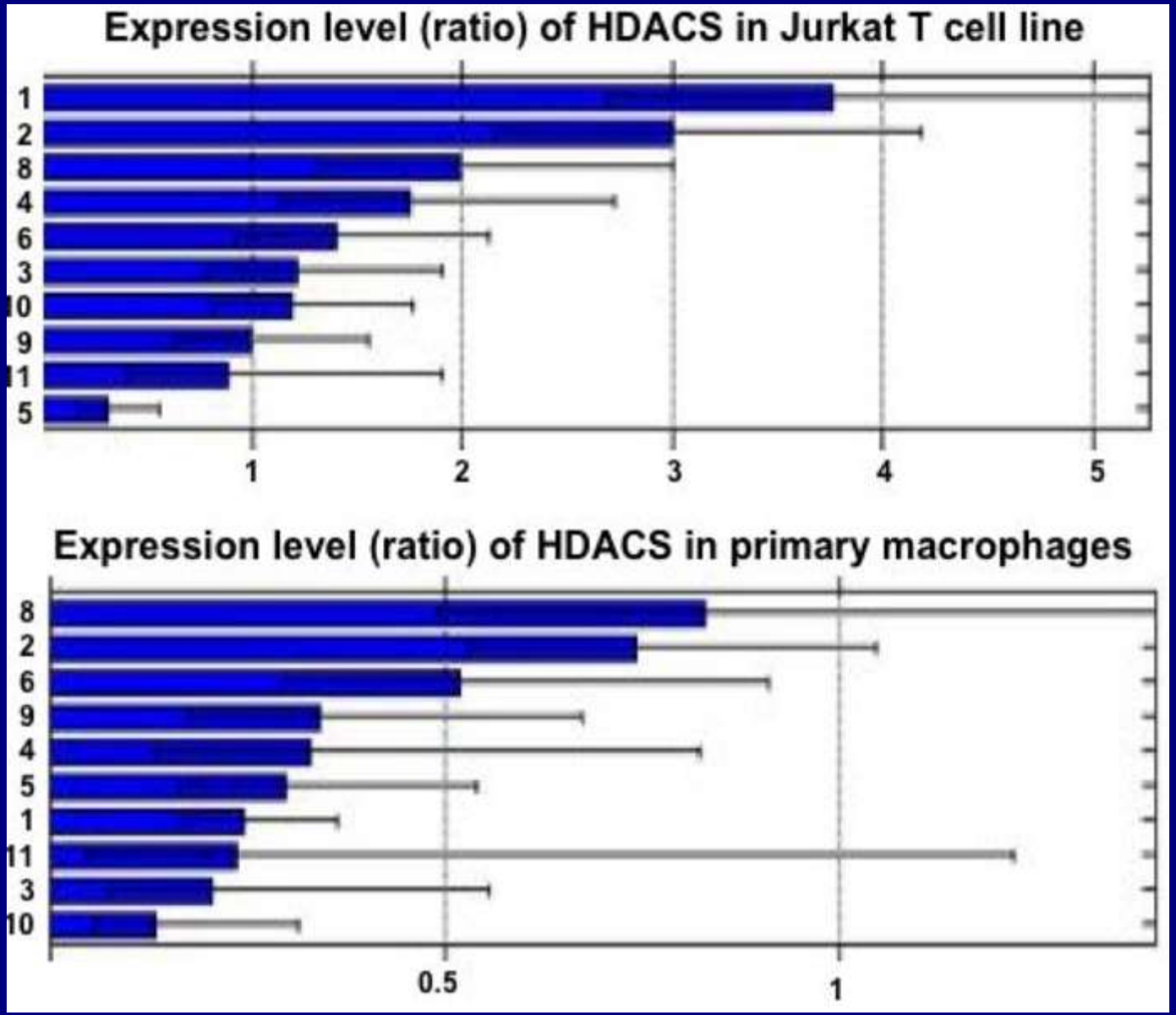




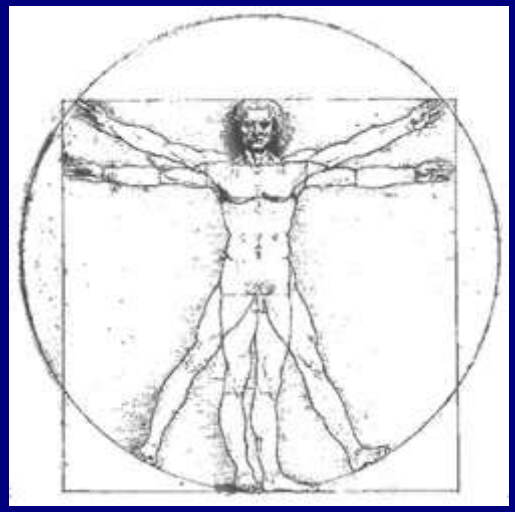


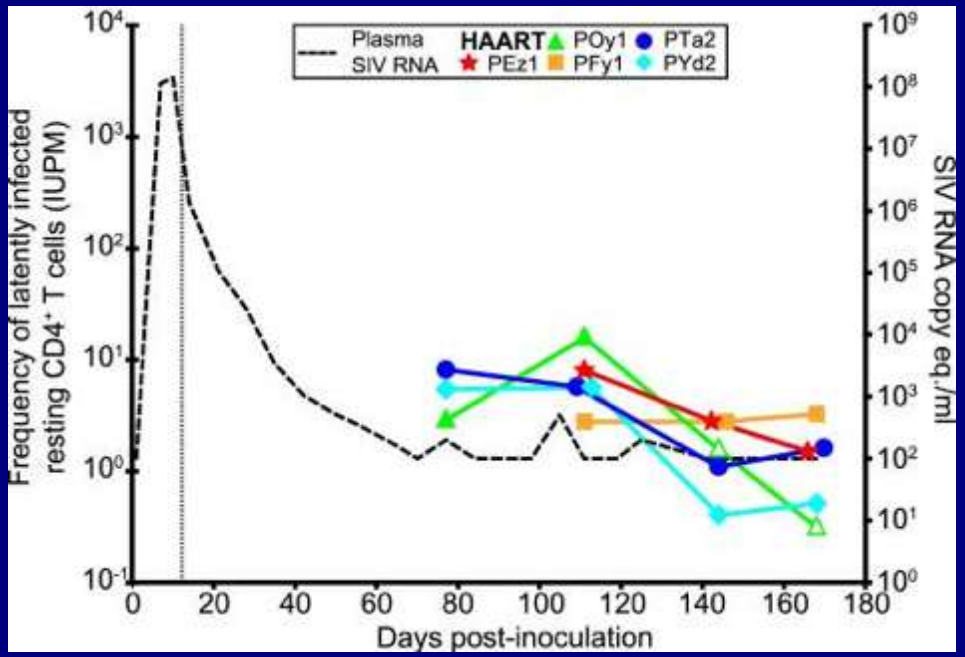



Free virions
Productively infected CD4+ T lymphoblasts
Resting CD4+ T cells with unintegrated HIV-1 DNA
Virions
DC
Infected macrophages
Resting CD4+ T cells with integrated provirus
Microglia
Astrocytes
0
30
60
90
120
?
Half-life (days)


Expression level (ratio) of HDACS in Jurkat T cell line
1
2
8
4
6
3
10
9
11
5
1
2
3
4
5
Expression level (ratio) of HDACS in primary macrophages
8
2
6
9
4
5
1
11
3
10
0.5
1

Where can HIV eradication approaches be studied?





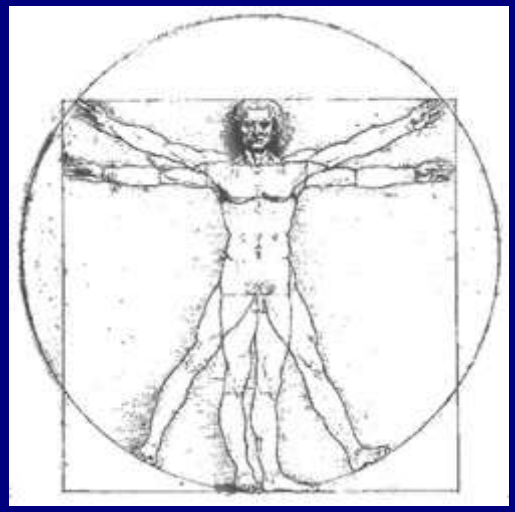
Dinoso *J Virol 2009*

The Journal of Infectious Diseases
1 July 2010, Vol. 202, No. 1: pp. 161-170

Simian Immunodeficiency Virus–Infected Macaques Treated with Highly Active Antiretroviral Therapy Have Reduced Central Nervous System Viral Replication and Inflammation but Persistence of Viral DNA. Zink et al.

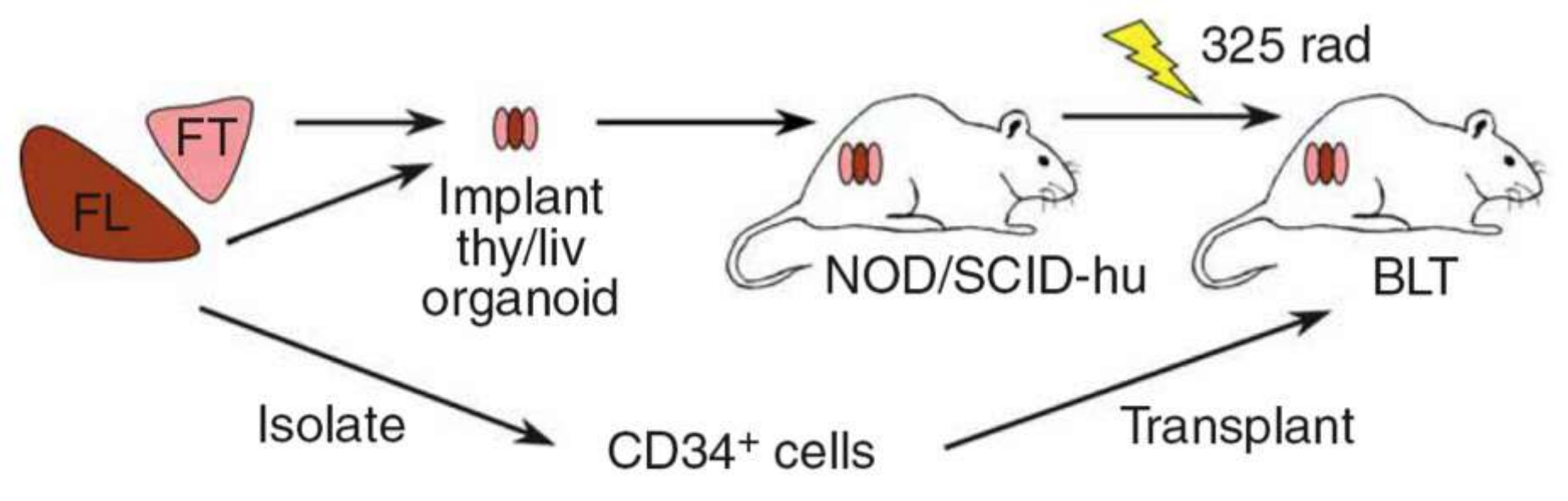
- Simian immunodeficiency virus in which all pigtail macaques develop AIDS and 90% develop CNS disease by 3 months
- Tenofovir disoproxil fumarate, saquinavir, atazanavir, and an integrase inhibitor starting at 12 days after inoculation and were euthanized at 175 days.
- Plasma and CSF viral loads declined rapidly
- Brain viral RNA was undetectable at necropsy
- Viral DNA levels were not different from untreated macaques.
- CNS inflammation was significantly reduced

Where can HIV eradication approaches be studied?



“Humanized” mice

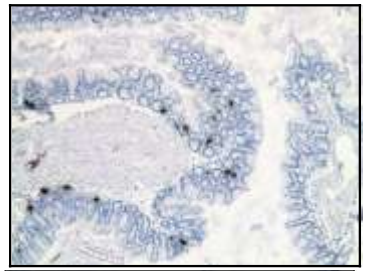
Generation of Bone Marrow/Liver/Thymus (BLT) Mice



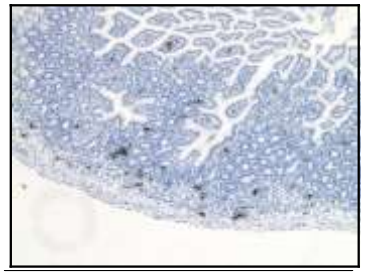
Laboratory group of Victor Garcia-Martinez, UNC

Systemic infection of BLT mice with HIV-1

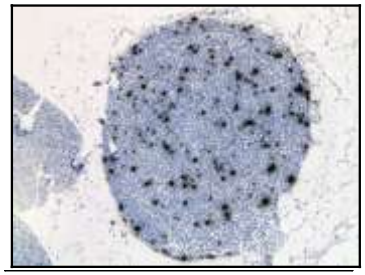
Large Intestine



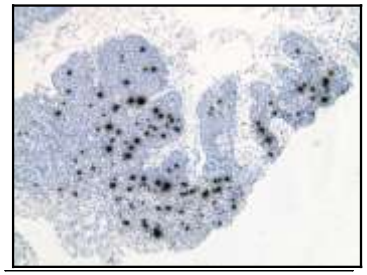
Small Intestine



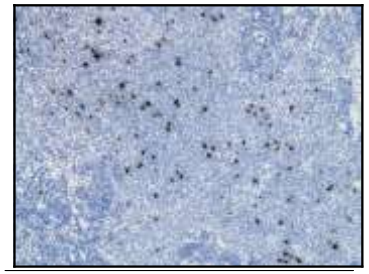
Mesenteric LN



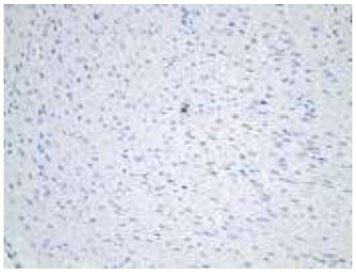
Thymic Organoid



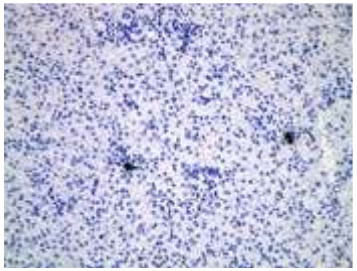
Spleen



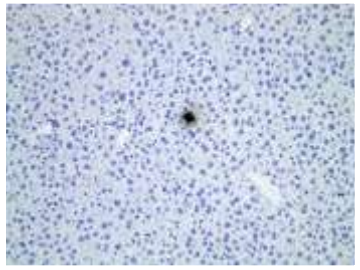
Heart



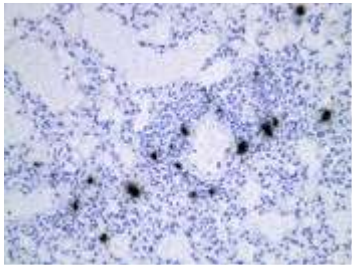
Kidney



Liver



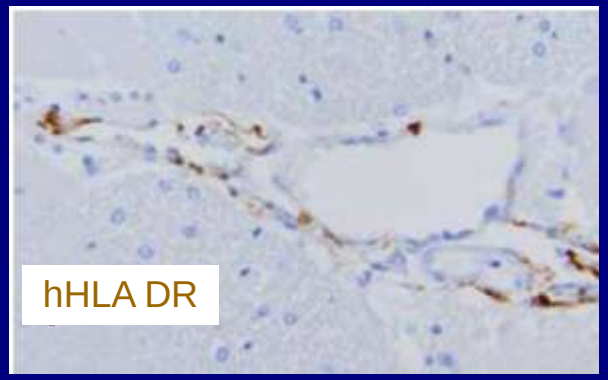
Lung

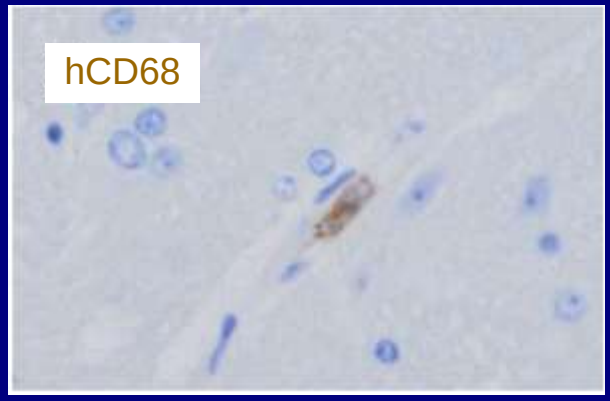


In situ hybridization analysis for the presence of HIV RNA in tissues from an infected mouse

Jake Estes

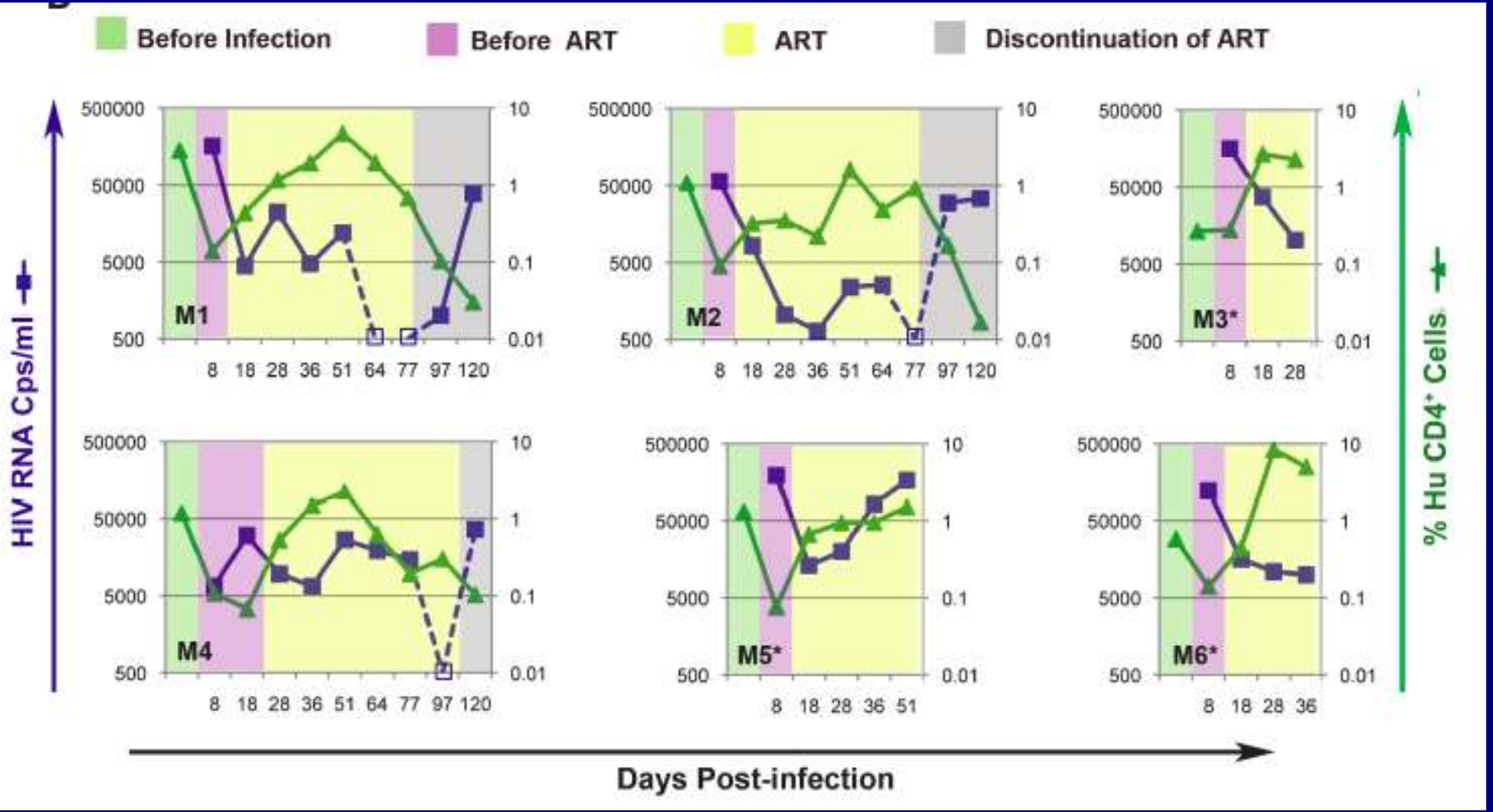
Reconstitution of BLT mice brain with human CD68^{+} macrophages and MHC II^{+} cells





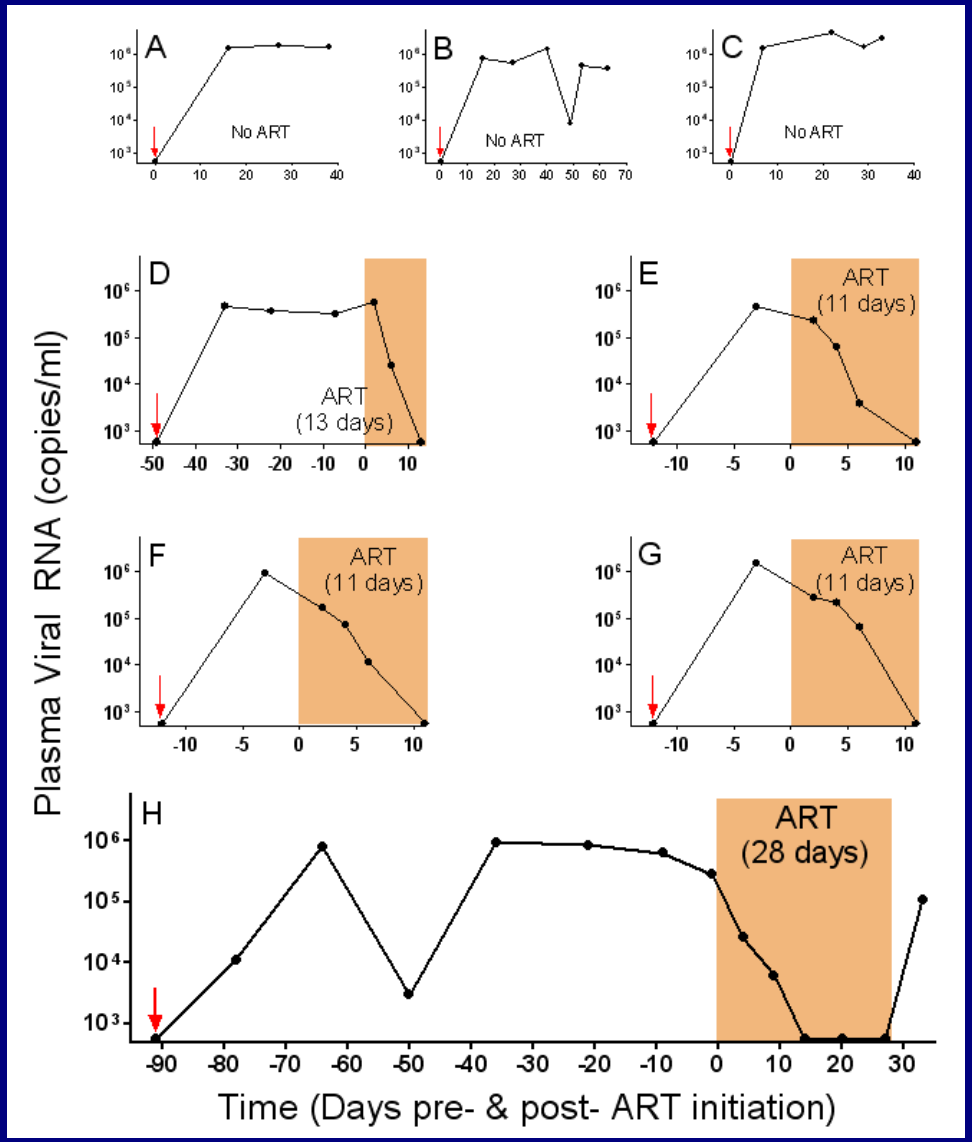
Victor Garcia-Martinez

Modeling ART to examine persistent CNS HIV infection in a small animal model?



Choudhary J Virol 2009

Suppression of plasma viremia in humanized mice

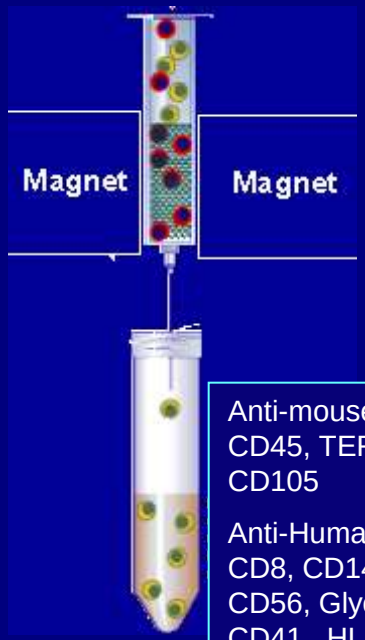


Raltegravir (2 mg)
FTC (3.5 mg)
TDF (5.2 mg)

Denton & Garcia *unpublished*

Purification of Resting hu-CD4$^+$ T cells from hu-Rag2$^{-/-}\gamma c^{-/-}$ Mice and Resting CD4$^+$ Cell Outgrowth Assay

Harvest lymphocytes from Blood, LN, Spleen, BM, FRT, Lung, Liver and Thymus



Anti-mouse cocktail:
CD45, TER119, CD31, CD105

Anti-Human cocktail:
CD8, CD14, CD16, CD19, CD56, Glycophorin A, CD41 , HLA-DR, CD25

97-99% pure resting CD4$^+$ T cells
0.5-1.0 million cells/mouse

Rest cells for 2 days in media with HIV RT and Integrase Inhibitors

24 hr maximal activation with PHA, IL-2, allogeneic cells

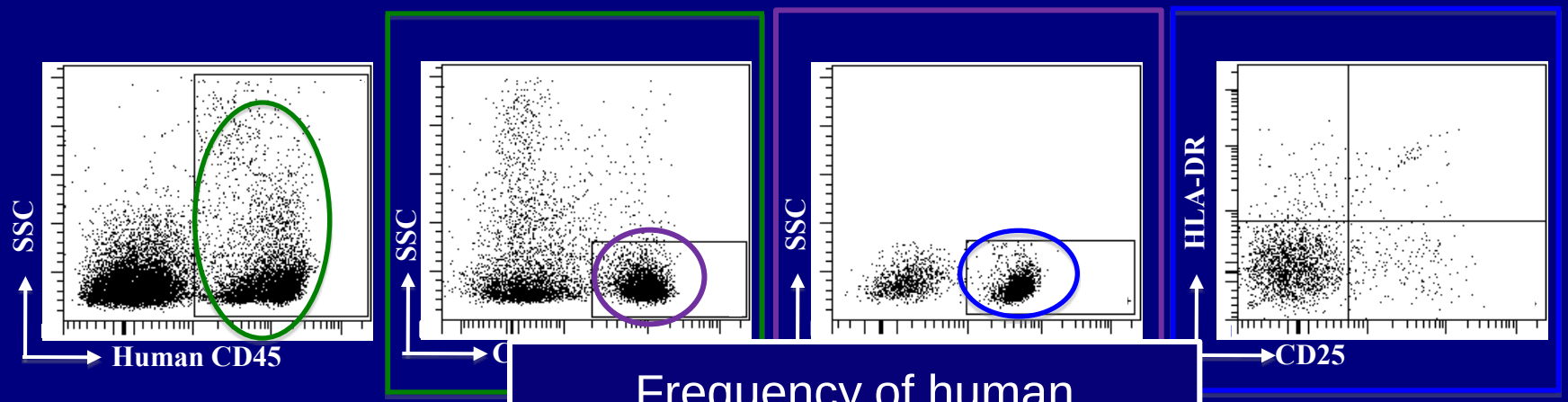
HIV outgrowth assay in limiting dilution of activated CD4$^+$ T feeder cells

Calculate frequency of resting cell infection by maximum likelihood method

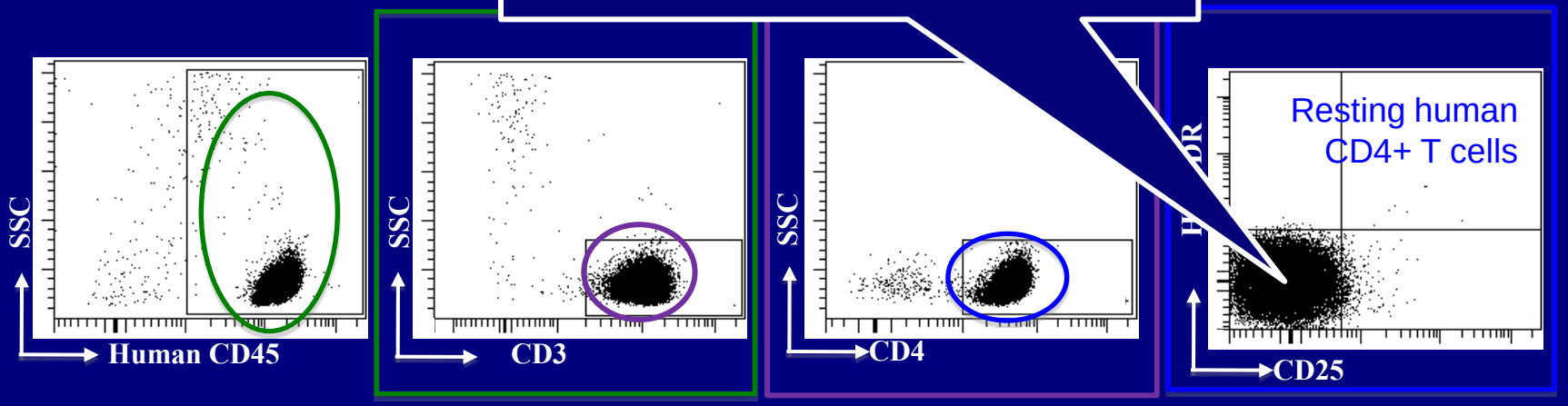
Choudhary , Margolis CROI 2011

Purification of Resting Human CD4+ T Cells from Humanized BLT Mice

A. Before Column Purification



B. After Column P



Frequency of human resting CD4+ T cell infection: 7 to < 3 per million

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

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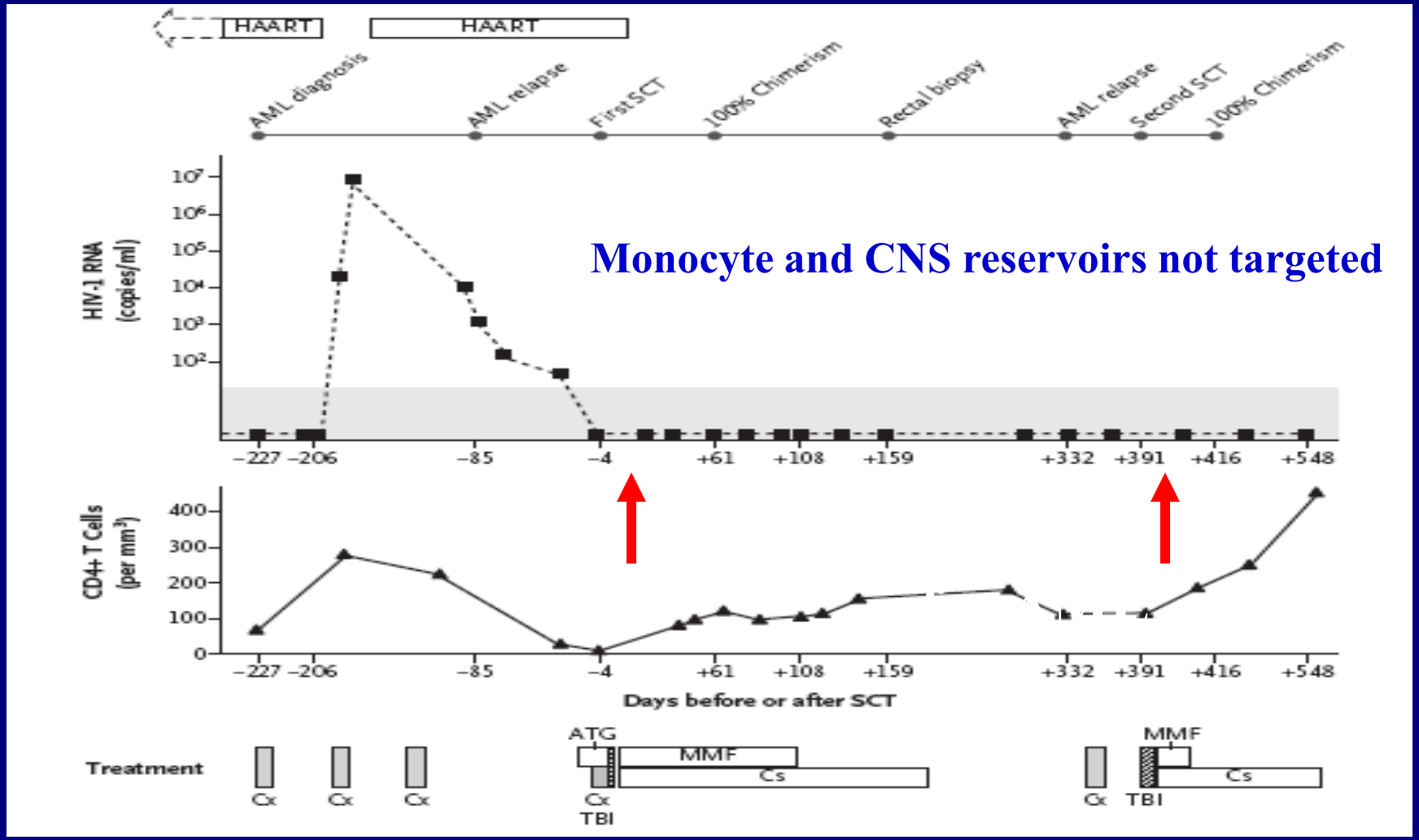

NEJM 2009; 360: 692

How to attack persistent neuro HIV infection?

- **New and better measures of persistence in patients**
- **Studies across all models: cell lines, primary cell models, patient cells, animal models, and patients**
- **Approaches to persistent viremia**
  - –Resting CD4+ T cells
  - –Are there other persistent cells with HIV
  - –Do we need better drugs
- **Approaches to persistent provirus**
  - –In resting CD4+ T cells
  - –In other cells?
  - –HDAC inhibitors, other epigenetic drugs
  - –Combination therapies to induce expression and cell death
  - –Paradox of oncologic approach

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National Institute of Allergy and Infectious Diseases

NIDA NATIONAL INSTITUTE ON DRUG ABUSE

NIMH National Institute of Mental Health

amfAR AIDS RESEARCH