# Finding HIV's Hiding Places

**Jake Estes, PhD**
**Professor and Chief**
**VGTI and ONPRC, OHSU**
**September 19, 2019**

OHSU

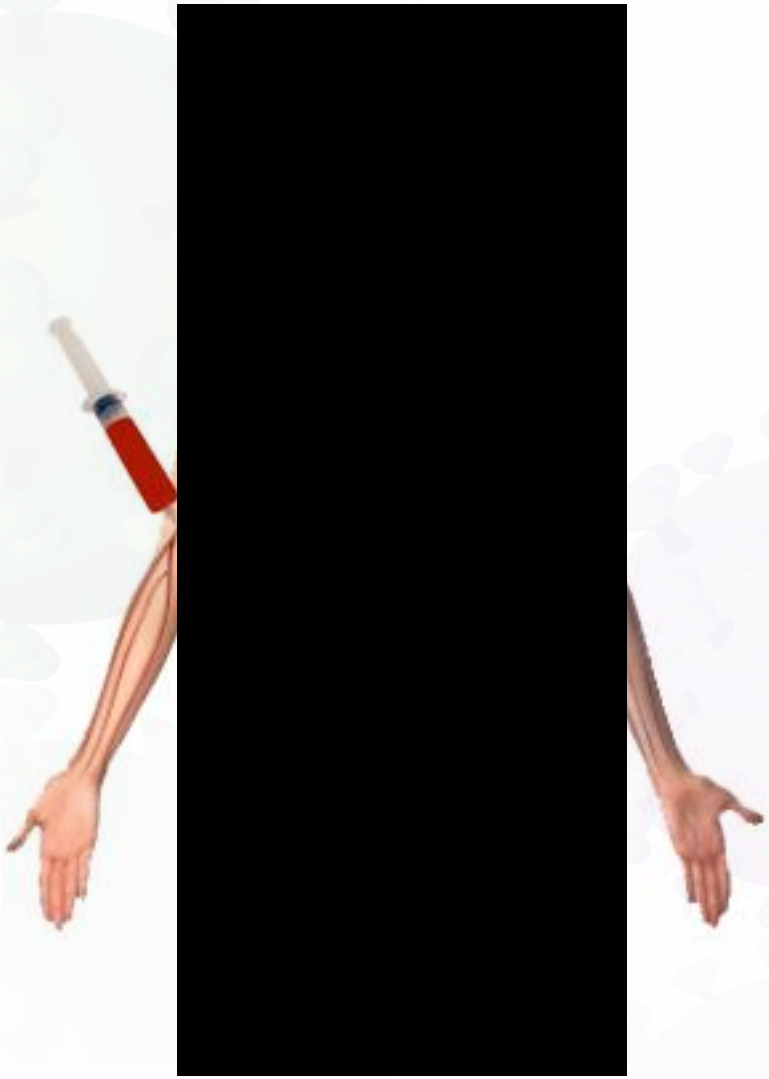
# BACKGROUND

- **Despite the extraordinary successes of ART, it is clear that the vast majority of HIV-infected individuals who are receiving clinically effective therapy will need to remain on continuous and uninterrupted treatment for the remainder of their lives**
  - Inevitably, when an HIV+ individual comes off suppressive ART, the virus rapidly rebounds
- **Although lifelong suppression of HIV replication with ART seems possible, side effects, need for strict adherence, resistance, stigma and cost all contribute to the necessity of finding an 'HIV cure'**
- **Long-term persistence of HIV reservoirs remains the *key obstacle* to achieving an HIV cure ('functional cure' - sustained ART-free virologic remission or eradication) in HIV-infected individuals after ART is discontinued**
- **Successful 'HIV cures' (Timothy Ray Brown + London and Barcelona patients) have galvanized the field to seek a 'functional cure' or HIV eradication through safer and more scalable approaches**

# BACKGROUND

- **Limited possibility of complete eradication or durable ART-free remission will be achieved before we understand the *nature* and *vulnerabilities* of virus that persists and is capable or reigniting systemic infection when ART is discontinued**
  - In order to achieve safe and scalable 'HIV cure' strategies, we must better understand HIV reservoir establishment and persistence *in vivo*
- **While extensive studies have identified and defined "resting" memory $CD4^+$ T cells as an important (dominant) viral reservoir in HIV-infected individuals, many questions regarding reservoir biology and viral persistence remain unanswered:**
  - Where does HIV predominately persist before and during ART?
  - What are the cellular and anatomic compartments from which infection might rebound after ART is stopped that would need to be impacted to achieve a 'functional cure' or HIV eradication?
  - What are the important cellular phenotypes and characteristics of HIV reservoirs?
  - What are the mechanisms for viral persistence during ART?
  - What role does sustained inflammation play in viral persistence?

# LIMITATIONS OF HUMAN STUDIES



**Very limited tissue sampling in human studies**

# NHP Models of HIV Disease

**Nonhuman primate (NHP) research has provided many critical insights into HIV infection and disease that are simply NOT feasible in a clinical setting**

- Immune systems, anatomy, and physiology are highly similar
- Transmission and longitudinal studies are possible
- Environmental factors can be controlled (i.e. virus, route, etc.)
- Any anatomic site can be sampled
- Effects of additional infections can be studied
- Preventative efficacy can be studied
- Therapeutic intervention / cure strategies can be studied

**Extensive tissue analysis**

**SIV+ macaques**

≈

**HIV+ humans**

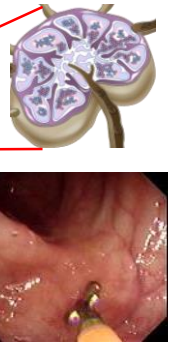
# TALK OVERVIEW

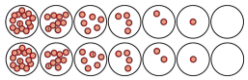
1. **Studies to define the dominant tissue locations and size of the AIDS-virus burden before and during ART within each major organ system and throughout the body**

2. **Studies to understand the size and importance of the "non-T cell" FDC viral reservoir within lymphoid tissues**

# QUANTIFYING THE HIV RESERVOIR:
## Focus on Tissues

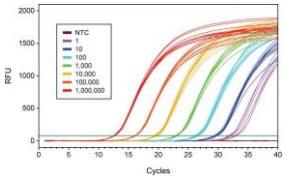
**Host** **Tissue** **Tissue Processing** **FACS**



**Reservoir**

**Analysis**




**Quantification**

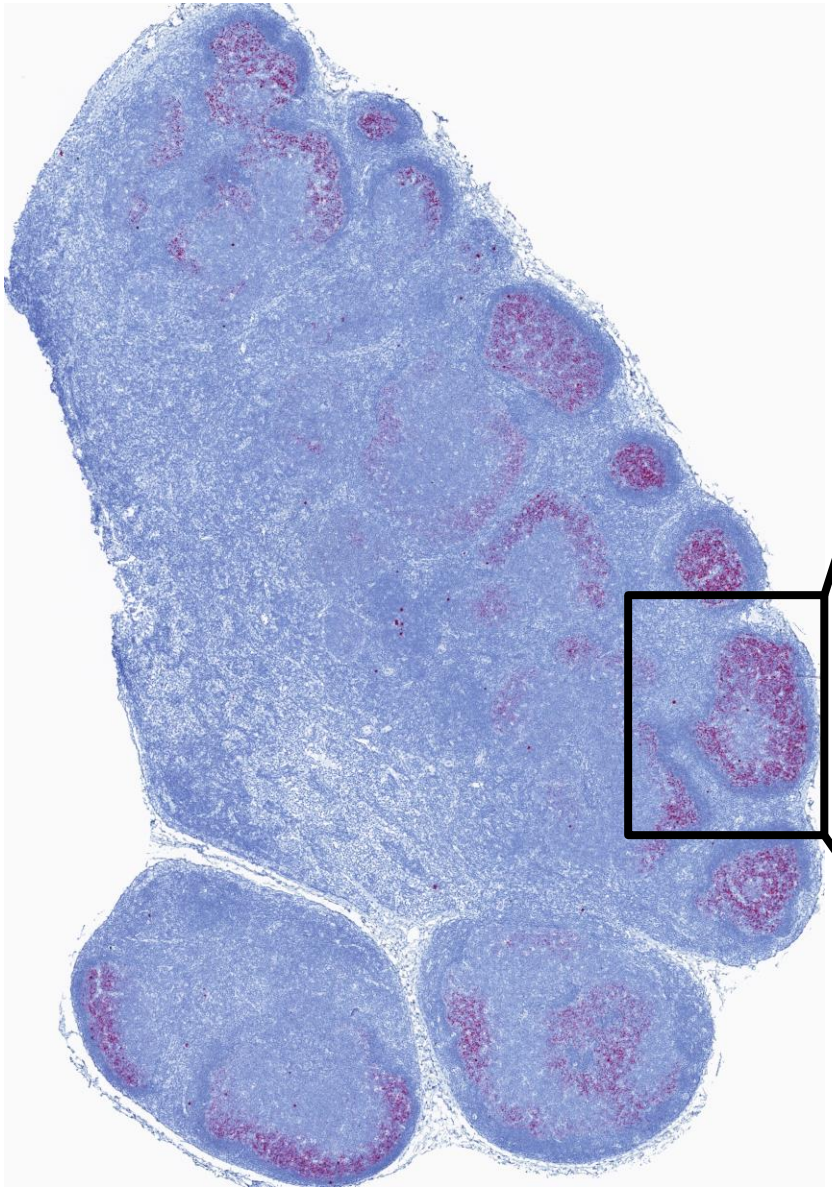
**Defining HIV/SIV Reservoirs**

qPCR TILDA IPDA QVOA ISA scRNAseq In situ Imaging

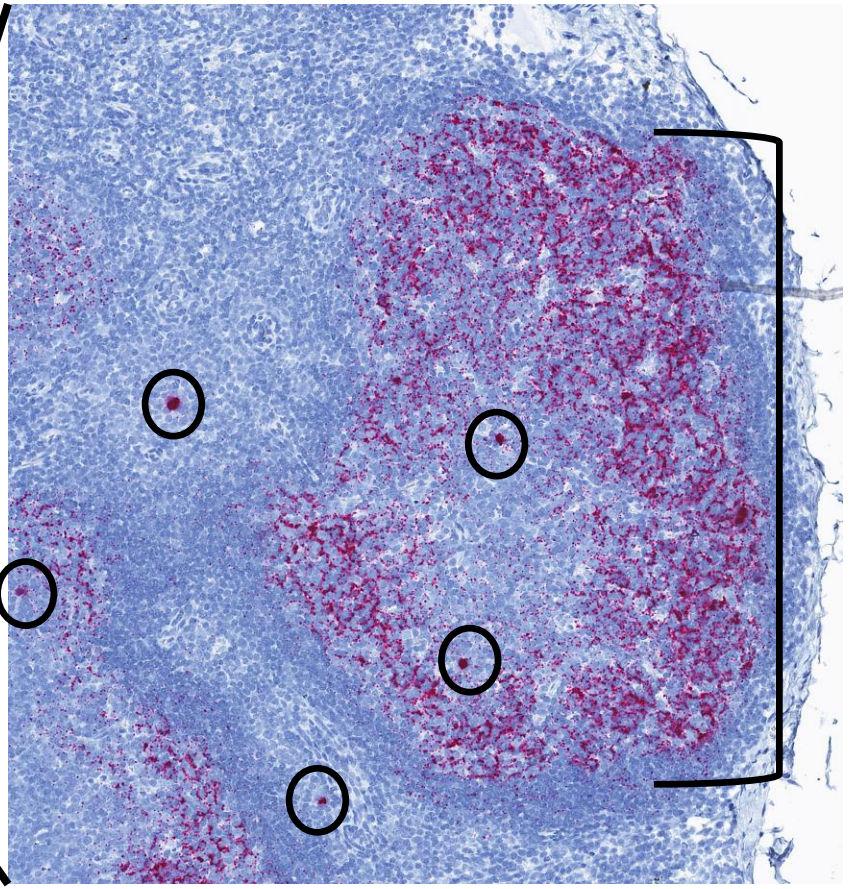
***Important spatial and contextual information about viral reservoirs, neighboring cells, and microenvironments are lost in this process***

- **Anatomical locations of viral reservoirs**
- **Status of infected cells (vRNA+ or vRNA-)**
- **Phenotypic assessment: T cell subsets and "non-T cell" reservoirs that are challenging to extract from tissues**

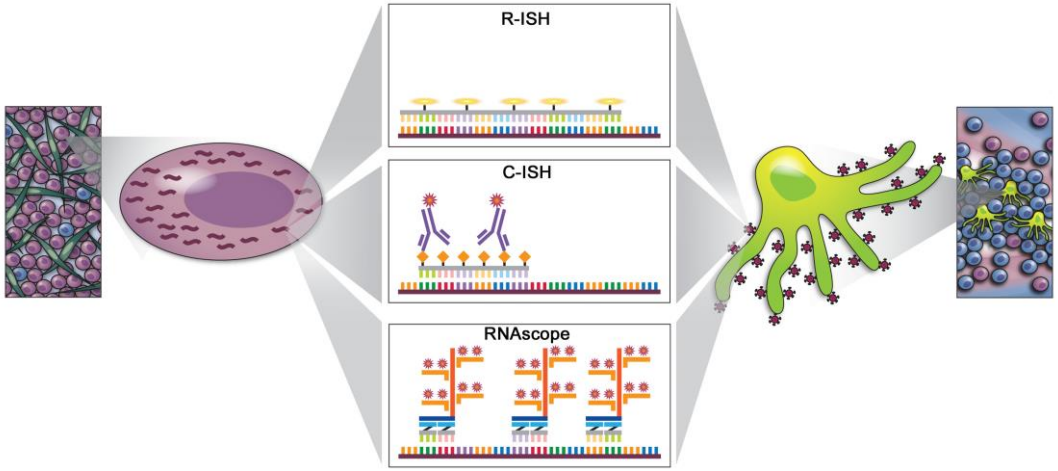
# CONTEXT OF VIRUS IN TISSUES



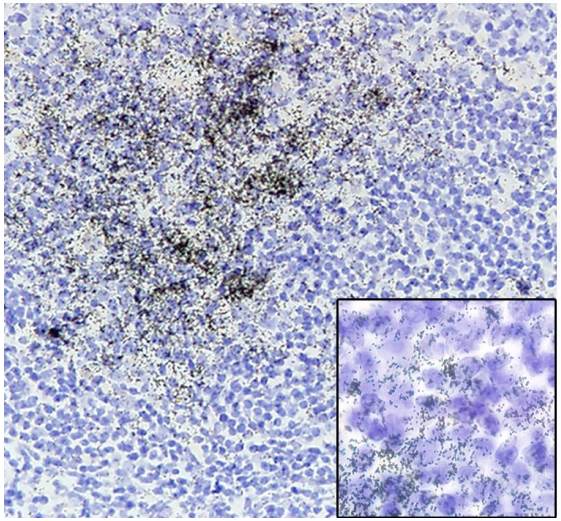
**pVL 6.6 x $10^6$ copies SIV RNA**
**LN = 5,300 SIV RNA/$10^5$ cell Eq.**
**522 SIV DNA/$10^5$ cell Eq.**



# ISH FOR THE DETECTION OF vRNA

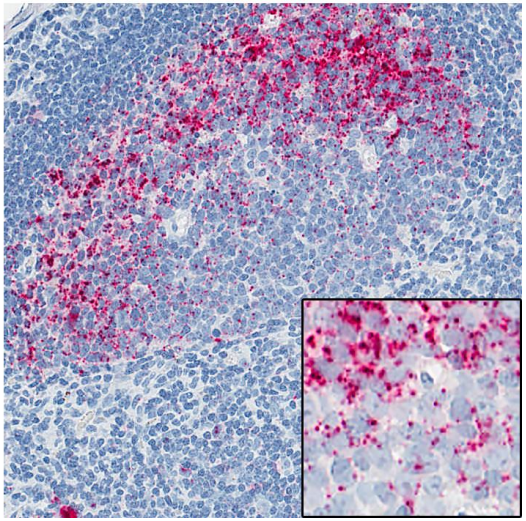



**R-ISH**



5 -21 Days

**RNAscope**



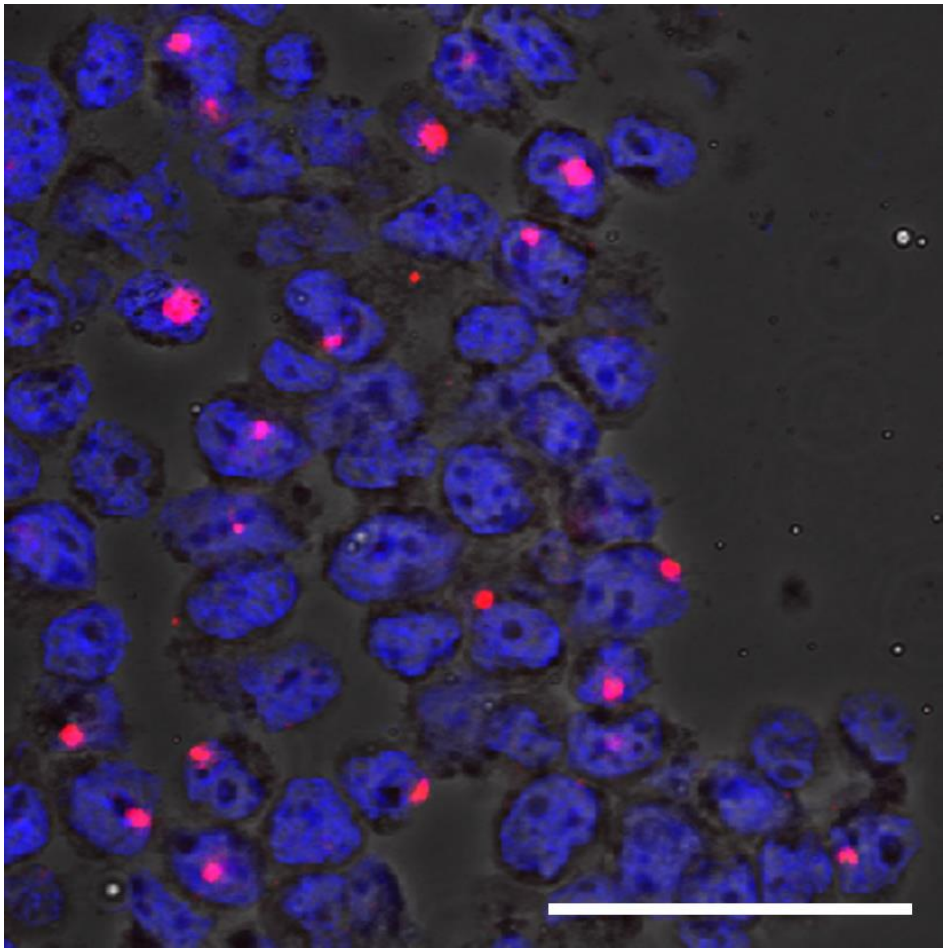
**1 Day**

Deleage C, et al. ***Pathog Immun*.** 2016 Spring;1(1):68-106

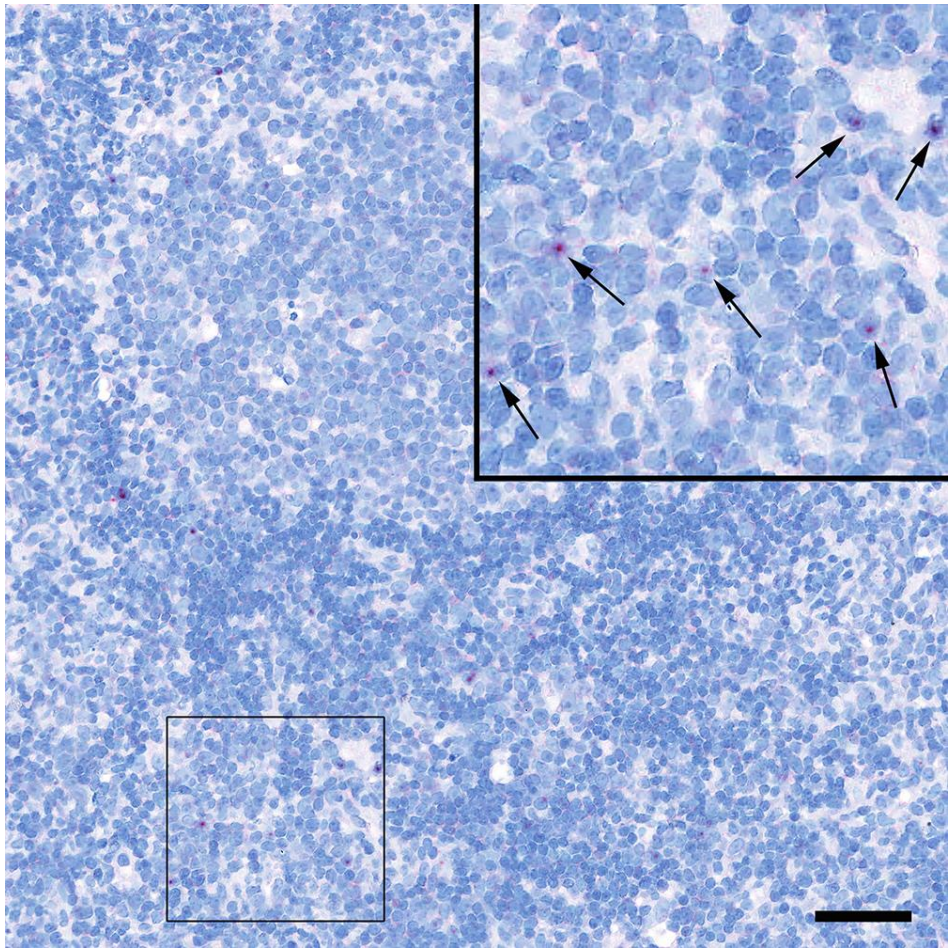
# DNAscope ISH FOR DETECTION OF vDNA

## SIV vDNA

**SIV+ 3D8 cells (1 vDNA copy/cell)**

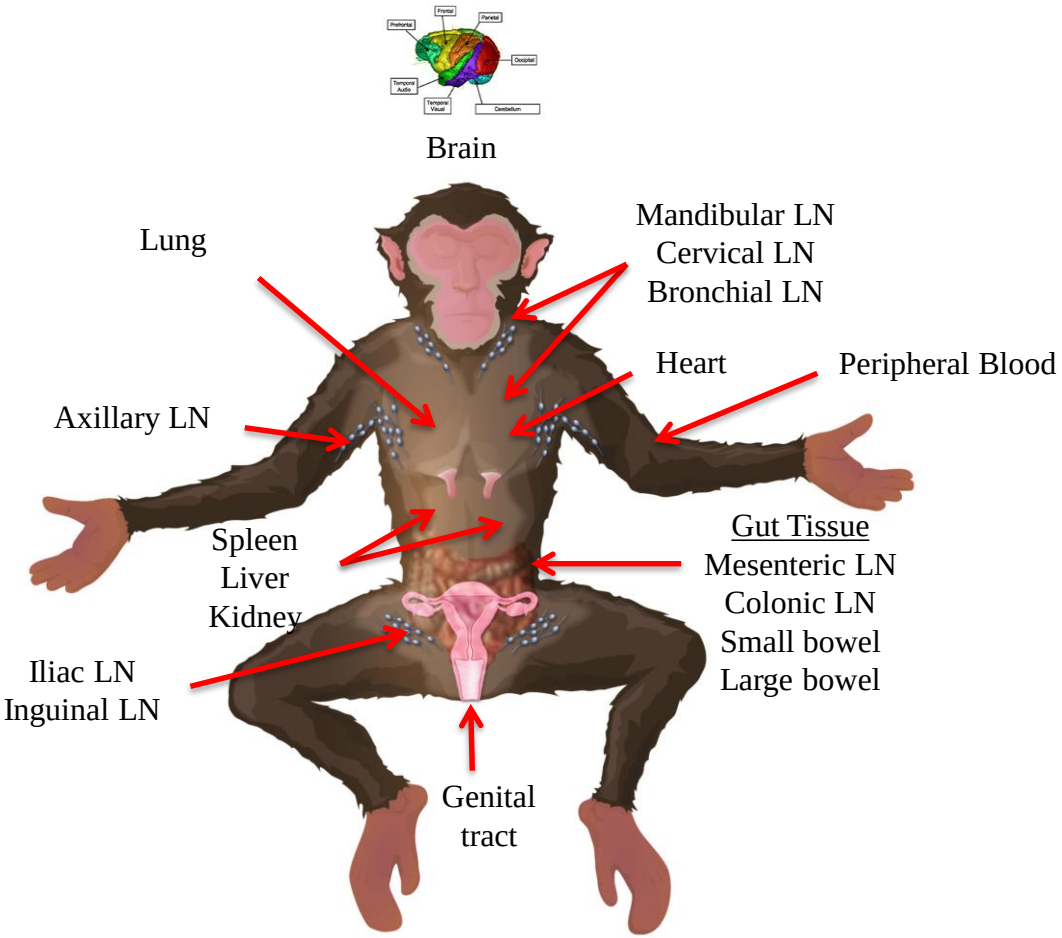


**Chronic SIV+ RM LN**

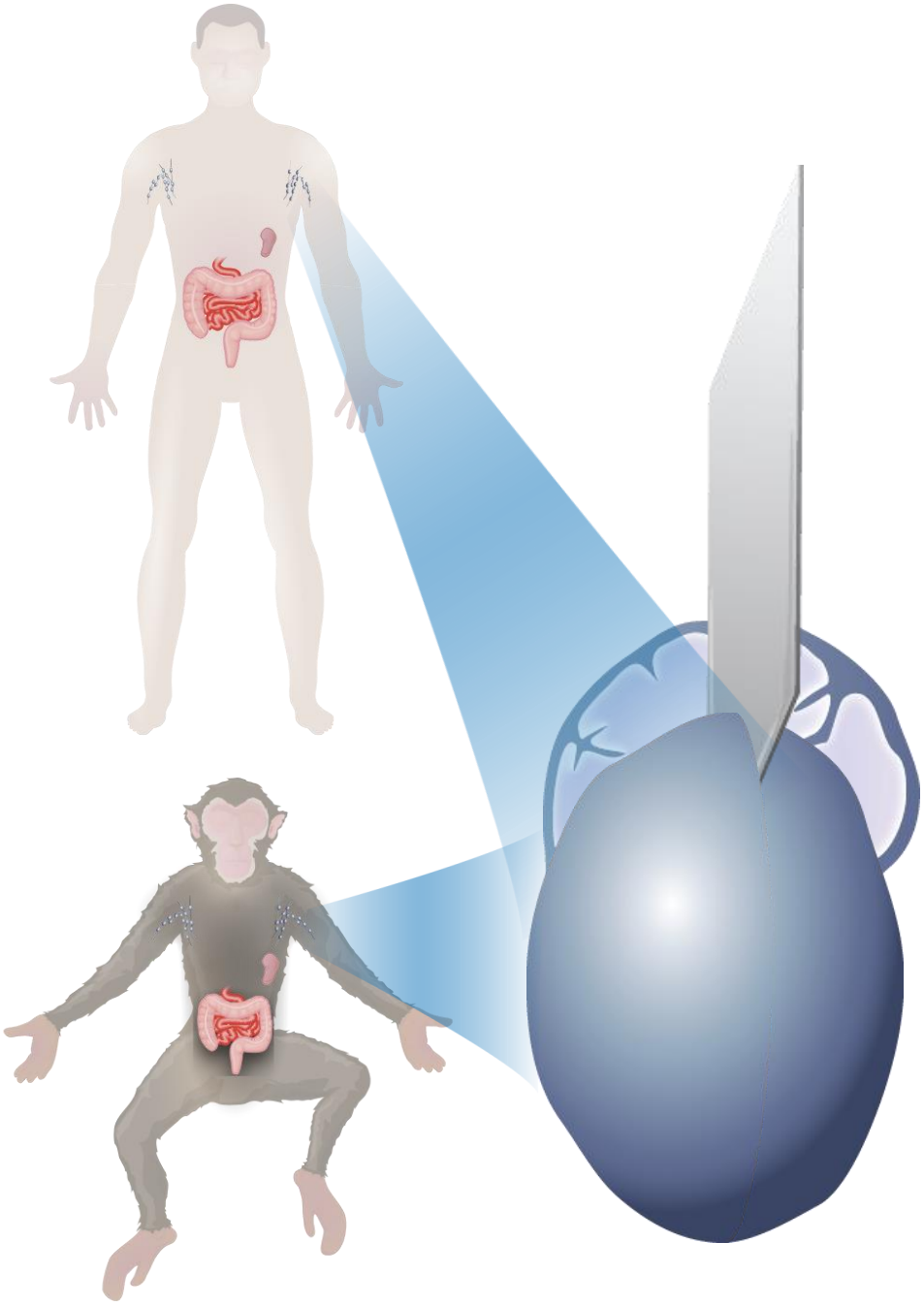


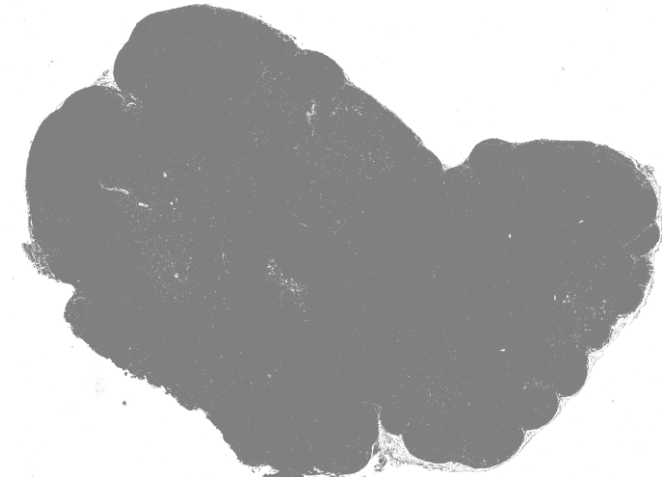
Deleage C, et al. ***Pathog Immun*.** 2016 Spring;1(1):68-106

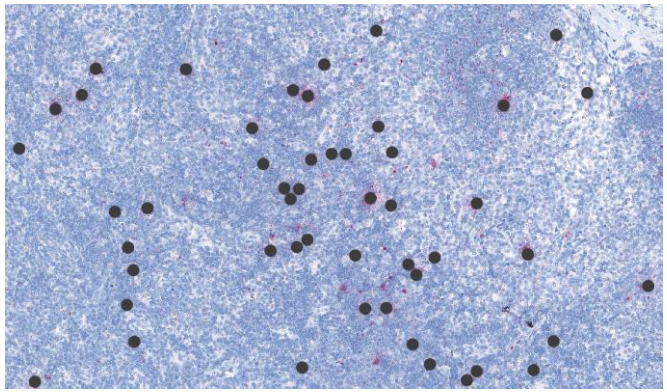
# COMPREHENSIVE SAMPLING IN NHPs




**We utilized NHP models of HIV infection and performed extensive tissue analysis to determine the key tissue locations and size of the total body cell reservoir burden before and during ART**







**Calculations**

The frequency of vRNA+ (vDNA+) cells/$\mu m^2$ area x 4 µm thick = vRNA+ (vDNA+) cells/$\mu m^3$ x $10^{12}$ $\mu m^3/cm^3$ x 1 $g/cm^3$ = Number vRNA+ (vDNA+) cells / g tissue

## Based on organ mass we can define:

- Number of infected cells / organ
- Proportional contribution of each organ system to total infected cells in the body
- Estimate the total number of infected cells in the body
- Estimate the total number of replication competent cells in HIV-infected individuals

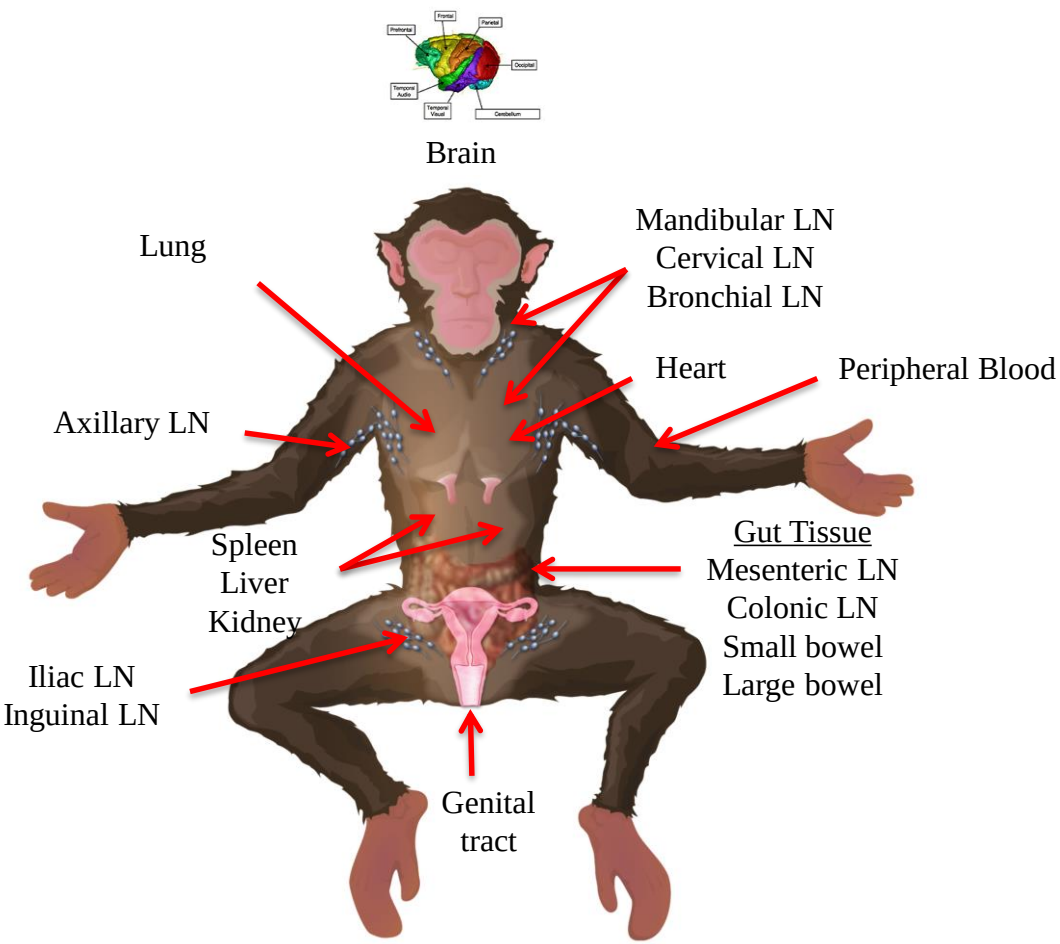
# ASSUMPTIONS

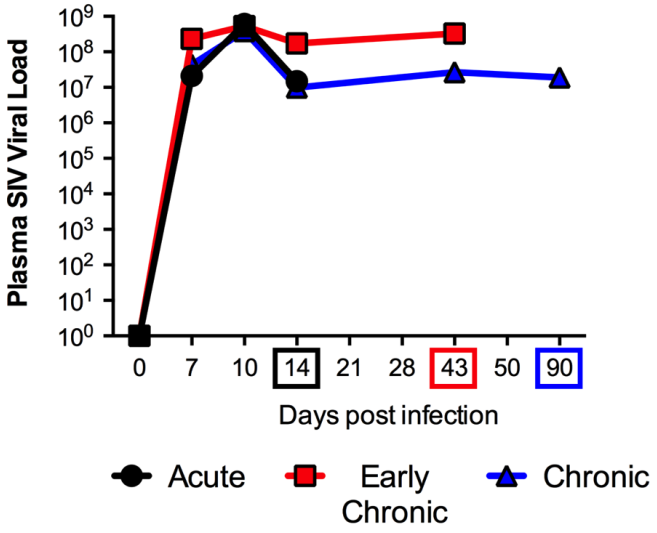
## Recognizing that cells within tissues are dynamic and not static:

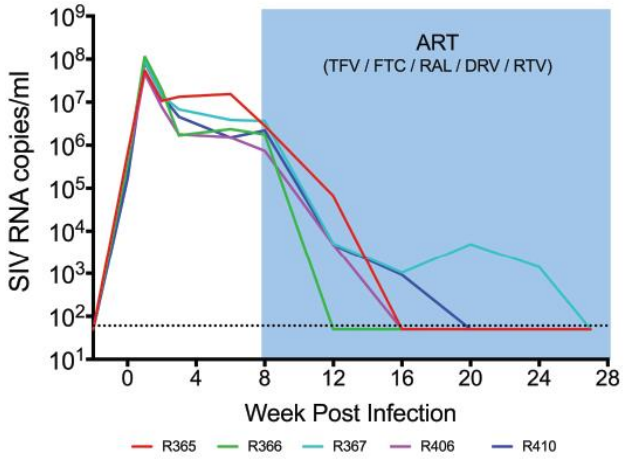
- Both active (vRNA+ cells) and latently infected cells (vDNA+/vRNA-) that persist during ART are potential sources of viral recrudescence after ART cessation
- We reason each organ contributes vRNA+ and vDNA+ cells to the total population of infected cells within the infected host in an amount proportional to: i) the frequency of the vRNA+ and vDNA+ cells measured by our ISH approaches, and ii) the total mass of the organs analyzed
- Reasonable estimates of the total size of the viral reservoir that contain potential replication competent virus can be made from our data based on the previously reported frequency of replication competent proviruses in HIV-infected patients (1-5%)

# VIRAL DISTRIBUTION IN ACUTE / CHRONIC SIV INFECTION

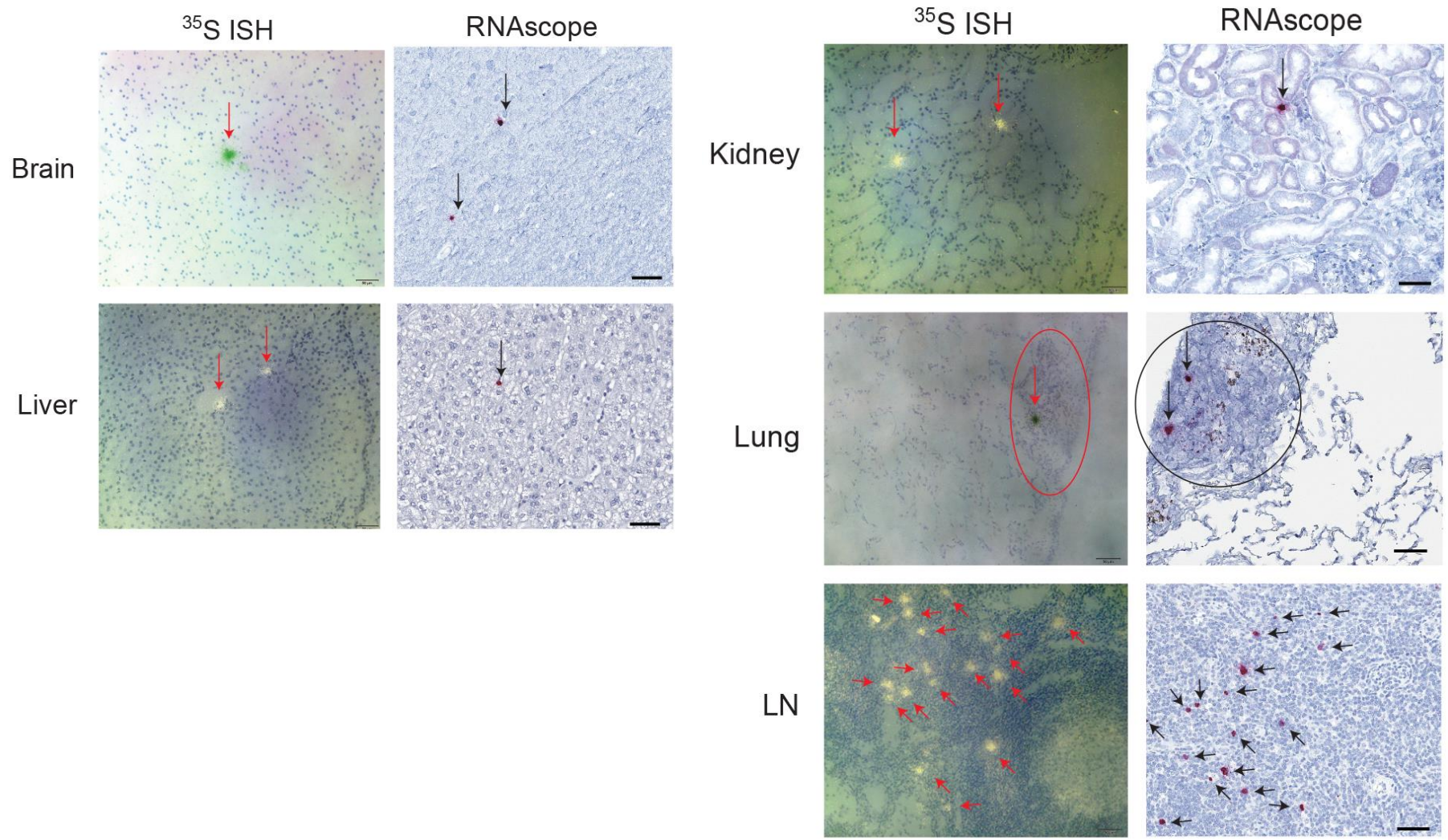
**Where do "active" (vRNA+) and total (vDNA+) reservoirs predominate in the body before and during cART?**



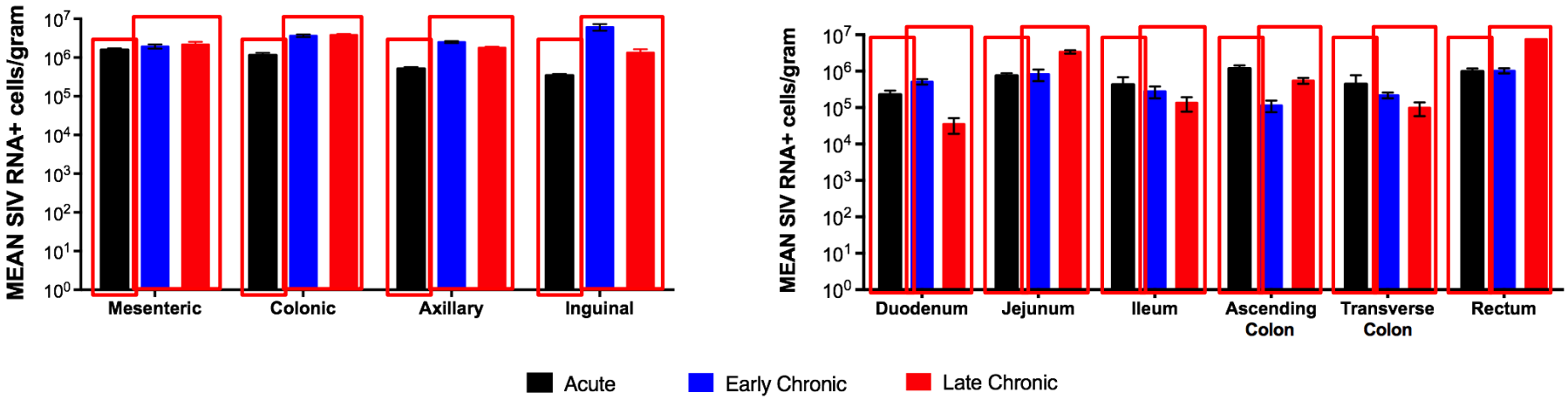
# INFECTED CELL DISTRIBUTION IN SIV INFECTION



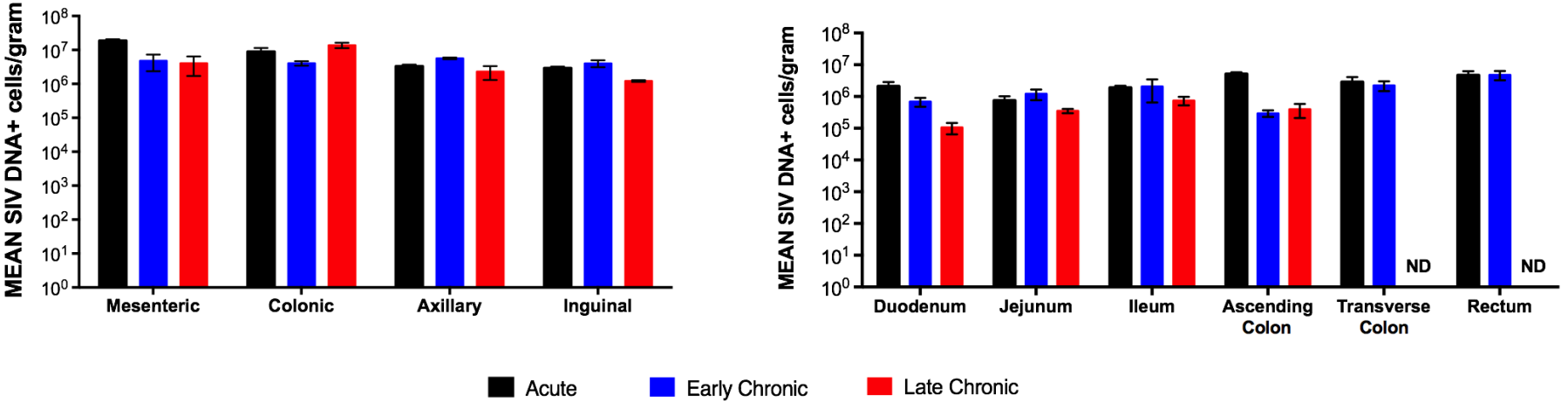

Estes JD, et al. ***Nat Med*****.** 2017 Nov;23(11):1271-1276.

# INFECTED CELL DISTRIBUTION IN SIV INFECTION

## vRNA+ Cells / gram



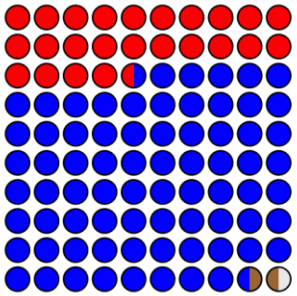

## vDNA+ Cells / gram




Estes JD, et al. ***Nat Med***. 2017 Nov;23(11):1271-1276.

# PROPORTION OF SIV vRNA+ CELLS THROUGHOUT THE BODY

**14 DPI**

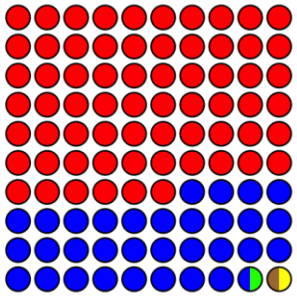


- Lymph Nodes (24.7%)
- Gut (74.0%)
- Spleen (0.07%)
- Brain (0.03%)
- Kidney (0.75%)
- Heart (0.15%)
- Lungs (0.084%)
- Liver (0.24%)

**98.8% LT**

**$2.71 \times 10^8$ vRNA+ cells**

**43 DPI**

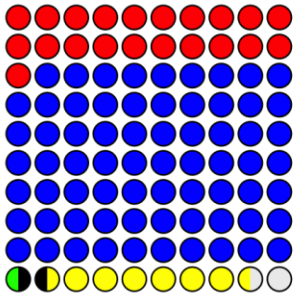


- Lymph Nodes (65.8%)
- Gut (32.9%)
- Spleen (0.27%)
- Brain (0.04%)
- Kidney (0.27%)
- Heart (0.06%)
- Lungs (0.55%)
- Liver (0.24%)

**99.5% LT**

**$2.82 \times 10^8$ vRNA+ cells**

**90 DPI**

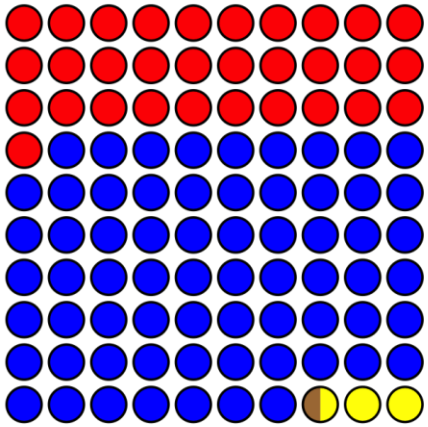


- Lymph Nodes (21.0%)
- Gut (69.2%)
- Spleen (0.14%)
- Brain (1.24%)
- Kidney (0.004%)
- Heart (0.008%)
- Lungs (7.0%)
- Liver (0.24%)

**97.4% LT**

**$9.37 \times 10^8$ vRNA+ cells**

**Average (n=3)**



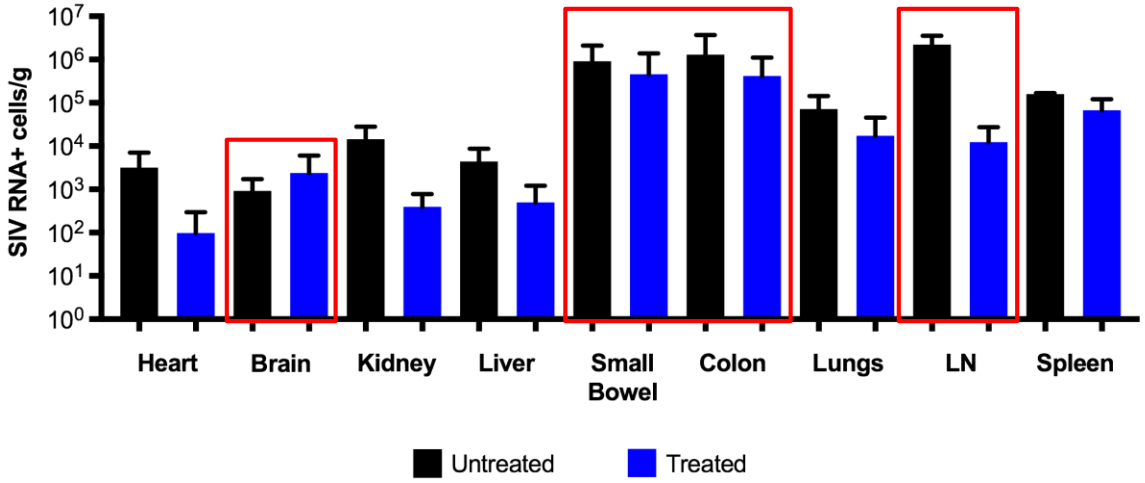
- Lymph Nodes (31.15%)
- Gut (65.7%)
- Spleen (0.22%)
- Brain (0.04%)
- Kidney (0.19%)
- Heart (0.04%)
- Lungs (2.4%)
- Liver (0.24%)

**99.5% LT**

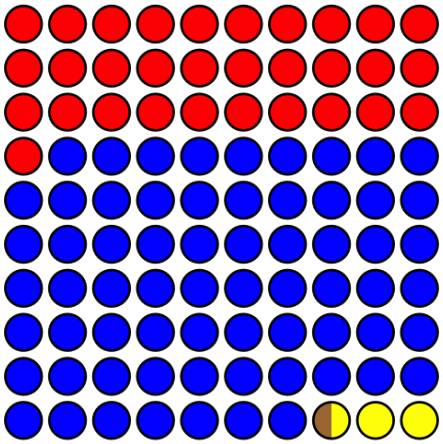
**$4.97 \times 10^8$ vRNA+ cells**

Estes JD, et al. ***Nat Med***. 2017 Nov;23(11):1271-1276.

# "ACTIVE" SIV RESERVOIRS (vRNA+) THROUGHOUT THE BODY BEFORE AND DURING cART




**Before cART (n=3)**

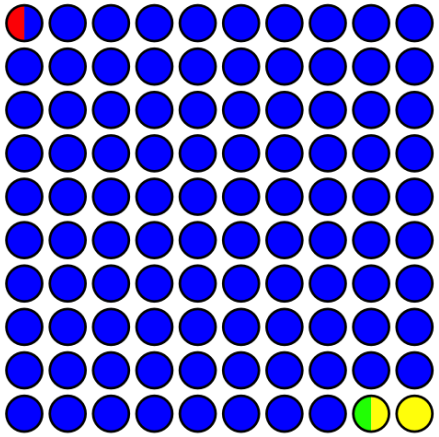


- Lymph Nodes (31.15%)
- Gut (65.7%)
- Spleen (0.22%)
- Brain (0.04%)
- Kidney (0.19%)
- Heart (0.04%)
- Lungs (2.4%)
- Liver (0.24%)

**4.97 x $10^8$ vRNA+ cells**

**99.5% LT**

**During cART (n=5)**



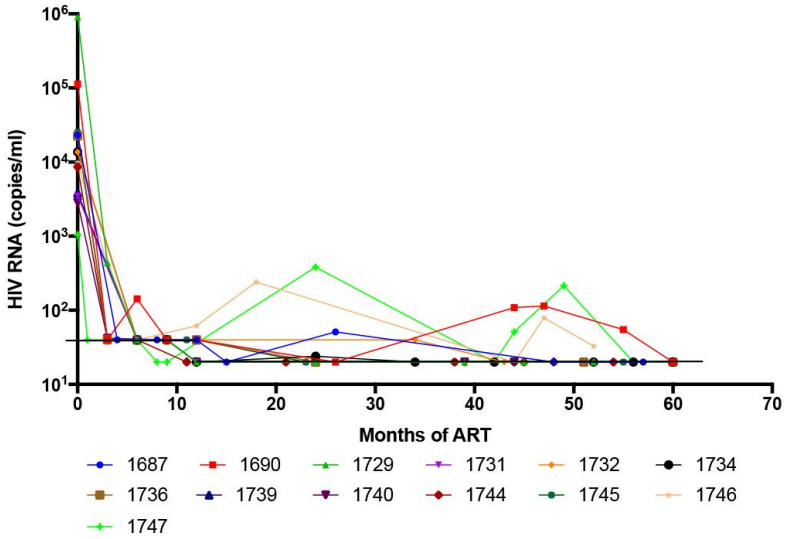
- Lymph Nodes (0.373%)
- Gut (97.5%)
- Spleen (0.18%)
- Brain (0.04%)
- Kidney (0.01%)
- Heart (0.03%)
- Lungs (1.5%)
- Liver (0.069)

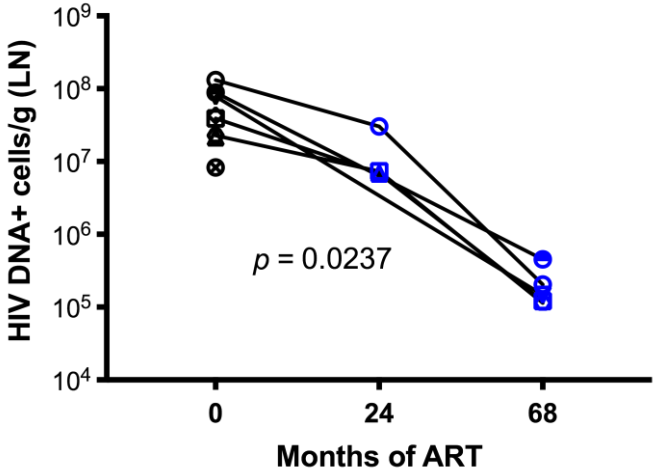
**1.2 x $10^8$ vRNA+ cells**

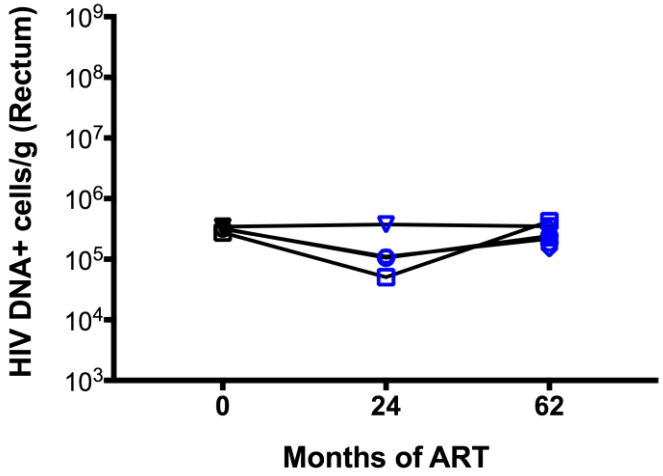
**99.6% LT**

Estes JD, et al. ***Nat Med***. 2017 Nov;23(11):1271-1276.

# DYNAMICS OF HIV-INFECTED CELLS DURING ART




**Estimated ~$10^7$ - $10^8$**
**Replication competent vDNA+ cells**

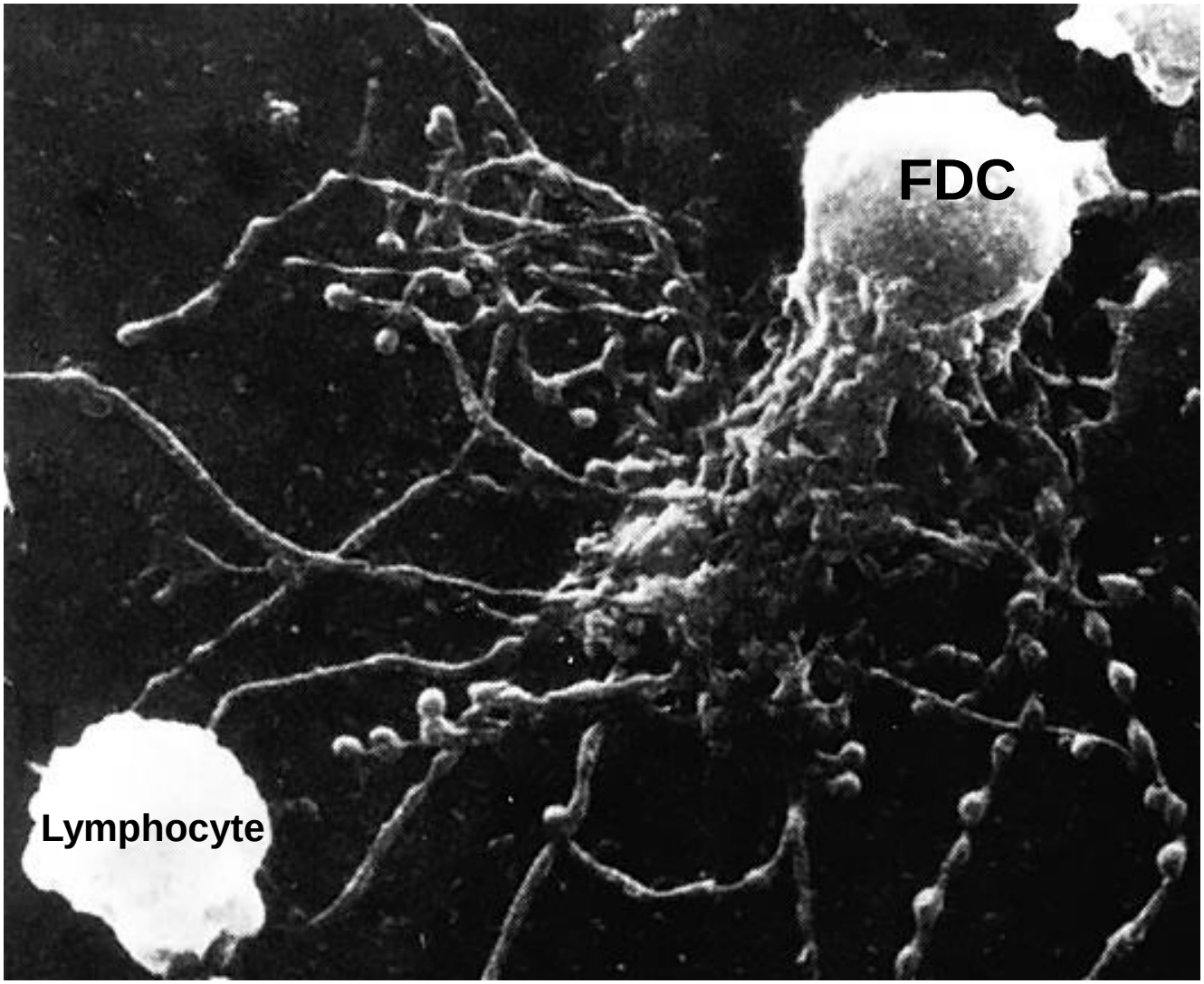
Estes JD, et al. ***Nat Med*****.** 2017 Nov;23(11):1271-1276.

# TALK OVERVIEW

1. Studies to define the dominant tissue locations and size of the AIDS-virus burden before and during ART within each major organ system and throughout the body

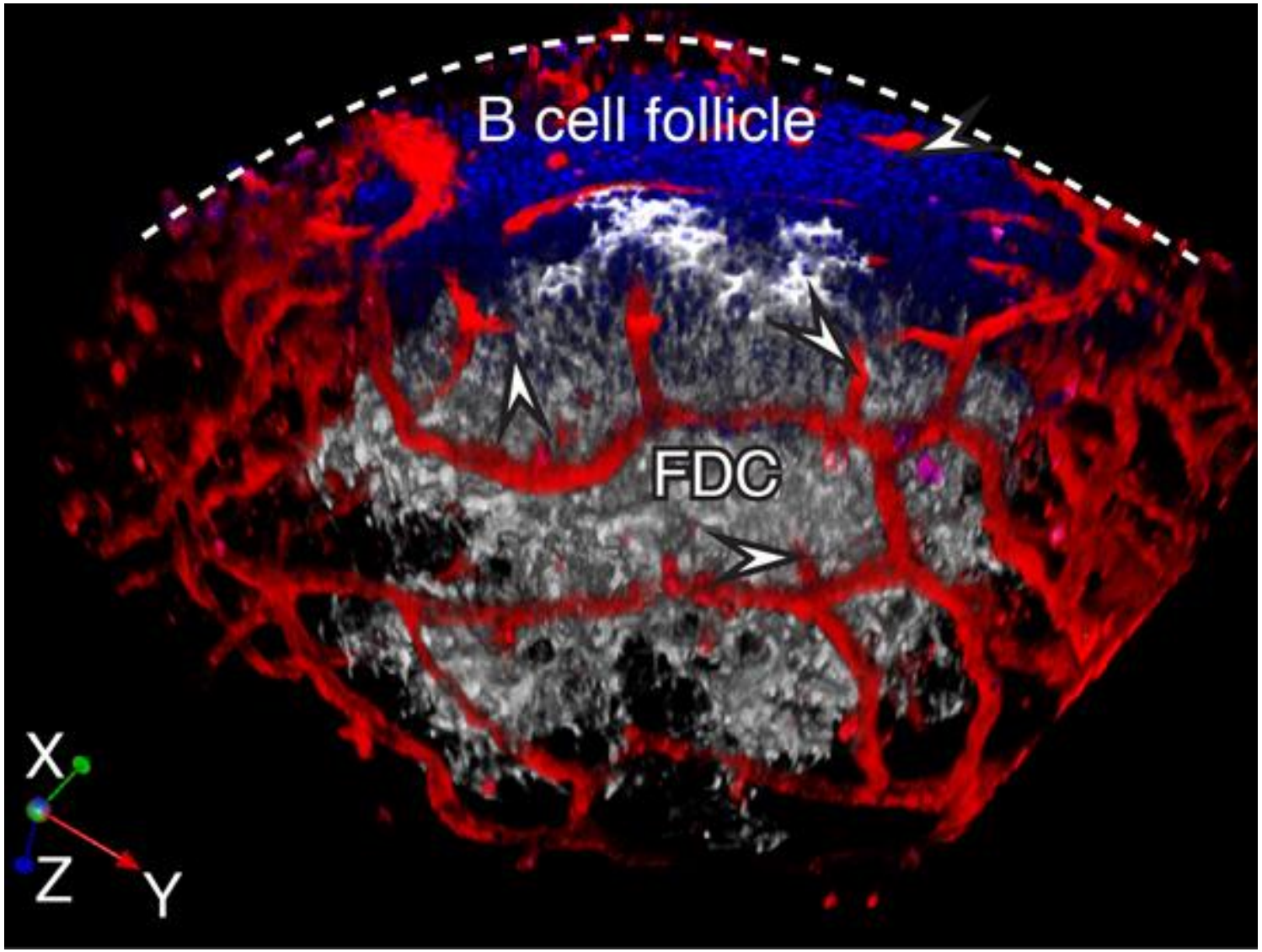
2. **Studies to understand the size and importance of the "non-T cell" FDC viral reservoir within lymphoid tissues**

# FOLLICULAR DENDRITIC CELLS (FDCs): Unique Morphology and Biological Function




Scanning Electron Micrograph of mouse FDC with lymphocytes surrounding FDC. *Immunobiology* 4th Edition, Janeway, Travers, Walport & Capra. Photograph provided by A.K. Szakal.

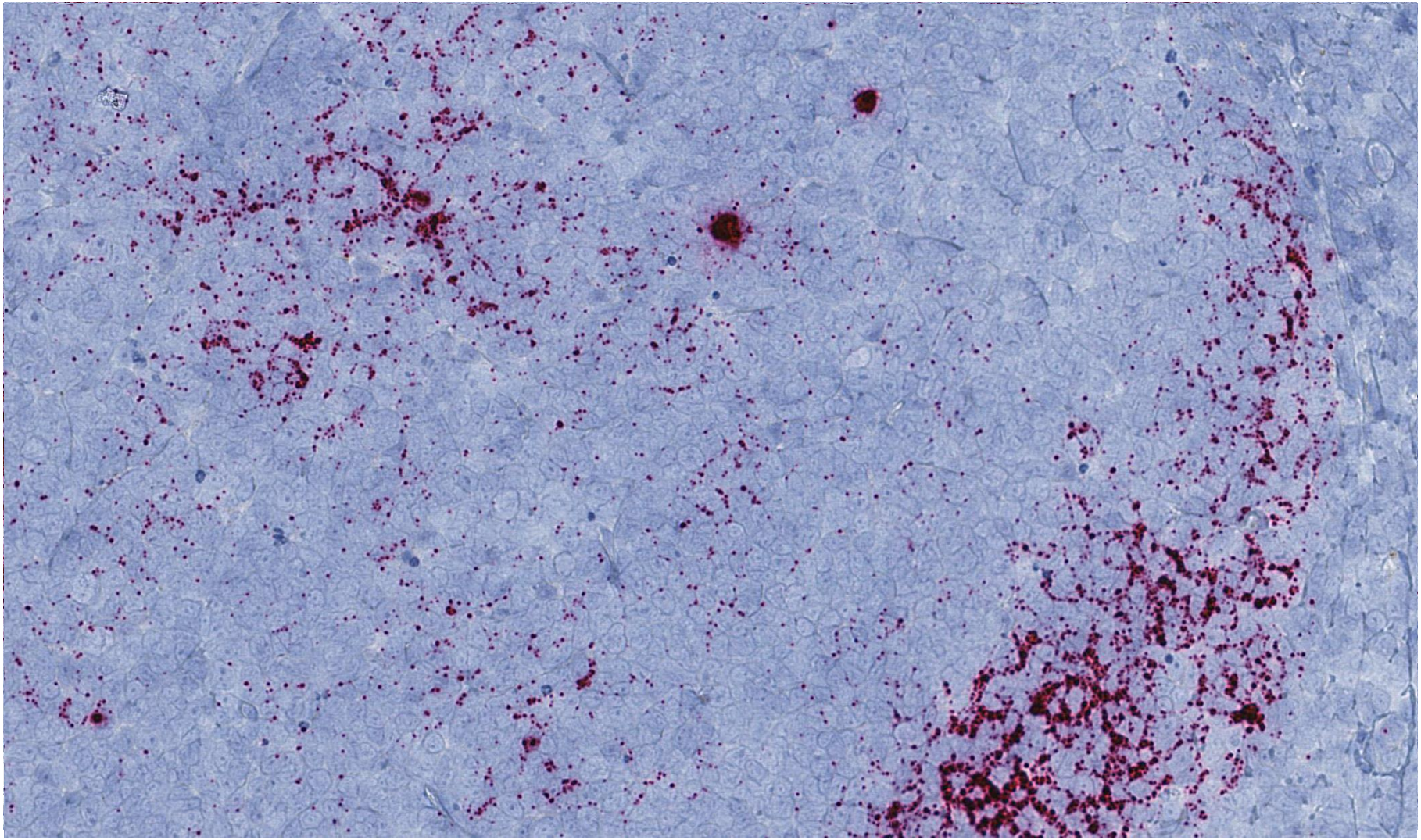
# FOLLICULAR DENDRITIC CELLS (FDCs): Unique Morphology and Biological Function



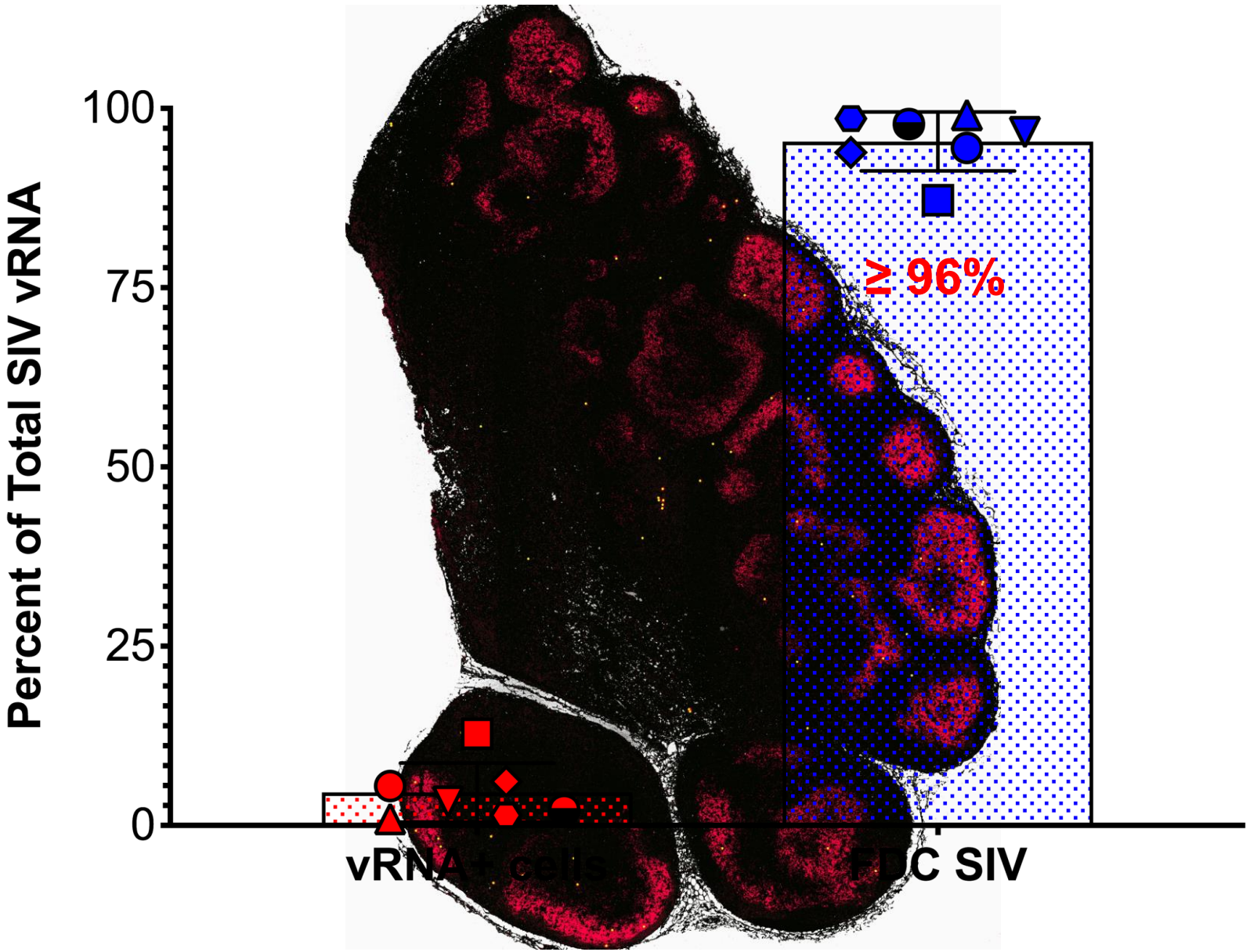

By Balthasar Heesters and Michael Carroll, IDI Harvard Medical School

# FDC – TRAPPED VIRAL PARTICLES

**RNAscope SIV vRNA (vRNA+ cells and Virions)**

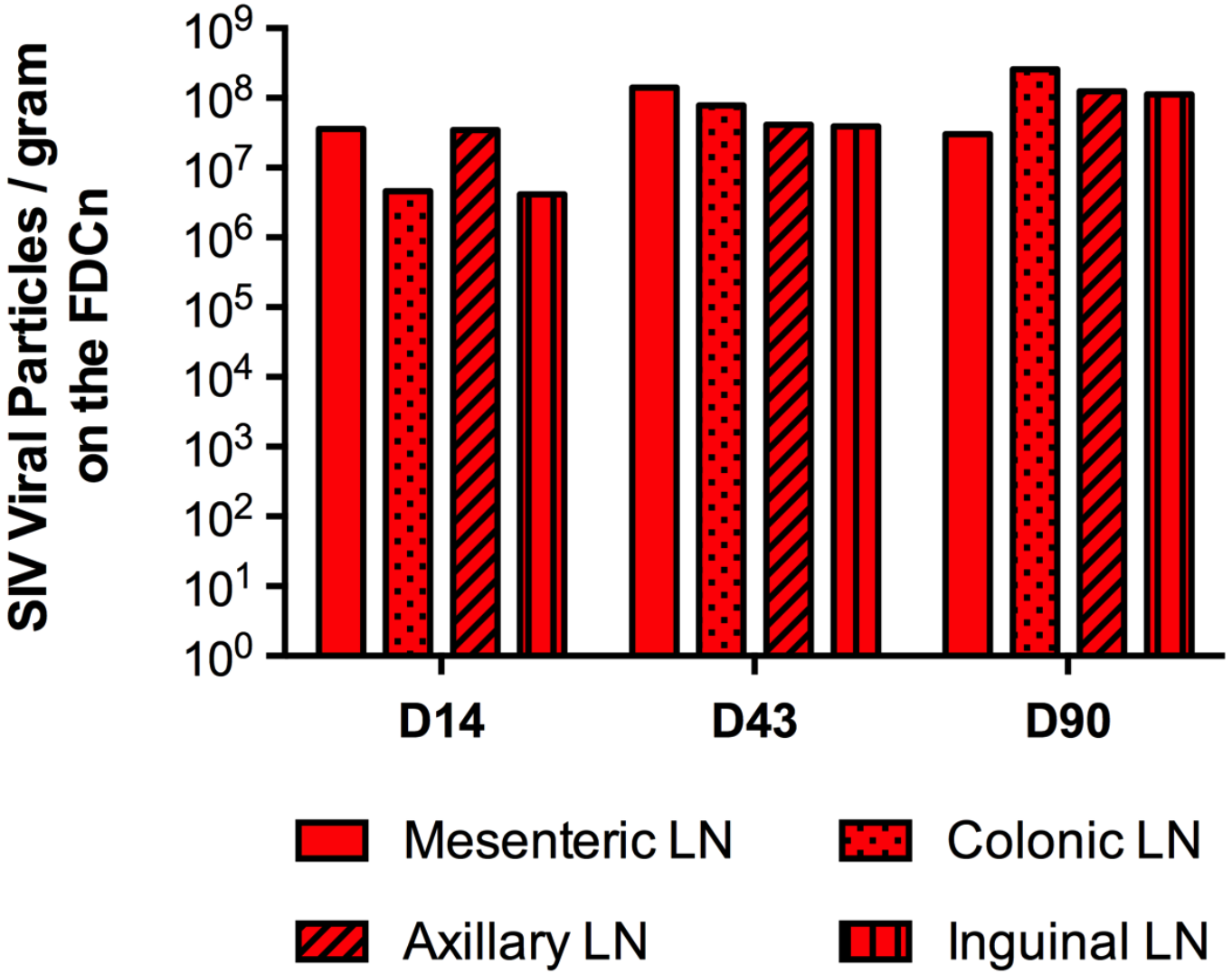


# CONTRIBUTION OF FDC-BOUND VIRUS vs. vRNA+ CELLS IN CHRONIC INFECTION



# FDC VIRAL RESERVOIR PRIOR TO cART

**~ $10^{10}$ Viral particles trapped on FDCs in LNs of RMs before ART**

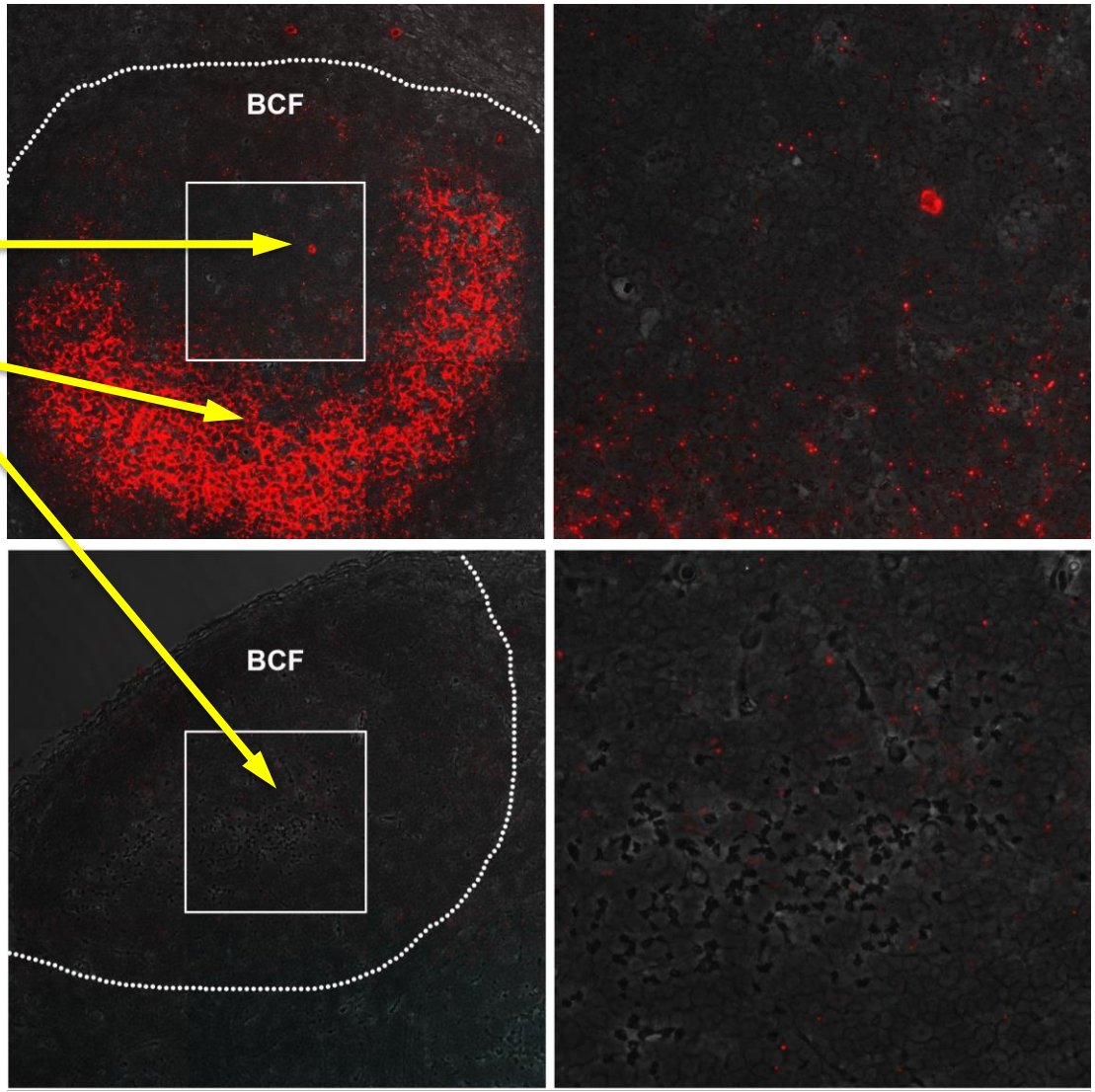


# FDC – TRAPPED VIRAL PARTICLES BEFORE AND DURING cART

**SIV vRNA (RNAscope)**

**vRNA+ cell**

**Viral Particles**

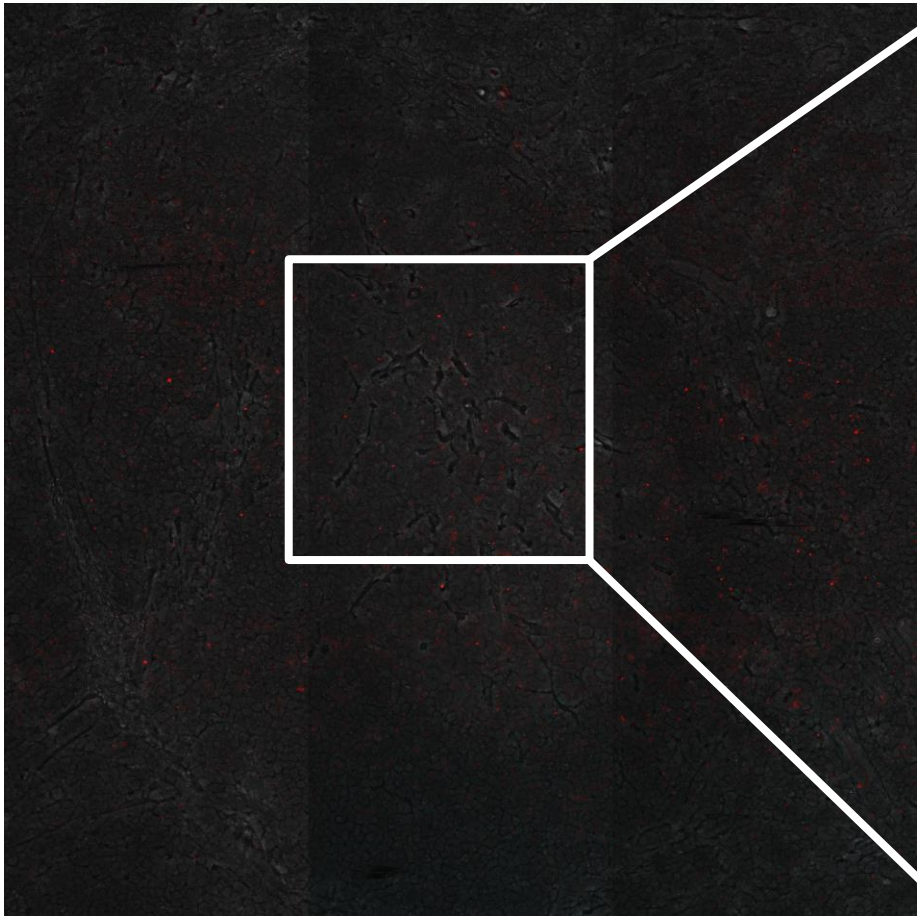


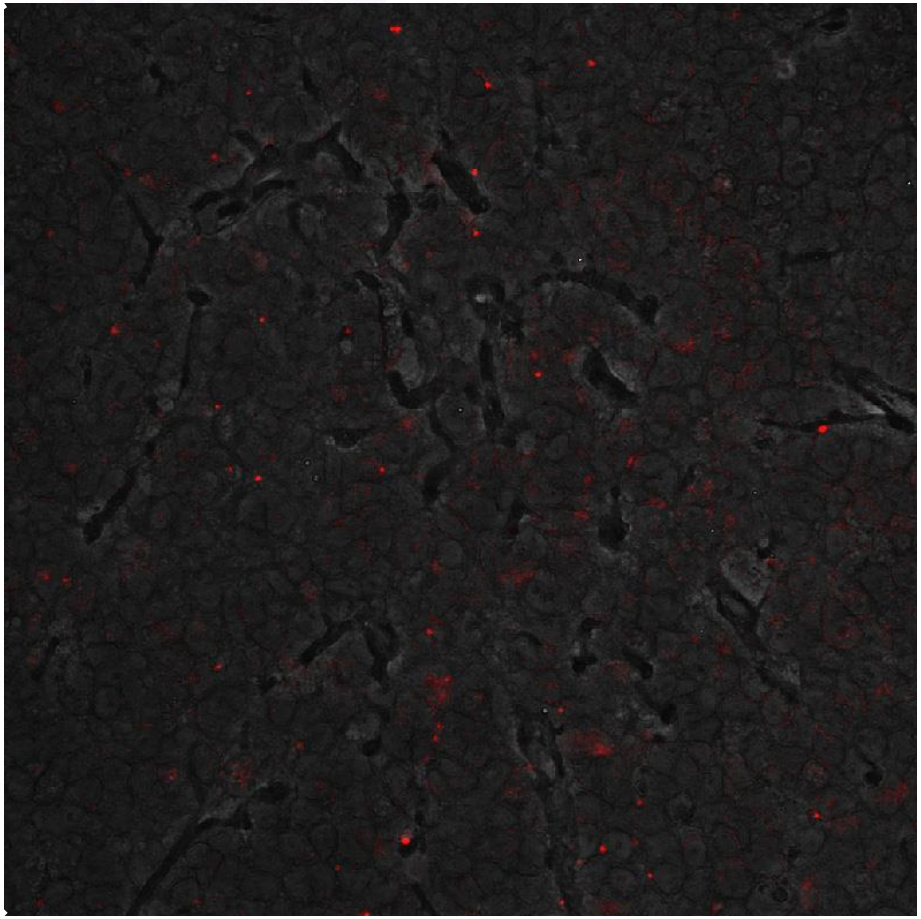

**Pre-ART**
**pVL 6.7 x $10^6$**

**During ART**
**pVL <30**
**LN VL 5.1/$10^5$ cells**

# FDC – TRAPPED VIRAL PARTICLES BEFORE AND DURING cART

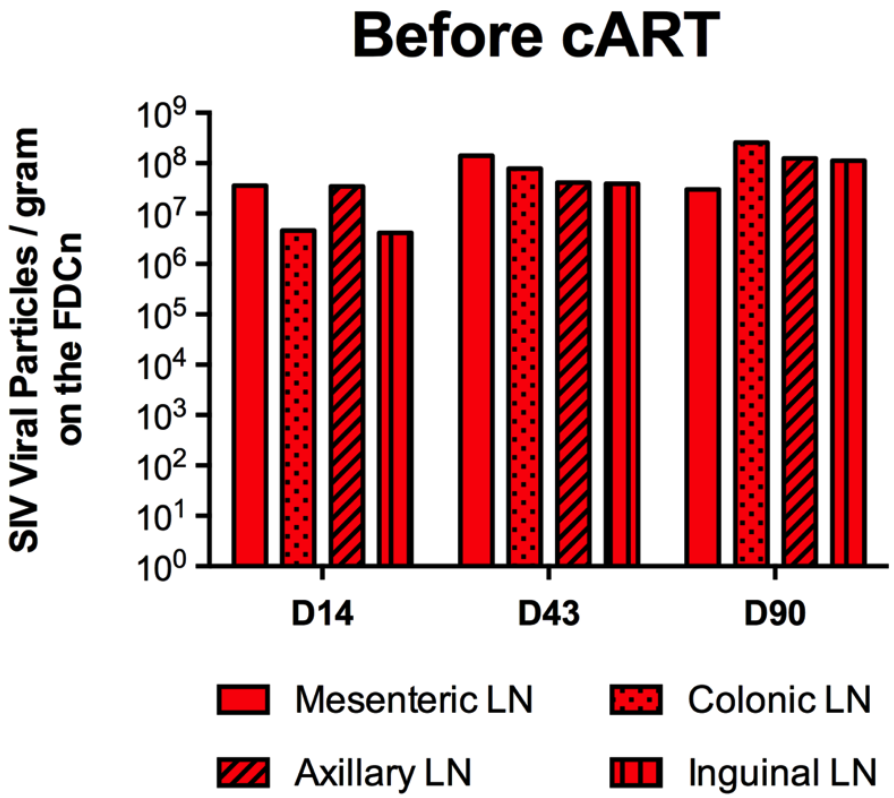
**pVL < 30 copies SIV RNA**
**LN = 8.2 SIV RNA/$10^5$ cell Eq.**

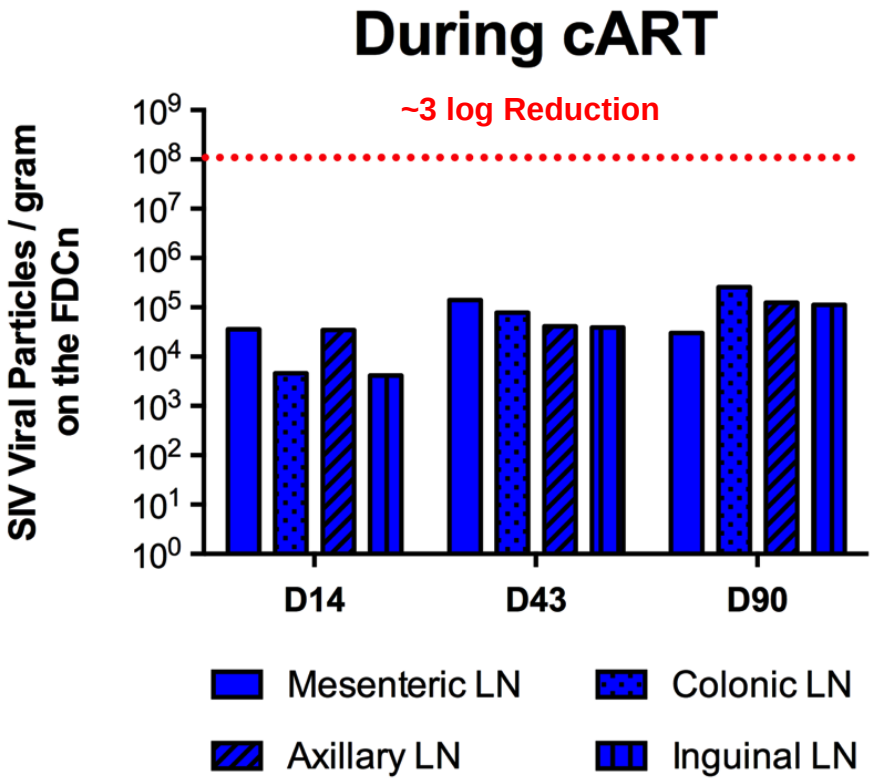




**~3 log Reduction in FDC-bound virions with ART (26 weeks)**

# CONTRIBUTION OF FDC RESERVOIR DURING ART?



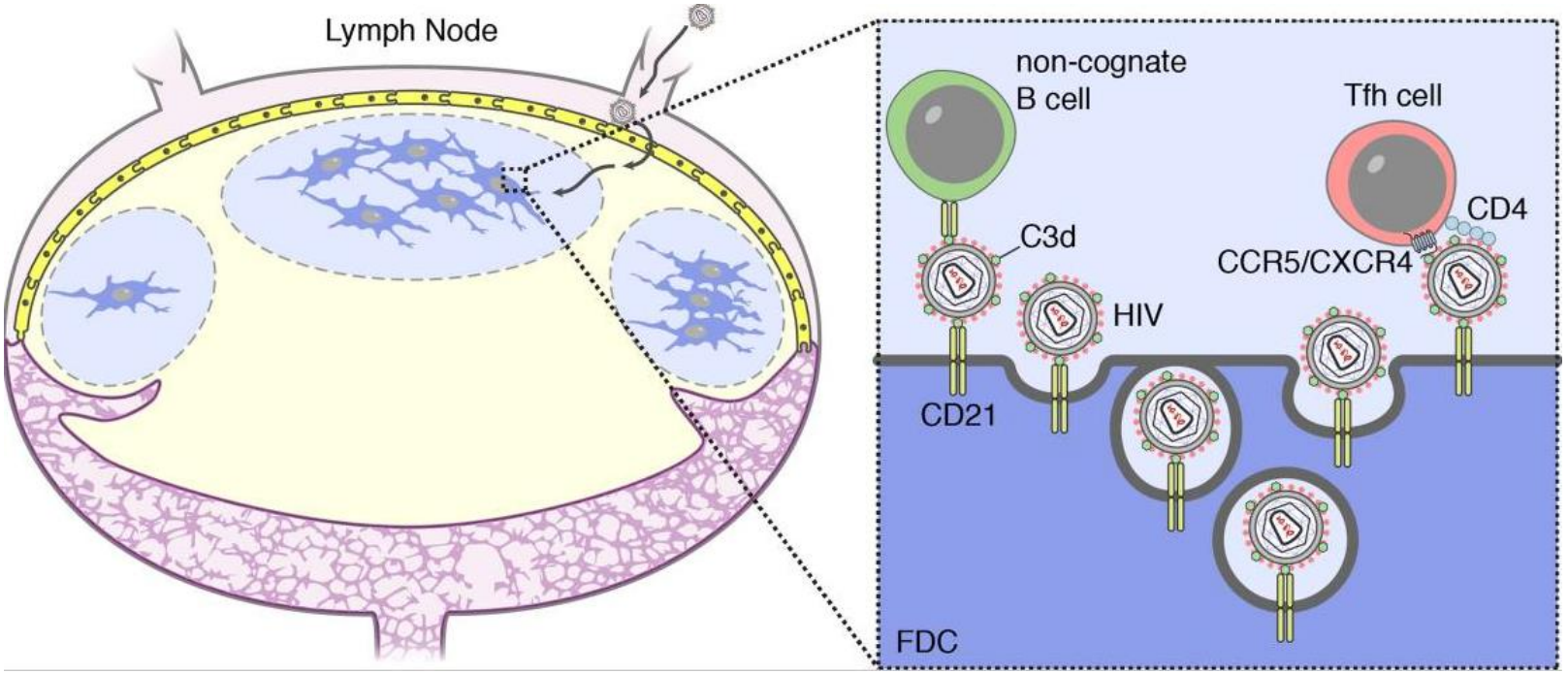




**~ $10^7$ Viral particles trapped on FDCs in LNs of RMs on ART**

RESEARCH ARTICLE

# Follicular Dendritic Cells Retain Infectious HIV in Cycling Endosomes

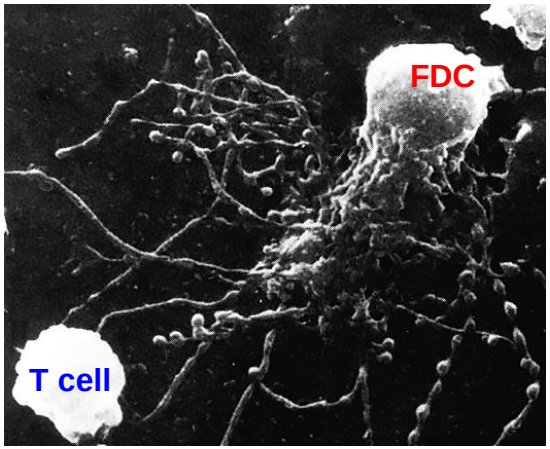
**Balthasar A. Heesters[1,2,3]*, Madelene Lindqvist[4,5], Parsia A. Vagefi[6], Eileen P. Scully[4,7], Frank A. Schildberg[2], Marcus Altfeld[4,8], Bruce D. Walker[4,5], Daniel E. Kaufmann[4,5,9], Michael C. Carroll[1,10]**

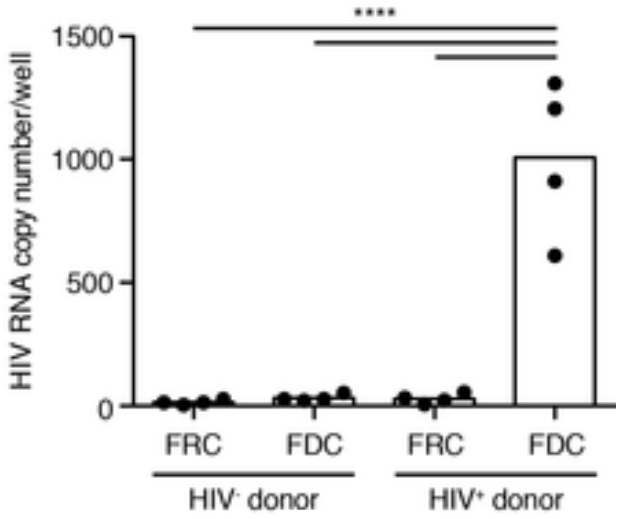


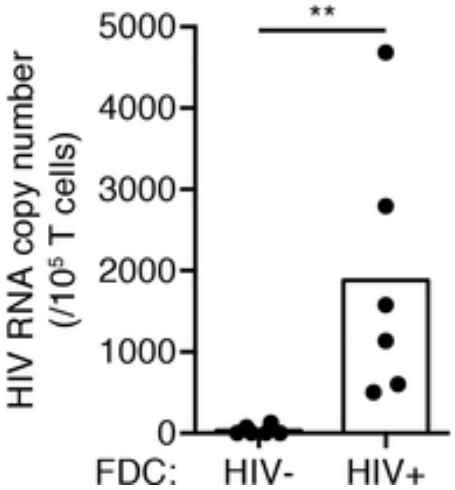

Heesters BA , et al. ***PLoS Pathog***. 2015

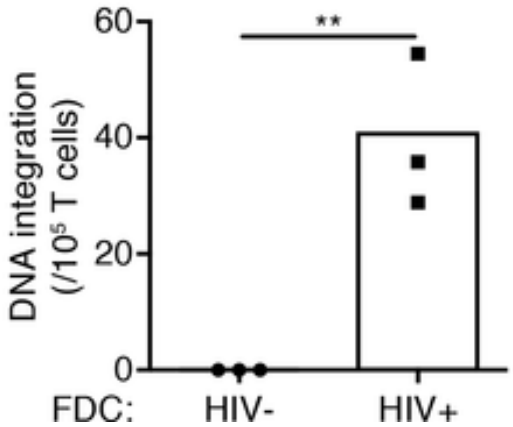
# THE FDC RESERVOIR IN HIV+ INDIVIDUALS

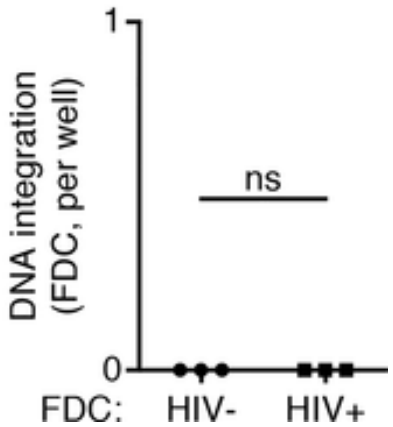
**HIV+ Individuals on ART for median of 8 years (1-24 years)**



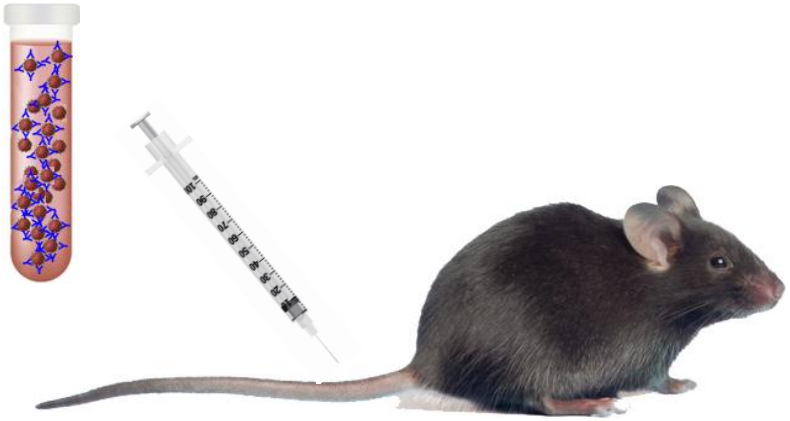



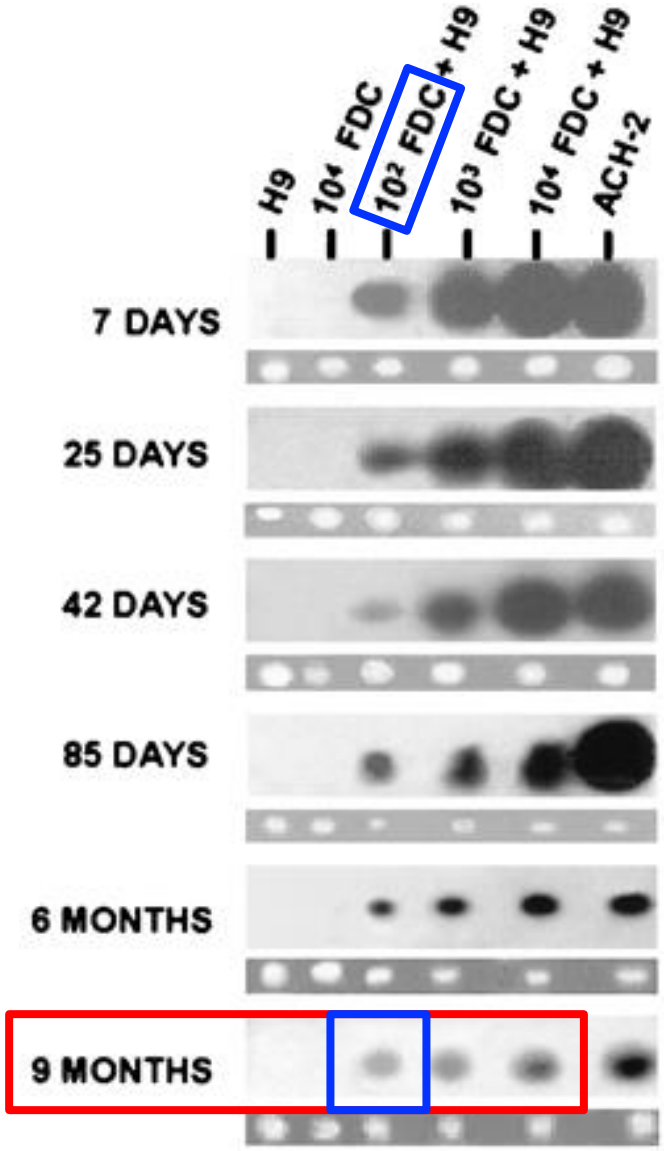

Heesters BA , et al. ***PLoS Pathog***. 2015

# THE FDC RESERVOIR: A HISTORY LESSON

**HIV Immune Complexes**



**Non-permissive Host**




Smith BA, et al. *J Immunol.* 2001 Jan 1;166(1):690-6

# SUMMARY

- **Importance of LT (GI tract) as predominant tissue sites of infected cells before and during ART (~99% of vRNA+ and vDNA+)**
  - "Active" reservoirs (vRNA+ cells) persist in SIV-infected RMs and HIV-infected individuals during long-term suppressive ART
- **BCFs within LTs represent the dominant source of vRNA before ART and FDCs continue to retain virus during ART**
- **"Active" (vRNA+) and total (vDNA+) reservoirs predominate in BCFs during ART**
- **Estimated between 2.3 x $10^7$ - 1.2 x $10^8$ replication competent vDNA+ cells in HIV-infected individuals**

**Significant challenges to overcome in developing effective 'HIV Cure' strategies:**

- **The large size of cellular viral reservoirs (both active and latent) containing replication competent proviruses represents two unique potential sources of viral rebound following treatment interruption**
- **The large repository of infectious virus trapped on FDCs within lymphoid tissues before and persisting during ART highlight a unique potential source of viral rebound following treatment interruption**

# ACKNOWLEDGMENTS


**FNLCR (OHSU*)**
W. Gregory Alvord
Kathleen Busman-Sahay*
**Chi Chan***
Vicky Coalter
**Claire Deleage**
Greg Del Prete
Xing-Pei Hao
Leslie Johnston
Brandon Keele
Rebecca Kiser
Jeff Lifson
Carissa Lucero
Rhonda Macallister*
David Morcock
Mike Nekorchuk*
Jeremy Smedley*
Brian Tabb
C. Mac Trubey

**NIH/NIAID**
Jason Brenchley
Danny Douek
Vanessa Hirsch
Nikki Klatt
Zach Klase
Krystelle Makamdop
J.C. Mudd
Alex Ortiz (O'Sick)
Srini Rao

**BCH/HMS**
**Michael Carroll**

**Emory**
Mirko Paiardini
Colleen McGary
Vincent Marconi
Luca Micci

**JCRC-Uganda**
Cissy Kityo
Francis Ssali

**UC-Davis**
Paul Luciw

**UCSF**
Steve Deeks
J. Mike McCune
Louis Swainson

**UMN**
Jodi Anderson
Greg Beilman
Jeff Chipman
Ashley Haase
**Tim Schacker**

**UN**
Courtney Fletcher


Frederick National Laboratory for Cancer Research

AIDS and Cancer Virus Program

Delaney AIDS Research Enterprise DARE to find a cure

National Cancer Institute

National Institutes of Health

Department of Health & Human Services USA