# News from the Reservoirs: Lymphatic Tissues

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# HIV Cure - Remission

- Goal: to find and diminish the size and achieve long-term control of a replication-competent HIV reservoir.
- Three preeminent challenges:
  - Identify the location of the anatomical reservoir/sanctuary from which the virus repopulates blood after ART discontinuation;
  - Define the mechanism(s) by which virus is maintained at low or undetectable levels in such locations;
  - Develop treatment(s) that will eradicate or silence virus without damaging nearby sensitive or irreplaceable tissues (eg, CNS).

Rose R, Nolan D, Maidji E, Stoddart C, Singer E, Lamers S, McGrath MS. AIDS Res Hum Retro 2018; 34:3-8.

# Defining the total AIDS virus burden: SIV RNA+ cells are mainly in lymphoid tissues

- Tissue virus burden was assessed in 27 macaques infected with 3 distinct simian AIDS viruses.
- 98.4% of vRNA+ cells were in lymphoid tissues (gut, LN, spleen).

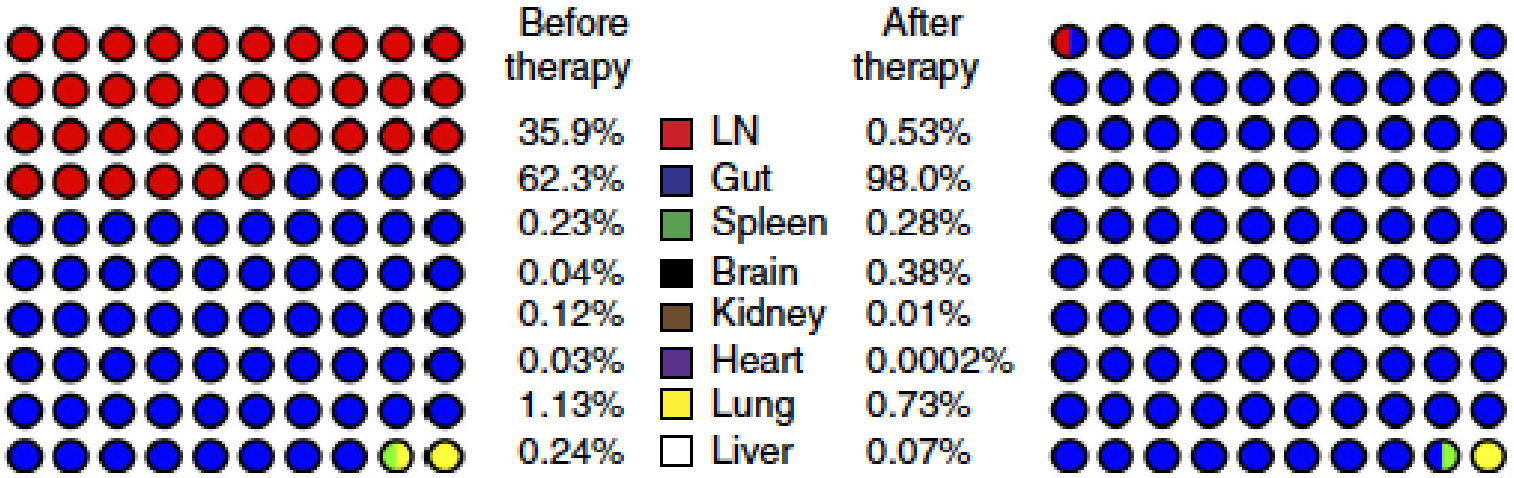
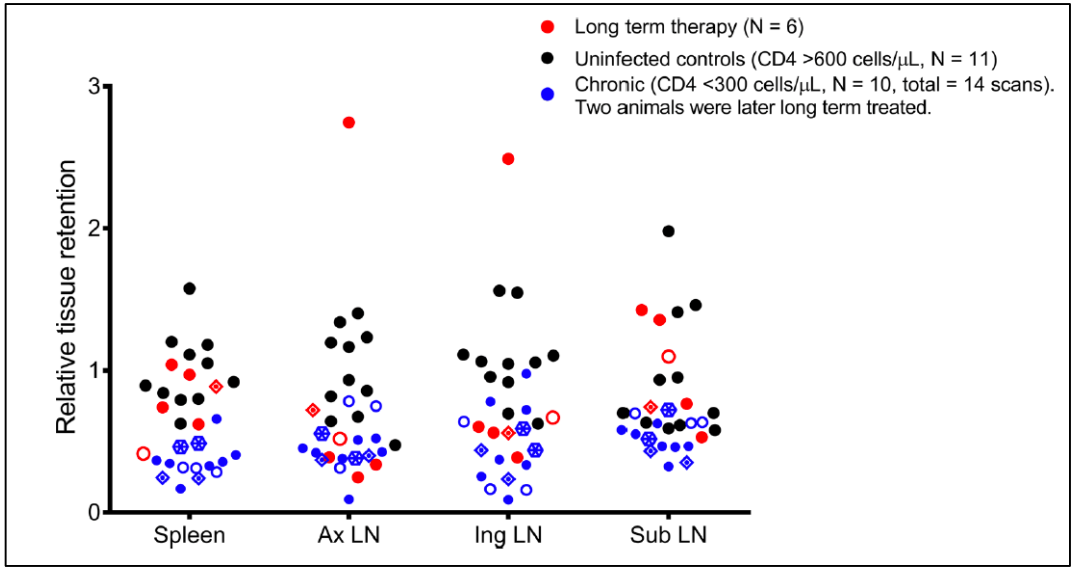



**Figure 1** Graphical representation of the proportion of vRNA+ cells in each organ system before and during suppressive ART.

Estes JD et al. Nature Medicine 2017;23:1271-76.

# Select Recent Lymphoid Tissue Literature - 1

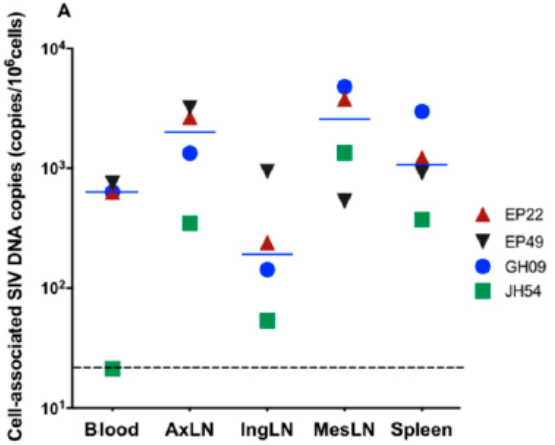
- Histoarchitectural Deterioration of Lymphoid Tissues in HIV and Aging.
  Furler R, Nixon D. AIDS Res Hum Retro 2020. DOI: 10.1089/AID.2019.0156
  - Histoarchitectural changes through fat accumulation and/or LT fibrosis are common themes help explain the decline of immunity in the elderly and HIV-infected persons.
- In Vivo Imaging of CD4 T Cell Dynamics.
  DiMascio M. JCI Insight 2018. doi.org/10.1172/jci.insight.97880
  - Non-invasive whole-body imaging of CD4 T cell pool in SIV-infected NHPs, revealed that the axillary and inguinal LN pools were suboptimally reconstituted during suppressive ART compared with splenic pools.
  - These data highlight the limitation of peripheral CD4 cells to infer whole body ART response.



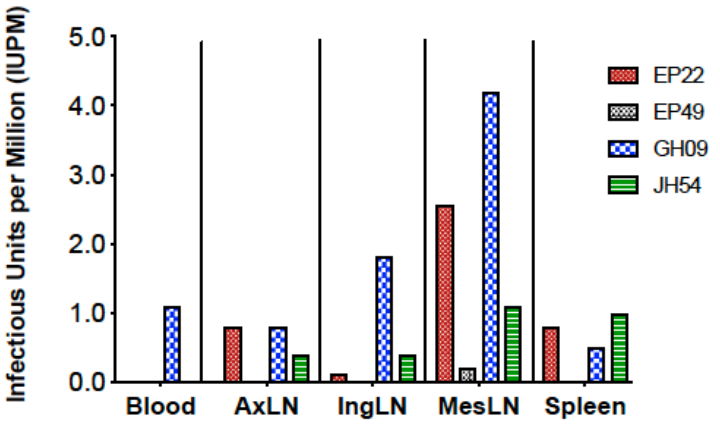
# Select Recent Lymphoid Tissue Literature - 2

- Cellular HIV Reservoirs & Rebound from Lymphoid Compartments. Maidji E . . Stoddart C. Viruses 2019;11:256. doi:10.3390/v11030256
  - State of art technology to quantitatively investigate composition and distribution of HIV reservoirs in lymphoid tissues in hu-mice.
  - LT harbored very rare transcription/translation competent HIV during suppressive EFdA therapy that may enable viral rebound. A small subset of CD163+ macrophages with RNA or DNA was also found.
- Persistent Viral Reservoirs in Lymphoid Tissues of NHPs. Siddiqui S. Viruses 2019;11:105. doi:10.3390/v11020105
  - Determined cell-associated DNA and RNA to determine size and distribution of HIV reservoirs, and viral outgrowth assays (QVOA) to determine replication-competence.

**Cell-Associated SIV DNA**




**Size of Replication-Competent Viral Reservoirs**



nature medicine

# B cell follicle sanctuary permits persistent productive simian immunodeficiency virus infection in elite controllers

Yoshinori Fukazawa[1,2], Richard Lum[1,2], Afam A Okoye[1,2], Haesun Park[1,2], Kenta Matsuda[3], Jin Young Bae[1,2], Shoko I Hagen[1,2], Rebecca Shoemaker[4], Claire Deleage[4], Carissa Lucero[4], David Morcock[4], Tonya Swanson[1,2], Alfred W Legasse[1,2], Michael K Axthelm[1,2], Joseph Hesselgesser[5], Romas Geleziunas[5], Vanessa M Hirsch[3], Paul T Edlefsen[6], Michael Piatak, Jr[4], Jacob D Estes[4], Jeffrey D Lifson[4] & Louis J Picker[1,2]

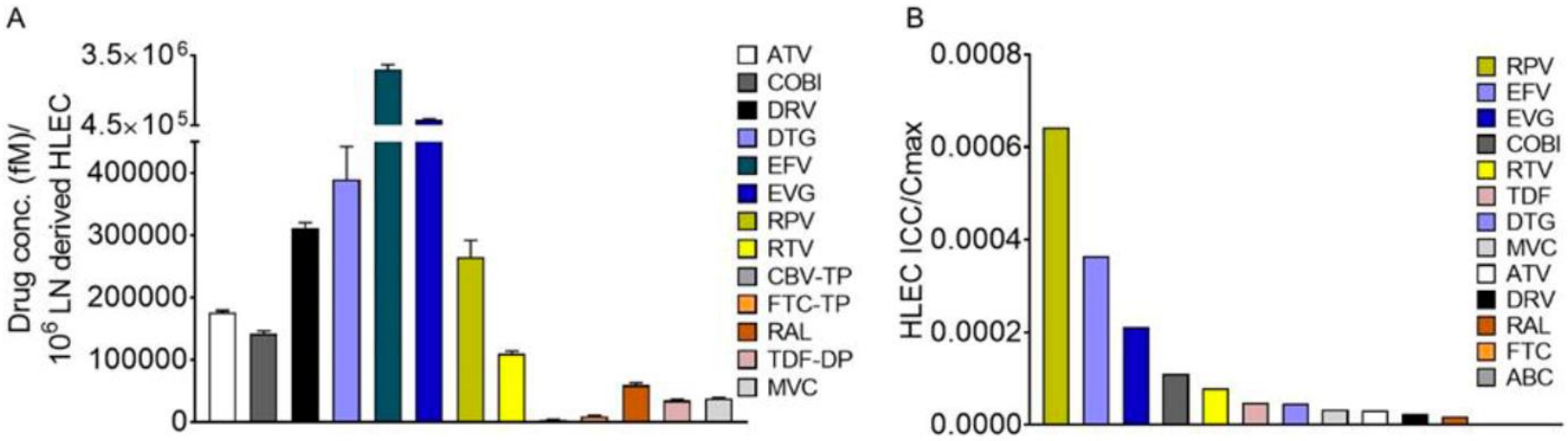
**Chronic-phase HIV and simian immunodeficiency virus (SIV) replication is reduced by as much as 10,000-fold in elite controllers (ECs) compared with typical progressors (TPs), but sufficient viral replication persists in EC tissues to allow viral sequence evolution and induce excess immune activation. Here we show that productive SIV infection in rhesus monkey ECs, but not TPs, is markedly restricted to $CD4^+$ follicular helper T ($T_{FH}$) cells, suggesting that these EC monkeys' highly effective SIV-specific $CD8^+$ T cells can effectively clear productive SIV infection from extrafollicular sites, but their relative exclusion from B cell follicles prevents their elimination of productively infected $T_{FH}$ cells. $CD8^+$ lymphocyte depletion in EC monkeys resulted in a dramatic re-distribution of productive SIV infection to non-$T_{FH}$ cells, with restriction of productive infection to $T_{FH}$ cells resuming upon $CD8^+$ T cell recovery. Thus, B cell follicles constitute 'sanctuaries' for persistent SIV replication in the presence of potent anti-viral $CD8^+$ T cell responses, potentially complicating efforts to cure HIV infection with therapeutic vaccination or T cell immunotherapy.**

Nature Medicine 2015;21:132-39.

# Mechanisms and Determinants of ARV Penetration into Lymphatic Tissues

- Portal vein vs. lymph blood flow is 500:1.
  - Most absorbed drugs are preferentially diverted to portal blood.
- Physicochemical characteristics associated with greater lymphatic system penetration: hi molecular weight, larger particle size, log P value > 5, hi long chain TG solubility.
  - All ARVs are low MW compounds and few have log P values > 5.
- Pharmacologic characteristics: distribution and expression of membrane transporters and CYPs in lymphatic endothelial cells and along the GI tract, and ARV substrate specificity.
  - P-gp increases from duodenum to ileum; CYP3A4 decreases from duodenum to colon.
- HIV-associated pathology: lymph node fibrosis.
  - Collagen deposition and resulting fibrosis are correlated with progression to AIDS and less immune reconstitution.

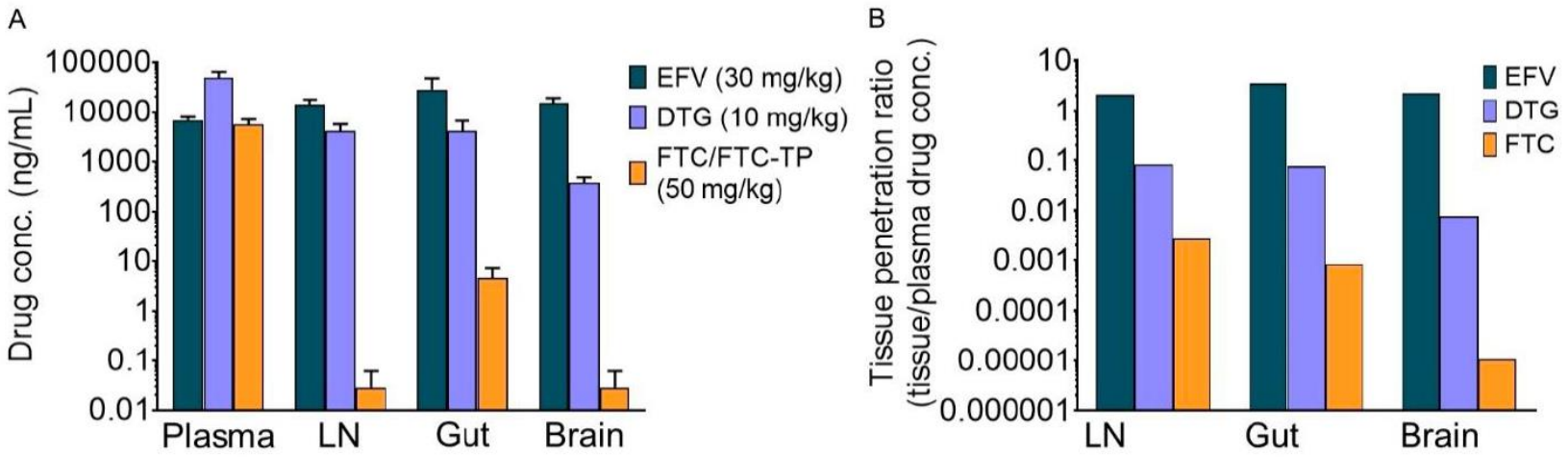
# Lymphoid Tissue Bioavailability of ARVs: lymphatic endothelial cell in vitro model




| Hi (> 67th %ile) | | Medium (67->33rd %ile) | | Low (<33rd %ile) | |
|---|---|---|---|---|---|
| RPV | 4.5 | RTV | 5.2 | ATV | 5.6 |
| EFV | 4 | TDF | 1.6 | DRV | 2.9 |
| EVG | 5.3 | DTG | 2.4 | RAL | 1.1 |
| Cobi | 5.7 | MVC | 5.1 | FTC | -0.6 |
| | | | | ABC | 0.9 |
| Avg LogP | 4.9 | | 3.6 | | 1.9 |

Dyavar R, et al. J Antimicrob Chemother 2019; 74:2974-78

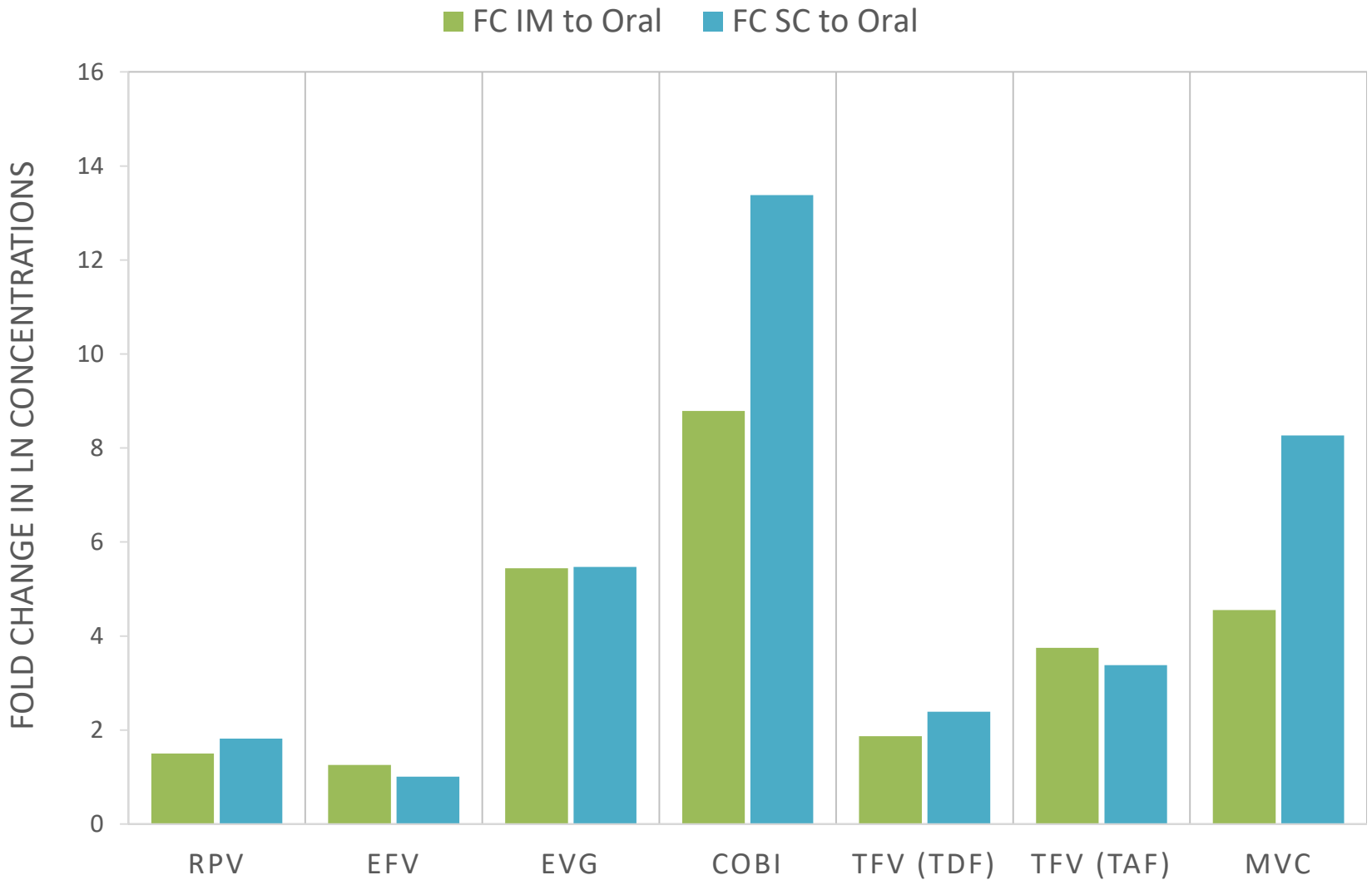
# Lymphoid Tissue Bioavailability of ARVs: bioavailability studies in mice




| | Hi (EFV) | | Medium (DTG) | | Low FTC) | |
|---|---|---|---|---|---|---|
| **Log P** | EFV | 4 | DTG | 2.4 | FTC | -0.6 |
| **pKa** | EFV | 10.2 | DTG | 8.2 | FTC | 2.65 |
| **$H_2O$** | EFV | 0.093 | DTG | 0.095 | FTC | 112 |

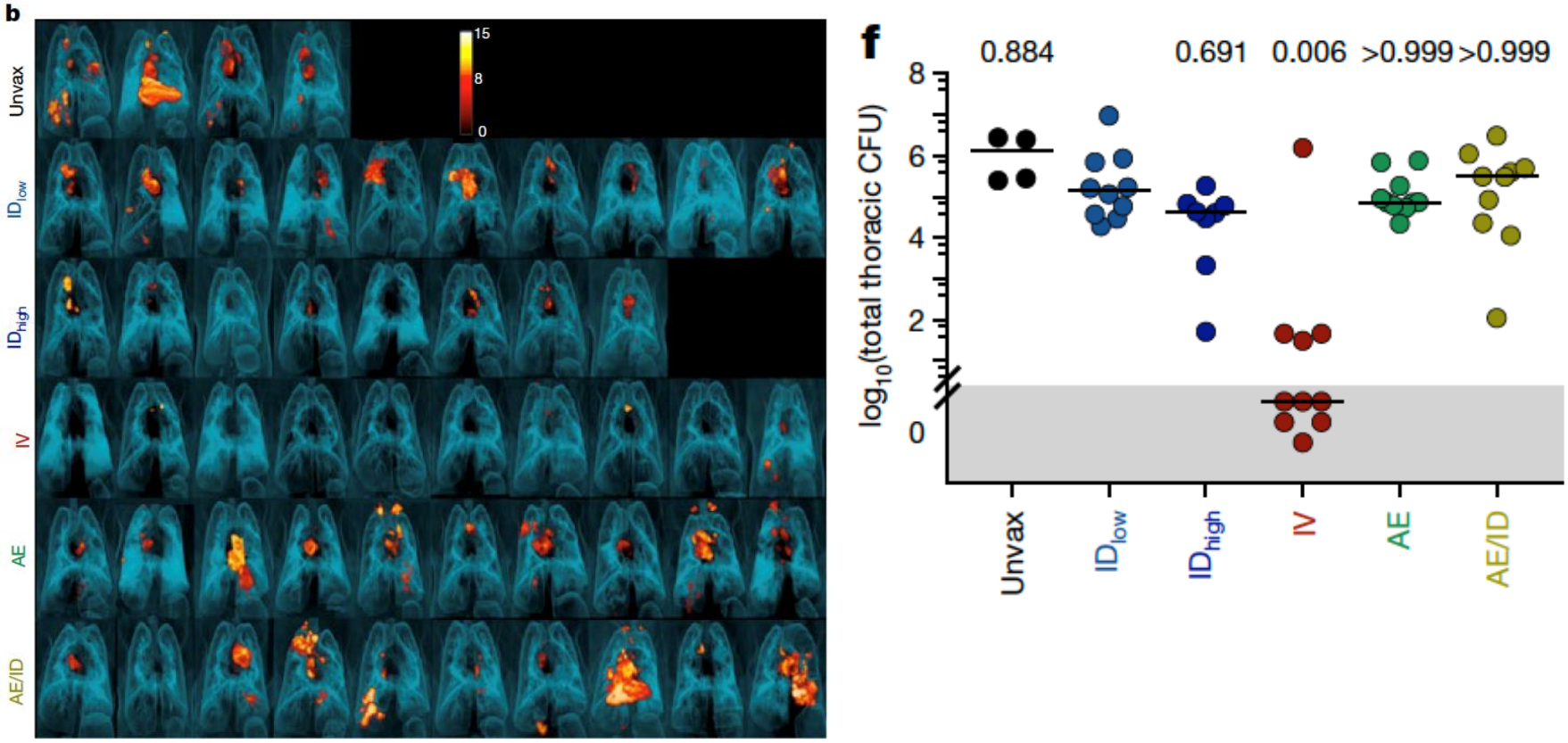
Dyavar R, et al. J Antimicrob Chemother 2019; 74:2974-78

# Lymph Node Concentrations in Mice: Comparison of Oral, IM and SC Dosing




Dyavar RS . . Fletcher CV. HIV Persistence Workshop 2019. Miami, FL, December 10-13, 2019

# Prevention of Tuberculosis after Intravenous BCG Immunization




- 9 of 10 NHPs that received IV BCG were protected against Mtb challenge, with 6 showing no sign of infection, vs. none who received low-dose intradermal administration.

Darrah PA et al. Nature 2020; 577:95-102.

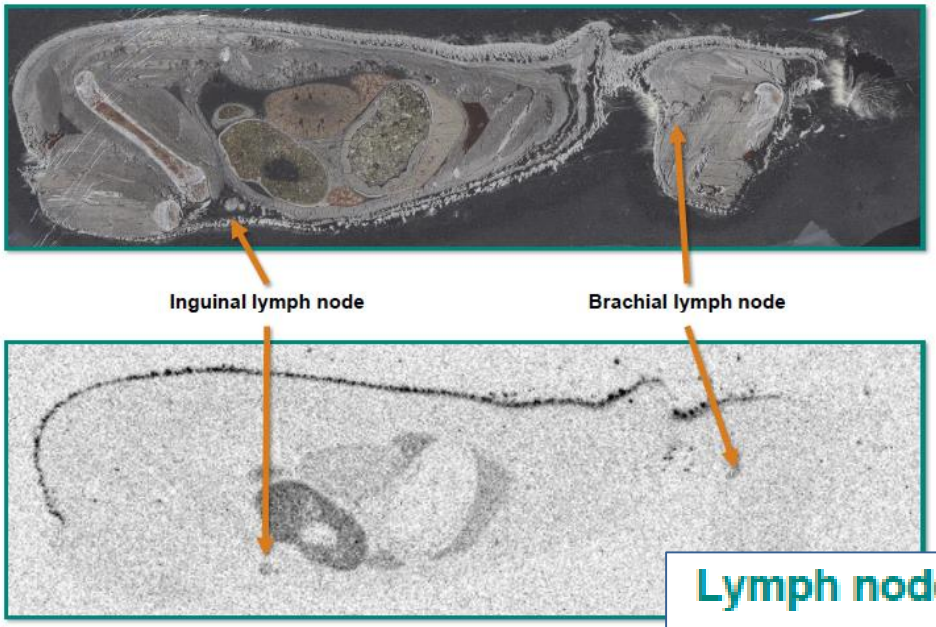
# Lymphoid Tissue Pharmacokinetics of TAF vs. TDF

| Matrix | Tenofovir-diphosphate (fmol/$10^6$ cells) Median (and Interquartile Range) | |
|---|---|---|
| | **TAF (n=13)** | **TDF (n=45)** |
| PBMC | 497 (384, 639) | 63 (44, 91) |
| LN | 136 (88, 156) | 22 (8, 27) |
| Ileum | 82 (17, 250) | 3056 (458, 5835) |
| Rectum | 47 (31, 102) | 441 (287, 985) |

Fletcher CV, Thorkelson A, Campbell K, Winchester L, Mykris T, Weinhold J, Anderson J, Zulk J, Moshele P, Jorstad S, Podany A, Baker J, Schacker TA.
Abstract 130, CROI 2019.

# Islatravir (EFdA, MK-8591) Lymphoid Tissue Penetration

**QWBA Study [$^{14}$C]MK-8591**
**in Male Rats at 24 Hours After 50 mg/kg P.O. Single Dose**




**Top:** Scanned optical image of 40 µm male rat whole-body sagittal section
40 µm male rat whole-body sagittal sections. Dark areas represent presenc

**Lymph node:blood ratio**

| Lymph Nodes | 0.5 hr | 24 hr |
|---|---|---|
| Lymph node – brachial | 2.69 | 7.80 |
| Lymph node – inguinal | 2.63 | 8.18 |
| Lymph node – sciatic | 3.06 | ND |
| Lymph node – submandibular | 2.47 | 5.39 |

Grobler JA et al.
Abstract # 435, CROI 2017

# HIV Cure - Remission

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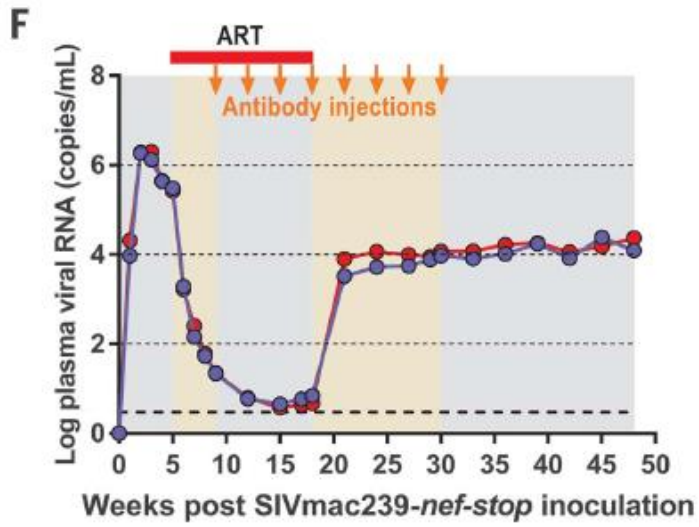
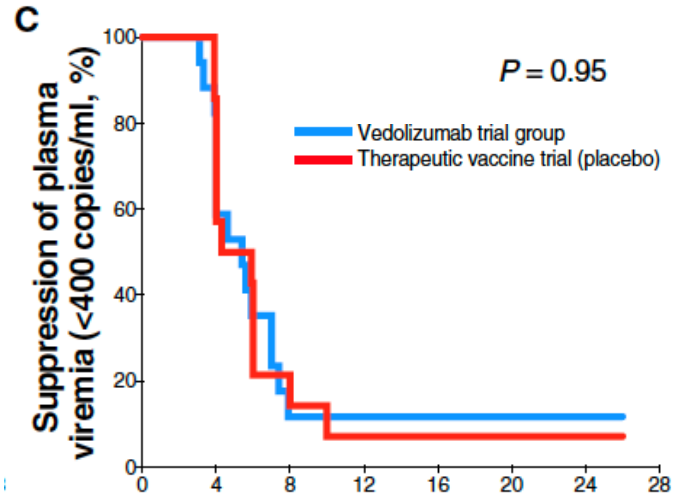
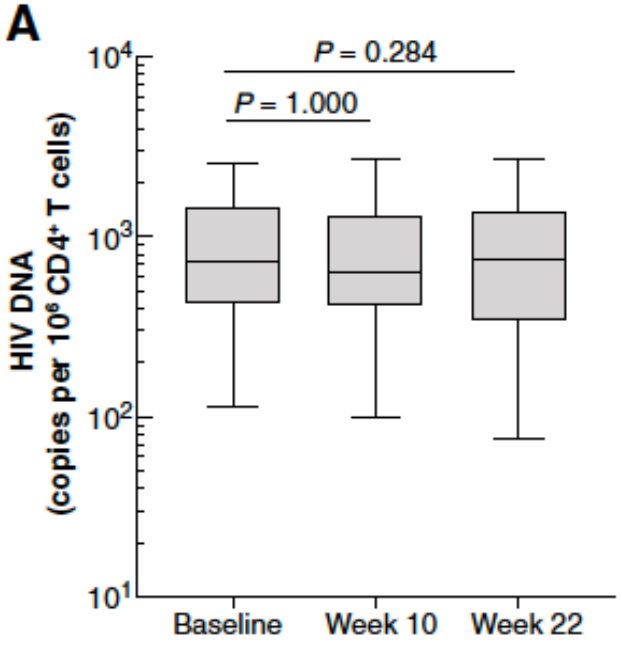
Rose R, Nolan D, Maidji E, Stoddart C, Singer E, Lamers S, McGrath MS. AIDS Res Hum Retro 2018; 34:3-8.

# Evaluation of α4β7 antibody in NHPs and HIV-Infected Persons.



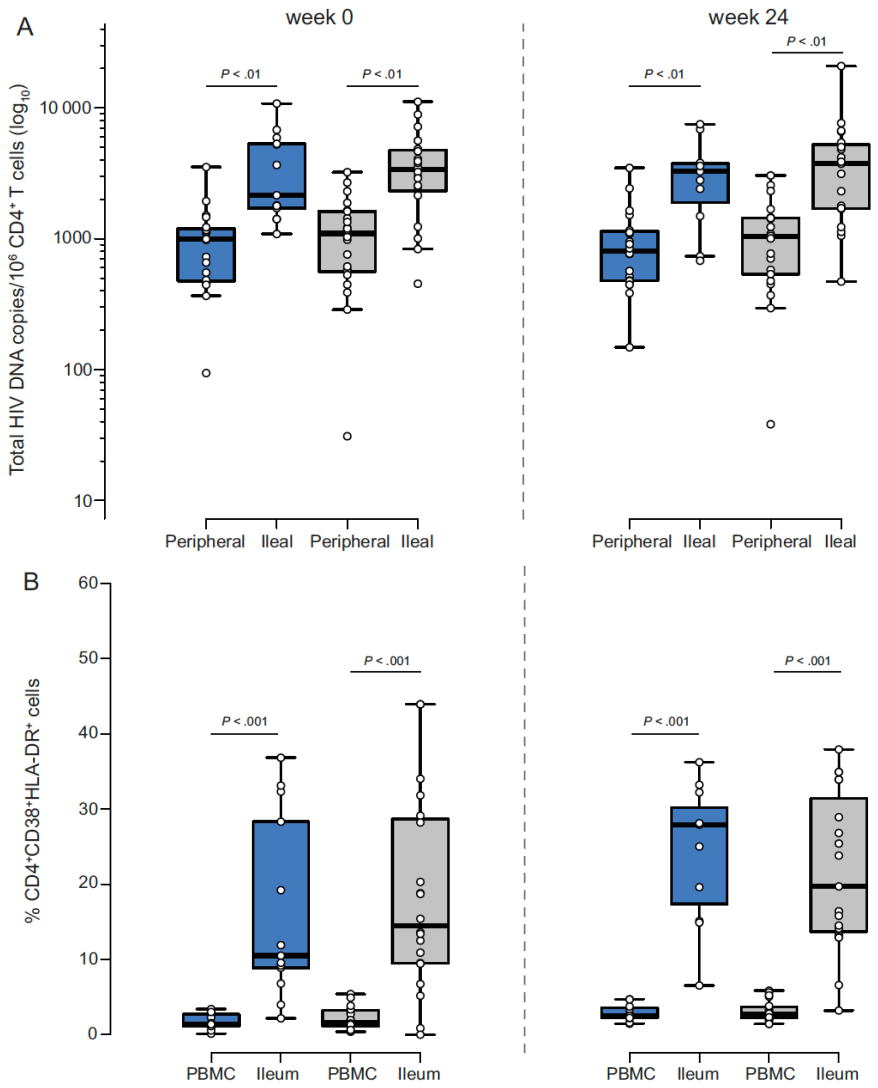




- In NHPs, plasma viremia, CD4 T cell counts and lymph node and rectal tissue viral loads were not different between anti- α4β7 and control mAb groups.
- In humans, only 1/20 who rec'd vedolizumab had suppression of plasma viremia after ART was d/c'd. There was no effect on the HIV reservoir.


1. Di Mascio M, et al. Science 2019; 365, 1025–1029.
2. Sneller MC, et al. Sci Transl Med 2019;11:eaax3447.

# Effect of a Switch from PI/r to DTG on Peripheral Blood and Ileal Biopsies

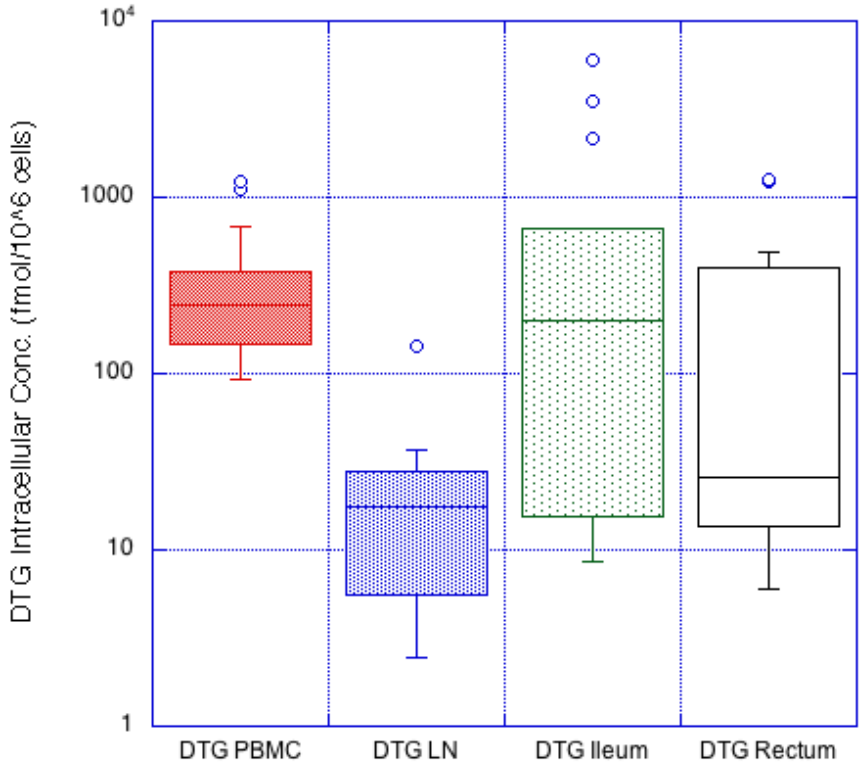
- 42 HIV-infected persons on PI-based ART (median 4yrs) with plasma VL < 50 cpm
- 22 remained on PI-ART and 20 were switched to DTG-ART.
- *Residual plasma viremia decreased in the switch group, but after adjustment for multiple comparisons, was NS.*
- HIV reservoir was larger in the ileum than peripheral blood; there was no change in reservoir size in the switch group.
- Similarly, there were more activated cells in tissues and the switch did not reduce this activation.




Moron-Lopez S et al. Clinical Infectious Diseases 2019; 69:1320-8.

# Dolutegravir Concentrations in Ileal and Rectal Tissues

- DTG concentrations in the ileum approximate those in PBMCs, while those in rectal tissues are reduced (median PBMC, 242; rectal, 56 fmol/$10^6$ cells). [1]



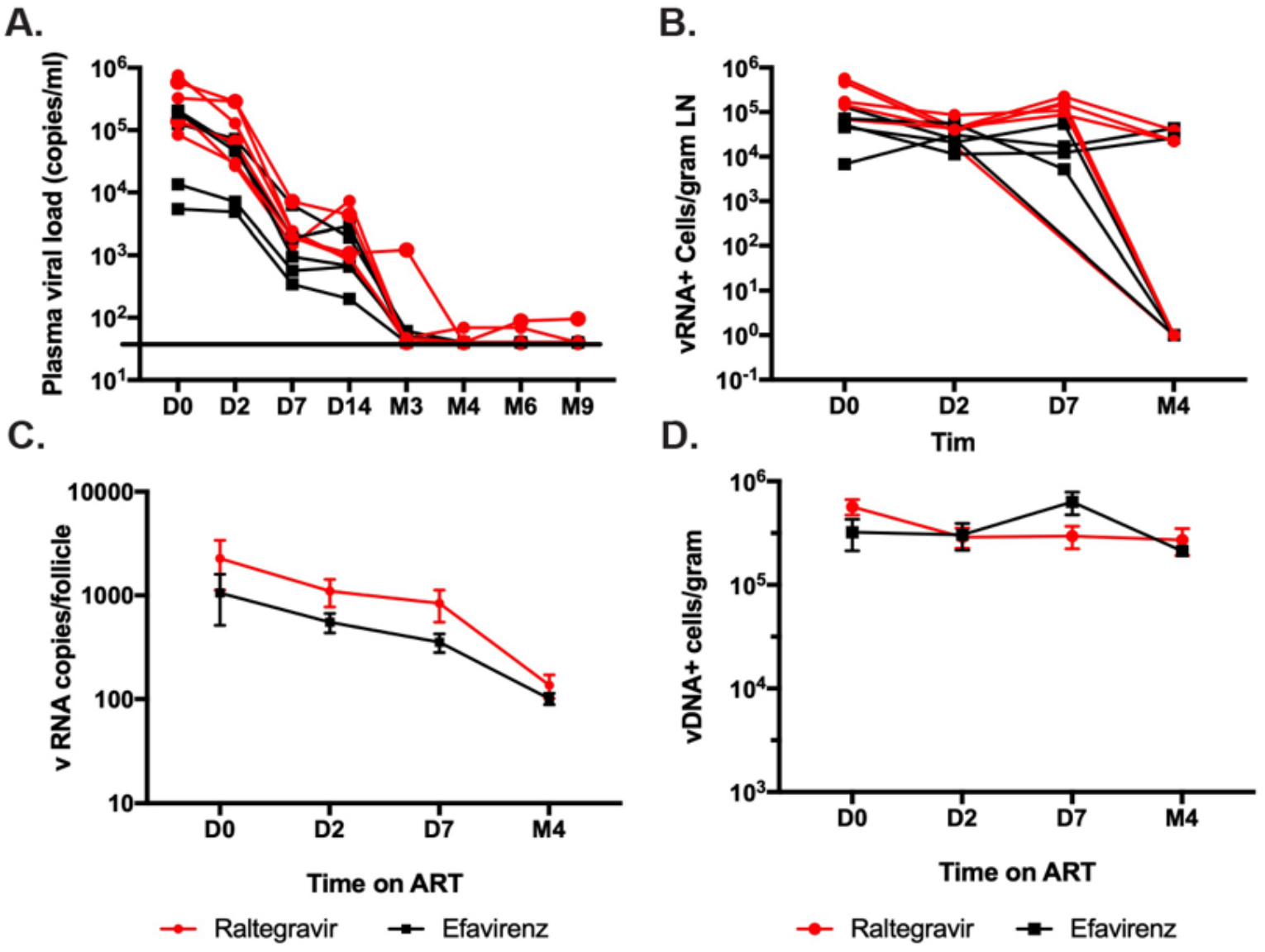

1. Fletcher CV, Thorkelson A, Winchester L, ... Schacker TW. Abstract, CROI 2018, March 4-7, 2018.

# Impact of Raltegravir vs. Efavirenz on Lymphoid Tissue (LT) Reservoirs

- Objective: to compare the kinetics of virus decay in LT and to measure cell-associated ARV concentrations in HIV-infected persons initiating RAL or EFV-based ART in Kampala, Uganda.
- 11 persons randomized to RAL- (n=6) or EFV-based (n=5) ART x 3 months; participants were changed to national standard (EFV+TDF + 3TC or FTC) and followed for another 3 months.
- Peripheral blood was obtained at days 2, 7, 14 and months 3, 4, and 6 on ART. LN biopsies were collected at baseline and days 2 and 7 and month 4. Rectal biopsy was done at mo. 4.
- Plasma VL and CD4 cells were measured in PB; vRNA and vDNA were measured in LT by in situ hybridization and quantitative image analysis; ARV concentrations were measured in PB and tissue MNCs by MS/MS.

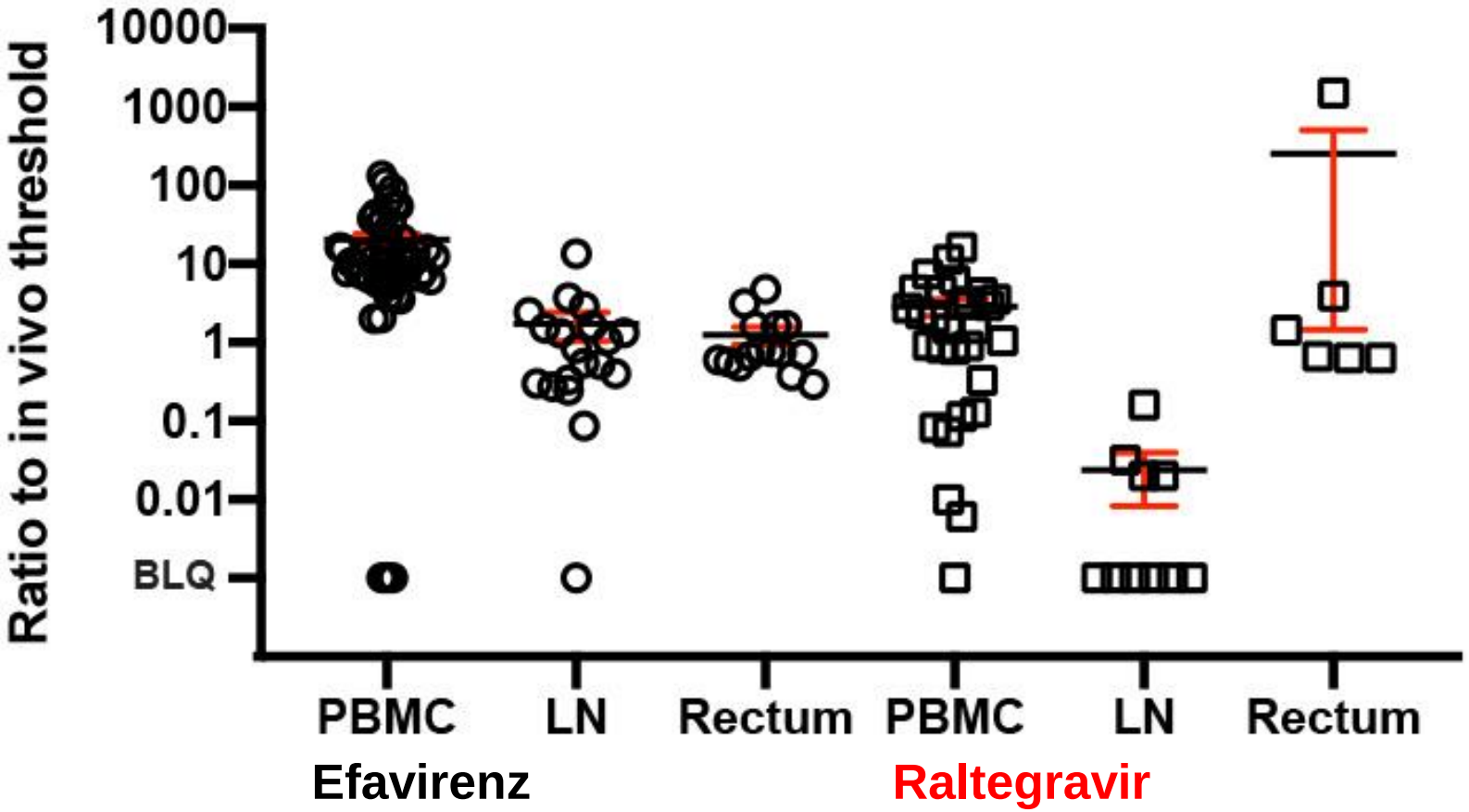
Rothenberger M, … Fletcher CV and Schacker TW. JAIDS 2019; 81:355-60.

# RAL vs. EFV: Virus Decay in Plasma and LT




Rothenberger M, … Fletcher CV and Schacker TW. JAIDS 2019; 81:355-60.

# RAL vs. EFV: PBMC and LT IQs (IQ = cell assoc. conc / clinical threshold)




Rothenberger M, … Fletcher CV and Schacker TW. JAIDS 2019; 81:355-60.

# Impact of Raltegravir vs. Efavirenz on Lymphoid Tissue (LT) Reservoirs

- We found no difference in the rate of virus decay in plasma or in LT in persons randomized to RAL- or EFV-based ART.
- RAL and EFV concentrations in plasma were consistent with expected concentrations. In PBMCs, cell-associated concentrations of RAL and EFV were both greater (1.4x and 10x, respectively) than putative clinical efficacy thresholds (60ng/mL and 700ng/mL, respectively).
- In the LN and rectum, the cell-associated concentrations of RAL and EFV did not exceed these clinical thresholds. The relative, equivalent sub-optimal LT concentrations of RAL and EFV may explain the lack of difference in LT virus decay.

Rothenberger M, … Fletcher CV and Schacker TW. JAIDS 2019; 81:355-60.

# General Pharmacokinetic Characteristics of Therapeutic Antibodies

| Process | Characteristics |
|---|---|
| Size | Large; ≈ 150kDa (vs <1 for small molecule drug) |
| Route of delivery | Parenterally (IV, SC, IM) |
| Absorption | Limited to none after oral administration; absorption after SC or IM administration involves uptake by the lymphatic system |
| Distribution | Lymphatic system involved is distribution throughout body;<br>Slowly distributes to tissues, can be minutes to hours;<br>Nonlinear binding to target of interest may occur, as can nonspecific binding within tissues;<br>Volume of distribution is dependent on affinity for tissue sites;<br>Clearance mechanisms may be present in tissues |
| Elimination | Elimination is largely by catabolism in endosomal space of cells (vs. hepatic metabolism or renal elimination);<br>Target mediated drug disposition often results in nonlinear pharmacokinetics |

# 3 Examples of Antibody Pharmacokinetics

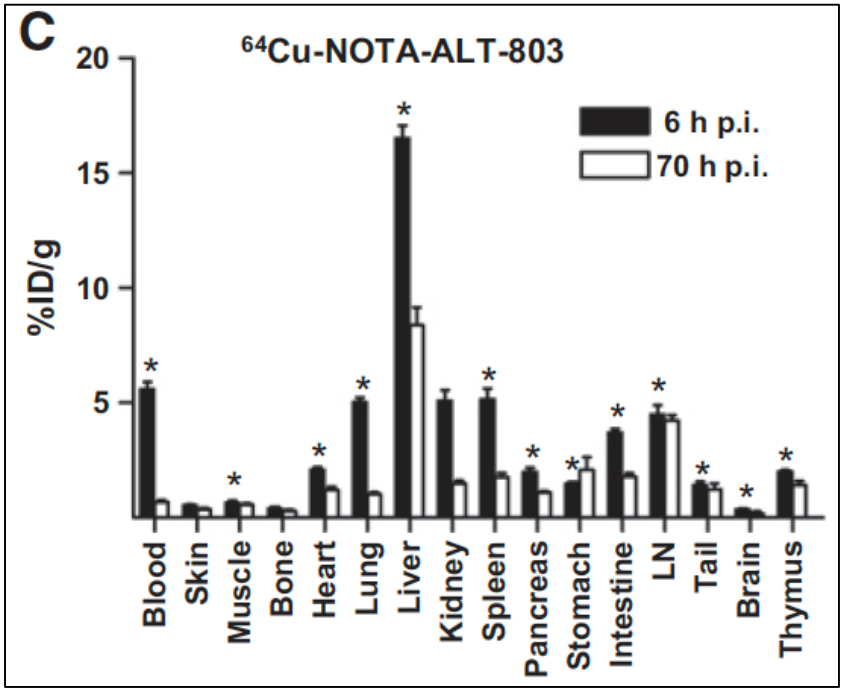



**Table 4. VRC01LS mean PK parameter values.**

| Group and dose | $C_{max}$ | $T_{max}$ | CL | $t_{1/2\beta}$ days | AUC |
|---|---|---|---|---|---|
| | | | Mean (s.d.) | | |
| **IV dosing** | | | | | |
| 5 mg/kg ($n$ = 3) | 246 (78) | 0.07 (0.08) | 40 (7) | 83 (11) | 4896 (499)* |
| 20 mg/kg ($n$ = 8) | 1,221 (397) | 0.2 (0.3) | 33 (8) | 76 (19) | 23,368 (5279)* |
| 40 mg/kg ($n$ = 5) | 2,234 (548) | 0.05 (0.02) | 38 (9) | 55(7) | 57,099 (13679) |
| Overall IV ($n$ = 16) | | | 36 (8) | 71 (18) | |
| **SC dosing** | | | | | |
| 5 mg/kg ($n$ = 9) | 69 (15) | 9.0 (7.9) | 61 (5)+ | 66 (24) | 3,777 (814) |

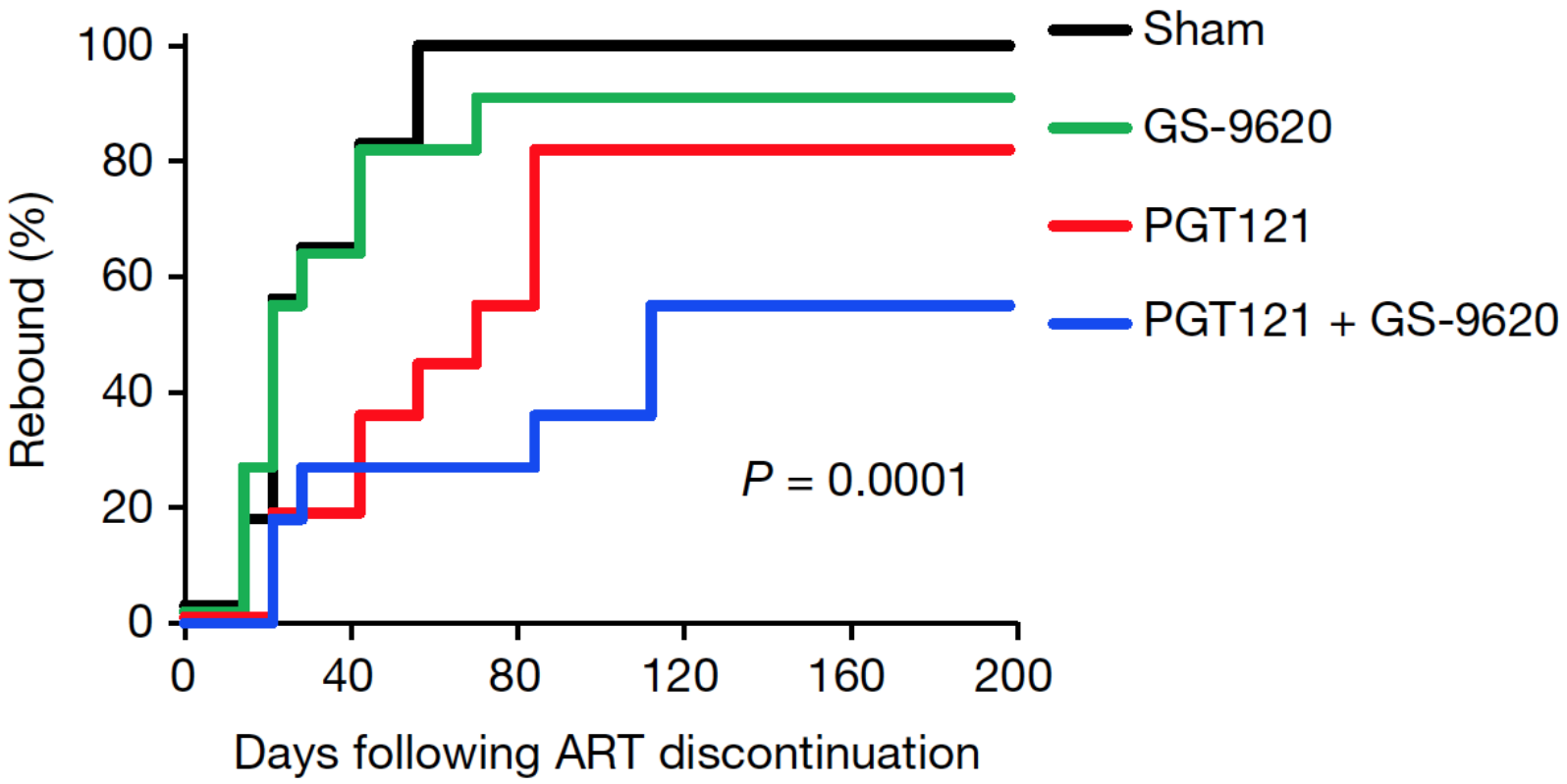
**TABLE I.** Pharmacokinetic characteristics (median and range) of PRO 542 after multiple doses every 7 days

| | PRO 542 20 mg/kg (n = 13) | PRO 542 10 mg/kg (n = 6)† | *P* value |
|---|---|---|---|
| AUC | 11,714 (5964-17,870 μg*h/mL | 11,362 (8531-13,124) μg*h/mL | .7257 |
| CL | 1.71 (1.12-3.35) mL/h/kg | 0.88 (0.76-1.17) mL/h/kg | .0009 |
| $T_{½}$ | 1.82 (1.22-2.43) days | 2.13 (1.54-2.58) days | .1144 |
| Cmax | 337 (84.8-517.8) μg/mL | 274 (229-322) μg/mL | .2926 |
| C-7 days | 8.77 (1.90-22.3) μg/mL | 6.95 (2.87-14.7) μg/mL | .5393 |


Rhode PR et al. Cancer Immunol Res 2015; 4:49-60.
Gaudinski MR, et al. PLoS Medicine 2018;15:1-20.
Fletcher CV, et al. J Allergy Clin Immunol 2007;119: 747-50.

# PGT121 + vesatolimod (GS-9620)

- 5 of 11 monkeys showed no rebound for > 6 months
- bNAb plus an innate immune stimulant may represent a strategy to target the viral reservoir; the mechanism includes cell activation and binding/elimination of virally-infected cells.




Borducchi E, et al., Barouch D. Nature 2018; 563:360-79.

Open access Protocol

BMJ Open

# Assessing the safety and pharmacokinetics of the monoclonal antibodies, VRC07-523LS and PGT121 in HIV negative women in South Africa: study protocol for the CAPRISA 012A randomised controlled phase I trial

Sharana Mahomed,[1] Nigel Garrett,[1] Edmund Capparelli,[2] Cheryl Baxter,[1] Nonhlanhla Yende Zuma,[1] Tanuja Gengiah,[1] Derseree Archary,[1] Penny Moore,[1,3] Natasha Samsunder,[1] Dan H Barouch,[4] John Mascola,[5] Julie Ledgerwood,[5] Lynn Morris,[1,3] Salim Abdool Karim[1,6]

ClinicalTrials.gov Identifier: NCT02568215

Mahomed S, et al. BMJ Open 2019;9:e030283. doi:10.1136/bmjopen-2019-030283

# My Crystal Ball: ARV Clinical Pharmacology Contributions to HIV Cure-Remission

- Formulation:
  - *Prodrugs, nanoformulations*
- Mechanism of action:
  - *Potent, selective agents with novel mechanisms of action and additive-to-synergistic with existing agents*
- Pharmacokinetics
  - *Improved tissue/organ distribution ($C_t$ or $C_c = C_p$)*
- Pharmacodynamics
  - *Full suppression of viral replication in all tissues, compartments, reservoirs*
  - *Novel, highly synergistic combinations and new, precision medicine approach to combinatorial therapeutics*

*Thank You*