CONVEGNO NAZIONALE

Let's stop HIV

Nuove prospettive
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Hotel Savoia
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Chairs:
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Come scappa il virus

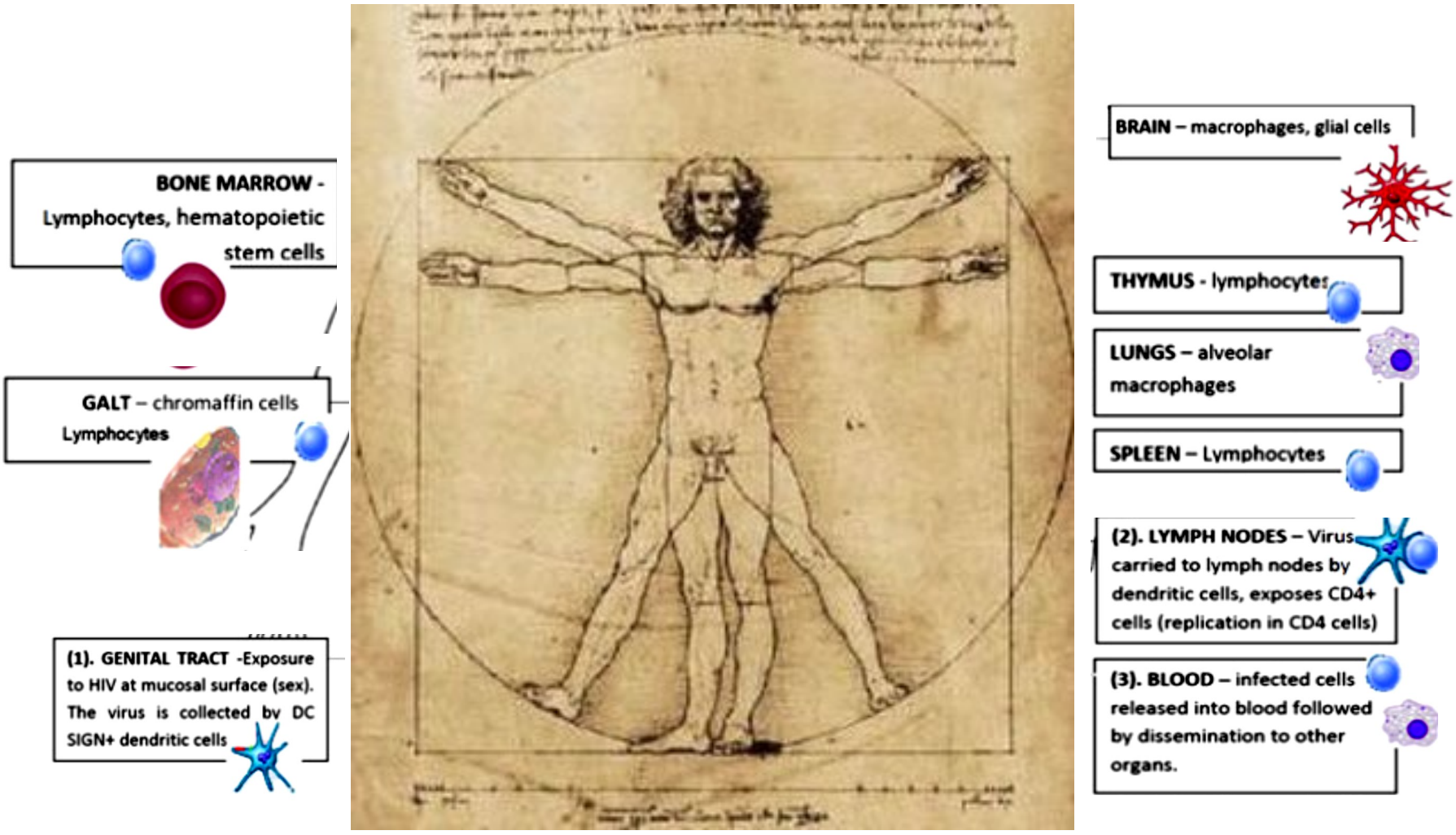
Prof. Carlo Federico Perno

Ospedale Niguarda

Sistema Socio Sanitario
Regione Lombardia

UNIVERSITÀ DEGLI STUDI DI MILANO

The HIV hiding places



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

AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Virology

2016

HIV DNA Is Frequently Present within Pathologic Tissues Evaluated at Autopsy from Combined Antiretroviral Therapy-Treated Patients with Undetectable Viral Loads

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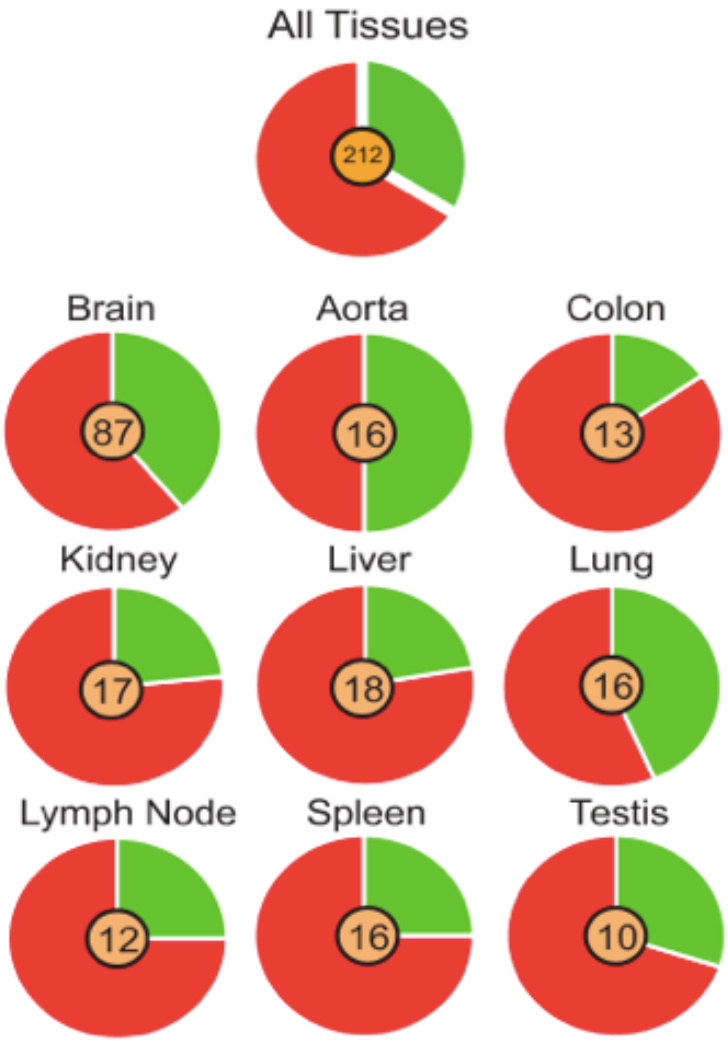
ABSTRACT

HIV infection treatment strategies have historically defined effectiveness through measuring patient plasma HIV RNA. While combined antiretroviral therapy (cART) can reduce plasma viral load (pVL) to undetectable levels, the degree that HIV is eliminated from other anatomical sites remains unclear. We investigated the HIV DNA levels in 229 varied autopsy tissues from 20 HIV-positive (HIV^+) cART-treated study participants with low or undetectable plasma VL and cerebrospinal fluid (CSF) VL prior to death who were enrolled in the National Neurological AIDS Bank (NNAB) longitudinal study and autopsy cohort. Extensive medical histories were obtained for each participant. Autopsy specimens, including at least six brain and nonbrain tissues per participant, were reviewed by study pathologists. HIV DNA, measured in tissues by quantitative and droplet digital PCR, was identified in 48/87 brain tissues and 82/142 nonbrain tissues at levels >200 HIV copies/million cell equivalents. No participant was found to be completely free of tissue HIV. Parallel sequencing studies from some tissues recovered intact HIV DNA and RNA. Abnormal histological findings were identified in all participants, especially in brain, spleen, lung, lymph node, liver, aorta, and kidney. All brain tissues demonstrated some degree of pathology. Ninety-five percent of participants had some degree of atherosclerosis, and 75% of participants died with cancer. This study assists in characterizing the anatomical locations of HIV, in particular, macrophage-rich tissues, such as the central nervous system (CNS) and testis. Additional studies are needed to determine if the HIV recovered from tissues promotes the pathogenesis of inflammatory diseases, such as HIV-associated neurocognitive disorders, cancer, and atherosclerosis.

HIV DNA Is Frequently Present within Pathologic Tissues Evaluated at Autopsy from Combined Antiretroviral Therapy-Treated Patients with Undetectable Viral Loads

229 varied autopsy tissues from 20 ART-treated patients with low or undetectable plasma viremia and cerebral fluid (CSF) VL prior to death, were analysed. HIV-DNA (>200 cp/10^6 cell) was identified in 48/87 brain tissues and 82/142 non-brain tissues. Abnormal histological findings were identified in all partecipants (brain, spleen, lung, lymph node, liver, aorta and kidney).



Tissues assayed with the number of HIV+ (red) and HIV- (green) tissues identified

S.L. Lamers 2016; 20:8968-83

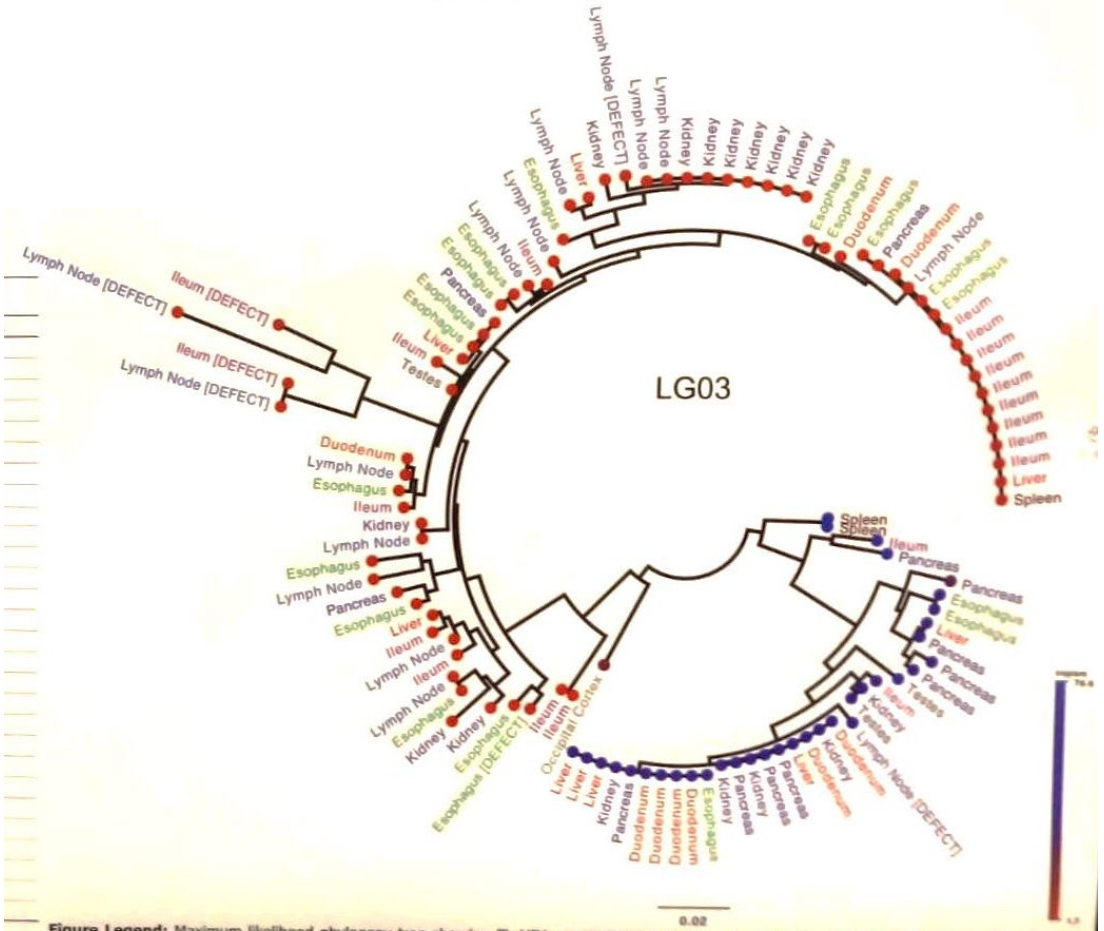
Abstract Number:
327

CROI 2019

CHARACTERIZING THE HIV DNA RESERVOIRS IN WHOLE-BODY TISSUES IN THE "LAST GIFT" COHORT

Author(s):
Michelli Faria de Oliveira[1], Benjamin Murrell[1], Thomas Vollbrecht[1], Susanna Concha-Garcia[1], Venkatesh Kumar[1], Magali Porrachia[1], Brianna Scott[1], Laura Layman[1], Caroline Ignacio[1], Sara Gianella[1], Davey M. Smith[1]

HIV DNA was detected in most body tissues despite long-term ART and confirmed undetectable HIV RNA at the time of death. Based on the FL HIV-env sequencing, most HIV reservoirs appeared to be intact provirus and may present different viral tropisms



Defining total-body AIDS-virus burden with implications for curative strategies

In the quest for a functional cure or eradication of HIV infection, we need to know how large the reservoirs are from which infection rebounds when treatment is interrupted. To that end, we quantified SIV and HIV tissue burdens in tissues of infected non-human primates and lymphoid tissue (LT) biopsies from infected humans. Before antiretroviral therapy (ART), LTs harbor more than 98 percent of the SIV RNA+ and DNA+ cells.

While ART substantially reduced their numbers, vRNA+ cells were still detectable and their persistence was associated with relatively low drug concentrations in LT compared to peripheral blood. Prolonged ART also reduced the level of SIV and HIV-DNA+ cells, but the estimated size of the residual tissue burden of 10^8 vDNA+ cells that potentially harbor replication competent proviruses, along with the evidence for continuing virus production in LT despite ART, identify two important sources for rebound following treatment interruption.

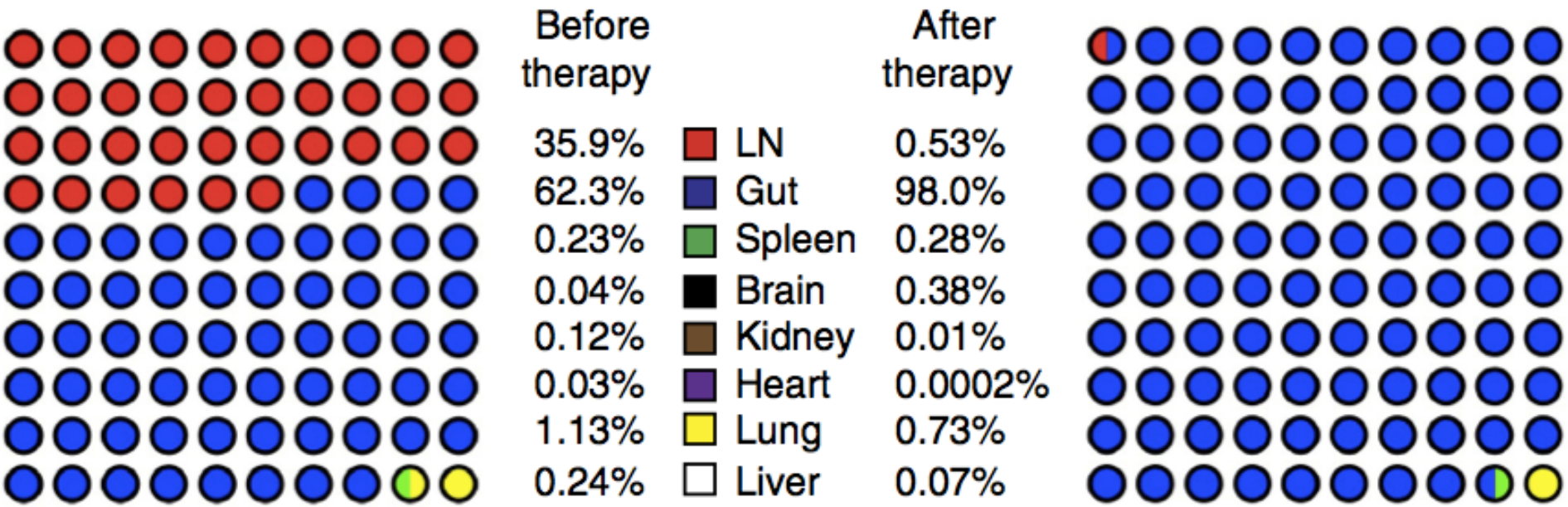
The large sizes of these tissue reservoirs underscore the challenges in developing "HIV cure" strategies that target multiple sources of virus production

Jacob D Estes Nature Medicine 2017

Defining total-body AIDS-virus burden with implications for curative strategies

During ART the numbers of virus (v) RNA+ cells substantially decreased but remained detectable.

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



Jacob D Estes Nature Medicine 2017

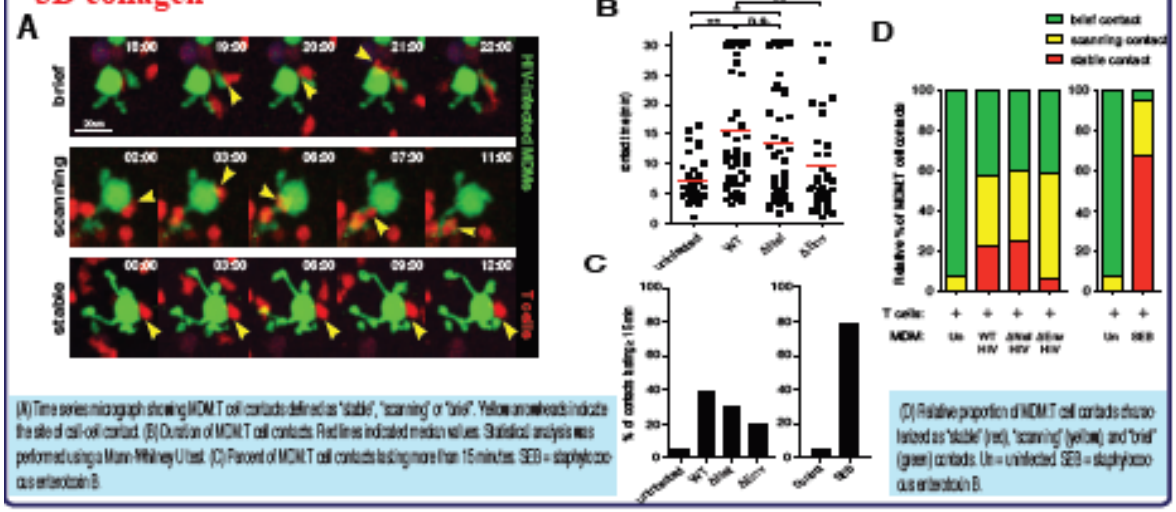
HIV infection alters dynamic macrophage:T-cell interactions to promote viral spread

#0221

Paul Lopez, Wan Koh, Ryan Hnatiuk, and Thomas T. Murooka

University of Manitoba, Departments of Immunology and Medical Microbiology and Infectious Diseases, Apotex Center, 750 McDermot Ave, Room 433; Winnipeg, MB, Canada R3E 0T5

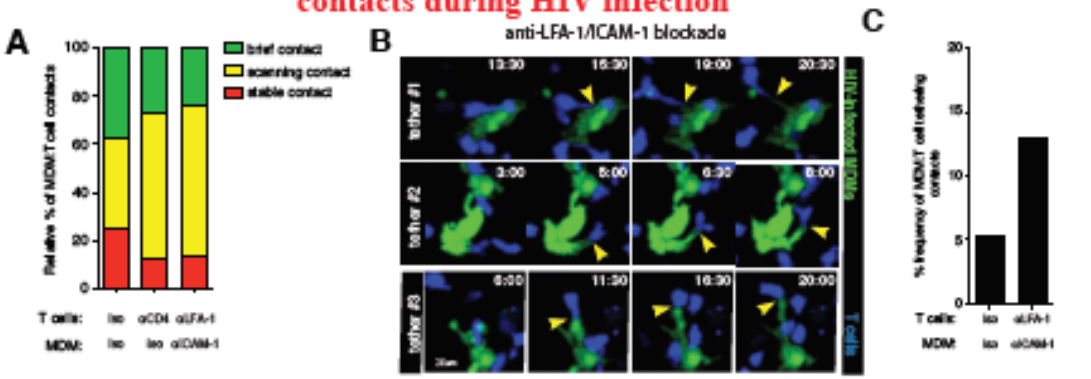
4) Dynamic visualization of HIV-infected macrophage:T cell interactions in 3D collagen



(A) Time series micrograph showing MDM:T cell contacts defined as "stable", "scanning" or "brief". Yellow arrowheads indicate the site of cell-cell contact. (B) Duration of MDM:T cell contacts. Red lines indicated median values. Statistical analysis was performed using a Mann-Whitney U test. (C) Percent of MDM:T cell contacts lasting more than 15 minutes. SEB = staphylococcus enterotoxin B.

(D) Relative proportion of MDM:T cell contacts characterized as "stable" (red), "scanning" (yellow), and "brief" (green) contacts. Un = uninfected. SEB = staphylococcus enterotoxin B.

5) Role of adhesive molecular interactions in stabilizing MDM:T cell contacts during HIV infection



(A) Relative proportion of MDM:T cell contacts characterized as "stable" (red), "scanning" (yellow), and "brief" (green) contacts. (B) Time series micrographs depicting three MDM:T cell tethering events after LFA-1/ICAM-1 dual antibody blockade. Yellow arrowheads indicate the point of contact and the membranous tether. (C) Percent frequency of MDM:T cell tethering events after antibody blockade. Tethers were defined as membranous extensions between MDM and T cells that were over 10 microns long.

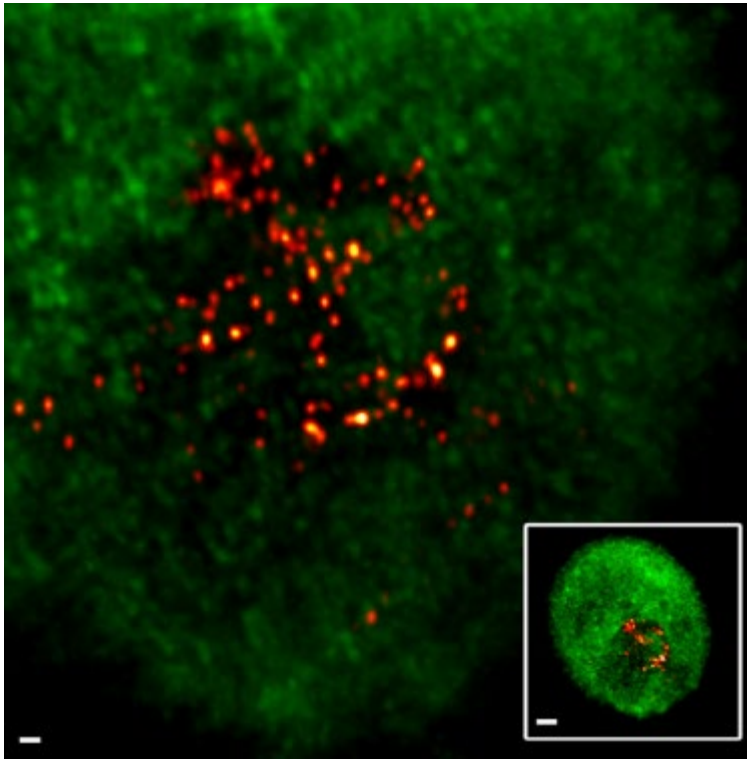
Visual characterization of macrophage: T cell interaction dynamics within a 3D environment reveals that HIV infection alters the morphology and cell-cell contact behaviors with susceptible T cells.

Env:CD4 and LFA-1:ICAM-1 contacts between infected macrophages and T cells are required for stable contact formation and optimal viral spread to T cells.

2019 CROI Conference on Retroviruses and Opportunistic Infections

Viral spread from macrophages to CD4+T lymphocytes

- in vitro infection of MDM leads to accumulation of infectious particles in a surface-connected vesicular compartment termed the virus-containing compartment (VCC) (Deneka et al., 2007; Jouve et al., 2007; Welsch et al., 2007)
- Infectious virus may be stored within the VCC for extended periods (Sharova et al., 2005) and then transferred rapidly to contacting CD4+ T cells (Giese and Marsh, 2014; Gousset et al., 2008; Groot et al., 2008).
- human CD169+ macrophages efficiently capture blood- or lymph-borne retroviruses in spleen and lymph nodes



Retroviruses use CD169-mediated trans-infection of permissive lymphocytes to establish infection

Xaver Sewald,[1*] Mark S. Ladinsky,[2†] Pradeep D. Uchil,[1†] Jagadish Beloor,[3] Ruoxi Pi,[1] Christin Herrmann,[1] Nasim Motamedi,[4‡] Thomas T. Murooka,[5] Michael A. Brehm,[6] Dale L. Greiner,[6] Leonard D. Shultz,[7] Thorsten R. Mempel,[5] Pamela J. Bjorkman,[2] Priti Kumar,[3*] Walther Mothes[1*]

Sciencexpress

2015

HIV infection alters dynamic macrophage:T-cell interactions to promote viral spread

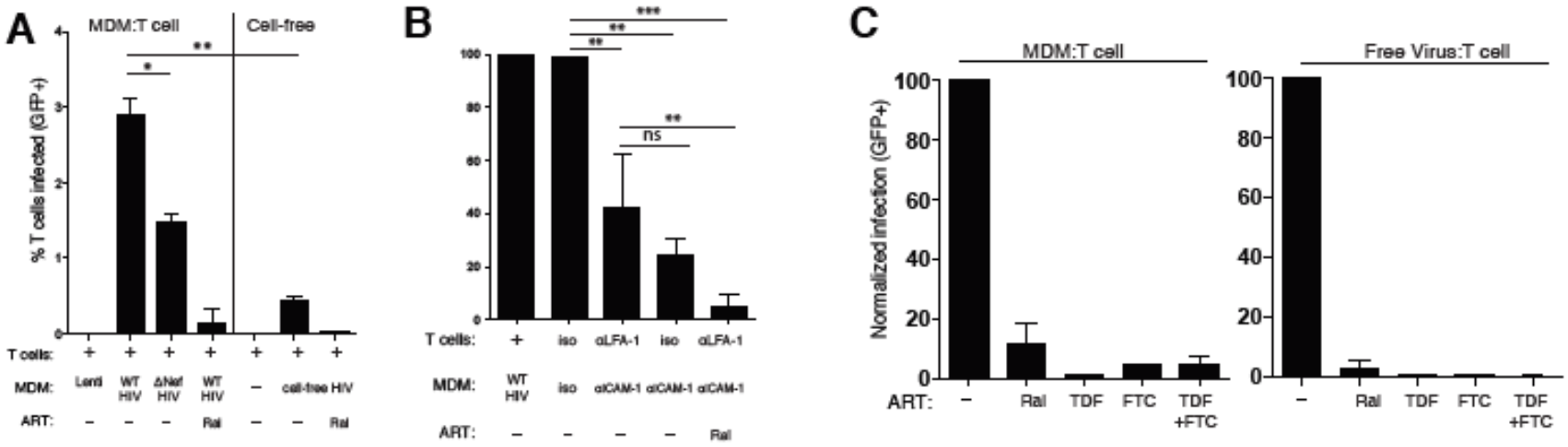
Paul Lopez, Wan Koh, Ryan Hnatiuk, and Thomas T. Murooka
University of Manitoba, Departments of Immunology and Medical Microbiology and Infectious Diseases, Apotex Center, 750 McDermot Ave, Room 433; Winnipeg, MB, Canada R3E 0T5

#0221

University of Manitoba

ART treatment is effective in preventing macrophage to T cell HIV spread at doses that inhibit infection by free virus.

6) HIV dissemination through dynamic MDM:T cell interactions in collagen



(A) Percent T cell infection, measured by GFP expression, after the indicated co-culture (MDM:T cell) or free virus alone (cell-free) conditions in collagen gels (n=5). Ral = Raltegravir. (B) T cell infection after antibody blockade (n=4). Normalized infection rates to HIV+MDM:T cell cocultures are shown. iso = isotype control. Ral = Raltegravir. (C) T cell infection after antiretroviral drug treatment. Normalized infection rates to untreated HIV+MDM:T cell co-cultures are shown. Ral = Raltegravir, TDF = Tenofovir, FTC = Emtricitabine.

2019 CROI Conference on Retroviruses and Opportunistic Infections

AMERICAN SOCIETY FOR MICROBIOLOGY | mBio®

2017

Mechanisms of CNS Viral Seeding by HIV$^+$ CD14$^+$ CD16$^+$ Monocytes: Establishment and Reseeding of Viral Reservoirs Contributing to HIV-Associated Neurocognitive Disorders

Mike Veenstra,[a] Rosiris León-Rivera,[a] Ming Li,[b] Lucio Gama,[b,c] Janice E. Clements,[b] Joan W. Berman[a,d]

IMPORTANCE HIV infects different tissue compartments of the body, including the central nervous system (CNS). This leads to establishment of viral reservoirs within the CNS that mediate neuroinflammation and neuronal damage, contributing to cognitive impairment. Our goal was to examine the mechanisms of transmigration of cells that contribute to HIV infection of the CNS and to continued replenishment of CNS viral reservoirs, to establish potential therapeutic targets. We found that an HIV-infected subset of monocytes, mature HIV$^+$ CD14$^+$ CD16$^+$ monocytes, preferentially transmigrates across the blood-brain barrier. This was mediated, in part, by increased junctional proteins JAM-A and ALCAM and chemokine receptor CCR2. We

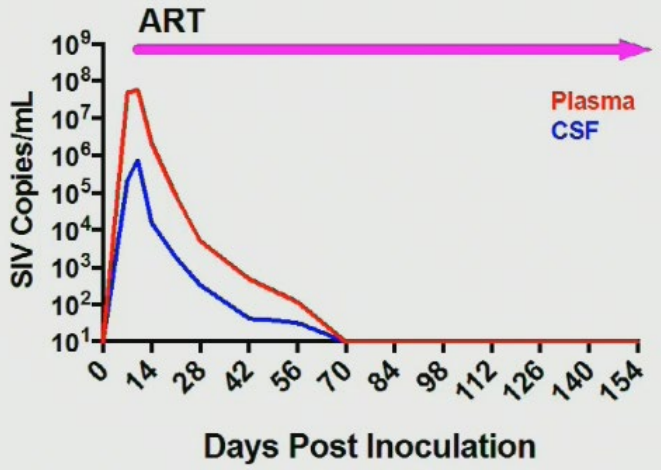
- **The HIV-1 sanctuary: new insights**

CROI 2018

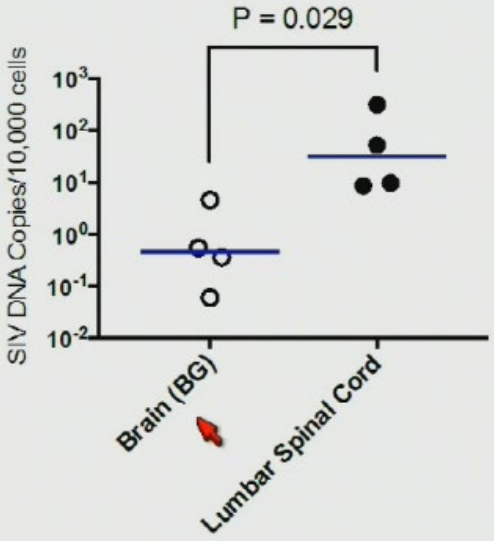
SIV REBOUND IN THE SPINAL CORD AFTER STOPPING ART: A NOVEL CNS RESERVOIR

Joseph Mankowski
Johns Hopkins University School of Medicine
Baltimore, MD, USA

SIV + ART: Suppression in Plasma and CSF



SIV DNA Levels: Brain vs Spinal Cord



CROI 2018

SIV REBOUND IN THE SPINAL CORD AFTER STOPPING ART: A NOVEL CNS RESERVOIR

Joseph Mankowski
Johns Hopkins University School of Medicine
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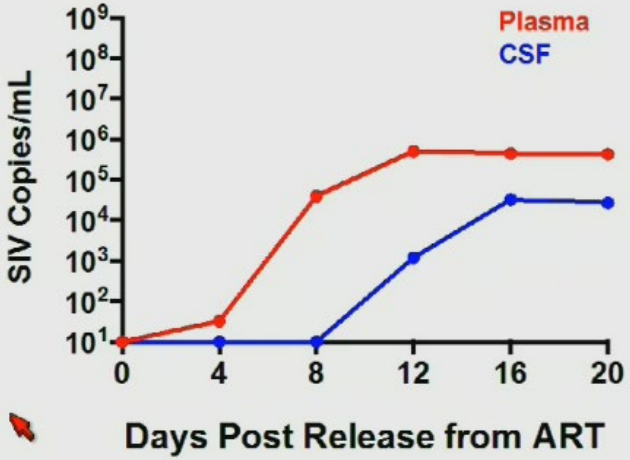
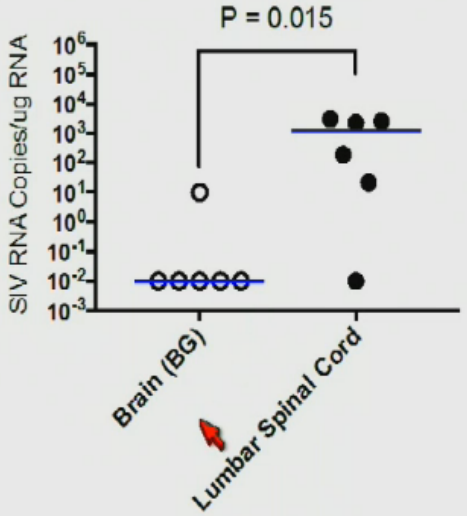
SIV Rebound after Stopping ART: Plasma vs CSF



SIV RNA Levels: Brain vs Spinal Cord



CROI 2018

SIV REBOUND IN THE SPINAL CORD AFTER STOPPING ART: A NOVEL CNS RESERVOIR

Joseph Mankowski
Johns Hopkins University School of Medicine
Baltimore, MD, USA

Spinal Cord Macrophages as an SIV Reservoir

- High levels of SIV DNA in spinal cord with ART
- After stopping ART, SIV RNA rebounds in CSF
- In CNS, highest SIV RNA levels in spinal cord macrophages
- SIV cultured from spinal cord macrophages is replication competent
- Unique spinal cord features: Neuroimmune responses, pharmacodynamics
- HIV cure strategies need to target CNS reservoirs including the spinal cord

HIV RNA and proviral HIV DNA can be detected in semen after 6 months of antiretroviral therapy although HIV RNA is undetectable in blood

Table 2. HIV RNA in blood plasma and seminal plasma and HIV DNA in PBMCs and seminal cells after six month ART

	HIV RNA copies/mL		Total HIV DNA copies/10^6 cells		Integrated HIV DNA copies/10^6 cells		2LTR circular HIV DNA copies/10^6 cells	
Patient	Blood plasma	Seminal plasma	PBMCs	Seminal cells	PBMCs	Seminal cells	PBMCs	Seminal cells
1	<LDL	14,300	109.45	743.44	26.44	190.53	16.97	0.50
2	<LDL	34,900	892.14	25.64	538.57	15. 00	185.67	0.27
3	14,996	8790	43.26	687.76	16.91	389.91	1.05	3.19
4	<LDL	60,500	30.90	64.87	13.57	11.61	8.08	1.17
5	<LDL	<LDL	54.40	575.35	43.83	435.94	2.58	2.97
6	<LDL	600	337.97	121.32	19.77	97.74	6.61	17.54
7	<LDL	1500	20.21	29.70	10.01	4.60	3.54	2.79
8	<LDL	6230	44.18	83.02	9.41	23.04	29.58	10.59
9	<LDL	9900	248.87	71.14	83.9	27.70	8.6	7.95
10	<LDL	2800	19.51	27.57	8.50	15.52	2.34	2.42
11	<LDL	860	58.36	28.54	35.8	15.25	1.90	1.05
12	<LDL	4780	115.22	477.55	18.03	64.23	0.60	31.62
13	178,161	74,300	48.65	21.03	36.24	10.98	7.87	1.24
14	<LDL	<LDL	29.29	618.87	19.01	315.55	2.21	15.05
15	<LDL	618	51.94	32.84	19.16	15.07	5.70	1.86
16	<LDL	6847	409.88	684.75	57.56	101.01	20.21	34.19
17	<LDL	4098	63.00	29.08	33.28	17.55	2.53	2.25
18	<LDL	306	80.63	41.74	363.11	17.38	1.48	3.31
19	<LDL	<LDL	62.45	23.01	8.47	11.02	0.56	1.35

LDL, lower than limit of detection

Du et al Microbiol Immunol 2016

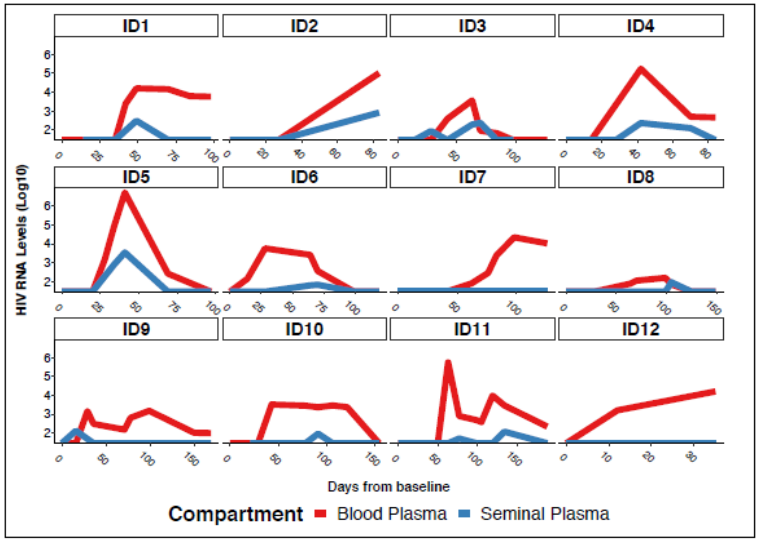
Poster Session # E10
Abstract Number 0392
26th Conference on Retroviruses and Opportunistic Infections
March 4 - 7, 2019
Seattle, WA

HIV Rebound in the Male Genital Tract after ART Interruption

Sara Gianella[1], Antoine Chaillon[1], Tae-Wook Chun[2], Caroline Ignacio[1], Colin Kovacs[3,4], Erika Benko[3], Sanja Huibner[3], Rupert Kaul[3]

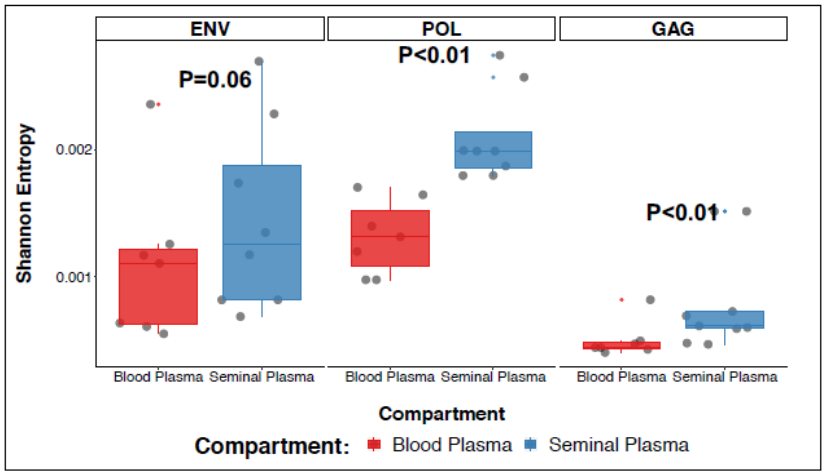
[1]University of California, San Diego, La Jolla, CA, USA, [2]National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH), Bethesda, MD, USA, [3]Maple Leaf Medical Clinic, Toronto, Ontario, Canada, [4]Department of Medicine, University of Toronto, Toronto, Ontario, Canada.

Compared to blood, HIV RNA rebound in semen occurred significantly later (median of 66 versus 42 days post ART interruption) and reached lower levels (164 versus 16,224 copies/ml).



Legend. HIV RNA Rebound after ART interruption in blood (**red**) and seminal plasma (**blue**).
Participant ID1-4 were randomized to the placebo group. Participants ID5-12 were randomized in the vaccine group.

Despite ART started during early infection, HIV diversity was higher in semen compared to blood in all three coding regions.



Legend. Viral diversity (Shannon entropy) was assessed after adjusting for haplotype frequency for all three regions in blood (**red**) and semen (**blue**).

Higher diversity in the genital compartment suggests distinct evolutionary dynamics.

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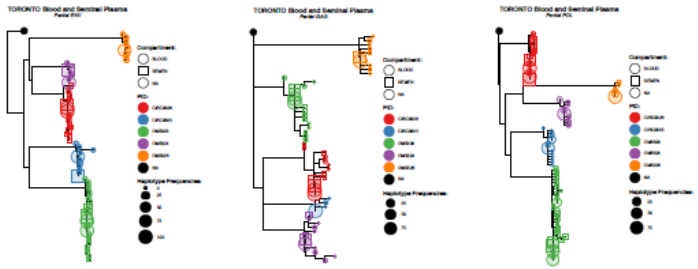
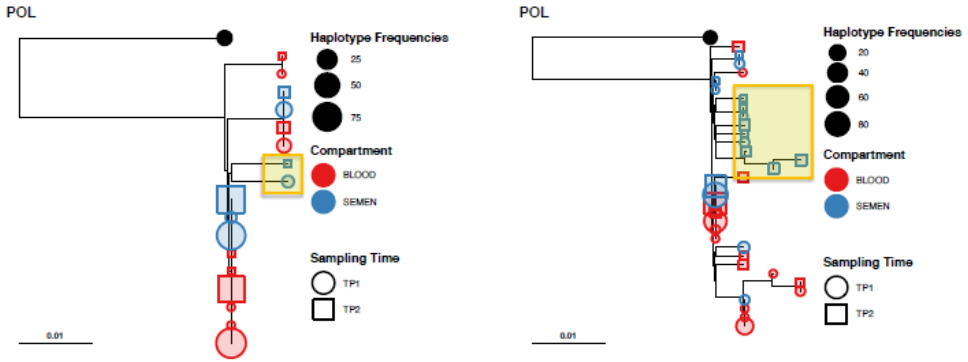
Unique viral populations are observed in seminal plasma

Paired sequence data were available for 5 participants.



Phylogenetic analysis confirmed the presence of compartment-specific monophyletic HIV RNA populations in at least one HIV region in 2 out of the 5 participants in longitudinal time-points.



Legend. **Approximate maximum likelihood phylogenetic reconstruction of sequences generated from longitudinally collected HIV-1 RNA Populations in blood and semen from 5 participants.** HIV haplotypes above a minimal frequency threshold of 0.01 were extracted from cleaned reads and were used to construct approximate maximum likelihood phylogenies using FastTree (Price et al., 2009).

Poster number 0249

HIV-1 diversity in gut is associated with residual mucosal virus production on ART

Manon Nayrac[1], Mary Requena[2], Nicolas Jeanne[2], Maud Mavigner[3], Claire Loiseau[4], Romain Carcenac[2], Michelle Cazabat[2], Jacques Izopet[1,2], Pierre Delobel[1,2]

[1]U1043, INSERM, Toulouse, France, [2] Toulouse University Hospital, Toulouse, France, [3] Emory Vaccine Center, Atlanta, GA, USA, [4] Australian Institute of Tropical Health and Medecine, Cairns, Australia

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INTRODUCTION : HIV-1 persists in cellular reservoirs and some anatomical compartments despite antiretroviral therapy (ART). We compared HIV-1 in gut and blood compartments on ART, regarding differences in target cells, residual HIV-1 DNA and RNA, coreceptor usage, and virus diversity.

METHODS : Peripheral blood and duodenum samples were obtained from 17 HIV-1-infected subjects with sustained plasma VL of <50copies/ml for 5 years.

Blood and duodenal CD4$^+$ T cells were phenotyped by flow cytometry (BD LSRII).

HIV-1 DNA was quantified in sorted blood and duodenal CD4$^+$ T cells by qPCR, HIV-1 RNA was quantified in duodenal tissue by qRT-PCR. Virus quasispecies were characterized by next-generation sequencing of C2V3C3 *env* (454 GS Junior), with data cleaning and coreceptor usage prediction by Pyrovir software. Viral diversity in blood and duodenum compartments was assessed by haplotype numbers, adjusted-Shannon entropy, and Hill numbers. Phylogenetic analyses were preformed using CLUSTAL W. A non-parametric test for panmixia was used to assess compartmentalization.

RESULTS:

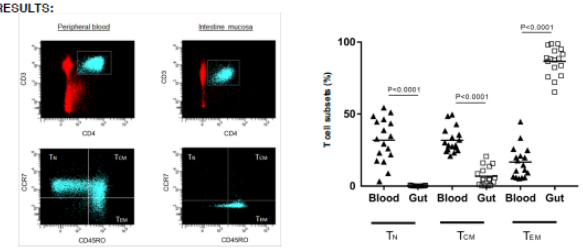


Figure 1. Characterization of T cell subsets in peripheral blood and duodenal mucosa (*lamina propria*)

Naïve T cells (**TN**, CD3$^+$CD4$^+$CD45RO$^-$CCR7$^+$), central memory T cells (**TCM**, CD3$^+$CD4$^+$CD45RO$^+$CCR7$^+$), and effector memory T cells (**TEM**, CD3$^+$CD4$^+$CD45RO$^+$CCR7$^-$) in peripheral blood and duodenal *lamina propria* were characterized by flow cytometry. **TN** and **TCM** were predominant in peripheral blood while they were scarce in the duodenum compartment (T_N 32.8% [3.3-54.45] vs. 0.1% [0-0.7], and T_{CM} 12.55% [5.15-42.35] vs. 6.2% [0.2-27], respectively). **TEM** were preponderant in the duodenal *lamina propria*, 88.5% [65.2-98.8] vs. 28.35% [20.85-49.5] in peripheral blood.

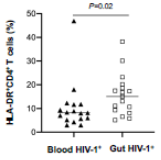


Figure 2. Frequency of HLA-DR$^+$CD4$^+$ T cells in blood and duodenum compartments from HIV-1-infected individuals

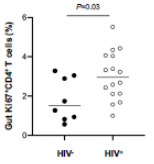


Figure 3. Frequency of Ki67$^+$ in duodenal CD4$^+$ T cells of HIV-1-infected individuals vs. uninfected controls

Activated CD4$^+$ T cells were found at a higher frequency in duodenum than in blood compartment (HLA-DR$^+$ CD4$^+$ T cells, 15% vs. 8.2%, P<0.05). Proliferating Ki67$^+$CD4$^+$ T cells were found at higher frequency in the gut mucosa of HIV-1-infected subjects than in uninfected controls (2.9% vs 1.5%, P=0.03).

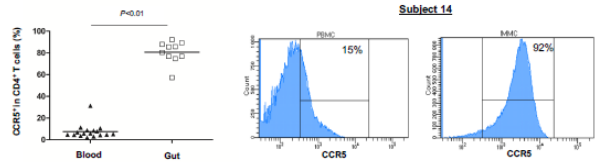


Figure 4. CCR5 expression on CD4$^+$ T cells from blood and duodenum of HIV-1-infected individuals (example : subject 14)

CCR5 is highly expressed on duodenal compared to blood CD4$^+$ T cells in HIV-1-infected individuals (83% vs 5.7%, P<0.01)

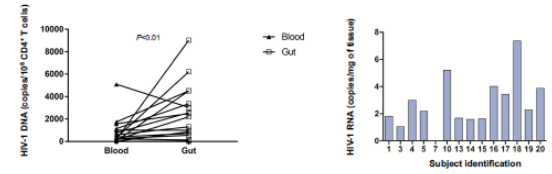


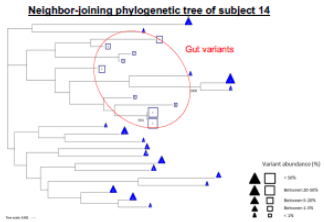
Figure 5. Quantification of HIV-1 DNA and RNA in CD4$^+$ T lymphocytes and duodenal tissue of HIV-1-infected individuals

HIV-1 DNA was 6.7-fold higher in duodenum than in blood CD4$^+$ T cells (328 vs 2197 copies/mL, P<0.01). Moreover, HIV-1 RNA was detected at low level (1-7 copies/mg) in duodenal tissue of 13/14 subjects despite being on sustained effective ART for a median duration of 5 years.

Subject ID	Blood compartment	Gut compartment
1	R5	R5
3	R5/X4	R5/X4
4	R5	R5
5	R5/X4	R5
6	R5/X4	R5
7	R5/X4	R5
8	R5/X4	R5
9	R5	R5/X4
10	X4	X4
13	R5/X4	X4
14	R5	R5
15	R5	R5
16	R5	R5
17	R5	R5
18	R5	R5
19	R5/X4	R5/X4
20	R5	R5

HIV-1 coreceptor usage was genotypicaly predicted at a clonal level from V3 sequences obtained by NGS to characterize each subject virus population. Genotypic prediction can only discriminate CCR5- vs. CXCR4-using clones. But dual-tropic R5X4 clones cannot be discriminated from pure X4 clones and both are thus classified as CXCR4-using viruses.
In the blood compartment, 9 subjects harbored only CCR5-using clones (blue) and 7 harbored both CCR5- and CXCR4-using clones (purple), while in the duodenum 13 subjects harbored only CCR5-using clones and 3 harbored both CCR5- and CXCR4-using clones. One subject harbored only CXCR4-using clones (orange) in both compartments.
The duodenum compartment was thus enriched in CCR5-using viruses.

Figure 6. Genotypic prediction of CCR5 and CXCR4 coreceptor usage of HIV-1 quasispecies characterized by NGS and PyroVir software in peripheral blood and duodenum CD4$^+$ T cells.



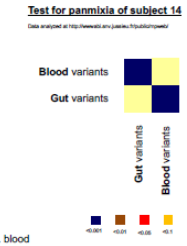


Figure 7. Compartmentalization of HIV-1 quasispecies in duodenal mucosa vs. blood

For all subjects, HIV-1 quasispecies displayed compartmentalization between the blood and duodenum (phylogenetic tree and test for panmixia, P<0.001).

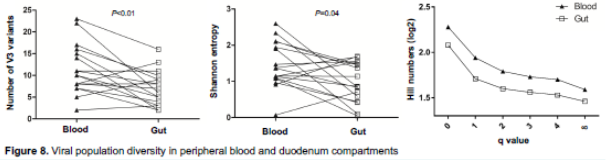


Figure 8. Viral population diversity in peripheral blood and duodenum compartments

Virus diversity in C2V3C3 *env* region was reduced in duodenum vs blood compartment. The median number of variants in the quasispecies was higher in peripheral blood than in duodenal mucosa (n=10 *vs.* 7 variants, respectively, P<0.01). Shannon entropy and hill numbers showed higher virus diversity in peripheral blood than in duodenal mucosa.

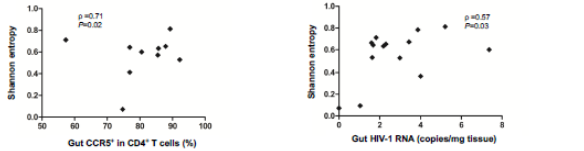
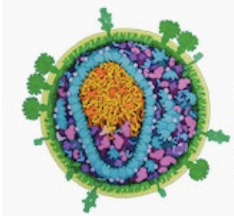


Figure 9. CCR5$^+$ target cell frequency and HIV-1 residual replication are associated with higher virus diversity in the duodenum

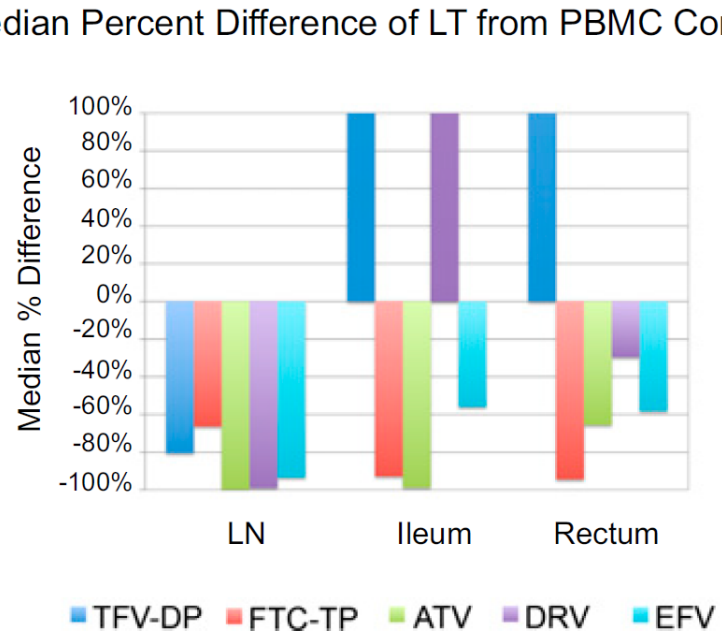
Despite being reduced in gut vs. blood, HIV-1 quasispecies diversity in the duodenum, assessed by adjusted-Shannon entropy of C2V3C3 *env*, correlated with mucosal CCR5$^+$CD4$^+$ T cell frequency (ρ=0.71, P<0.05), and residual mucosal HIV-1 RNA level (ρ=0.57, P<0.05).

CONCLUSION : HIV-1 persists in the duodenum mucosa on ART with increased levels of infected cells compared to blood CD4$^+$ T cells, and low-level mucosal HIV-1 RNA production. Virus diversity was reduced with enrichment in CCR5-using viruses and compartmentalization in duodenum vs. blood. However, the frequency of gut CCR5$^+$ target cells and the level of HIV-1 RNA were associated with a higher virus diversity in the gut compartment, suggesting residual virus production in the gut mucosa despite sustained ART.



- **The HIV-1 sanctuary: the meaning of compartmentalization**

Compared with concentrations in PBMCs, the **concentration** of **TFV, FTC, ATV, DRV and EFV** was **lower in the lymphatic tissue compartment**, particularly in the **lymph node**.

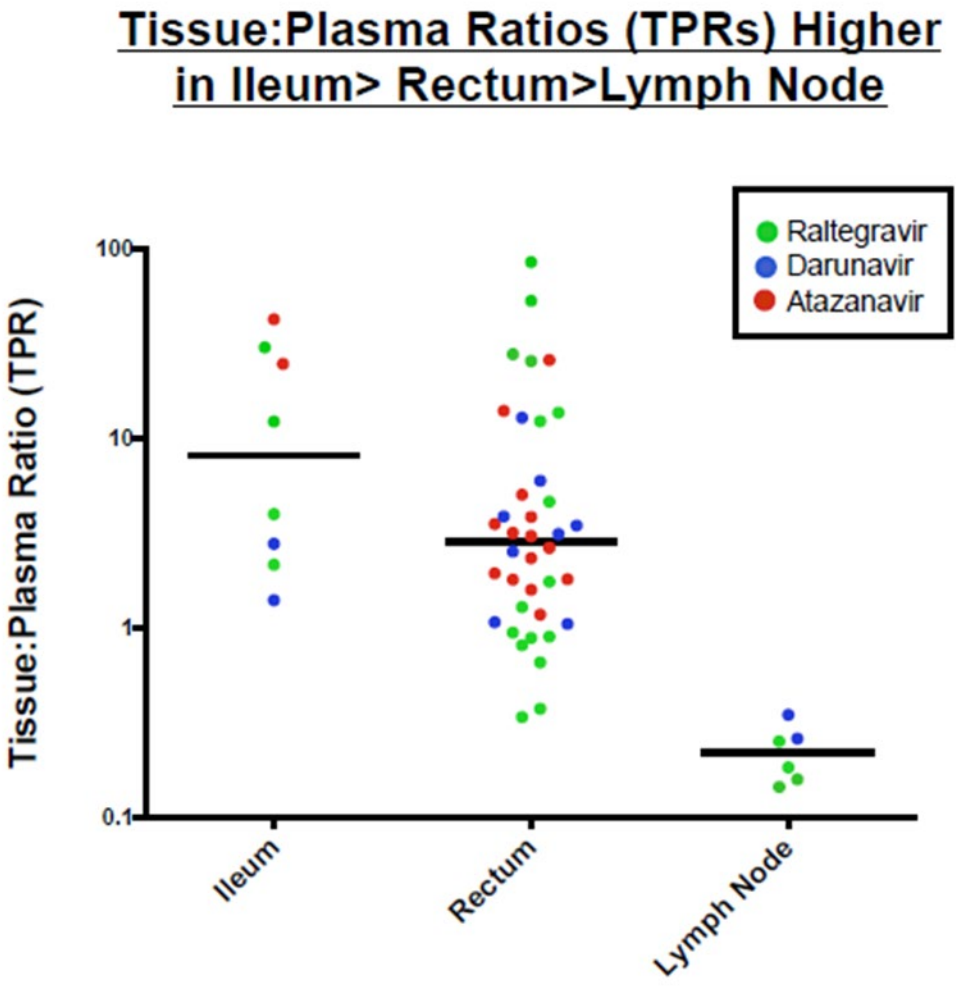


Significance

We show that HIV continues to replicate in the lymphatic tissues of some individuals taking antiretroviral regimens considered fully suppressive, based on undetectable viral loads in peripheral blood, and that one mechanism for persistent replication in lymphatic tissues is the lower concentrations of the antiretroviral drugs in those tissues compared with peripheral blood. These findings are significant because they provide a

Fletcher, PNAS 2014

INTEGRASE AND PROTEASE INHIBITOR CONCENTRATIONS IN LYMPH NODE AND GUT MUCOSAL TISSUE



This is the first study to evaluate GALT and N tissue concentrations in patients eceiving RAL and 800 mg daily DRV.

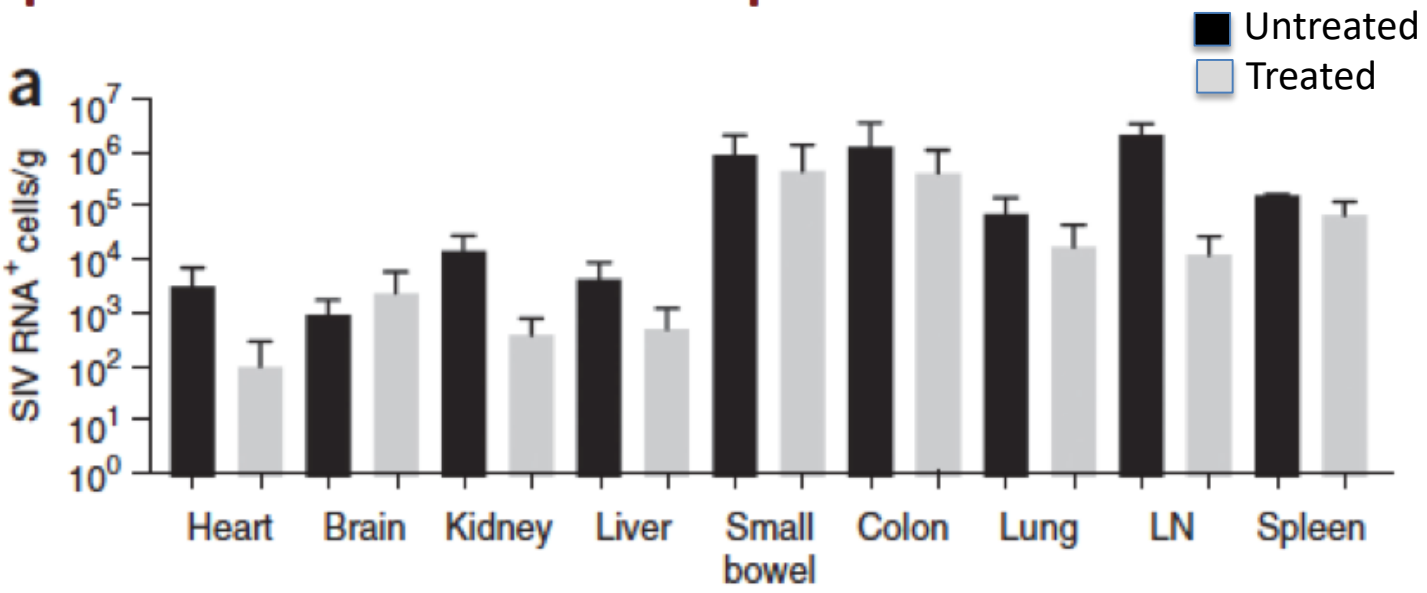
Tissue:plasma ratios were higher in eum>rectum as shown previously, and owest in lymph node.

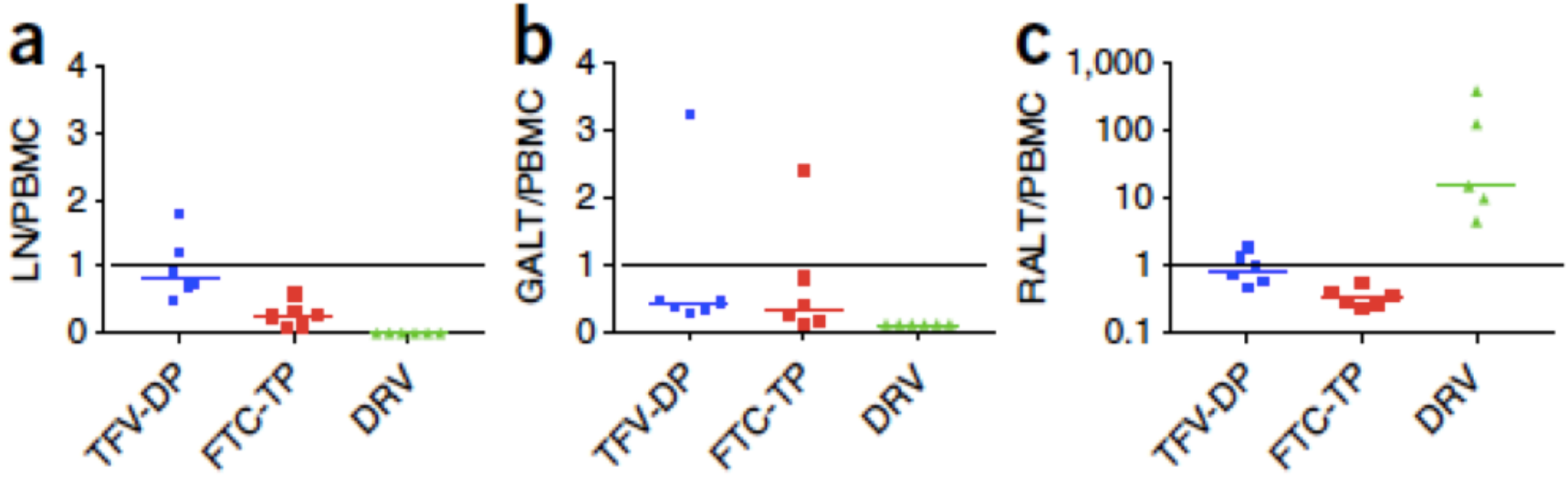
In a limited number of participants, oncentrations of RAL were significantly ower in lymph nodes vs. GALT, supporting rior observations.

These results support the current limited lata on tissue ART drug concentrations and ave potential implications on HIV cure trategies.

Lee, et al Abstr 407, CROI 2017

Low ART conc may contribute to incomplete suppression of viral replication





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

RALT: rectum

PLOS PATHOGENS 2017

Early Antiretroviral Therapy Is Associated with Lower HIV DNA Molecular Diversity and Lower Inflammation in Cerebrospinal Fluid but Does Not Prevent the Establishment of Compartmentalized HIV DNA Populations

Michelli F. Oliveira[1]*, Antoine Chaillon[1], Masato Nakazawa[1], Milenka Vargas[1], Scott L. Letendre[1,2], Matthew C. Strain[1], Ronald J. Ellis[2,3], Sheldon Morris[1], Susan J. Little[1], Davey M. Smith[1,4], Sara Gianella[1]*

Even when antiretroviral therapy (ART) is started early after infection, HIV DNA might persist in the central nervous system (CNS), possibly contributing to inflammation, brain damage and neurocognitive impairment. Paired blood and cerebrospinal fluid (CSF) were collected from 16 HIV-infected individuals on suppressive ART: 9 participants started ART <4 months of the estimated date of infection (EDI) ("early ART"), and 7 participants started ART >14 months after EDI ("late ART"). For each participant, neurocognitive functioning was measured by Global Deficit Score (GDS). HIV DNA levels were measured in peripheral blood mononuclear cells (PBMCs) and CSF cell pellets by droplet digital (dd)PCR. Soluble markers of inflammation (sCD163, IL-6, MCP-1, TNF-α) and neuronal damage (neurofilament light [NFL]) were measured in blood and CSF supernatant by immunoassays. HIV-1 partial *C2V3 env* deep sequencing data (Roche 454) were obtained for 8 paired PBMC and CSF specimens and used for phylogenetic and compartmentalization analysis. Median exposure to ART at the time of sampling was 2.6 years (IQR: 2.2–3.7) and did not differ between groups. We observed that early ART was significantly associated with lower molecular diversity of HIV DNA in CSF ($p<0.05$), and lower IL-6 levels in CSF ($p = 0.02$), but no difference for GDS, NFL, or HIV DNA detectability compared to late ART. Compartmentalization of HIV DNA populations between CSF and blood was detected in 6 out of 8 participants with available paired HIV DNA sequences (2 from early and 4 from late ART group). Phylogenetic analysis confirmed the presence of monophyletic HIV DNA populations within the CSF in 7 participants, and the same population was repeatedly sampled over a 5 months period in one participant with longitudinal sampling. Such compartmentalized provirus in the CNS needs to be considered for the design of future eradication strategies and might contribute to the neuropathogenesis of HIV.

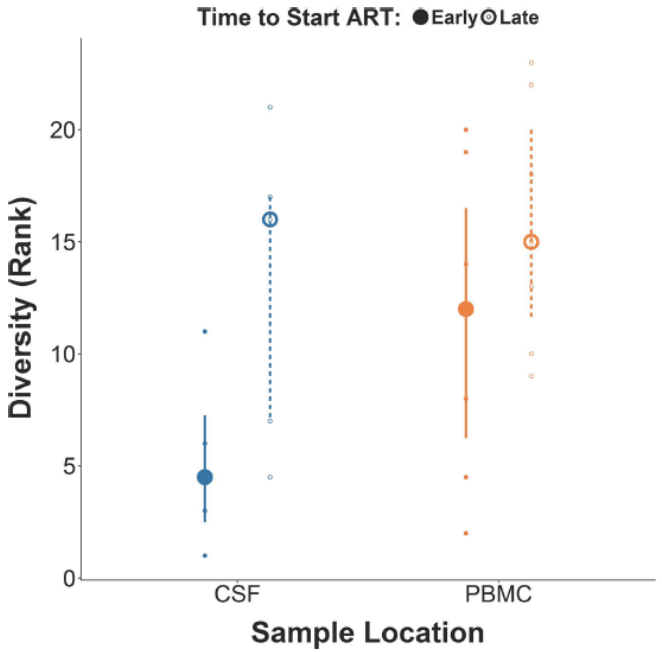


Fig 1. Comparison of molecular diversity for HIV DNA (partial *env* gene) in CSF cells and PBMC between early ART versus late ART groups. Mann Whitney comparison between early ART versus late

AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Virology

2016

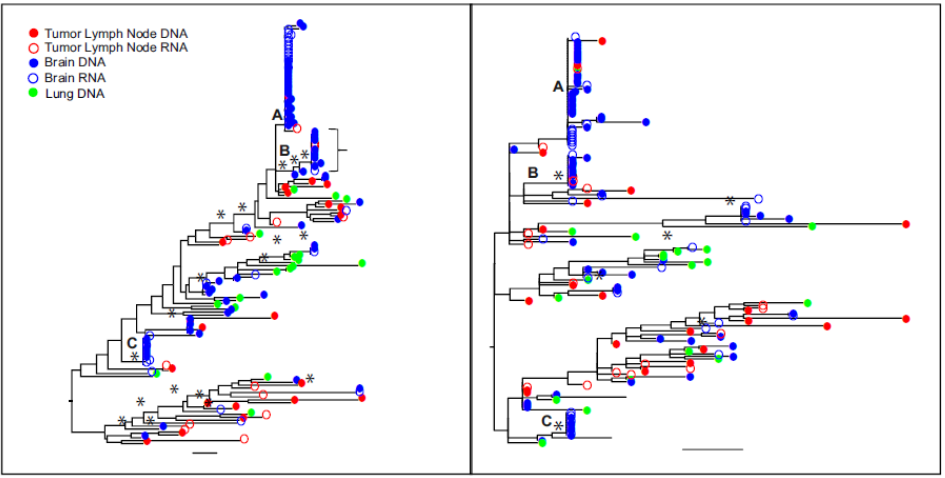
HIV Maintains an Evolving and Dispersed Population in Multiple Tissues during Suppressive Combined Antiretroviral Therapy in Individuals with Cancer

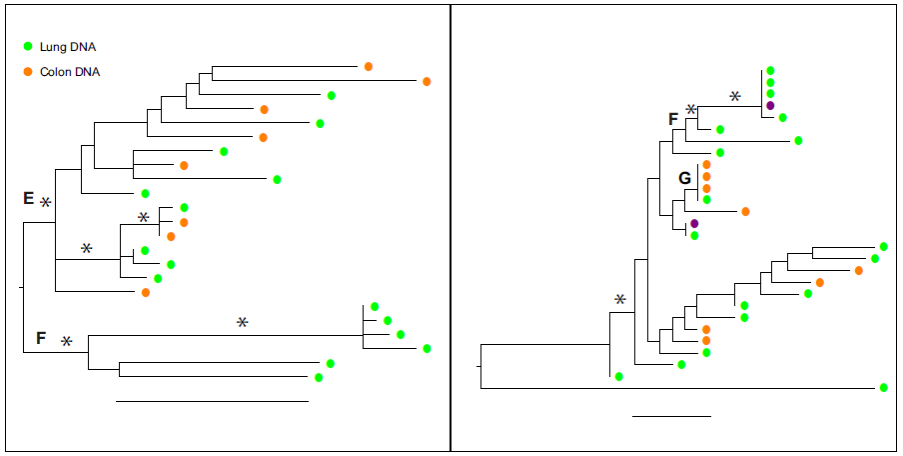
Rebecca Rose,[a] Susanna L. Lamers,[a] David J. Nolan,[a,b] Ekaterina Maidji,[c] N. R. Faria,[d] Oliver G. Pybus,[d] James J. Dollar,[b] Samuel A. Marunlak,[b] Andrew C. McAvoy,[b] Marco Salemi,[b] Cheryl A. Stoddart,[c] Elyse J. Singer,[e] Michael S. McGrath[f,g]

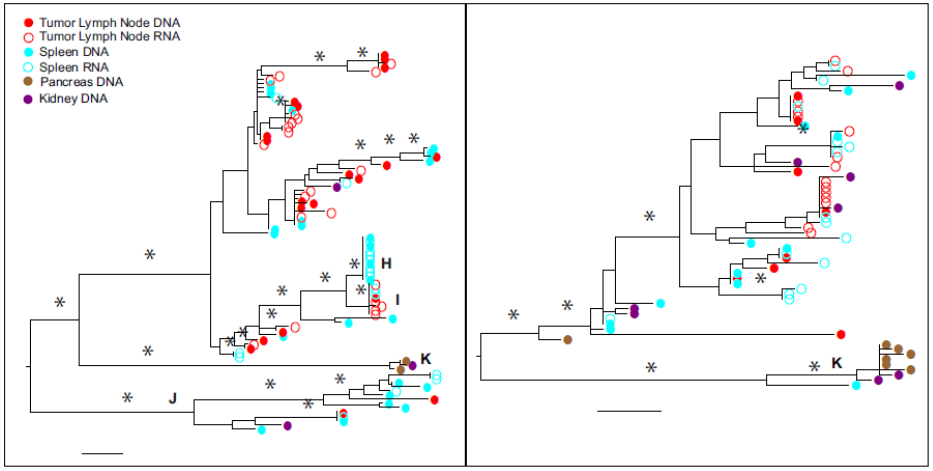
ABSTRACT

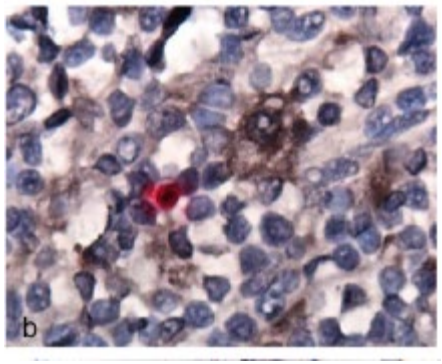
While combined antiretroviral therapy (cART) can result in undetectable plasma viral loads, it does not eradicate HIV infection. Furthermore, HIV-infected individuals while on cART remain at an increased risk of developing serious comorbidities, such as cancer, neurological disease, and atherosclerosis, suggesting that during cART, tissue-based HIV may contribute to such pathologies. We obtained DNA and RNA *env*, *nef*, and *pol* sequences using single-genome sequencing from postmortem tissues of three HIV^+ cART-treated ($cART^+$) individuals with undetectable viral load and metastatic cancer at death and performed time-scaled Bayesian evolutionary analyses. We used a sensitive *in situ* hybridization technique to visualize HIV *gag-pol* mRNA transcripts in cerebellum and lymph node tissues from one patient. Tissue-associated virus evolved at similar rates in $cART^+$ and cART-naive ($cART^-$) patients. Phylogenetic trees were characterized by two distinct features: (i) branching patterns consistent with constant viral evolution and dispersal among tissues and (ii) very recently derived clades containing both DNA and RNA sequences from multiple tissues. Rapid expansion of virus near death corresponded to wide-spread metastasis. HIV RNA^+ cells clustered in cerebellum tissue but were dispersed in lymph node tissue, mirroring the evolutionary patterns observed for that patient. Activated, infiltrating macrophages were associated with HIV RNA. Our data provide evidence that tissues serve as a sanctuary for wild-type HIV during cART and suggest the importance of macrophages as an alternative reservoir and mechanism of virus spread.

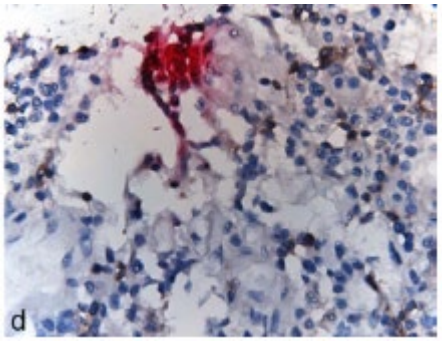
Dispersal of viral populations among tissue and evidence of activated macrophages surrounding HIV-1 expressing cells











Rose R et al., 2016

Factors that may influence compartmentalization, transcriptional potential, virus spread

- Physical isolation of a particular tissue/cell type
- Local concentrations of antiviral drugs and/or drug resistance
- Altered requirements for target cell entry and recognition (ENV variability, altered transcriptional potential, CTL escape…)

Conclusions

- ✓ Even if the most important hiding places for HIV have been characterized, **new sanctuaries and the trafficking among them** is still a focus of HIV research

- ✓ Unmet need: The attention should be posed on **viral factors influencing compartmentalization, reservoir seeding and persistence**.

CONVEGNO NAZIONALE

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Let's stop HIV

Nuove prospettive
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Grazie per l'attenzione!