Hot topics on CNS and HIV
(most relevant presentations in conferences or articles published recently)

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CNS relevance of HIV infection in treated suppressed patients

- CNS as target organ (clinical relevance)
  - Cognitive impairment
  - Symptomatic CSF escape
- CNS as viral reservoir (relevance for cure)
  - Symptomatic CSF escape
  - Compartmentalization
  - Potential obstacle to eradication

Clinical relevance of CNS HIV infection

Prevalence of neurocognitive disorders in a well-treated and aging Swiss HIV Cohort

Matthias Cavassini[1*], Melanie Metral[2*], Isabella Locatelli [3], Peter Brugger[4], Klemens Gutbrod[5], Andreas U. Monsch[6], Isaure Nadin[7], Marc Schwind[8], Riccardo Pignatti[9], Renaud Du Pasquier[2], and the NAMACO study group[**], a Swiss HIV Cohort Study

[1] Service of Infectious Diseases, CHUV, [2] Service of Neurology, CHUV, [3] Institute of Social and Preventive Medicine, University of Lausanne, [4] Division of Infectious Diseases and Hospital Epidemiology, University Hospital Zurich, [5] Division of Neurology, University Hospital of Bern, [6] Memory Clinic, Felix Platter Hospital, Basel & Faculty of Psychology, University of Basel, [7] Service of Neurology, University Hospital of Geneva, [8] Neurology Clinic, St. Gallen, [9] Service of Neurology, EOC Lugano, Switzerland.

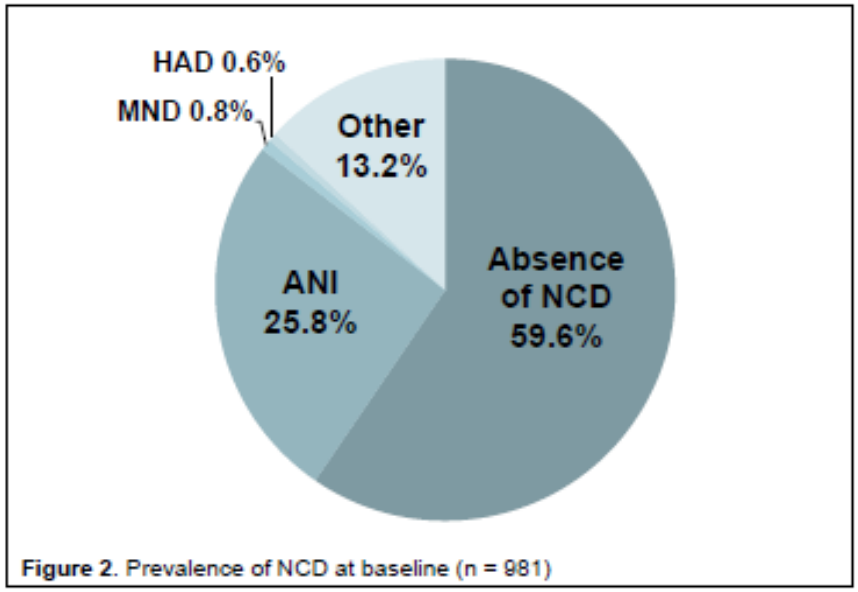


Figure 2. Prevalence of NCD at baseline (n = 981)

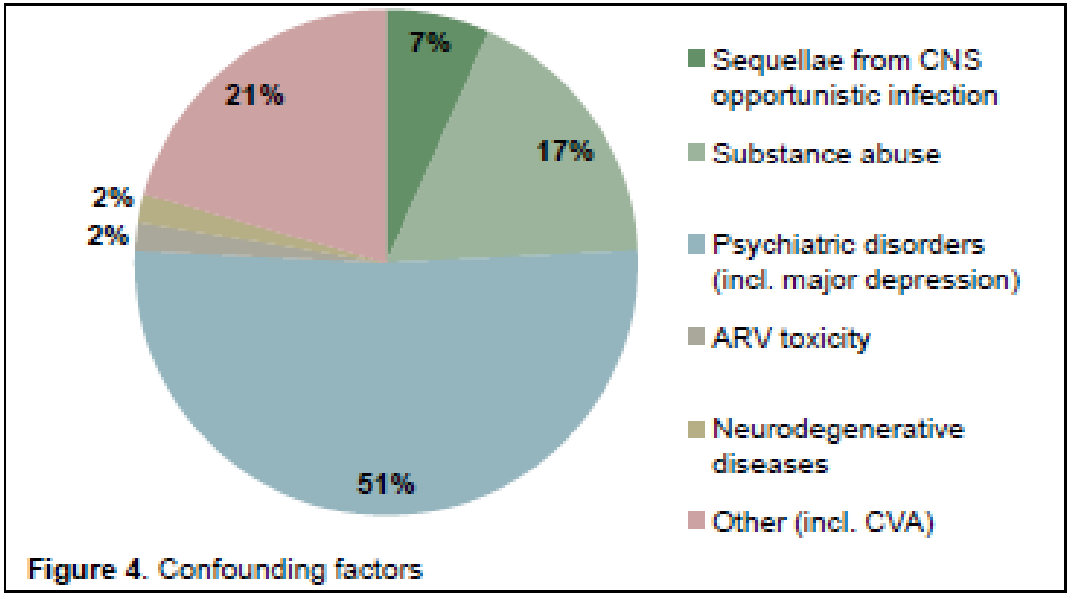
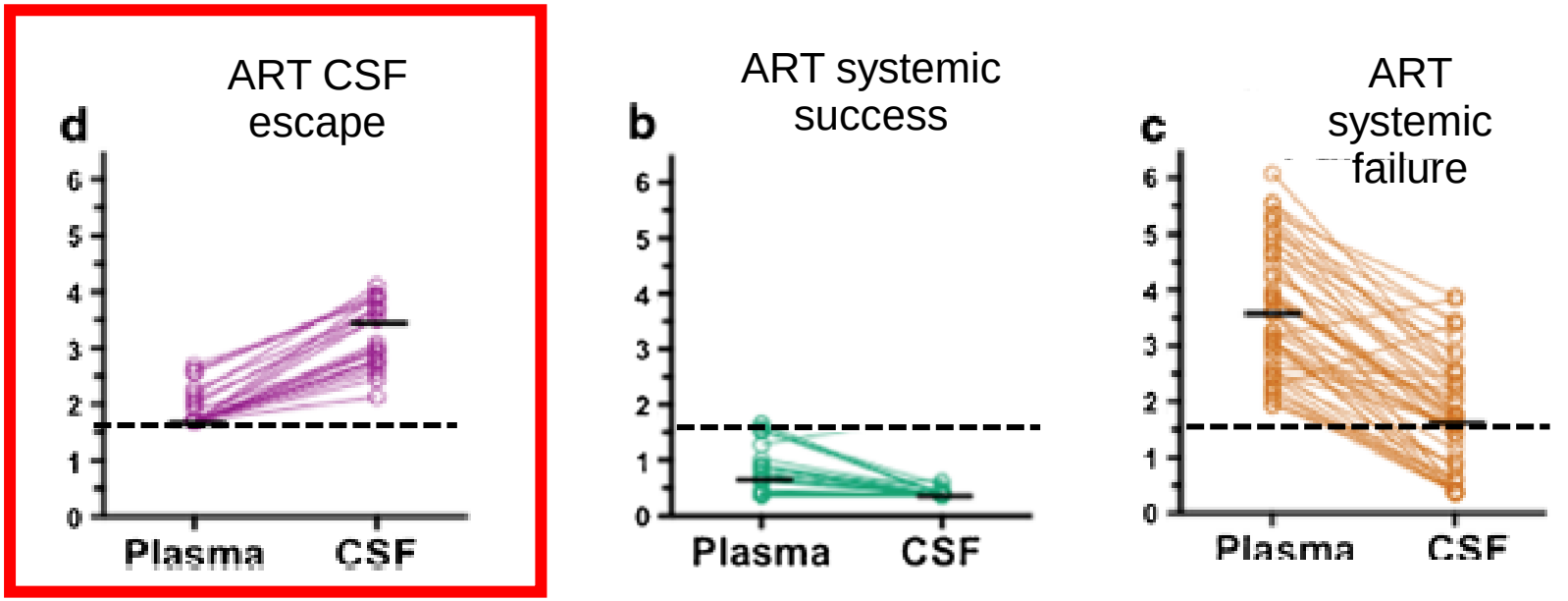


Figure 4. Confounding factors

CSF viral escape



Ferretti F et al. Curr HIV/AIDS Rep 2015

- On ART > 6/9 months
- CSF VL > LLD (if plasma VL suppressed) or CSF VL > plasma VL (if plasma VL >50)
- Symptomatic or asymptomatic

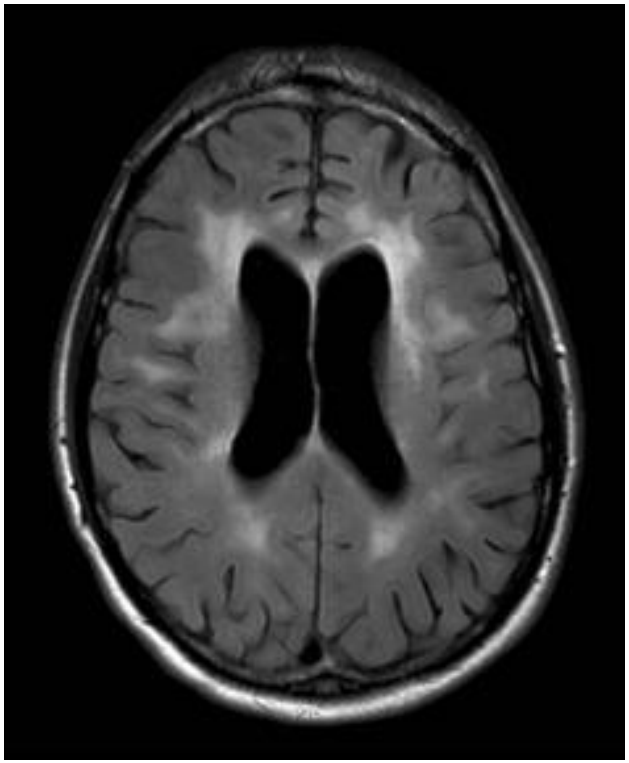
CSF escape: encephalitis with dementia

- M, 50
- **2008: Progressive dementia**

- History of HIV-D
- CD4 nadir: 145
- 1991: Starts ART
- Since 2005 **TDF,FTC,LPV/r**

- CD4 632
- **Plasma HIV 265 c/mL**
- **CSF HIV 750 c/mL**
- **CSF cells 26/µL**

→ CSF and plasma mutations to NRTIs (67,75,77,118,184,210,215,219) and PIs (46,54,82,90)



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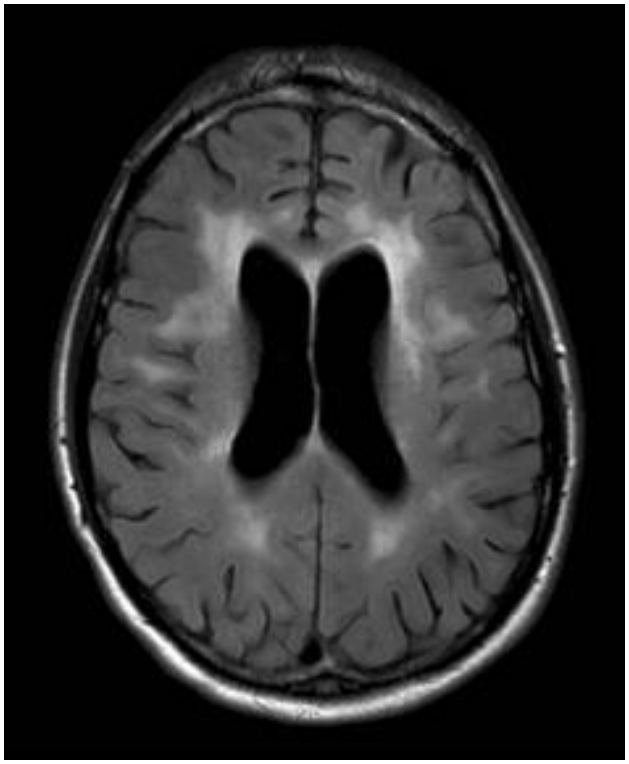


→ Resolution by cART optimization for genotypic profile

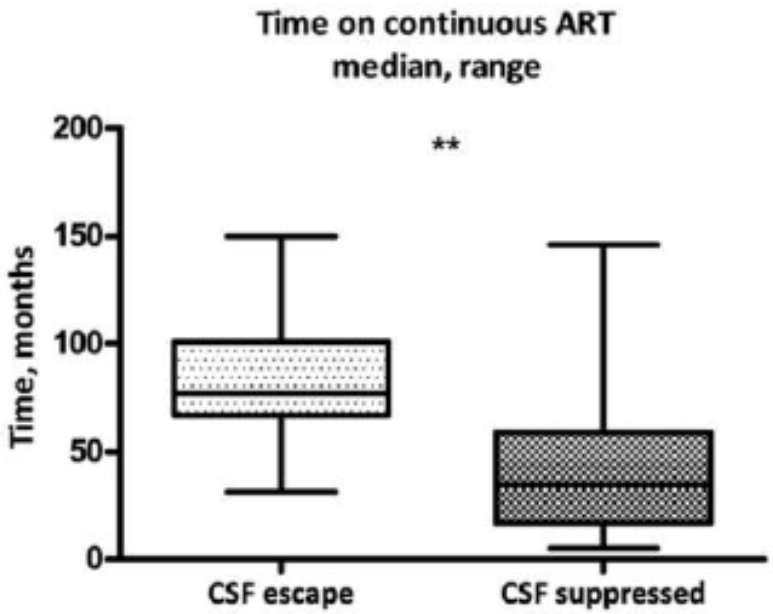
Asymptomatic CSF viral escape

69 pts with plasma HIV RNA<50 c/mL

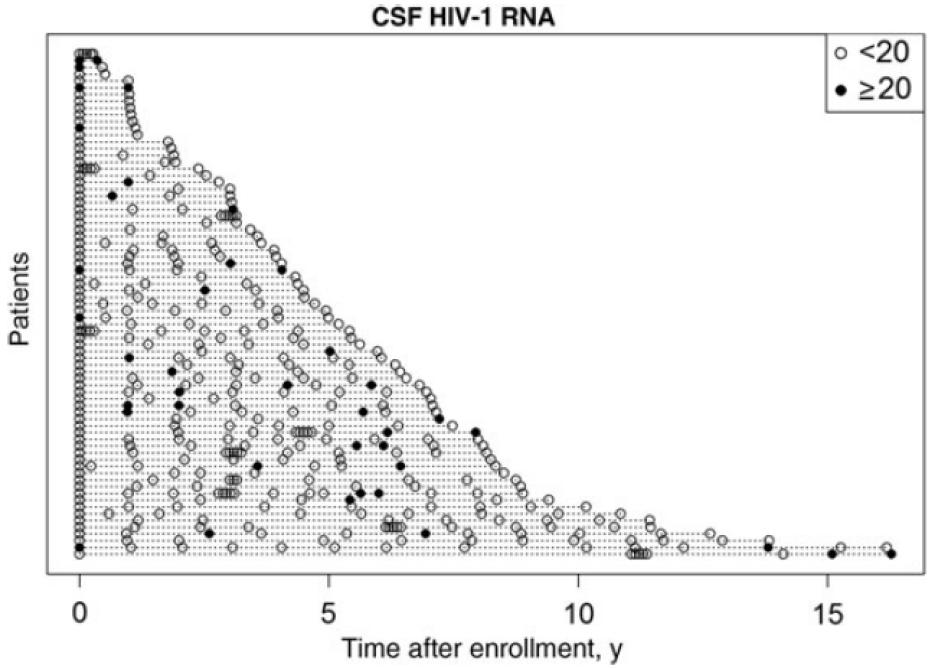
CSF escape (>50 c/mL) in 7 (10%), median 121 (range 52-860) c/mL

75 pts patients with longitudinal CSF samples (median, 5 samples/pt)

≥1 CSF escape (>50 c/mL) in 23%.



Edén A et al. *J Infect Dis* 2010

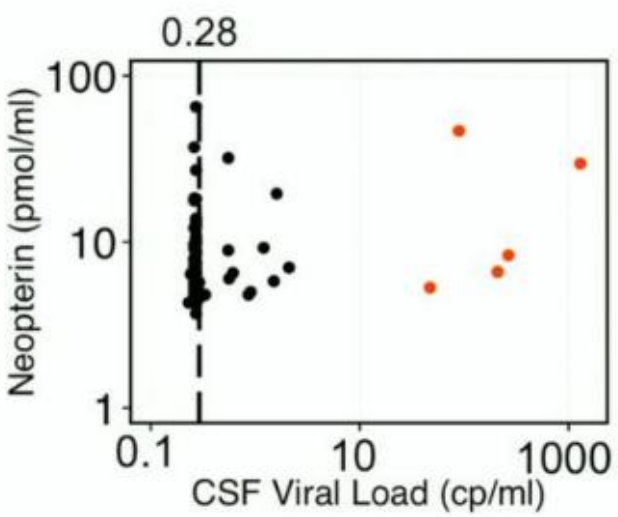


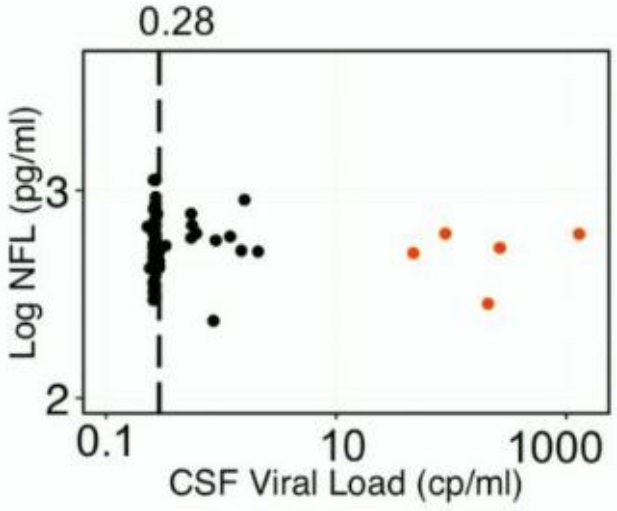
Edén A et al. *J Infect Dis* 2016

ASYMPTOMATIC HIV-1 CSF ESCAPE IS UNCOMMON AND NOT ASSOCIATED WITH NEURONAL DAMAGE (Joseph SB, CROI 2017, abs. # 70)

Frequency of asymptomatic escape: 6%

Asymptomatic CSF escape is not associated with elevated levels of neopterin or neurofilament light chain (NFL)





Highlights of the Global HIV-1 CSF Escape Consortium Meeting, 9 June 2016, Bethesda, MD, USA

Objective:

Gather investigators from diverse sites to discuss opportunities for future collaborative work on this emerging issue

→Reach a consensus set of definitions of the distinct forms of CSF escape

→Define clinical implications

→Investigate biological mechanisms

Joseph J et al, J Viral Eradication 2016

Table 1. Summary of CSF escape cohorts or cases presented at the Global HIV-1 CSF Escape Consortium meeting

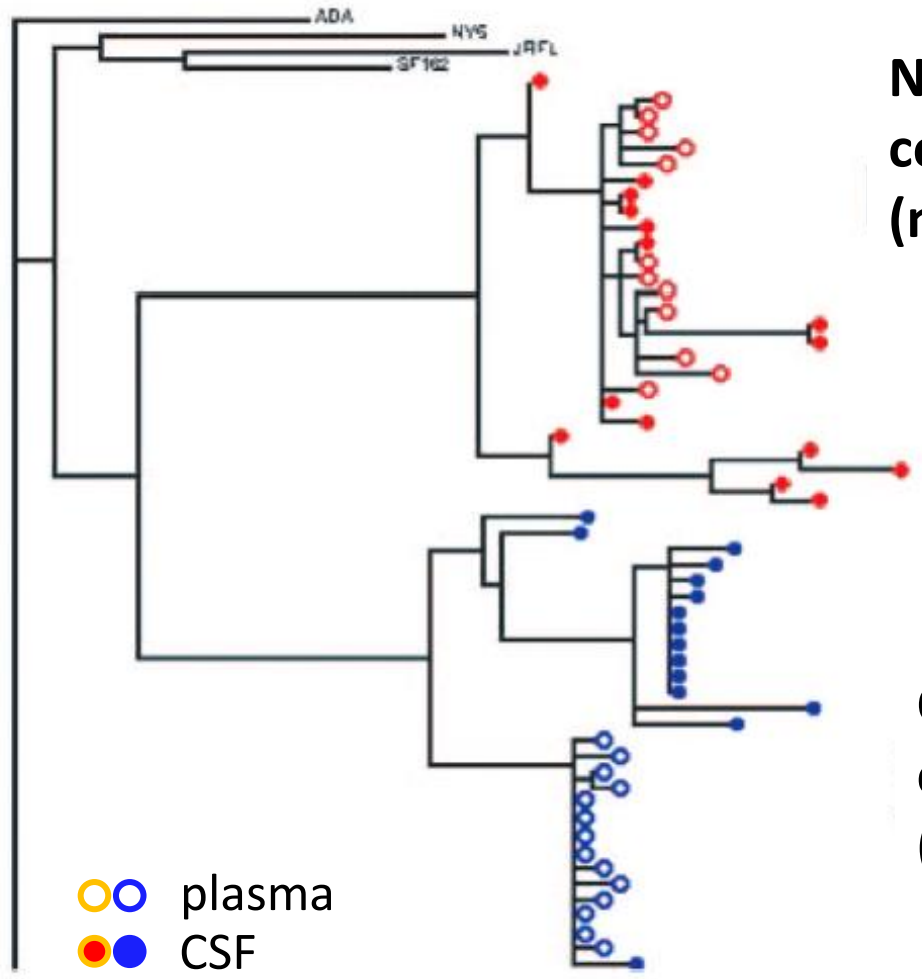
Speakers	Study site	Total number of cases	Number of cases of HIV-1 CSF escape	Neurosymptomatic	Asymptomatic	Criteria for determining CSF escape	Estimated prevalence[1]
Price, Gisslen, Cinque, Spudich, Joseph S	Multiple[2] (San Francisco, New Haven, Chapel Hill, USA; Sweden; Italy)	N/A	81	42	39	Symptomatic: PVL<50 & CVL>100 or PVL 50–100 & CVL 2 × PVL; or Asymptomatic: PVL<50 & CVL>50	N/A
Joseph S	THINC Study Sites (Chapel Hill, San Francisco, New Haven, USA)	97	6	N/A	6	PVL<40 & CVL>40 or CVL>PVL	6%
Winston (UK)	UK	142	30	3	27	PVL<50 & CVL>200 or $\log_{10}$ CVL>1.5 × $\log_{10}$ PVL	21%
Winston (Europe)	EU	134	1	1	N/A	CVL>PVL	0.7%
Ene	Romania/Adult	91	4	2	2	CVL>0.5 log of PVL	4.4%
Perez	Spain	125	4	4	N/A	PVL: not detectable; CVL: detectable	3.2%
Sacktor	Uganda	91	9	4	5	PVL: not detectable; CVL: detectable	10%
Wright	Australia	167	6	3	3	PVL: 6 months not detectable; CVL: detectable	3.5%
Dravid	India	62	17	17	0	CVL: detectable with PVL: not detectable; CVL>1 log of PVL	27.4%
Letendre	CHARTER/HNRC sites	849	60	23	37	CVL>PVL with PVL: not detectable; CVL>1 log of PVL	7%
Nath	Washington DC	56	11	7	4	PVL<40; CVL>20	20%
Gabuzda	Boston, MA/NNTC (four sites)	200/426 (626)	11/29 (40)	11/17	0/12	PVL<50, CVL>50; CVL>0.5 log of PVL	6.4%
Wojna	Puerto Rico**	380	10	3/9	6/9	CVL>PVL	2.6%

Table 2. Challenges to consortium studies of CSF HIV-1 escape

Need for common definitions of CSF escape
• Category of escape with 'undetectable' plasma viral load: which assay measurements (assay platform/method, lower limit of detection, cutoff for 'undetectable' definition?
• Category of escape with CSF/plasma HIV discordance in treated patients: what ratio considered 'discordant,' what plasma viral load is considered evidence of 'treatment'?
• Category of 'symptomatic' viral escape: which clinical manifestations fulfil criteria for 'symptomatic'?
• Category of 'asymptomatic' viral escape: what evaluation required to define as 'asymptomatic'?
Determination of ART regimens considered 'treatment': include 'old' regimens, 'atypical' regimens, 'simplified' (two-drug) regimens?
Enrolment/recruitment methods
• Include participants referred for LP for clinical reasons?
• Screening in research-only participants, clinical setting?
• Any requirement for screening for concomitant CNS infection/inflammation (to assess for 'secondary' CSF escape)?
Data collection, dissemination, interpretation.
• Agreement on common elements of clinical and demographic data to be interpreted across sites, including methods?
• Agreement on neuropsychological test and neuroimaging methods and standardisation across sites?
• Common open database?
• Willingness to share data across sites?
Samples to be collected
• Agreement on sample types (CSF supernatant, plasma, CSF pellets, PBMC, other tissues)?
• Common methods for sample collection, processing, storage?
• Willingness to share samples across sites for specialty assays?
Infrastructure and support
• Funding mechanism for research studies that required collaboration between investigators?
• Organisation of and support for consortium teleconferences and in-person meetings?

The CNS as a reservoir and virus compartmentalization

CNS compartmentalization of HIV infection



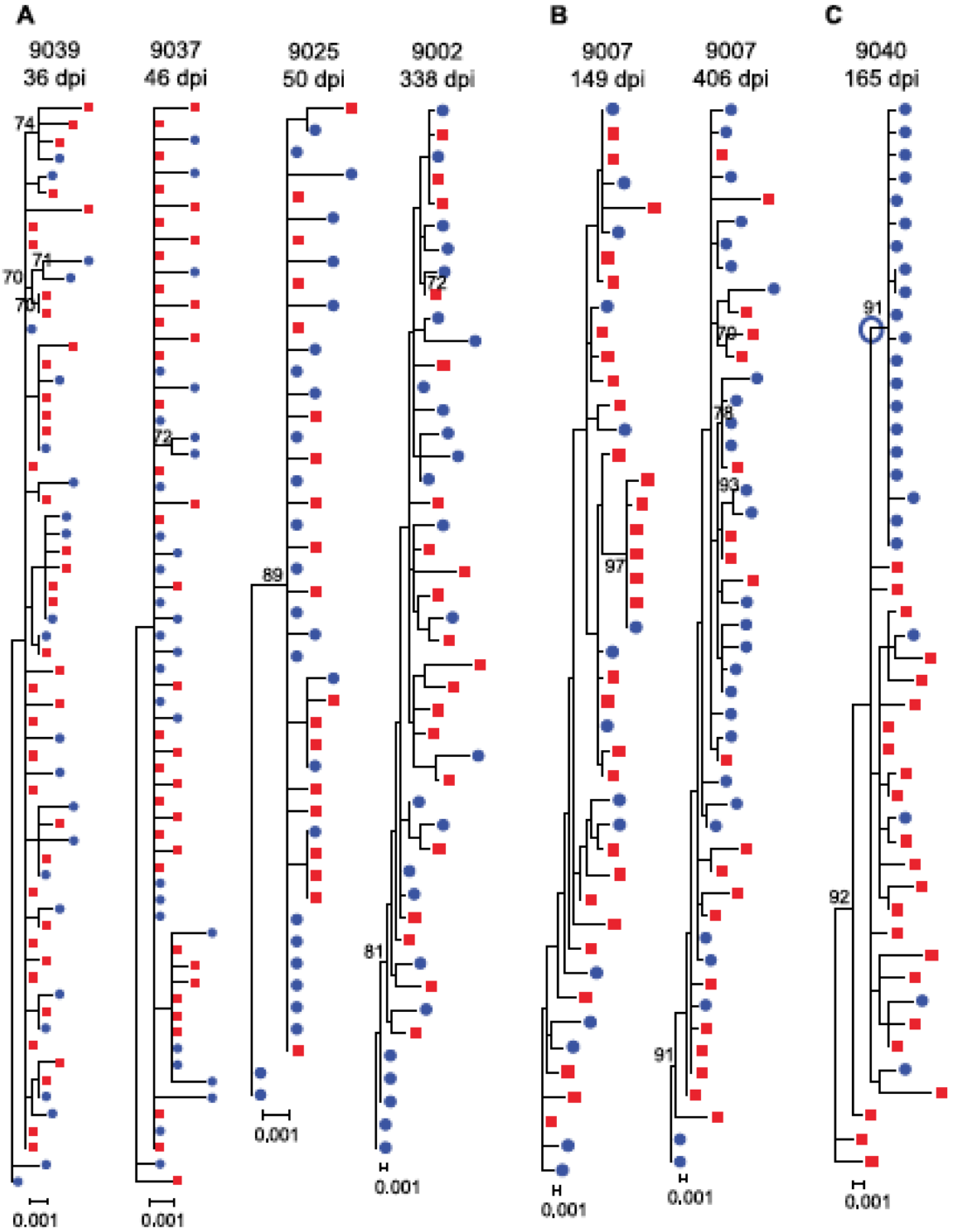
No CSF compartmentalization (neuroasymptomatic)

CSF compartmentalization (ADC)

Pillai SK et al., Brain 2006

Compartmentalization and Clonal Amplification of HIV-1 Variants in CSF during Primary Infection

Shnell G. et al, J Virol 2010



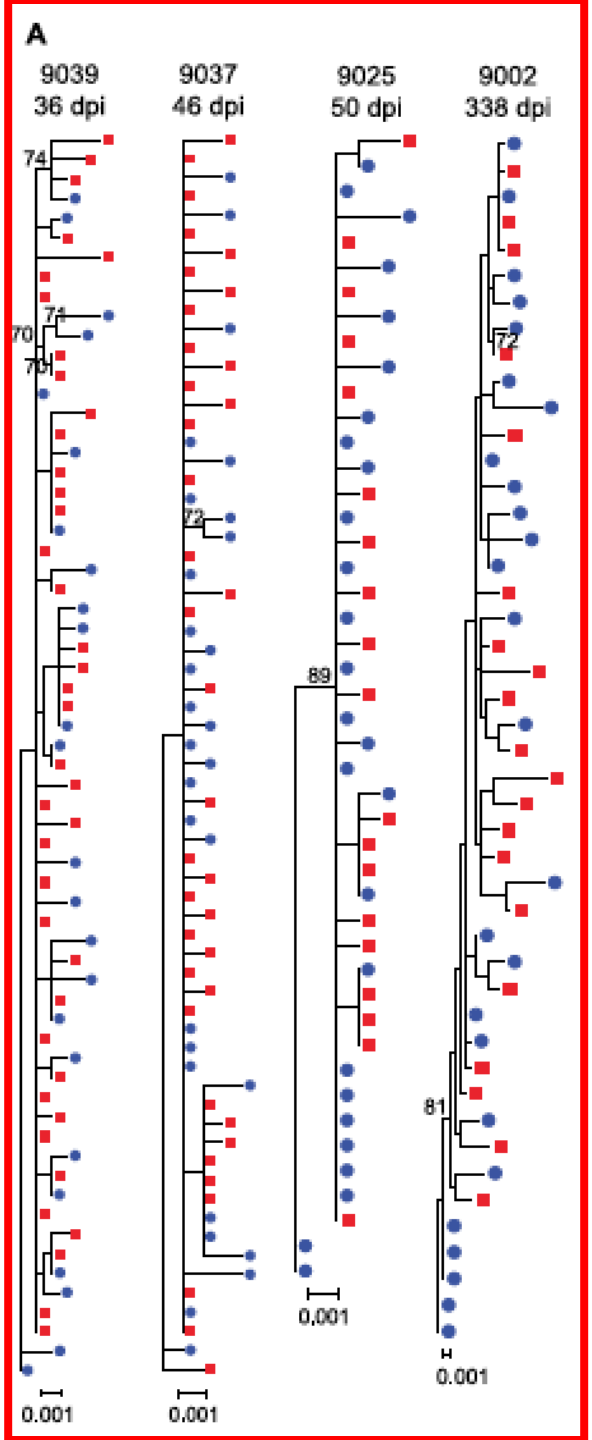
Compartmentalization and Clonal Amplification of HIV-1 Variants in CSF during Primary Infection

Shnell G. et al, J Virol 2010

Equilibration between blood plasma and CSF HIV-1 populations

CSF

plasma



Compartmentalization and Clonal Amplification of HIV-1 Variants in CSF during Primary Infection

Shnell G. et al, J Virol 2010

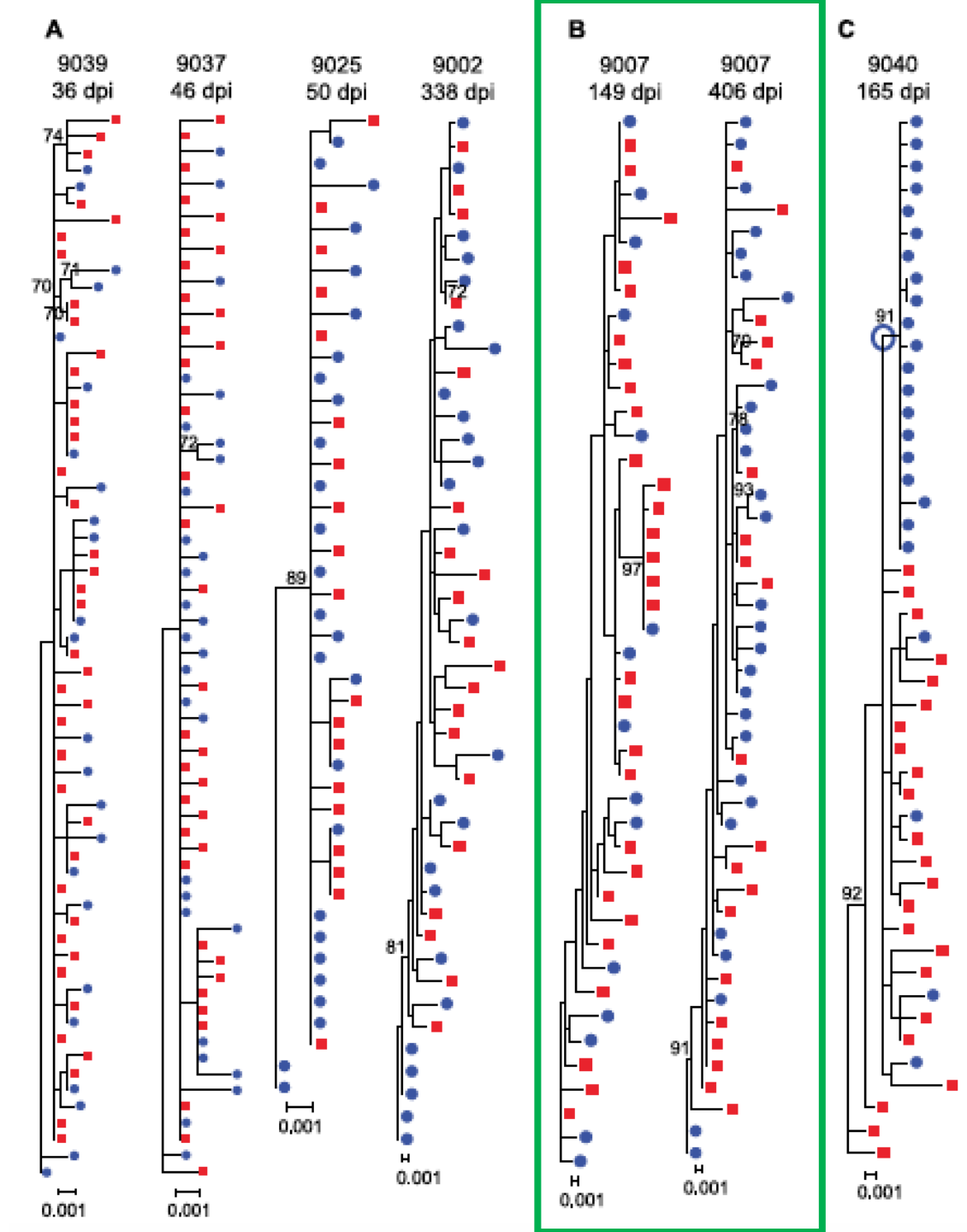
Equilibration

↓

Initial discordance

CSF

plasma



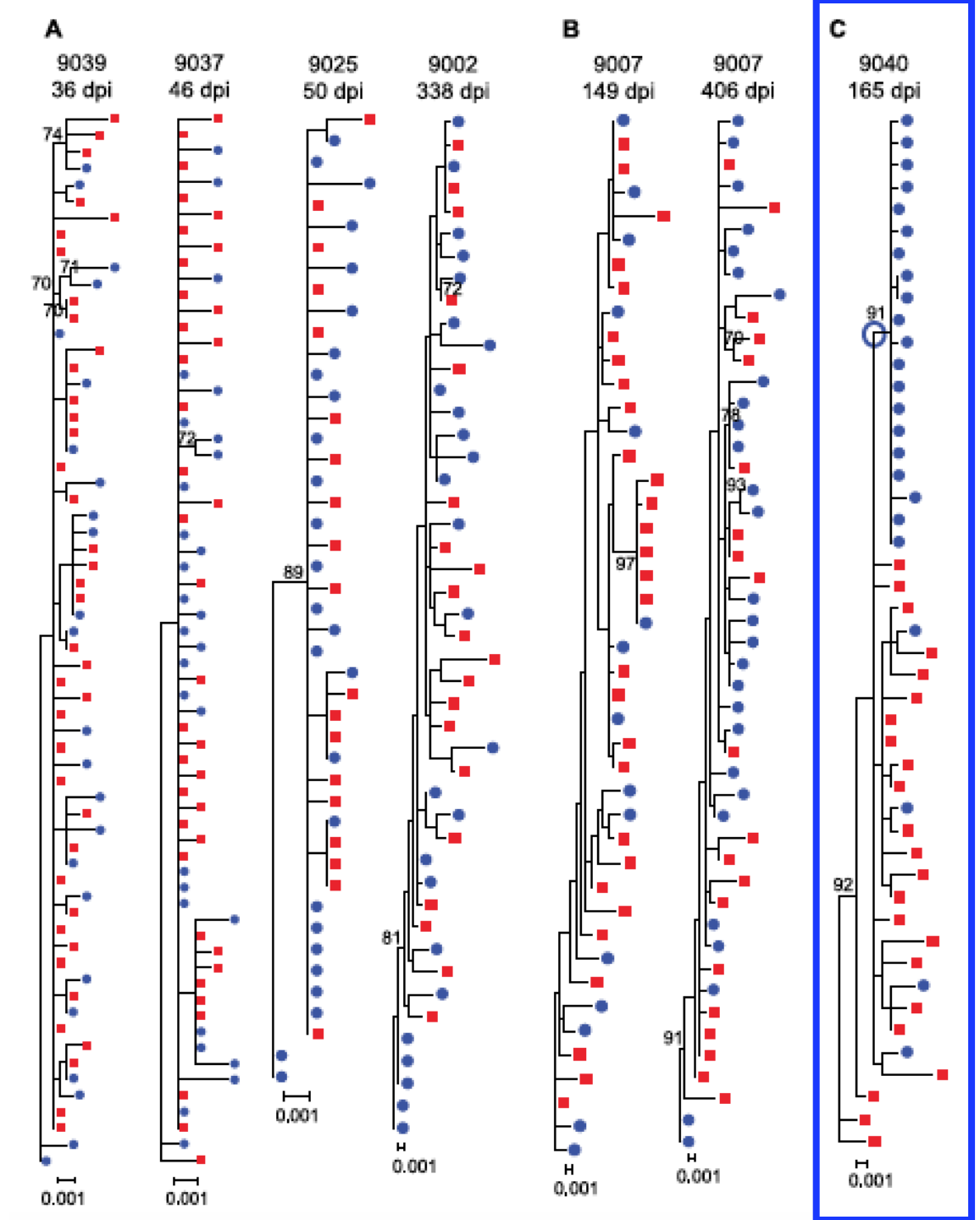
Compartmentalization and Clonal Amplification of HIV-1 Variants in CSF during Primary Infection

Shnell G. et al, J Virol 2010

Discordance = compartmentalization

CSF

plasma



Poster # 364

CSF HIV-1 compartmentalization by *env* deep sequencing: relation to neuronal injury

Richard W. Price[1], Magnus Gisslen[2], Laura P. Kincer[3], Ean Spielvogel[3], Amy Lin[2], Jasur Eusuff[2], Serena Spudich[4], Ronald Swanstrom[3], Sarah Beth Joseph[3], and the THINC Study Group[3]

[1]University of California, San Francisco, California; [2]University of Gothenburg, Sweden; [3]University of North Carolina Chapel Hill, Yale University, New Haven, Connecticut

Contact: Richard W. Price, M.D.
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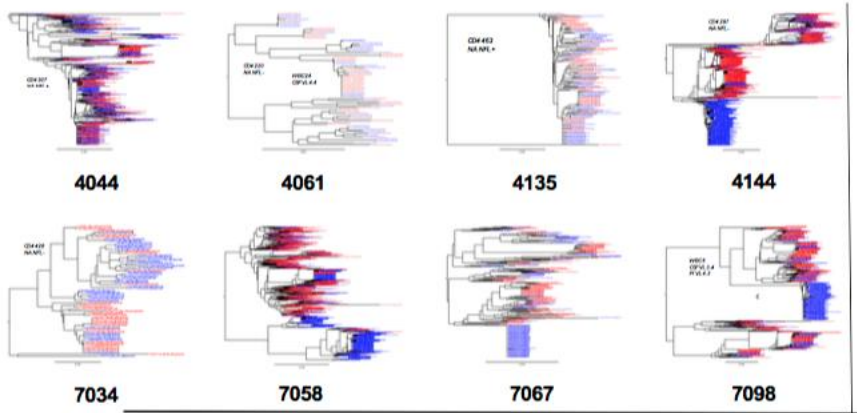
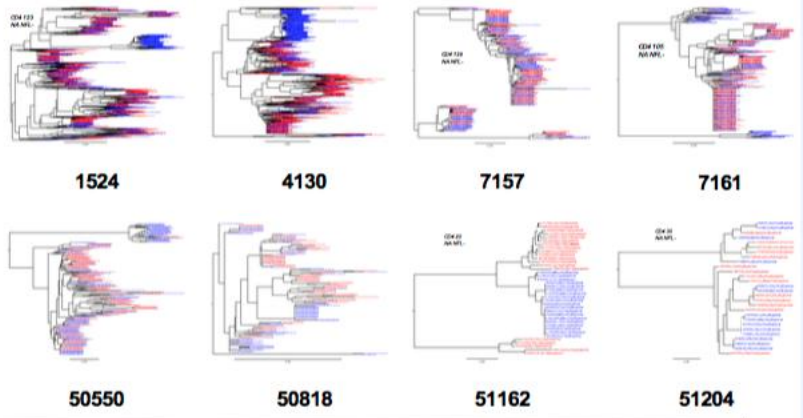
Tel: 415-206-4487

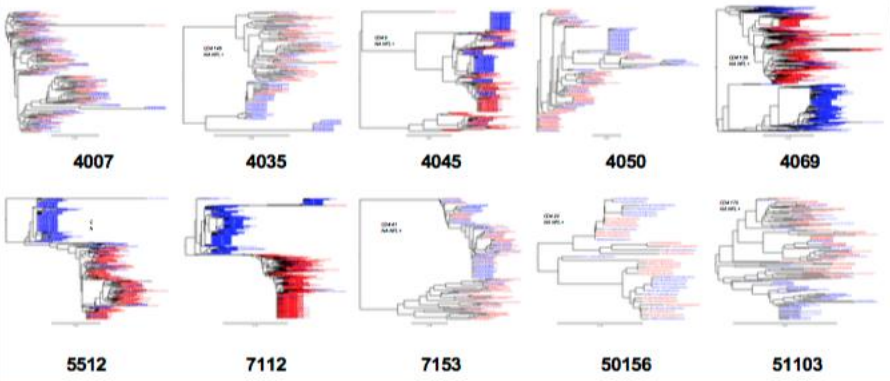
1. Neuroasymptomatic (NA) *CD4 >200* cells/µL (N=8)



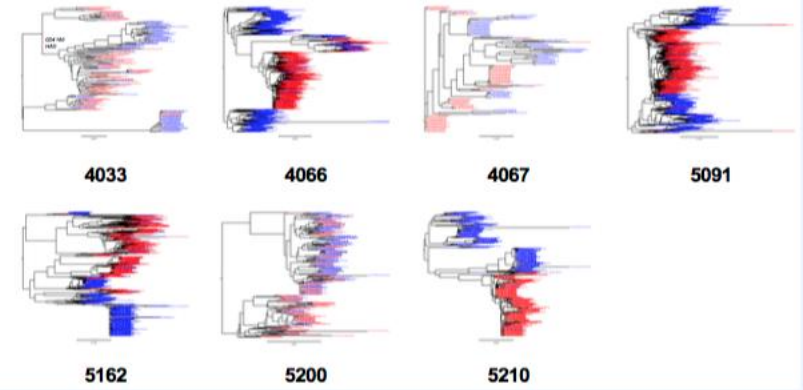
2. NA CD4 <200 with normal CSF NFL (*NFL-negative*) (N=8)



3. NA CD4 <200 with elevated NFL (*NFL+*) (N=10)



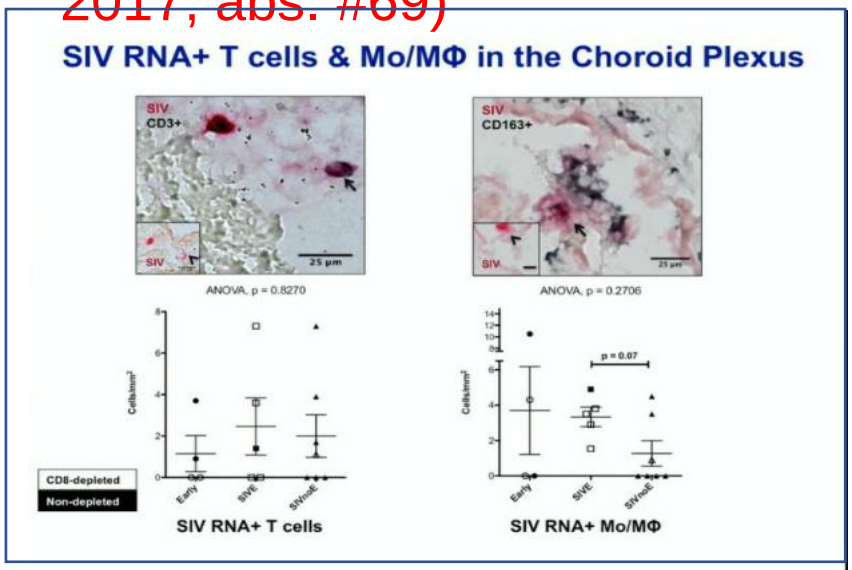
HAD (N=7), all with elevated CSF NFL

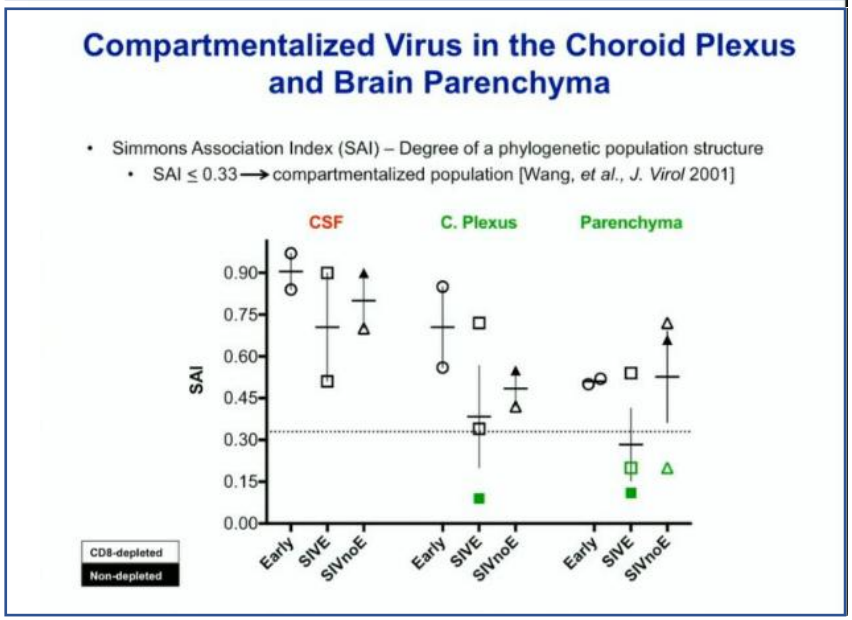


- Major (>30%) CSF *env* sequence compartmentalization in all of the 7 HAD subjects
- CSF *env* sequence compartmentalization also present in the other groups, including the two without evidence of ongoing CNS injury (normal CSF NFL)

→ CSF HIV-1 compartmentalization does not provide a simple biomarker of neuropathic infection

CNS PARENCHYMA AND CHOROID PLEXUS, NOT CSF, ARE VIRAL RESERVOIRS IN MONKEYS WITH AIDS (J Mallard, CROI 2017, abs. #69)





- Detection of SIV-RNA+ T cells and Mo/MΦ in CP

  → CP as a source of CSF virus.

- Dispersed phylogeny of CSF viral sequences among peripheral and CNS sequences

  → the CSF is not a viral reservoir.

- Mo/MΦ accumulation and compartmentalization of viral sequences in CP and CNS

  → infected Mo/MΦ in these tissues are the source of CNS viral reservoir.

P579

Discordant HIV RNA in Olfactory Mucosa of HIV-positive Patients

Calcagno A[1], Allice T[2], Bertero L[3], Amasio EM[4], Trunfio M[1], Imperiale D[5], Ghisetti V[2], Di Perri G[1], Cassoni P[3] and Bonora S[1].

[1]Unit of Infectious Diseases, Department of Medical Sciences, University of Torino; [2]Laboratory of Microbiology and Molecular Biology, Ospedale Amedeo di Savoia, ASL TO2; [3]Unit of Pathology, Department of Medical Sciences, University of Torino; [4]Unit of Otorhinolaryngology, Ospedale Maria Vittoria, ASL TO2; [5] Unit of Neurology, Ospedale Maria Vittoria, ASL TO2, Torino, Italy.

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Background

- The control of HIV in the CNS is of major importance for preventing neurological syndromes and, potentially, neurocognitive decline in HIV-positive subjects;[1]
- CSF HIV RNA may represent a suboptimal marker of brain tissue viral replication;[2]
- The olfactory mucosa (OM) is an easily accessible CNS-derived tissue located over the cribriform plate.[3] It is the way of entry into the CNS for several viruses and it may contain extracellular proteins in patients with dementias (tau, alpha-synuclein, etc.);[4-6]
- Nasal Brushing is a non-invasive technique that has been used for diagnosing ciliary dyskinesia, cystic fibrosis and it is now the gold standard for Creutzfeld-Jacob disease.[7,8]

Patients and Methods

Inclusion Criteria

- HIV-positive patients undergoing LPs for clinical reasons.

Procedure

- Patients underwent nasal brushing (<72 hours apart from the spinal tap) with a flocked swab (Copan, Brescia, Italy);
- After local epinephrine application the swabs were inserted and gently rolled (360°) over the nasal vault (2 swabs/nostril) by a trained Ear Nose Throat consultant;
- Swabs were then inserted in 4% formaldehyde (FA), Copan UTM viral transport medium (UTM) or 0.9% saline solution (SS).

Analysis

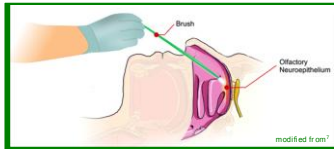
- FA samples were stained with monoclonal anti-olfactory marker protein (OMP, Santa Cruz Biotechnology), anti-CD3 e anti-CD20;
- UTM samples were used for quantifying HIV RNA with a CAP/CTM HIV-1 v2.0 procedure (1 mL of NB was used as CAP/CTM input and PCR processing was evaluated with the internal control quantitation standard of the assay). Plasma and CSF HIV RNA was quantified using CAP/CTM v.2.0 assay (Roche Molecular, USA, LOD 20 copies/mL);
- SS samples were vortexed at 900 rpm for 5 min and stored at -80°C.

Design

HIV-substudy in a cross-sectional, controlled, diagnostic study in CNS-affecting disorders ("SOLFAMU", NCT02951559);

Aim of the substudy

Comparing HIV RNA and biomarkers on OM with plasma and CSF



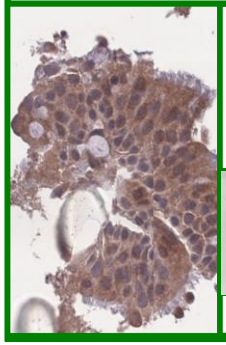
Results (n=19)

Tolerability

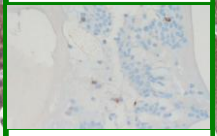
Short duration mild discomfort and sneezing were the only reported side effects.

n (%) or median (IQR)	Naive n=7	Treated n=12
Gender (male)	3 (42.9%)	7 (58.3%)
Age (years)	46.7 (33-51)	54.3 (45-59)
CD4 (cell/uL)	14 (5-174)	347 (109-729)
plasma HIV RNA (Log_{10} cps/mL)	5.2 (4.9-5.7)	<1.3 (<1.3-1.8)
CSF HIV RNA (Log_{10} cps/mL)	2.2 (1.3-3)	<1.3 (<1.3-1.7)
CSF cells (n/mm^3, median/range)	0 (0-2)	0 (0-7)
CSF serum albumin ratio	7.6 (5.9-8.5)	5.3 (3.5-8)
CSF neopterin (ng/mL)	1.6 (1-3.3)	1 (0.6-1.3)
Diagnosis:		
asymptomatic	5 (71.4%)	1 (8.3%)
HAND	2 (28.6%)	5 (41.7%)
CNS OIs	0	2 (16.6%)
Neurological symptoms (headache, neuropathy)	0	4 (33.3%)

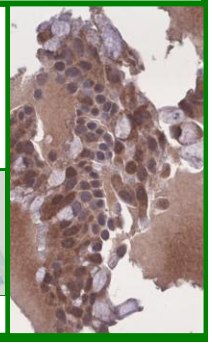
Pathology



All samples showed a high cellularity, anti-OMP immunoreactivity and a median of 45% (30-72) anti-OMP positive cells. Rare T-lymphocytes were observed.



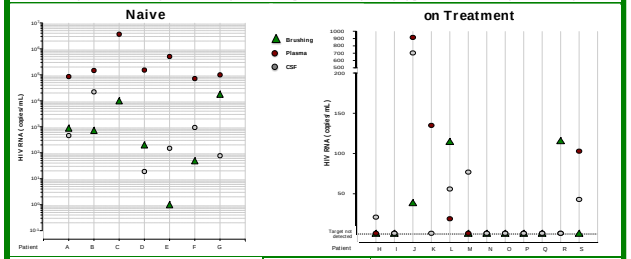
rare CD3+ cells

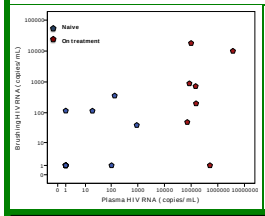


Molecular Biology

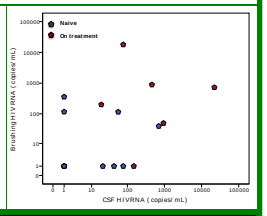
OM HIV RNA was detectable in 10 samples

725 (<20-17898) copies/mL [median (range)] <20 (<20-358)





OM HIV RNA showed a significant correlation with plasma (rho=0.67, p=0.001) but not CSF HIV RNA (rho=0.26, p=0.273)



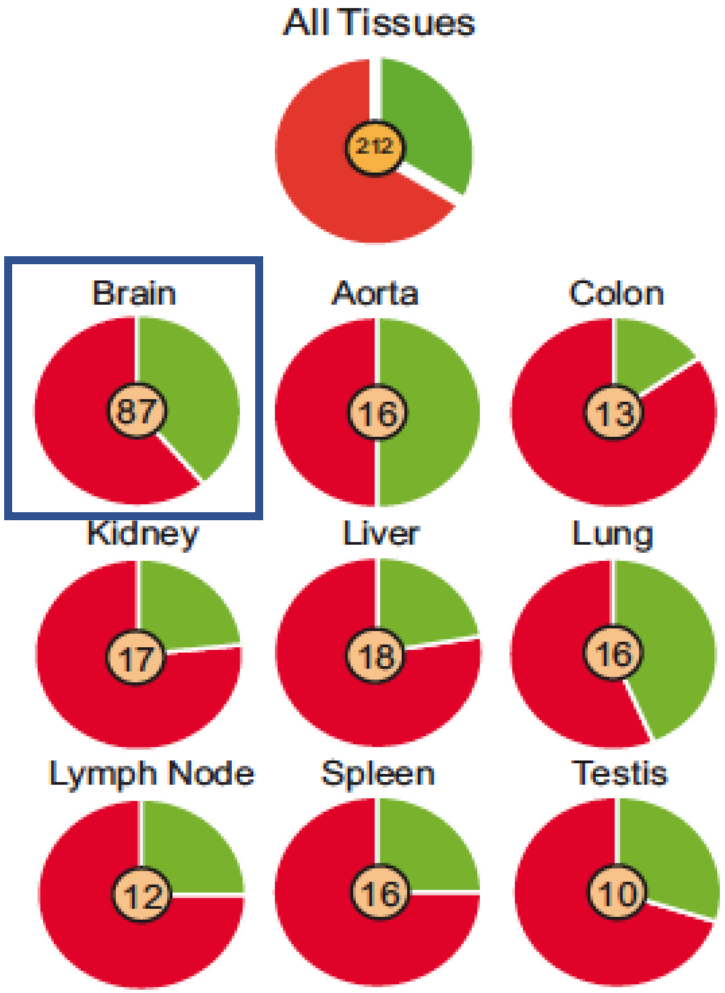
Conclusions and Further Developments

- Nasal brushing is a safe and promising procedure that allows a non-invasive collection of olfactory mucosa cells, including olfactory neurons:
  - *Immune-staining is currently ongoing*
- HIV RNA can be measured in most samples and it correlates with plasma viral load. Studies are ongoing to understand the clinical relevance and source of this mucosal HIV RNA:
  - *The comparison of OM viral sequences with plasma, CSF and lymphoid tissue viruses as well as the amplification of other viruses (CMV, EBV) is currently ongoing*

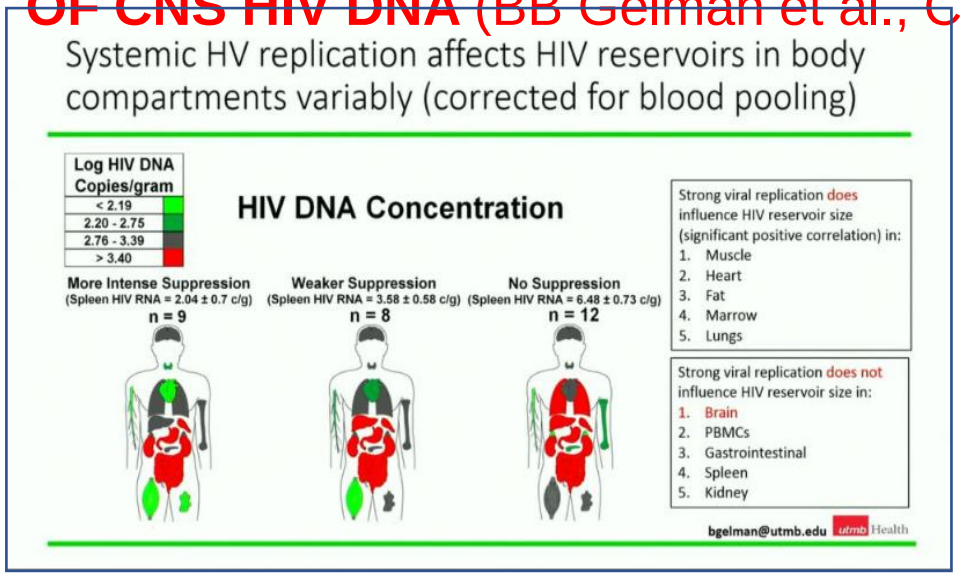
1. Nightingale S, et al. Lancet Neurol. 2014
2. Gelman BB, et al. JAIDS 2013
3. Chen CR, et al. J Neurol Surg B Skull Base. 2014
4. van Riel D, et al. J Pathol. 2015
5. Witt M. et al. Mov Disord. 2009
6. Ayala-Grosso CA, et al. Brain Pathology 2015
7. Orrù CD, et al. NEJM 2014
8. Bongianni M, et al. JAMA Neurology 2016

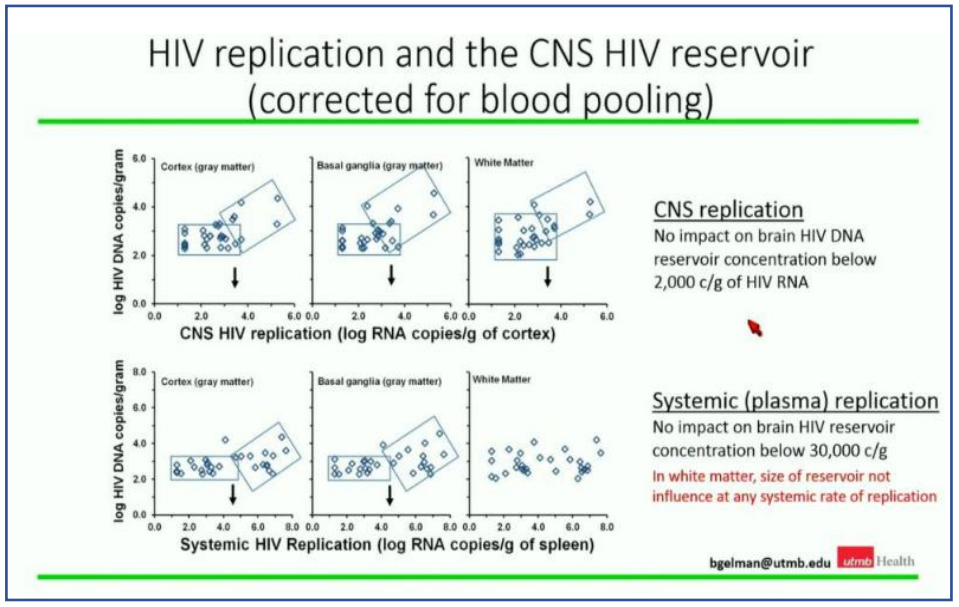
HIV DNA Is Frequently Present within Pathologic Tissues Evaluated at Autopsy from Combined Antiretroviral Therapy-Treated Patients with Undetectable Viral Loads (JV 1996 Lamers et al.)

- 229 autopsy specimens from 20 HIV pts who died while on cART with low or undetectable plasma and CSF VL (National Neurological AIDS Bank, NNAB)
- HIV-DNA measured in tissues by quantitative and droplet digital PCR
- HIV-DNA identified in 48/87 brain tissues and 82/142 non-brain tissues at >200 c/million cell equivalents
- No participant was completely free of tissue HIV
- Parallel sequencing studies from some tissues recovered intact HIV DNA and RNA.



INTENSITY OF SUPPRESSION LINKED WITH SHIFTING COMPARTMENTALIZATION OF CNS HIV DNA (BB Gelman et al., CROI 2017, abs #68)





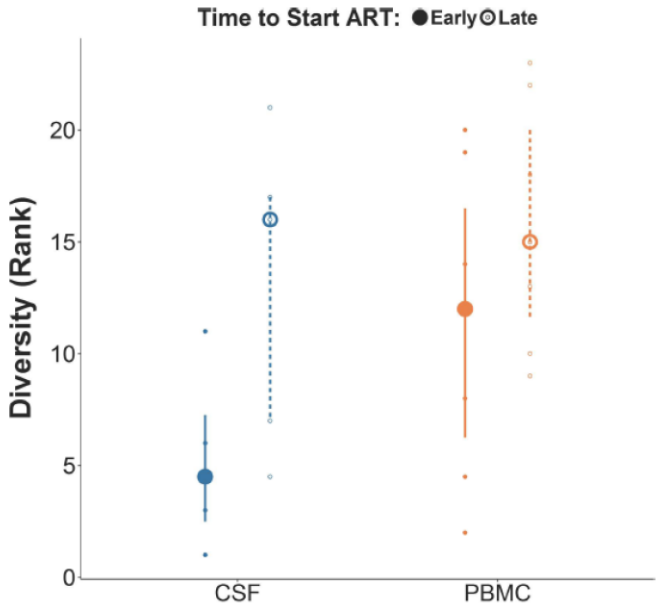
Tissue samples from 29 autopsy cases (NTTC)

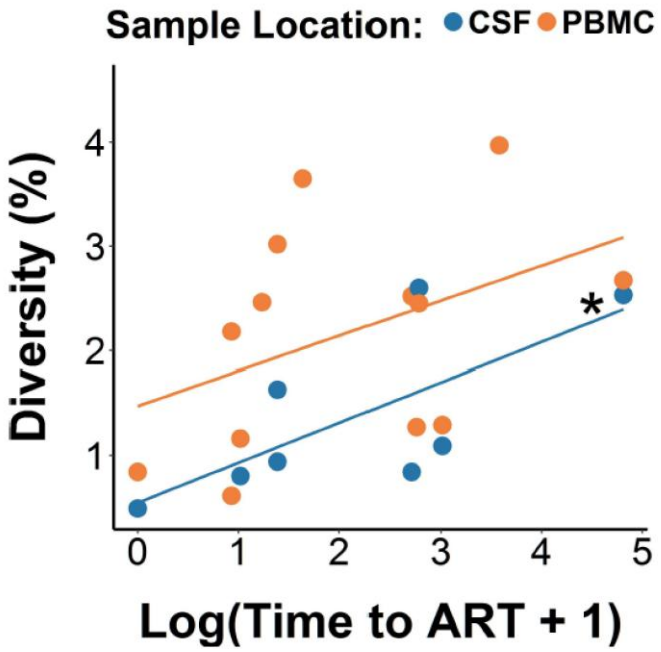
→ A more intense viral suppression, both within the CNS compartment and systemically will not diminish the total brain pool size

Early ART is Associated with lower HIV DNA Molecular Diversity and lower Inflammation in CSF but Does Not Prevent the Establishment of Compartmentalized HIV DNA Populations (Oliveira MF, PLOS Pathogens 2017)

Sequential paired blood and CSF from 16 ART-treated suppressed pts (after a median of 2.6 years from ART start):

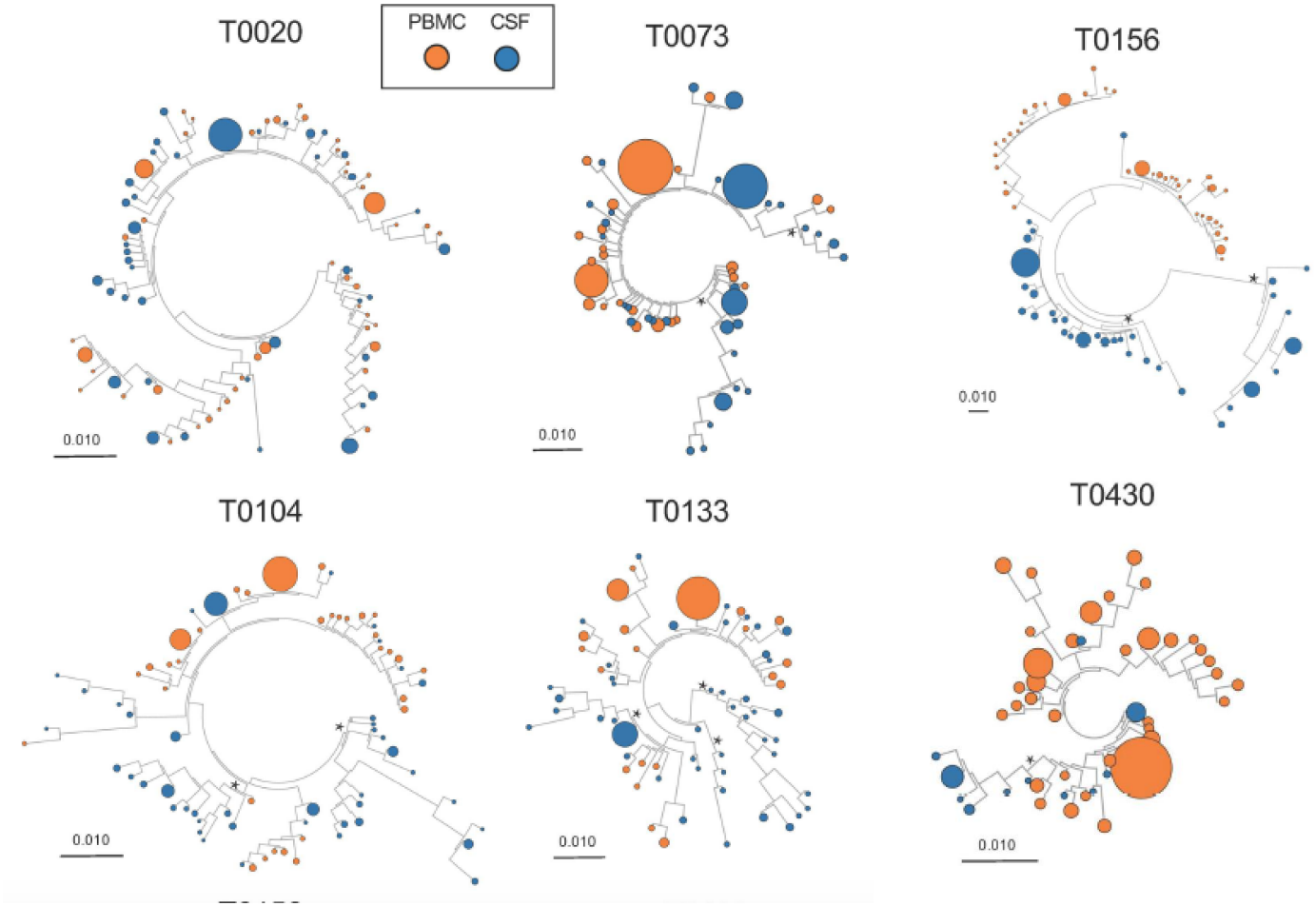
- 9 early ART (<4 months of infection)
- 7 late ART (>14 months after infection)





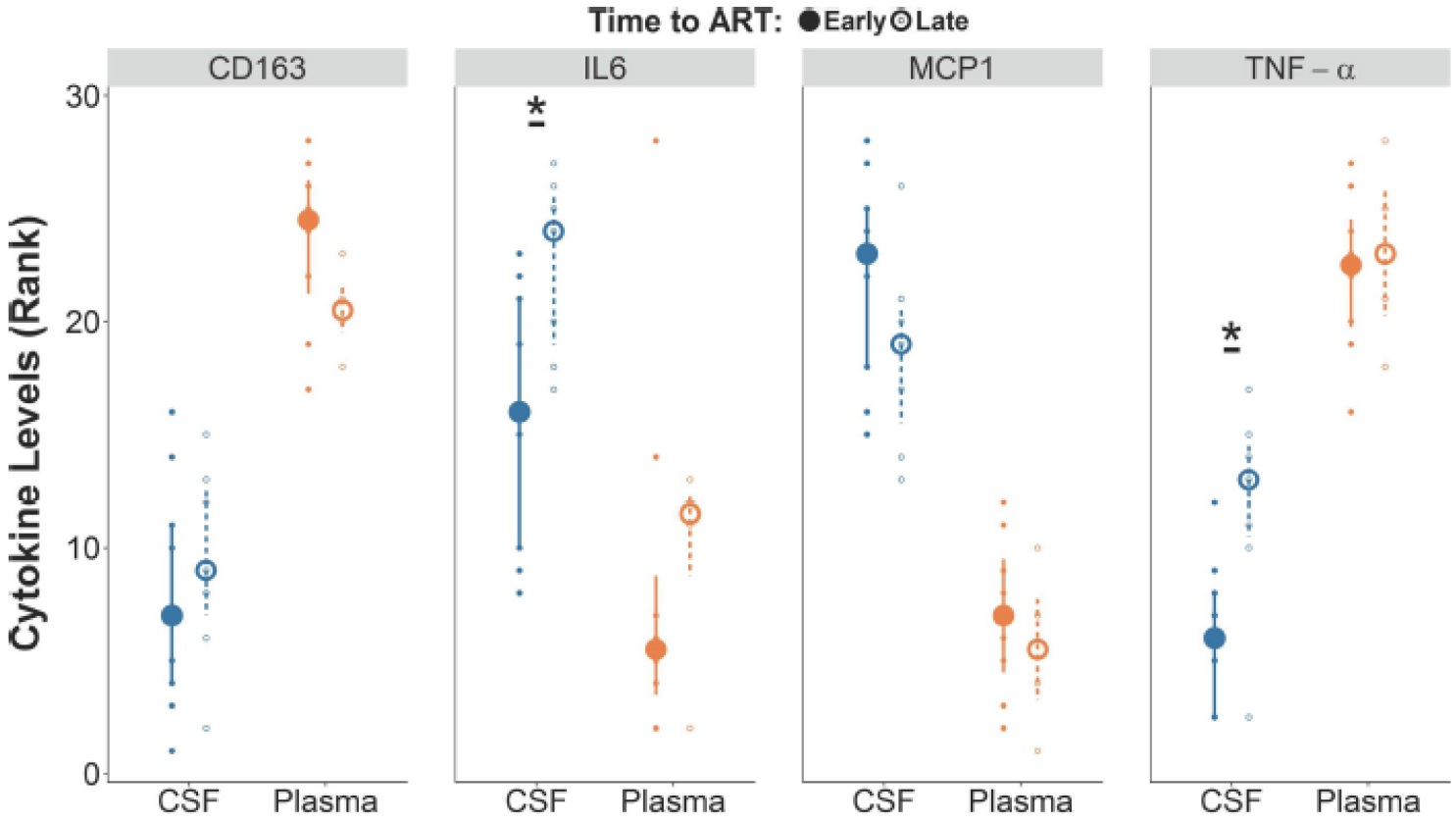
Early ART was associated with lower molecular diversity of HIV DNA in CSF in comparison to late ART

Early ART is Associated with lower HIV DNA Molecular Diversity and lower Inflammation in CSF but Does Not Prevent the Establishment of Compartmentalized HIV DNA Populations (Oliveira MF, PLOS Pathogens 2017)



CSF-blood HIV DNA compartmentalized in the majority (75%) of the participants with available paired sequences, including two (66%) early ART patients

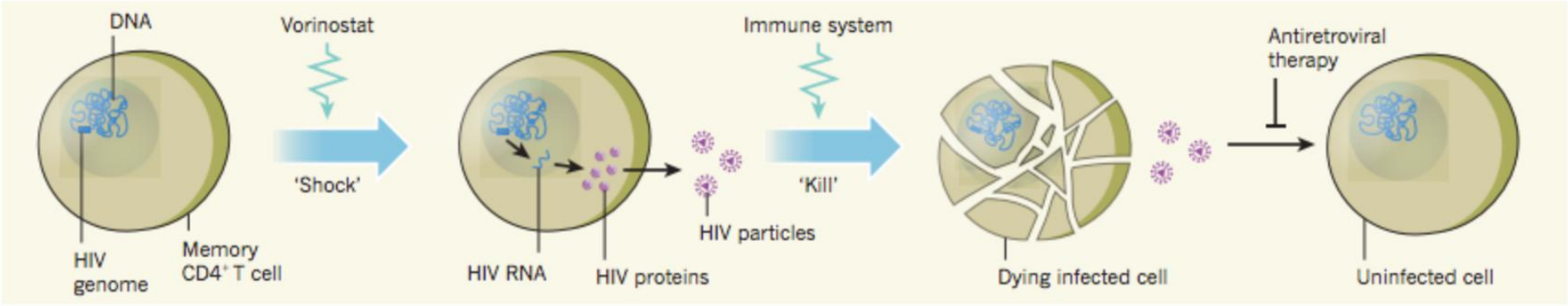
Early ART is Associated with lower HIV DNA Molecular Diversity and lower Inflammation in CSF but Does Not Prevent the Establishment of Compartmentalized HIV DNA Populations (Oliveira MF, PLOS Pathogens 2017)



Early ART was associated with lower level of IL-6 and TNF-alpha in CSF in comparison to late ART

CNS and eradication

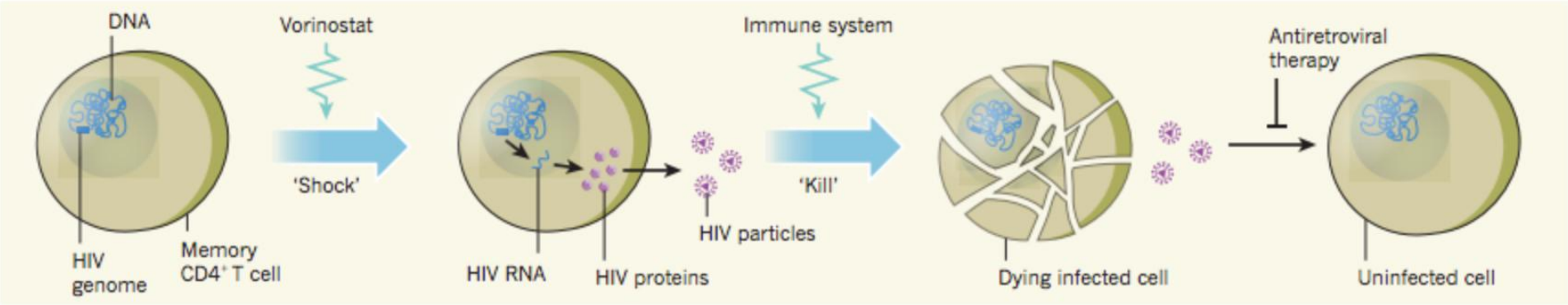
Latency reversing agents (LRA)



HIV: Shock and kill, SG Deeks, Nature 487, 439–440 (26 July 2012)

- Histone deacetylase inhibitors (HDACi, e.g., varinostat)
- Bromodomain inhibitors
- Protein kinase C agonists
- Cytokines, such as IL-2 and IL-15
- Others…

Latency reversing agents (LRA)



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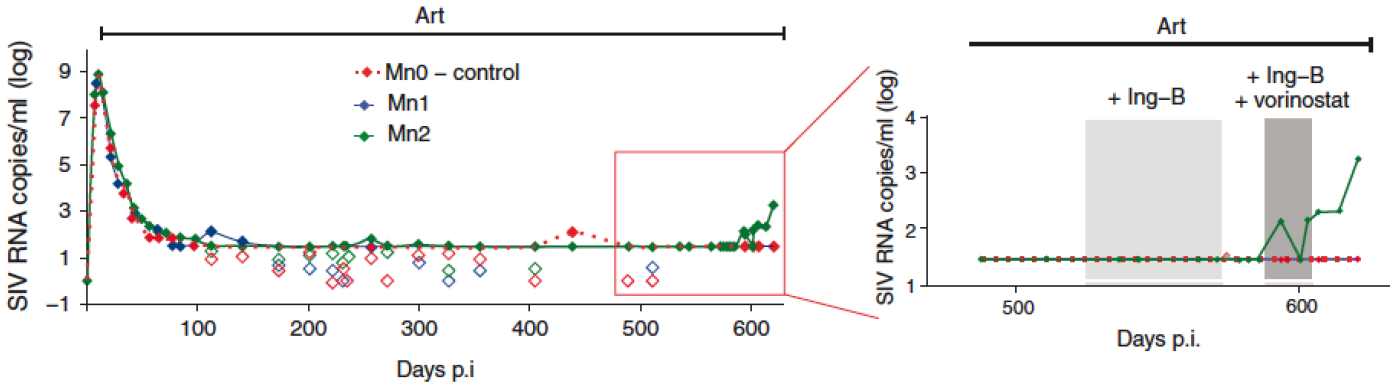
Initial trials of HIV eradication examine only viral load in peripheral blood as an indication of HIV reactivation or change in the latent reservoir,

→ But most latent HIV-1 genomes are in tissues and may respond differently to LRA

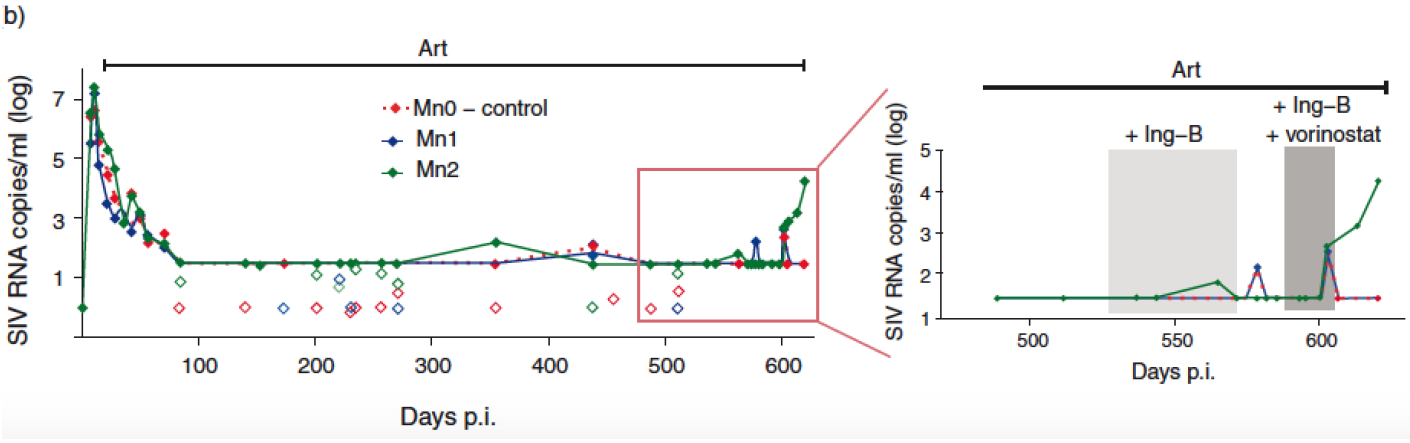
Reactivation of SIV reservoirs in the brain of virally suppressed macaques following administration of latency reversing agents (Gama L et al., AIDS 2017)

- 3 SIV-infected pigtailed macaques ART-treated since 12 days p.i.
- Macaque Mn0 (red): control
- Macaques Mn1 (blue) and Mn2 (green) treated with ingenol-B starting at 530 days p.i. with ingenol-B and ingenol-B plus vorinostat

Plasma



CSF



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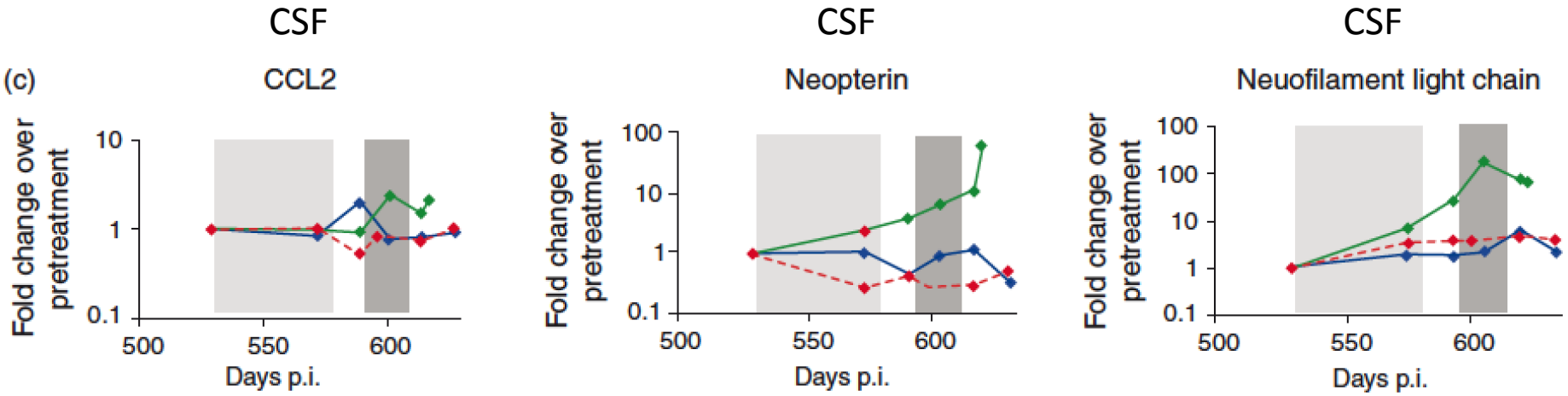
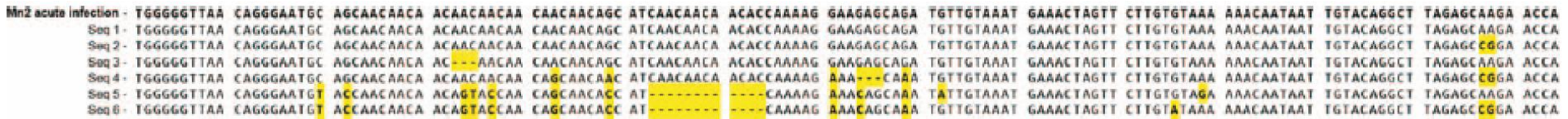


Reactivation of SIV reservoirs in the brain of virally suppressed macaques following administration of latency reversing agents (Gama L et al., AIDS 2017)

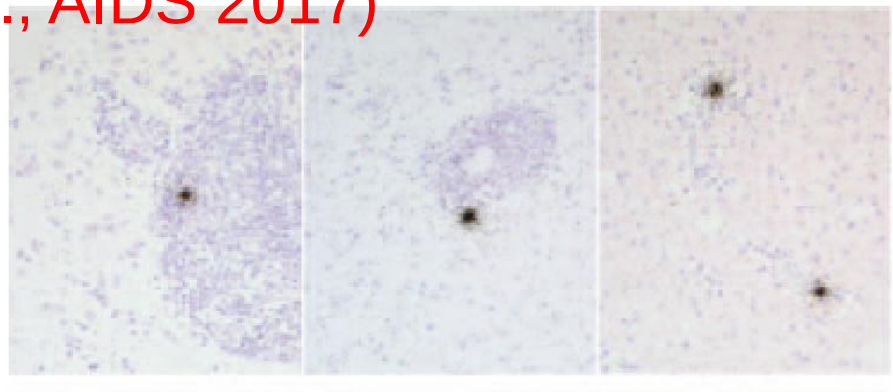
→ Unique SIV variant in CSF of macaque Mn2



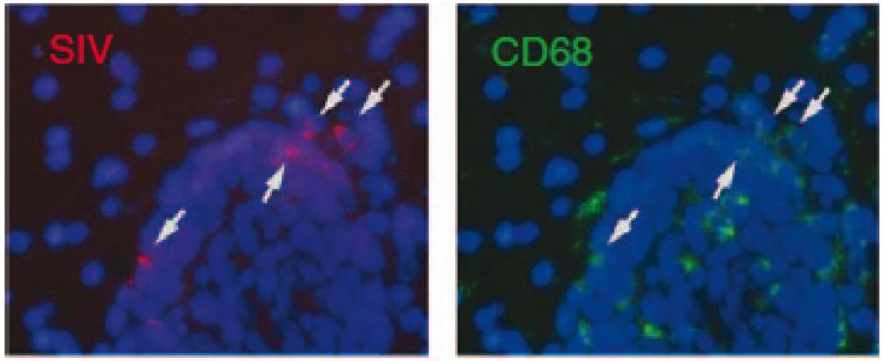
		Seq1	Seq2	Seq3	Seq4	Seq5	Seq6
	CSF	29	–	2	–	69	69
	Plasma	87	2	11	–	–	–
	PBMC	81	–	–	–	–	19
	OC	81	–	–	–	–	19
Brain	BG	86	–	14	–	–	–
	PC	91	–	9	–	–	–
	Spleen	100	–	–	–	–	–
	Liver	100	–	–	–	–	–
	Lung	90	–	10	–	–	–
	Kidney	80	–	12	8	–	–
	ALN	75	–	13	–	–	12
	BLN	77	–	11	–	–	12
	CLN	90	–	–	10	–	–
	RLN	34	–	–	66	–	–
	SLN	100	–	–	–	–	–

→ The most abundant SIV genotype in CSF was unique and expanded independent from viruses found in the periphery

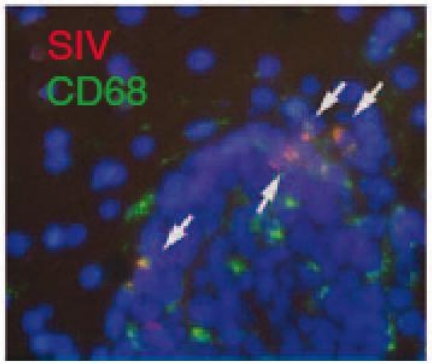
Reactivation of SIV reservoirs in the brain of virally suppressed macaques following administration of latency reversing agents (Gama L et al., AIDS 2017)



- Focal SIV RNA in the occipital cortex of macaques Mn2 (ISH)

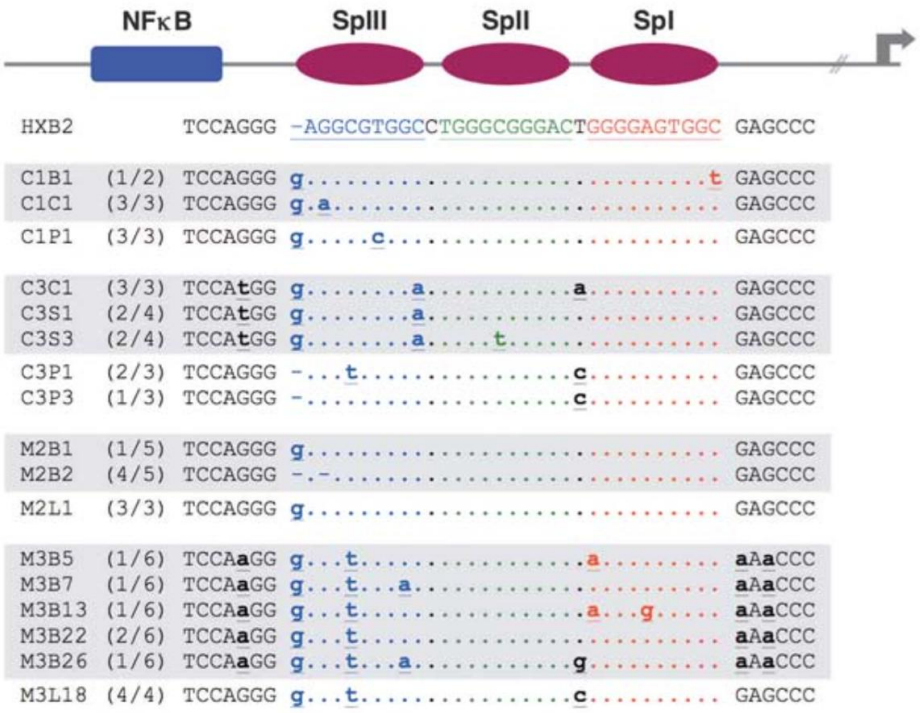


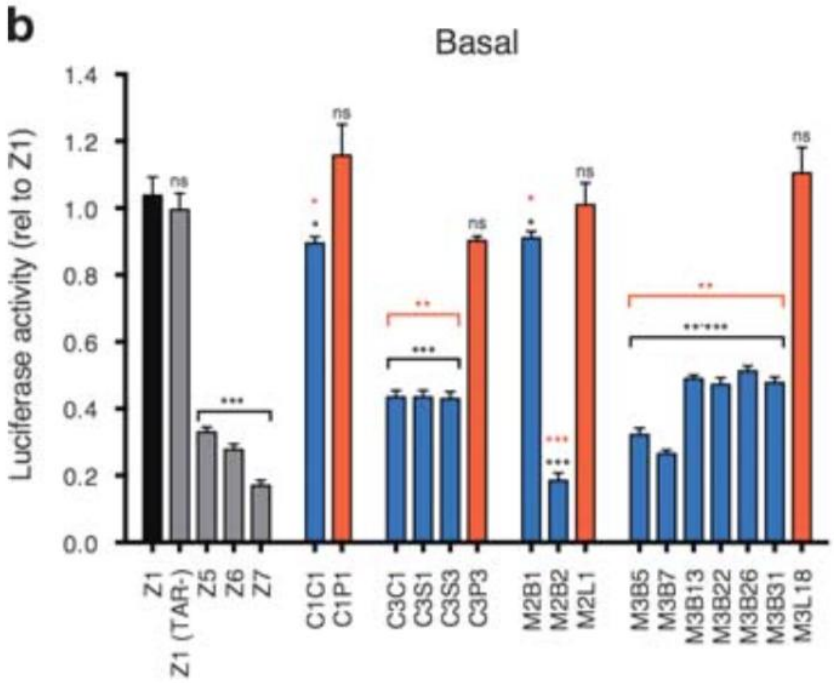
- Colocalization of SIV-RNA (ISH-red) and CD68 (IHC-green) in macaque Mn2



→ The CNS harbors latent SIV genomes after long-term suppression by ART, indicating that the brain represents a potential viral reservoir and should be seriously considered during AIDS cure

CNS-specific regulatory elements in brain-derived HIV-1 strains affect responses to latency-reversing agents with implications for cure strategies (LR Grey, Molecular Psychiatry, 2016)

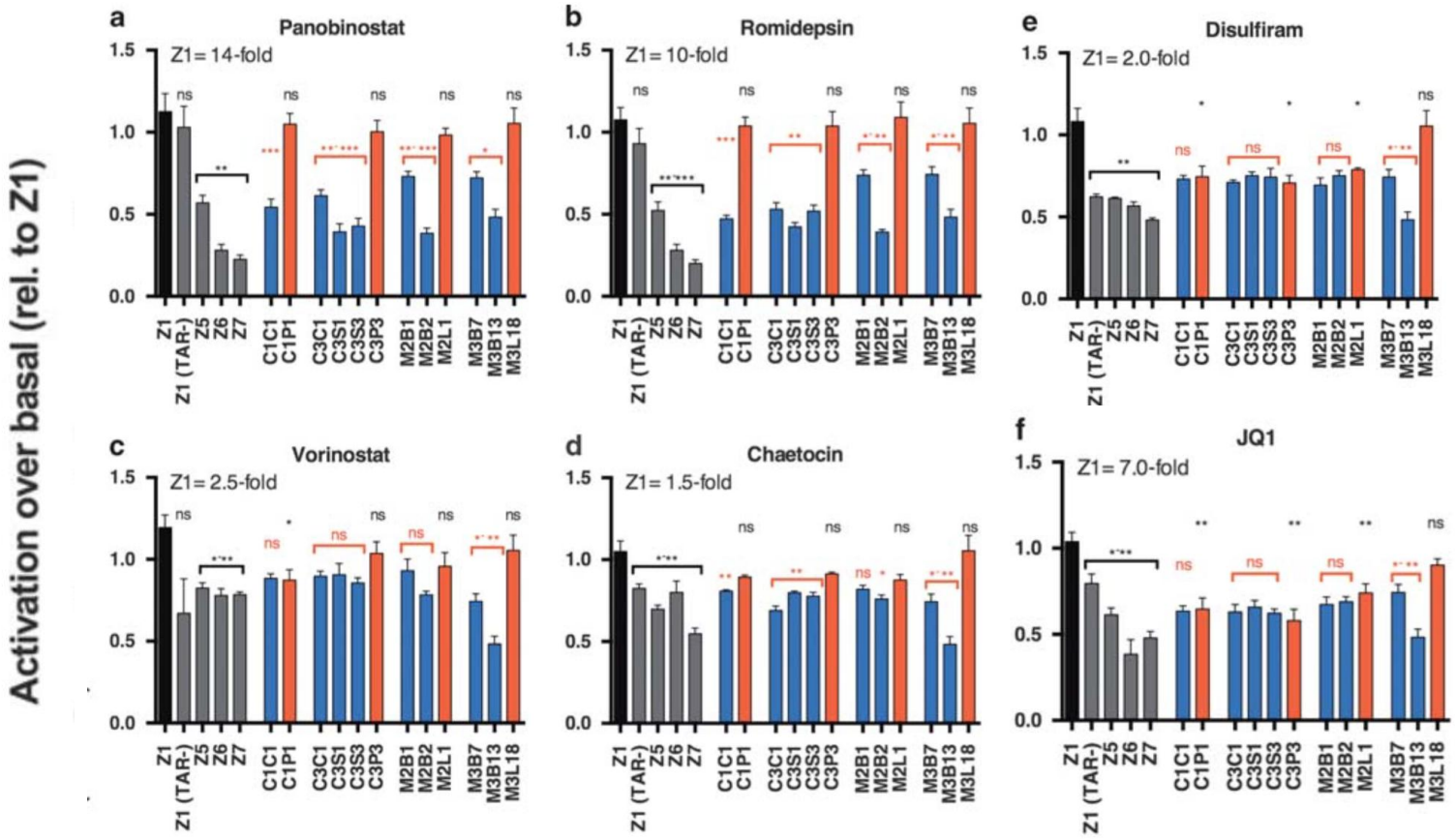




CNS-derived HIV-1 strains (grey) have LTR polymorphisms within and surrounding the Sp transcription factor motifs

LTR polymorphisms result in decreased binding to Sp1 and reduced transcriptional activity of CNS-derived HIV (orange) compared with lymphoid-derived LTRs (blue)

CNS-specific regulatory elements in brain-derived HIV-1 strains affect responses to latency-reversing agents with implications for cure strategies (LR Grey, Molecular Psychiatry, 2016)



CNS-derived viruses are less responsive to activation by the HDACi panobinostat and romidepsin compared with lymphoid-derived viruses.

→ CNS strains have unique transcriptional regulatory mechanisms, which impact the latency regulation

Conclusions

- CNS still relevant as target organ
- Additional evidence for CNS as tissue reservoir, which may have implications for HIV eradication