



UNIVERSITÀ
DI TORINO



Focus on neuropsychiatric disturbances in people living with HIV **What CSF can tell us?**

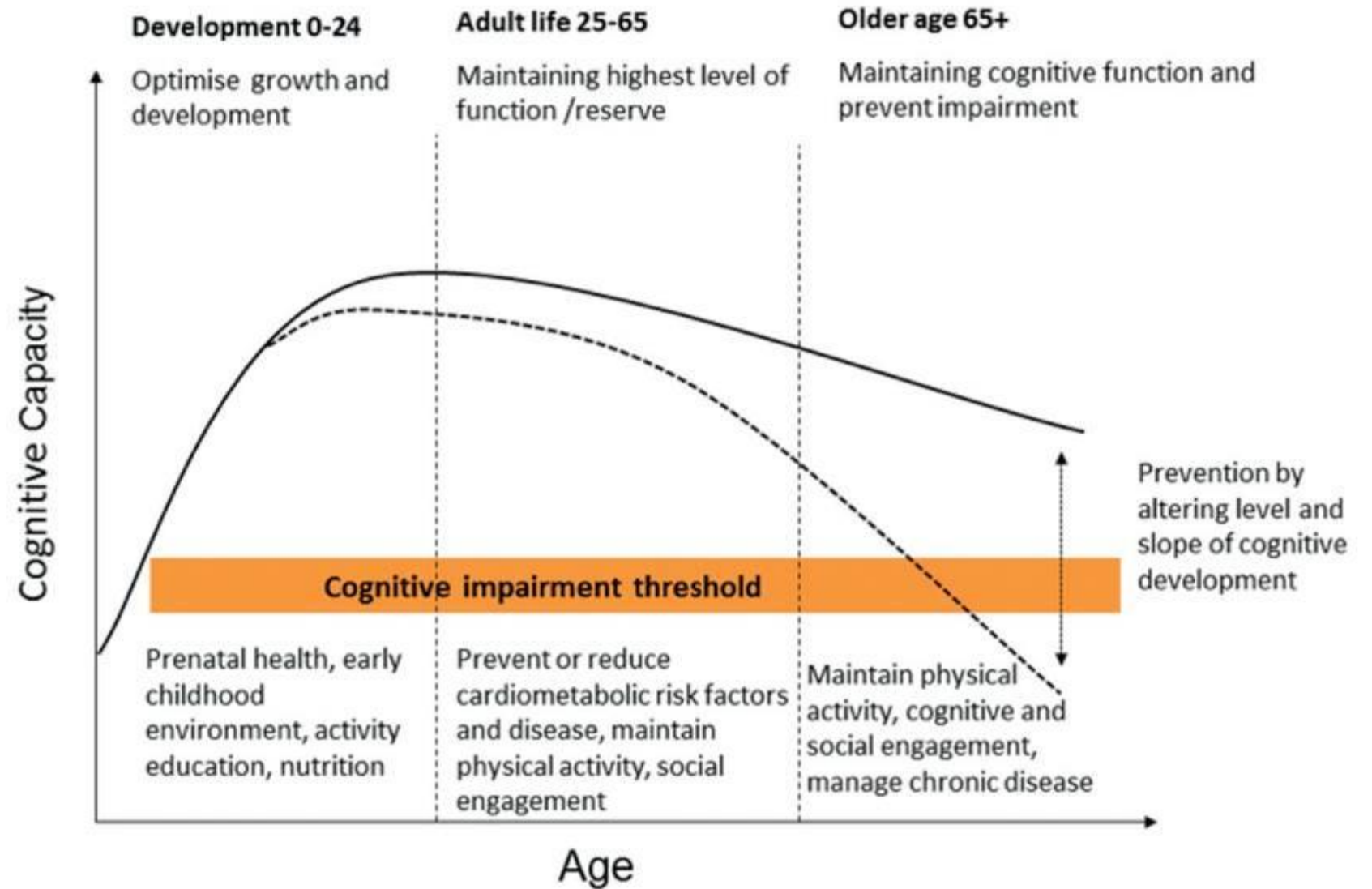
Andrea Calcagno
Università di Torino

DISCLOSURES

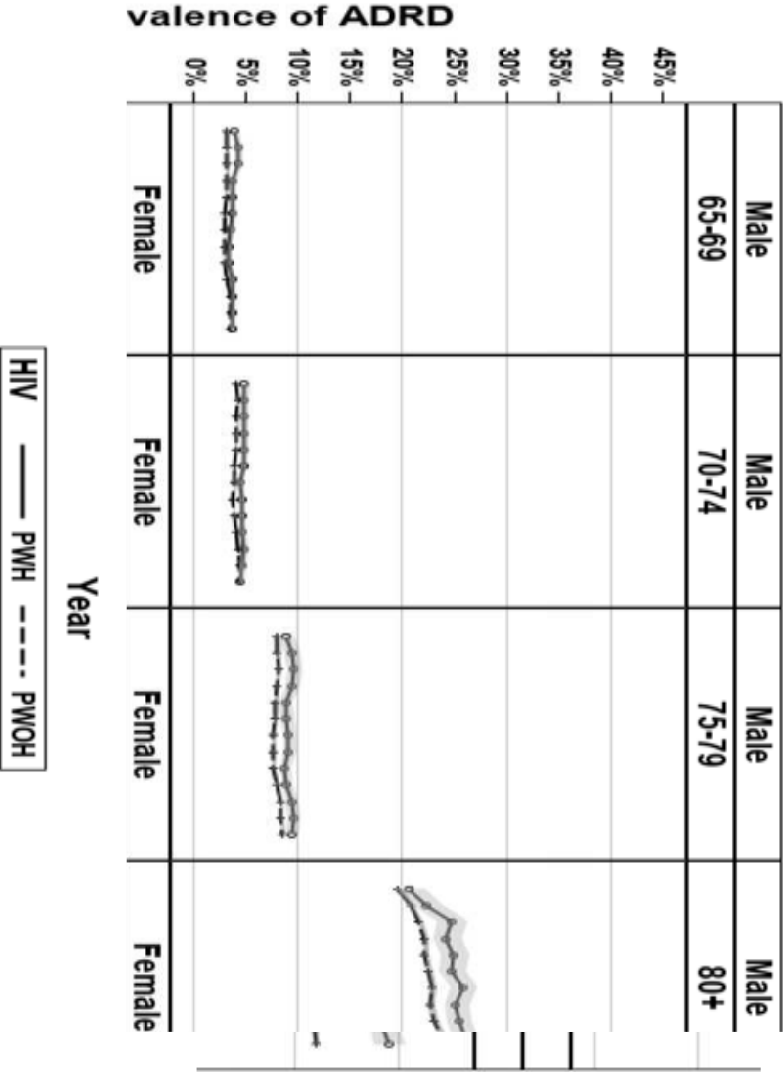
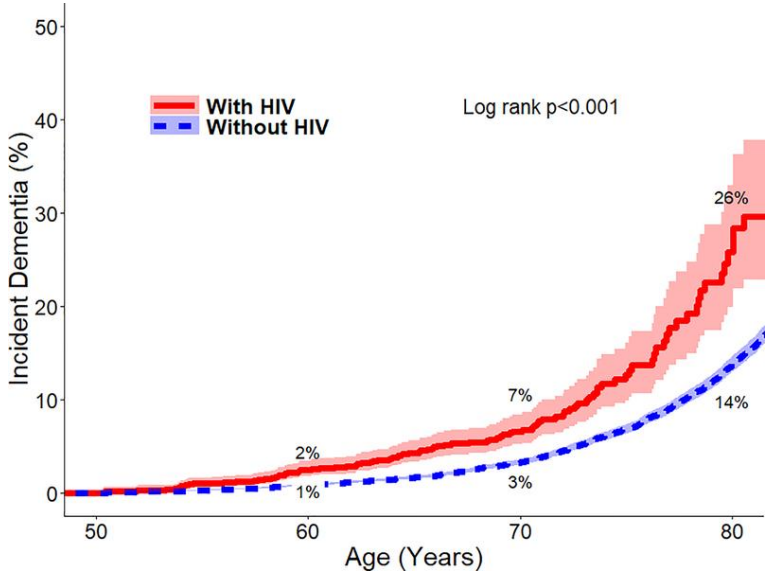
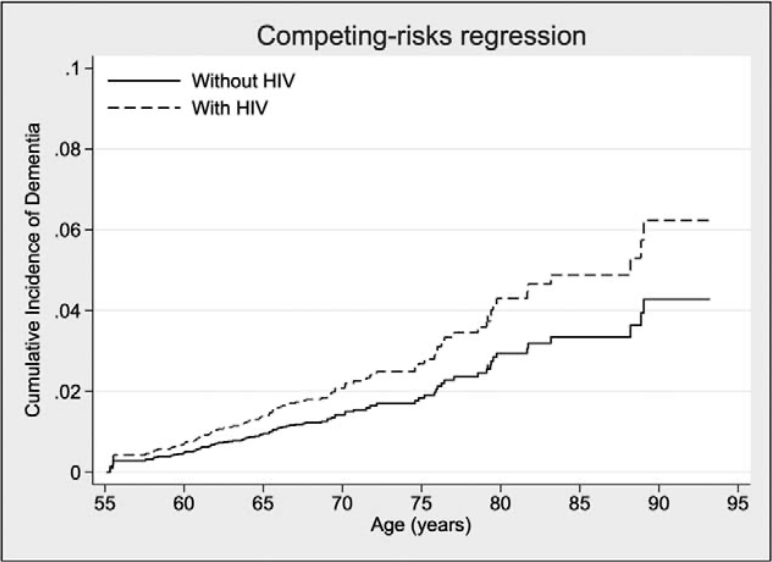
I have read and understood ICMJE policy on declaration of interest
and I declare that in the past five years

- My institution has received research grants from AbbVie, Gilead Sciences, Bristol Myers Squibb, Janssen-Cilag and ViiV Healthcare
- I received speaker and consultancy honoraria from Gilead Sciences, Insmed, Janssen-Cilag, MSD, Roche, Sobi and ViiV Healthcare

Cognitive trajectory



The incidence of dementia in OPLWH



What CSF can/can't tell us

Can demonstrate

Can't measure

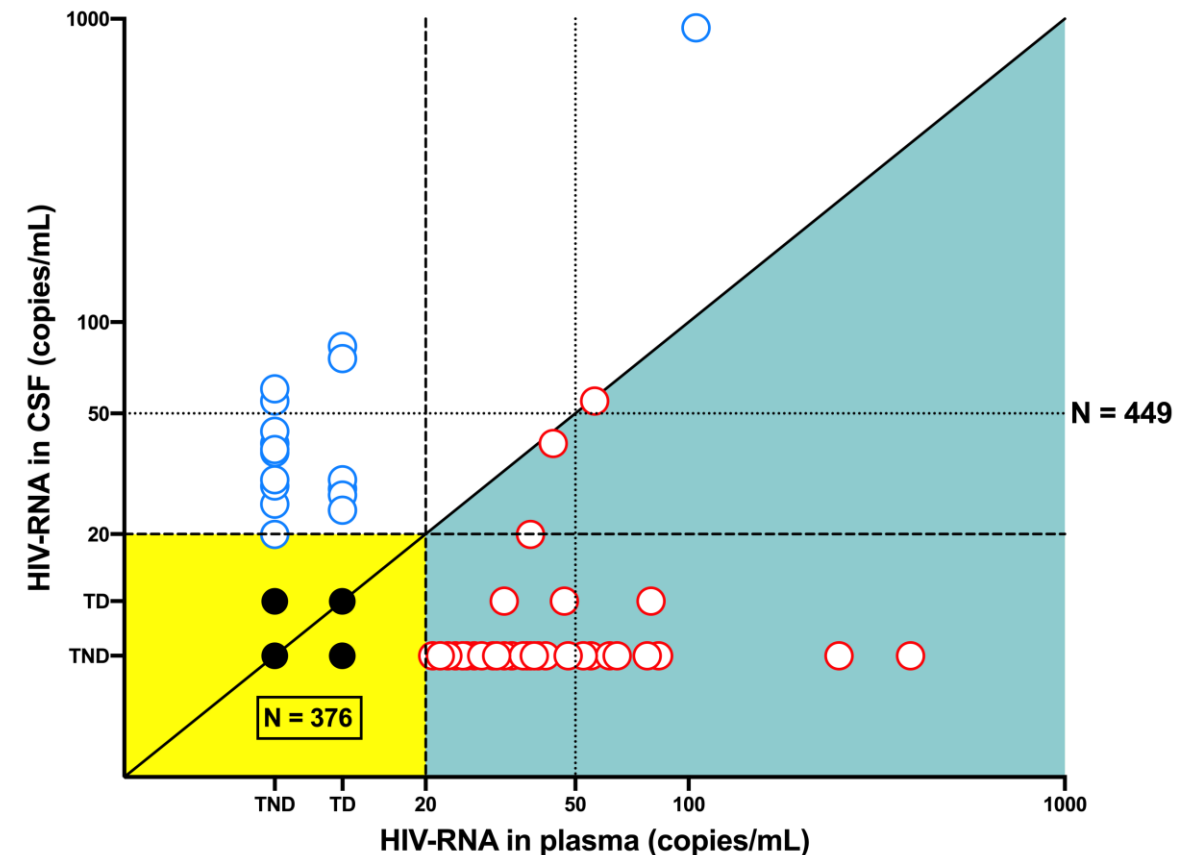
- HIV replication in the CSF

Symptomatic CSF escape with INSTIs

Drug	Symptoms	PI HIV RNA copies/mL	CSF HIV RNA copies/mL	CSF RAMs to INSTI	Ref
RAL + DRV/r + EFV + TDF/FTC +/- ENF	Asthenia, skin hyperesthesia, loss of memory and psychomotor slowing	50 - <50	340 - 3400	Q148R	Watanabe 2010
RAL qd + DRV/r	Headaches, fever and meningismus	<50	1438	N155H	Mora-Peris 2013
RAL + LPV/r	-	51	13K	N155H	Dravid 2018
RAL + PI + NRTIs	Neurological symptoms and convulsions	10.8 K	1.2 M	-	Bang 2022
EVG + TAF/FTC?	Progressive cognitive and gait decline	980	-	EVG	Muir 2021
DTG + DRV/r + MVC bid	Cerebellar symptoms	<40	200	R263K	Vassallo 2016
BIC/FTC/TAF	Lethargy, generalized tonic-clonic seizures	33	34.9 K	-	Al-Chalabi 2022

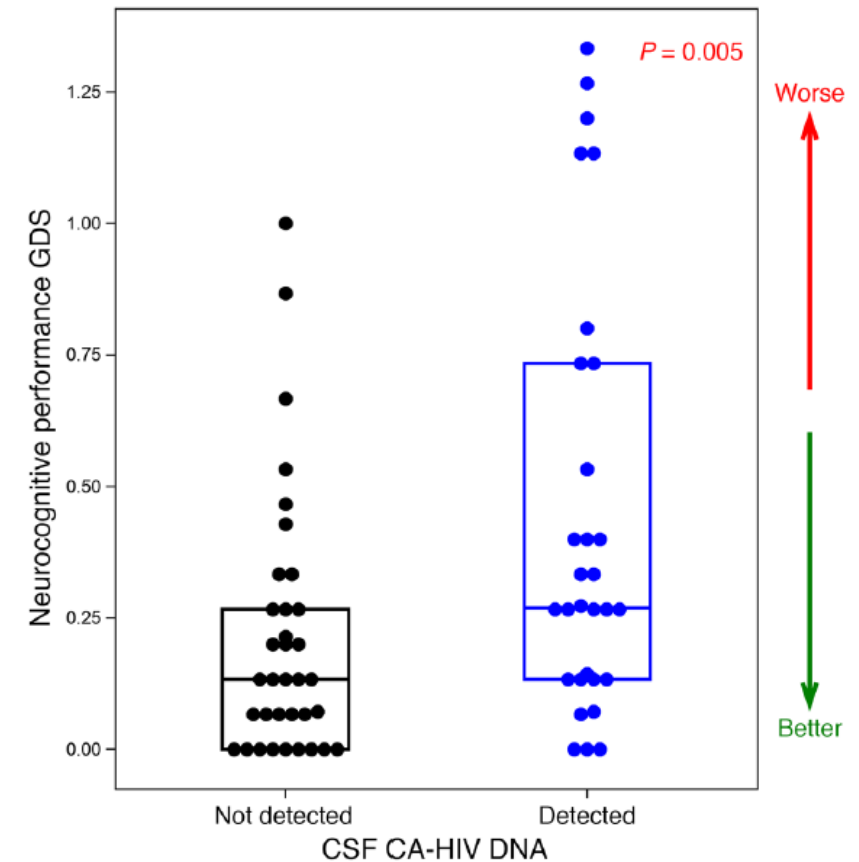
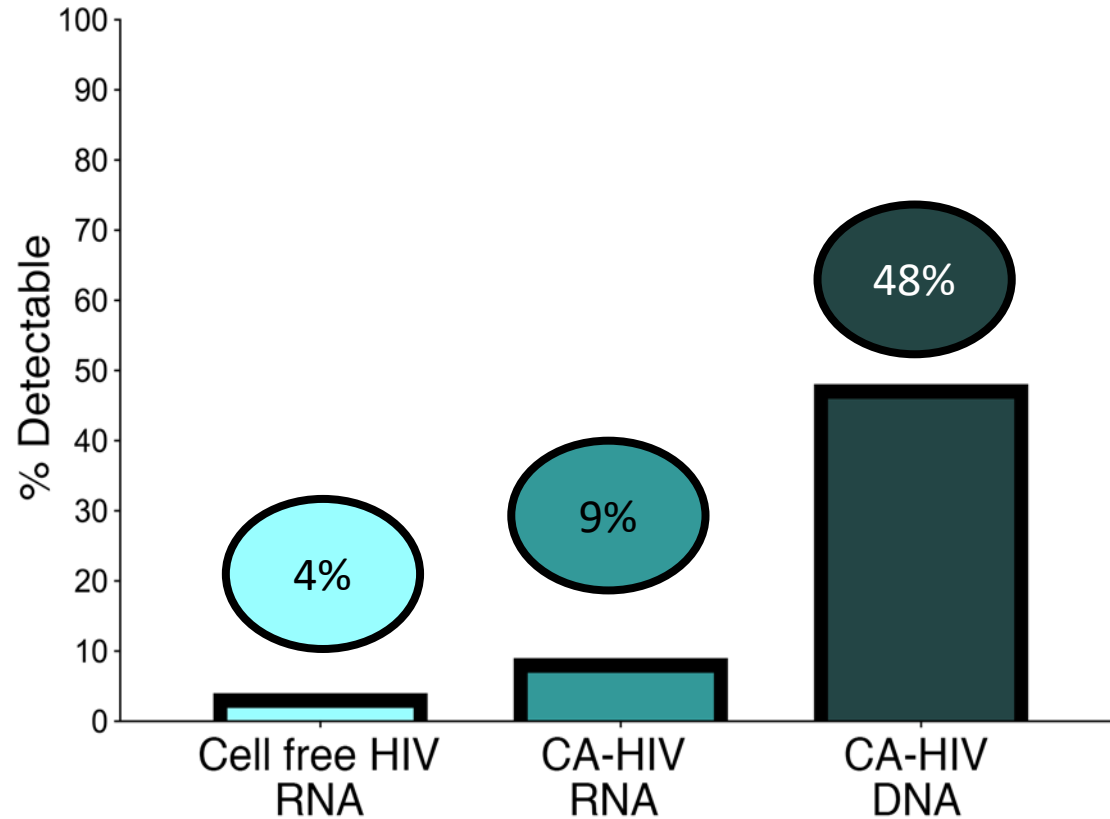
Asymptomatic CSF HIV-1 Escape: Incidence and Effects On CNS Biomarkers

- 449 samples from 173 PLWH
- CSF-E 8.1% with a median CSF HIV RNA of 30 (25–55) copies/mL.
 - Out of 449 samples, CSF-E was present in 19 (4%)
 - One individual had ≥ 2 consecutive samples with CSF-E but remained clinically stable
- Samples with CSF-E exhibited:
 - a 25% higher geometric mean for CSF neopterin compared to those without CSF-E ($P = 0.01$)
 - an 8% higher geometric mean albumin ratio ($P = 0.04$).
 - the odds ratio for detectable CSF WBC ($\geq 3 \times 10^6$ cells/L) with CSF-E was 3.9 ($P = 0.004$)
 - no significant differences in CSF NfL levels



Persistent infected cells in the CSF

Spudich S, et al. JCI 2019



Detection of CA-HIV DNA in CSF cells did not significantly associate with pre-ART CD4 count , HIV RNA level , CD4/CD8 ratio, or years on ART

What CSF can/can't tell us

Can demonstrate

- HIV replication in the CSF
- RAMs in the CSF

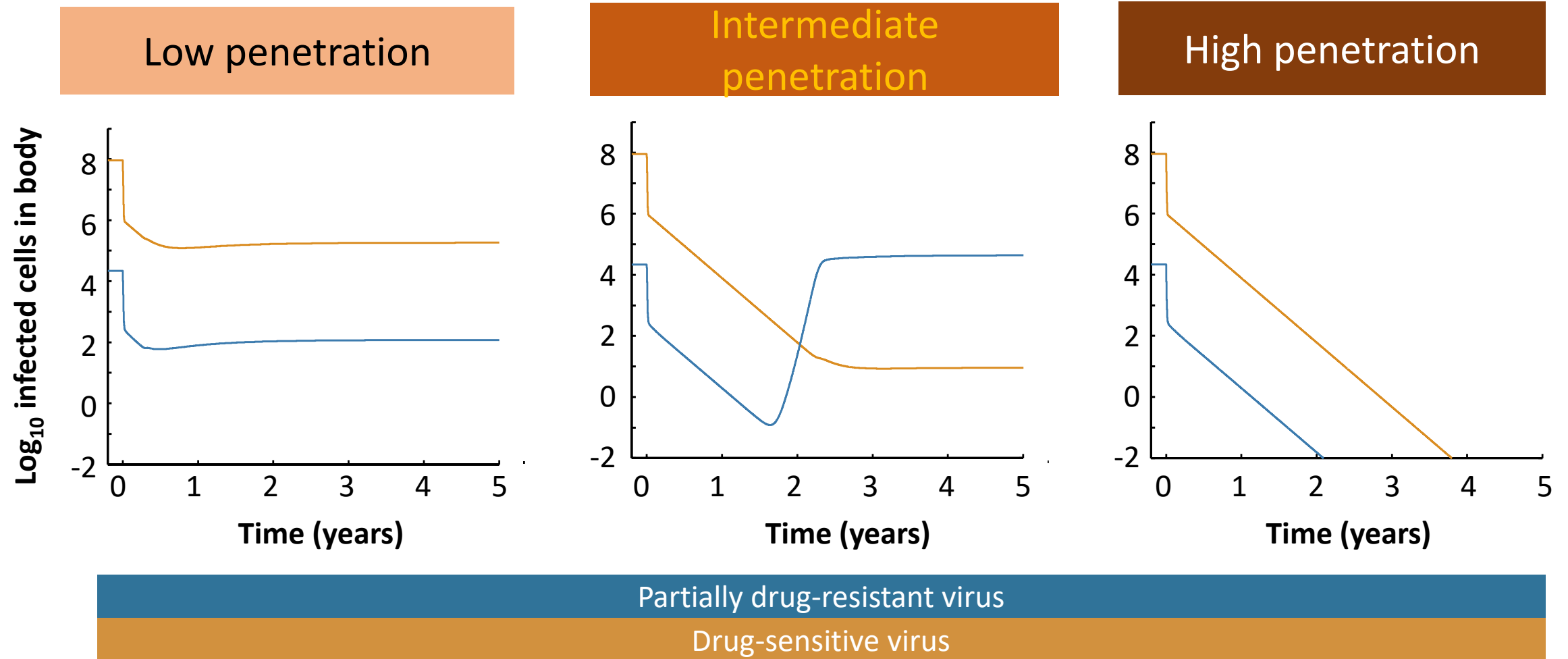
Can't measure

Resistance-associated mutations in the CSF

CD4⁺-T-cell counts, plasma, and CSF HIV-1 viral loads in individuals with CSF/Plasma HIV-1 RNA discordance.

Case ID	Current ART regimen	CD4 ⁺ count, cells/mm ³		Plasma VL at event, copies/mL	CSF VL at event, copies/mL	CSF HIV-1 resistance associated mutations			
		Nadir	At event			NRTI	NNRTI	PI	Integrase
1	TDF/FTC/NVP	83	108	<20	12,000	Not done	Not done		
2	TDF/FTC/ATV/r	32	187	210	16,000	Not done	Not done		
3	TDF/FTC/ATV/r	78	668	400	4600	M41L, L74V, M184V, T215Y	A98G, K103N, K238T	No	No
4	TDF/FTC/ATV/r	70	294	<20	35,200	Not done	Not done		
5	ABC/3TC/LPV/r	108	510	110	3100	D67N,K70R,L74I, M184V,T215V, K219Q	K101E, V108I, Y181C	V82A	No
6	TDF/3TC/ATV/r	8	521	240	3200	M41L, D67N, T69N, K70R, M184V, T215V, K219E	K101E Y181C, H221Y	I50L	No
7	TDF/FTC/ATV/r	132	509	890	8820	Not done	Not done		
8	TDF/3TC/ATV/r	65	562	500	5200	Not done	Not done		
9	LPV/r/Raltegravir	145	327	<20	120	Could not be amplified	Could not be amplified		
10	AZT/TDF/3TC/ATV/r	56	313	130	4440	D67N, T69N, K70R, V75I, F77L, Q151M, M184V, T215V, K219E	K101E, V108I, Y181C, H221Y	I50L	No
11	TDF/FTC/ATV/r	28	153	720	4800	D67I,T69N,K70R, V75M,M184V, T215E, K219E	K103S,G190A	M46I	
12	TDF/FTC/ATV/r	45	213	<20	900	M184I	K103N	No	No
13	TDF/FTC/LPV/r	22	148	310	3500	Not done	Not done		
14	AZT/TDF/FTC/ATV/r	168	496	590	34000	Not done	Not done	No	No
15	TDF/3TC/ATV/r	178	419	<20	150	Not done	Not done		
16	LPV/r/Raltegravir	16	413	51	13000	M41L,T215Y,M184V	K103N,P225H, K238T	V82A	N155H
17	TDF/FTC/ATV/r	127	210	47	380	K65R,M184V	V179D,Y181C, Y188L	No	No
18	LPV/r/Raltegravir	113	377	<20	78	Not done	Not done		
19	TDF/3TC/ATV/r	85	441	89	3500	Could not be amplified	Could not be amplified		
20	TDF/3TC/ATV/r	27	197	290	4100	M41L,D67N,M184V, T215Y,K219Q	K103N,Y181C, H221Y	No	No

How to select RAMs in a compartment?



What CSF can/can't tell us

Can demonstrate

- HIV replication in the CSF
- RAMs in the CSF
- The presence of other microorganisms

Can't measure

Latent infections and neuro inflammation and cognition in PWH



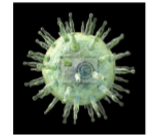
Syphilis

—————→ NCI → Marra C, et al. Int J STD AIDS 2013



Toxoplasma

—————→ NCI → Ene L, et al. J Neuroimmunol 2016; Bharti AR, et al. CID 2016; Escobar-Guevara EE, et al. JNV 2019



EBV

—————→ Neuroinflammation → Lupia T, et al. AIDS 2020
Neurocognition → Trunfio, M, et al. AIDS 2024



Cytomegalovirus

CMV

—————→ HANAs → Lichtner M, et al. JID 2015; Gianella S, et al. AIDS 2020
NCI → Letendre S, et al. CID 2018; Ballegaard V, et al. JAIDS 2018



HSV-1

—————→ Neuronal damage → Trunfio M, et al. JNV 2023



SARS-CoV-2

—————→ PCS in PLWH → Peluso MJ, et al. JCI 2023

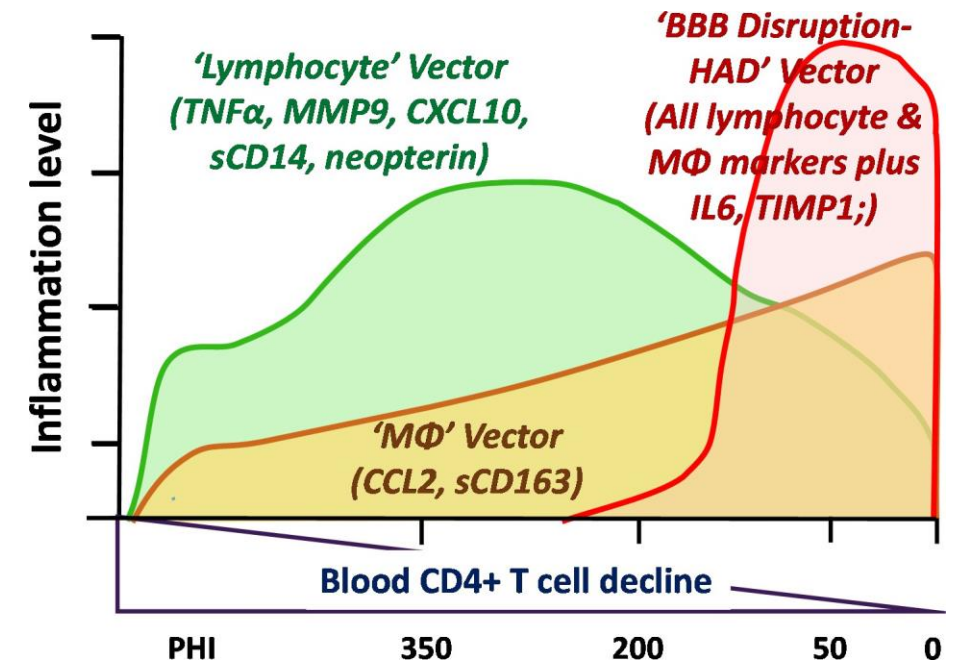
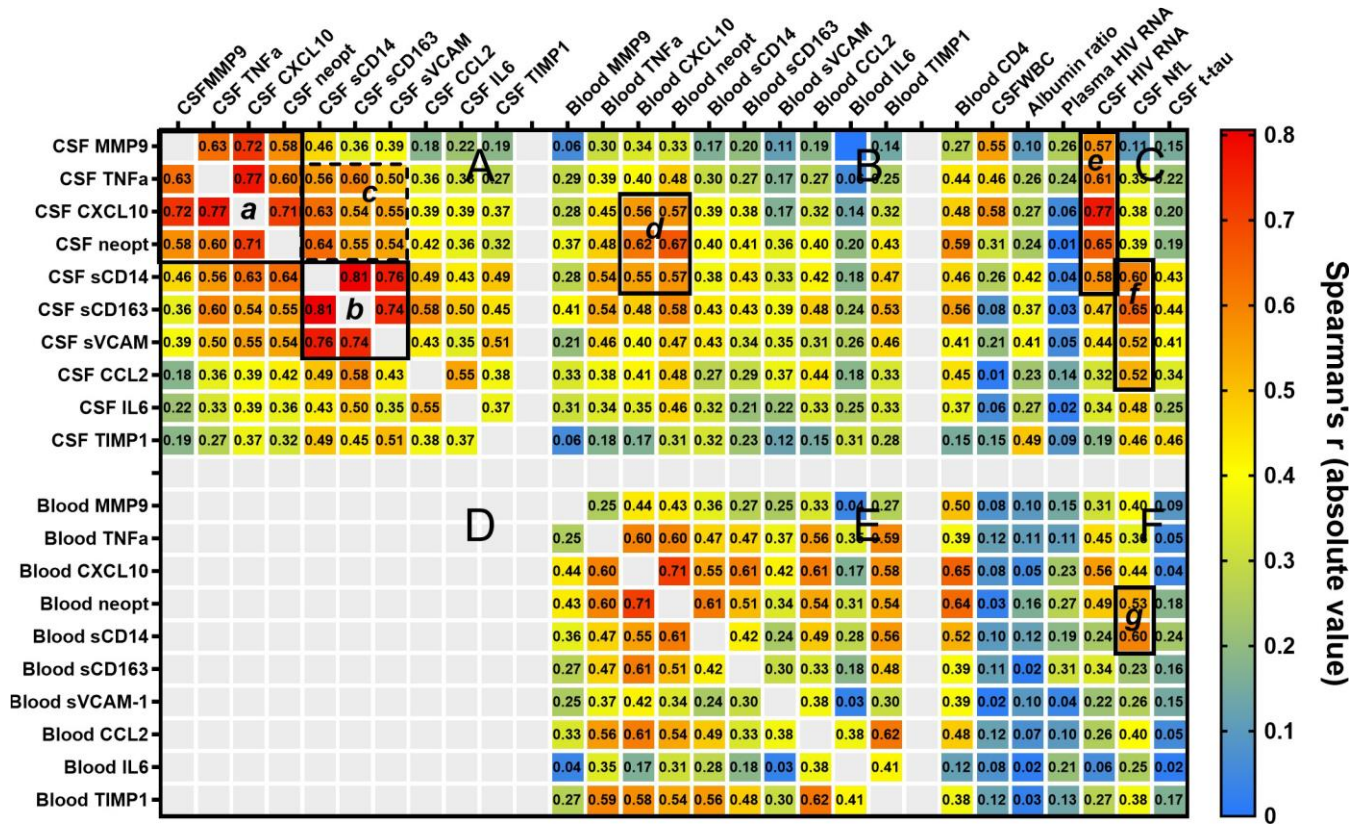
What CSF can/can't tell us

Can demonstrate

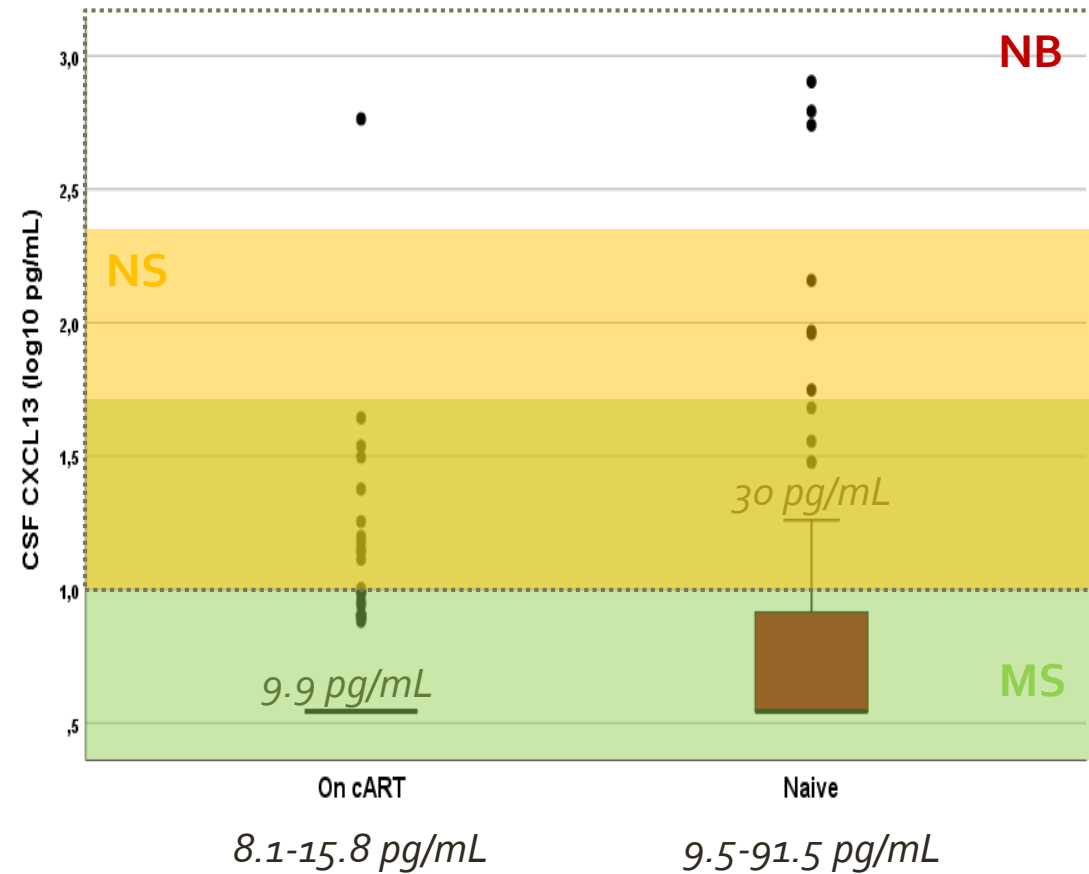
- HIV replication in the CSF
- RAMs in the CSF
- The presence of other microorganisms
- Intrathecal inflammation and immune activation

Can't measure

Biomarkers and phenotypes



CXCL₁₃ in the CSF



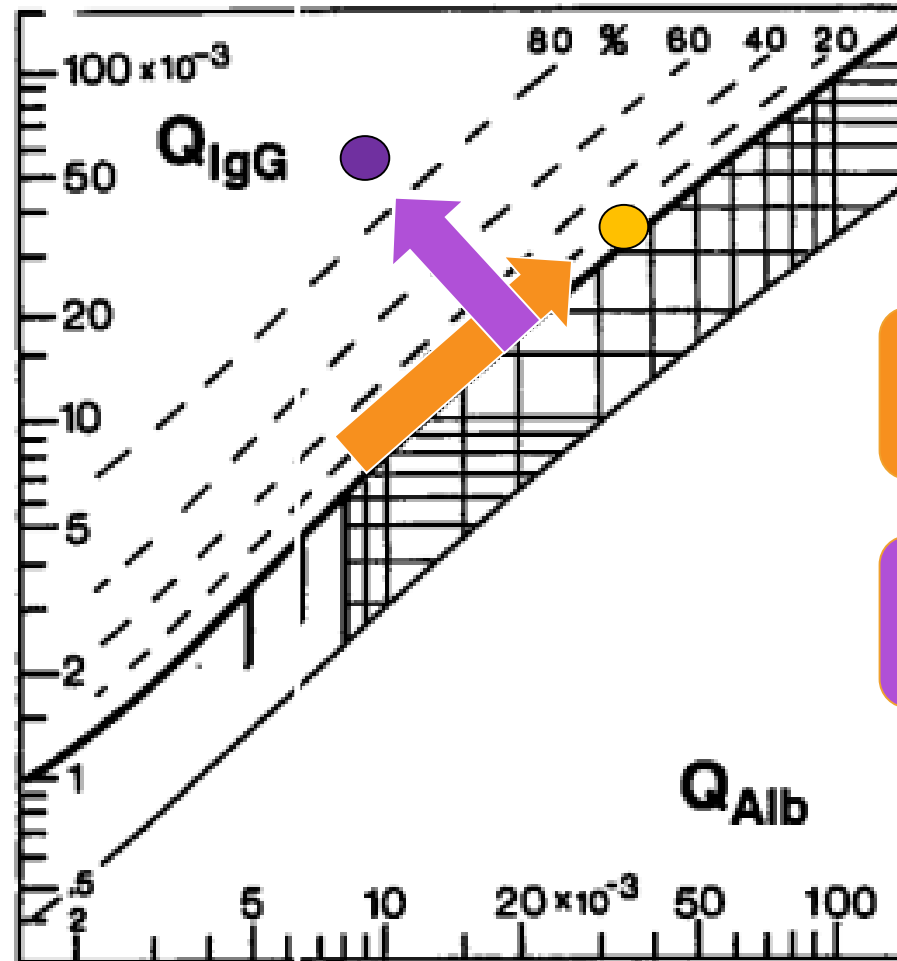
What CSF can/can't tell us

Can demonstrate

- HIV replication in the CSF
- RAMs in the CSF
- The presence of other microorganisms
- Intrathecal inflammation and immune activation
- Neuronal damage, abnormal proteins, BBB status

Can't measure

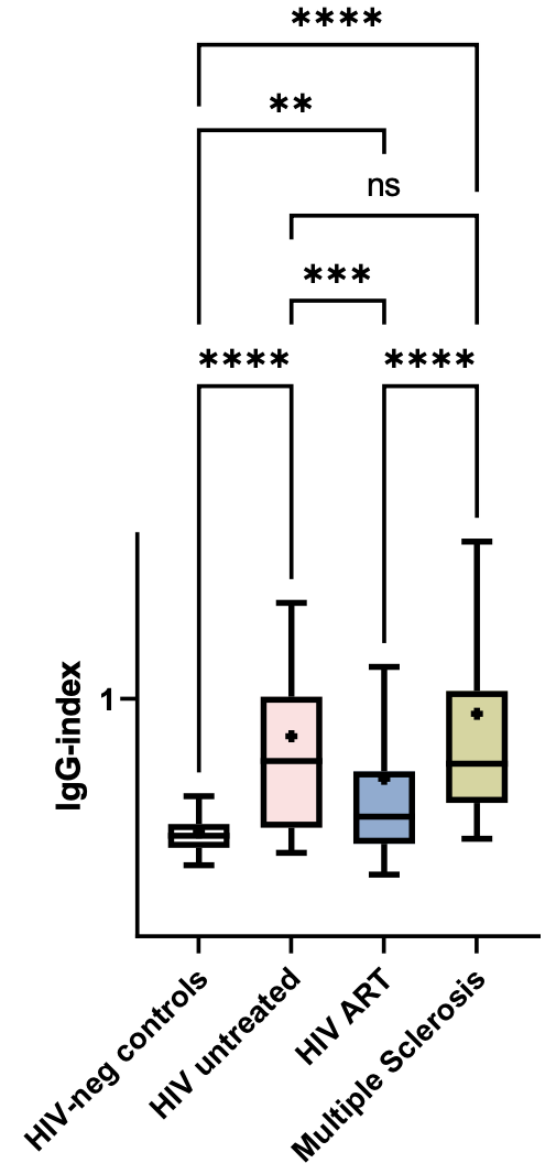
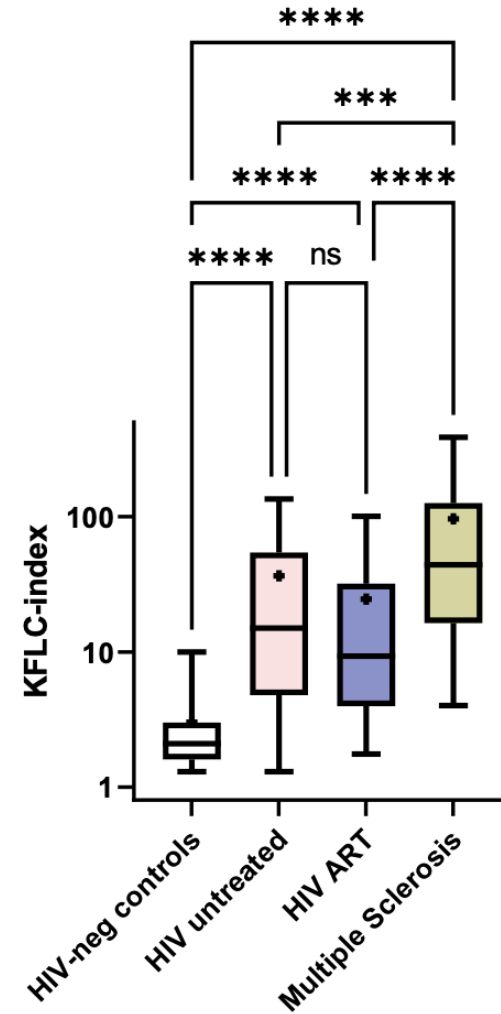
Reibergrams



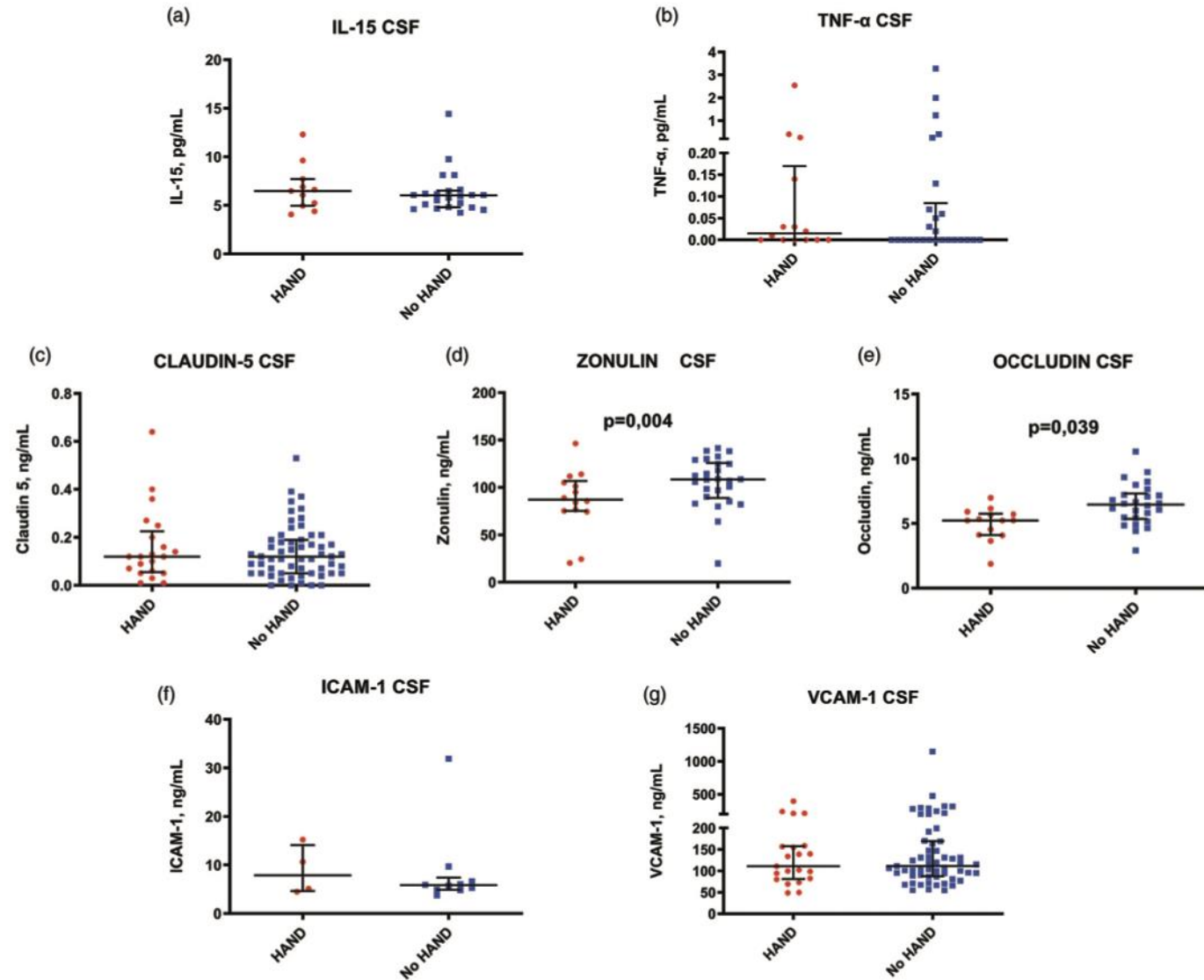
BBB IMPAIRMENT

INTRATHECAL
SYNTHESIS OF
IGGs

IgG index (and Kappa free light chain) in PLWH and patients with MS



Tight junctions and Cognition



What CSF can/can't tell us

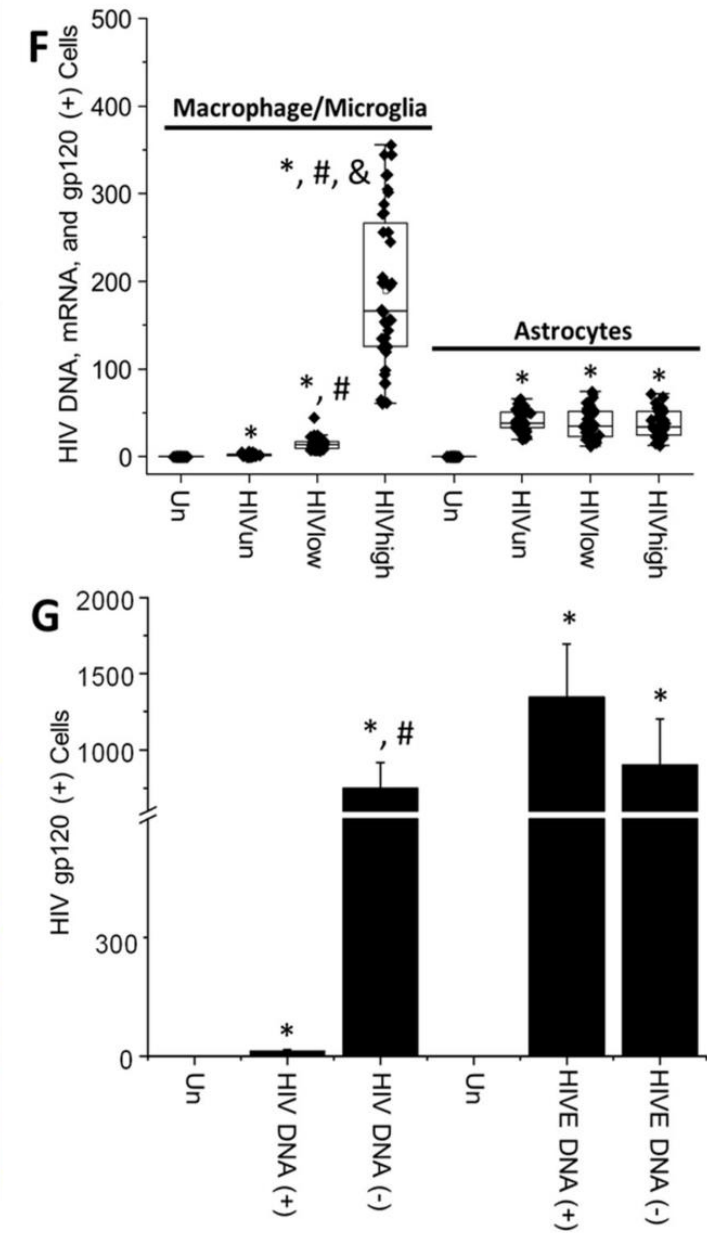
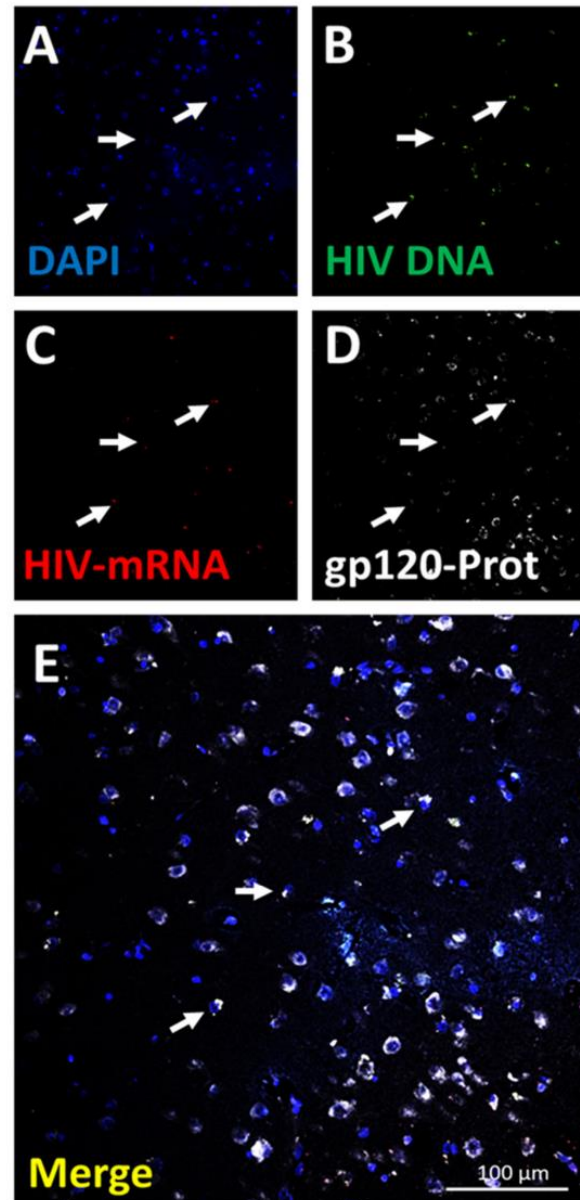
Can demonstrate

- HIV replication in the CSF
- RAMs in the CSF
- The presence of other microorganisms
- Intrathecal inflammation and immune activation
- Neuronal damage, abnormal proteins, BBB status

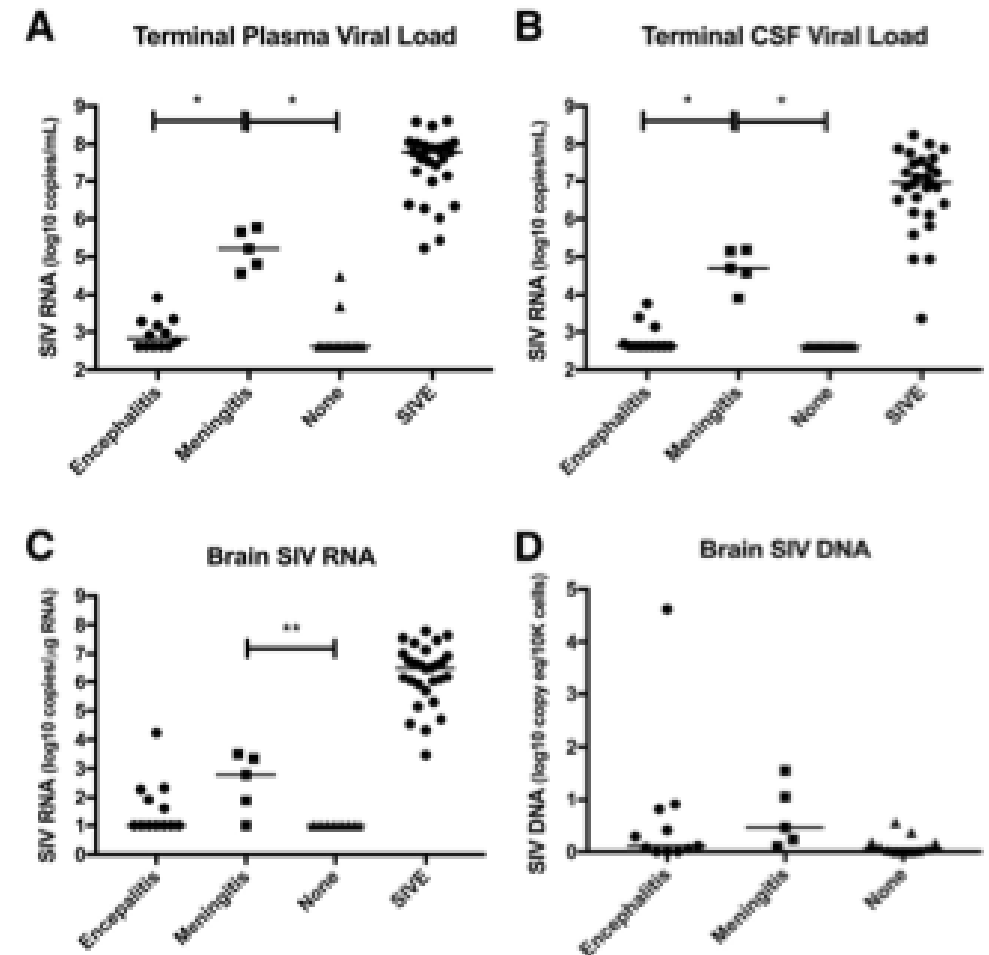
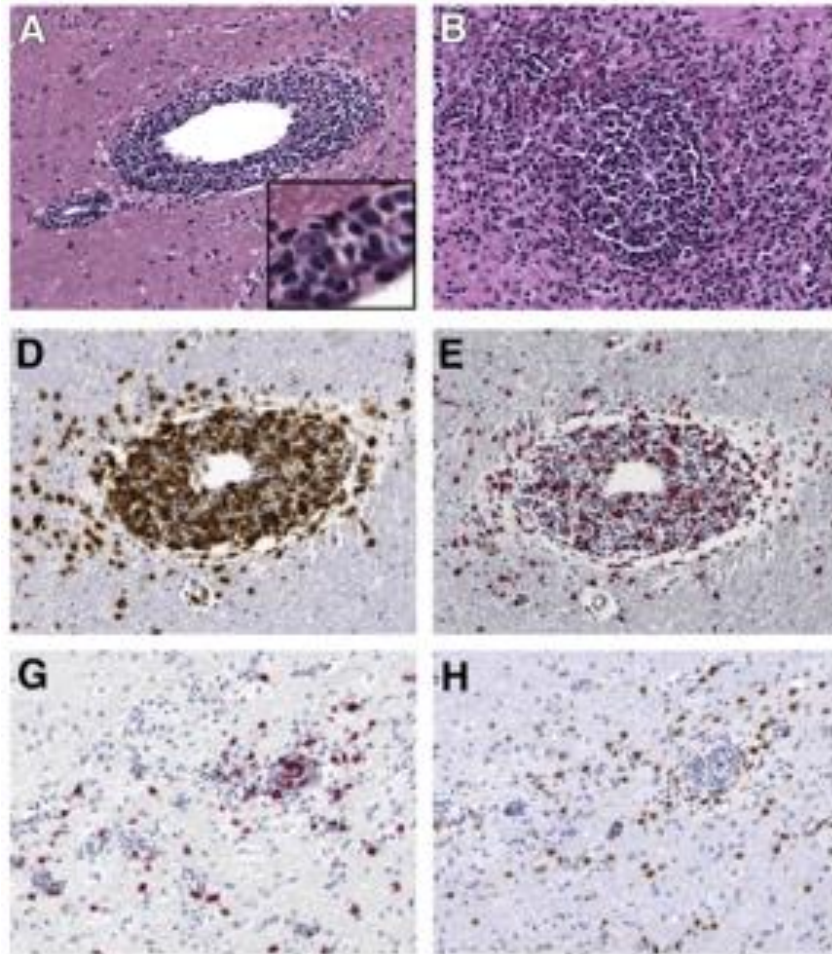
Can't measure

- HIV replication in the brain parenchyma

HIV reservoir in brain tissue



Lymphocyte-Dominant Encephalitis and Meningitis in SIV-Infected Macaques Receiving Antiretroviral Therapy



What CSF can/can't tell us

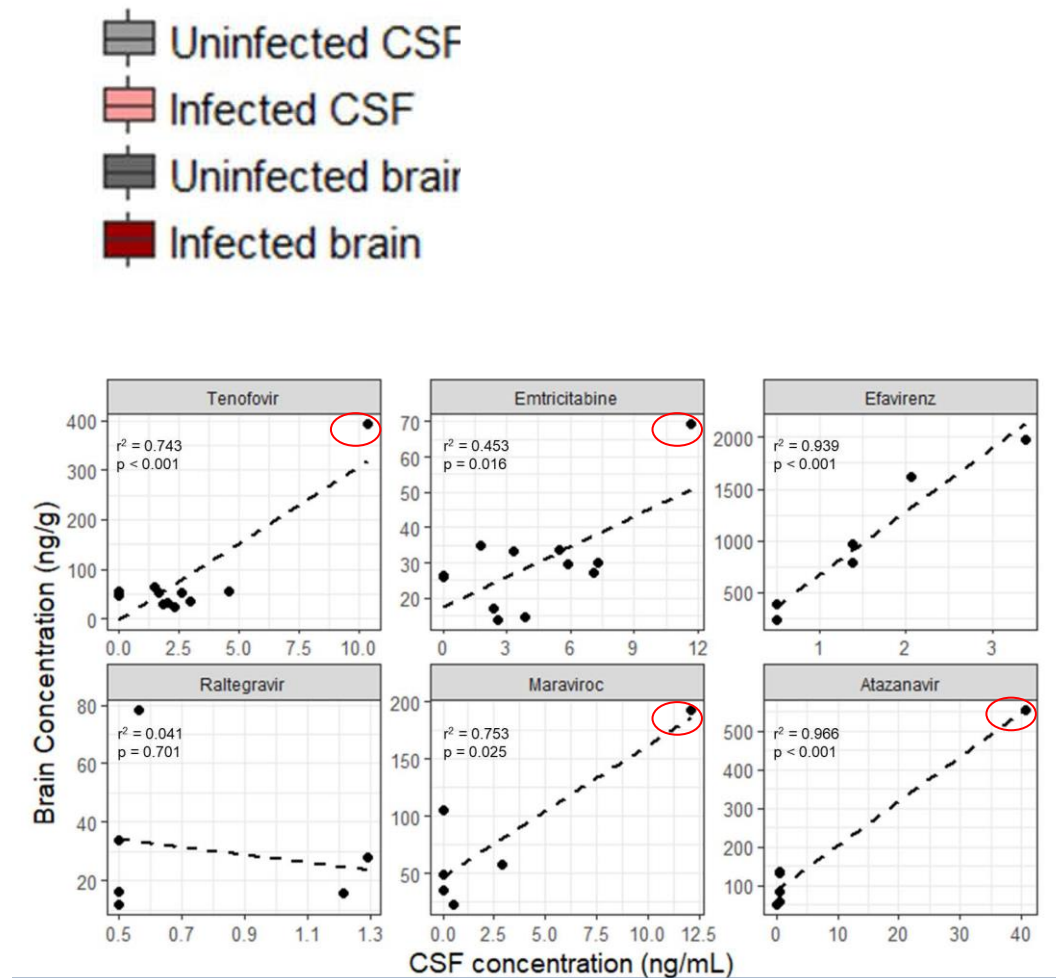
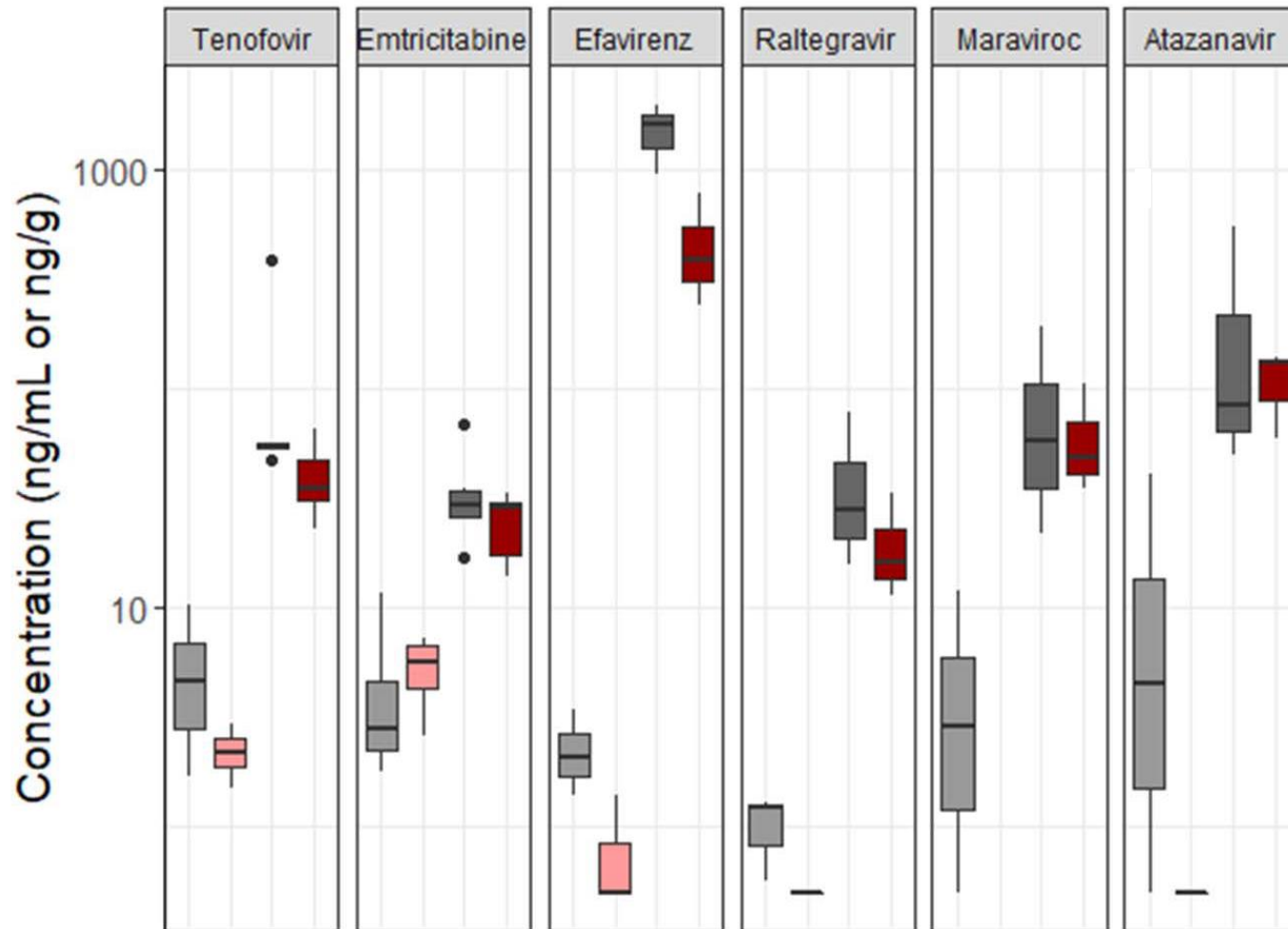
Can demonstrate

- HIV replication in the CSF
- RAMs in the CSF
- The presence of other microorganisms
- Intrathecal inflammation and immune activation
- Neuronal damage, abnormal proteins, BBB status

Can't measure

- HIV replication in the brain parenchyma
- PK of drugs in the the brain parenchyma

CSF and brain concentrations in macaques



“Last Gift” preliminary tissue PK results

Ferrara M, et al. CROI 2024 #608

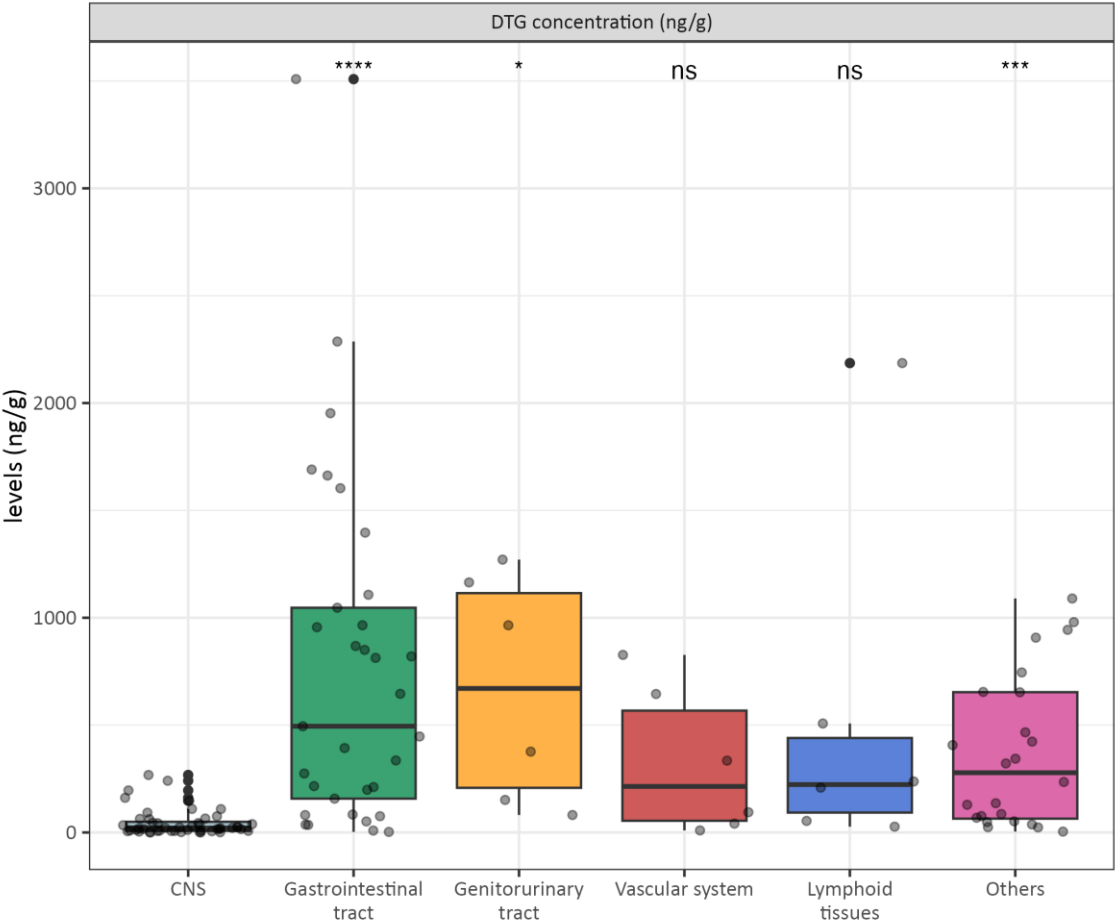


Table 2.DTG concentration (ng/g)					
Location	Drug measures				
	N Measures	Mean	Min	Max	Distribution
CNS					
Basal Ganglia	6	52.1	0.9	196.0	
Cerebellum	5	38.6	6.8	109.0	
Frontal Cortex (motor)	6	27.9	1.8	92.3	
Frontal Cortex (pre-motor)	6	24.4	0.1	75.5	
Hippocampus	6	61.3	0.1	267.8	
Medulla	3	68.5	20.0	161.5	
Occipital Cortex	6	63.7	1.0	240.9	
Parietal Cortex	5	45.2	1.7	147.7	
Pons	5	36.8	6.9	109.6	
Gastrointestinal tract					
Colon (left)	6	514.8	2.6	1046.7	
Colon (right)	4	843.3	81.1	1603.3	
Duodenum	6	850.1	36.4	3508.7	
Ileum	6	794.6	8.8	1952.7	
Jejunum	5	944.6	35.4	2286.7	
Rectum	6	704.1	198.8	1662.4	

What CSF can/can't tell us

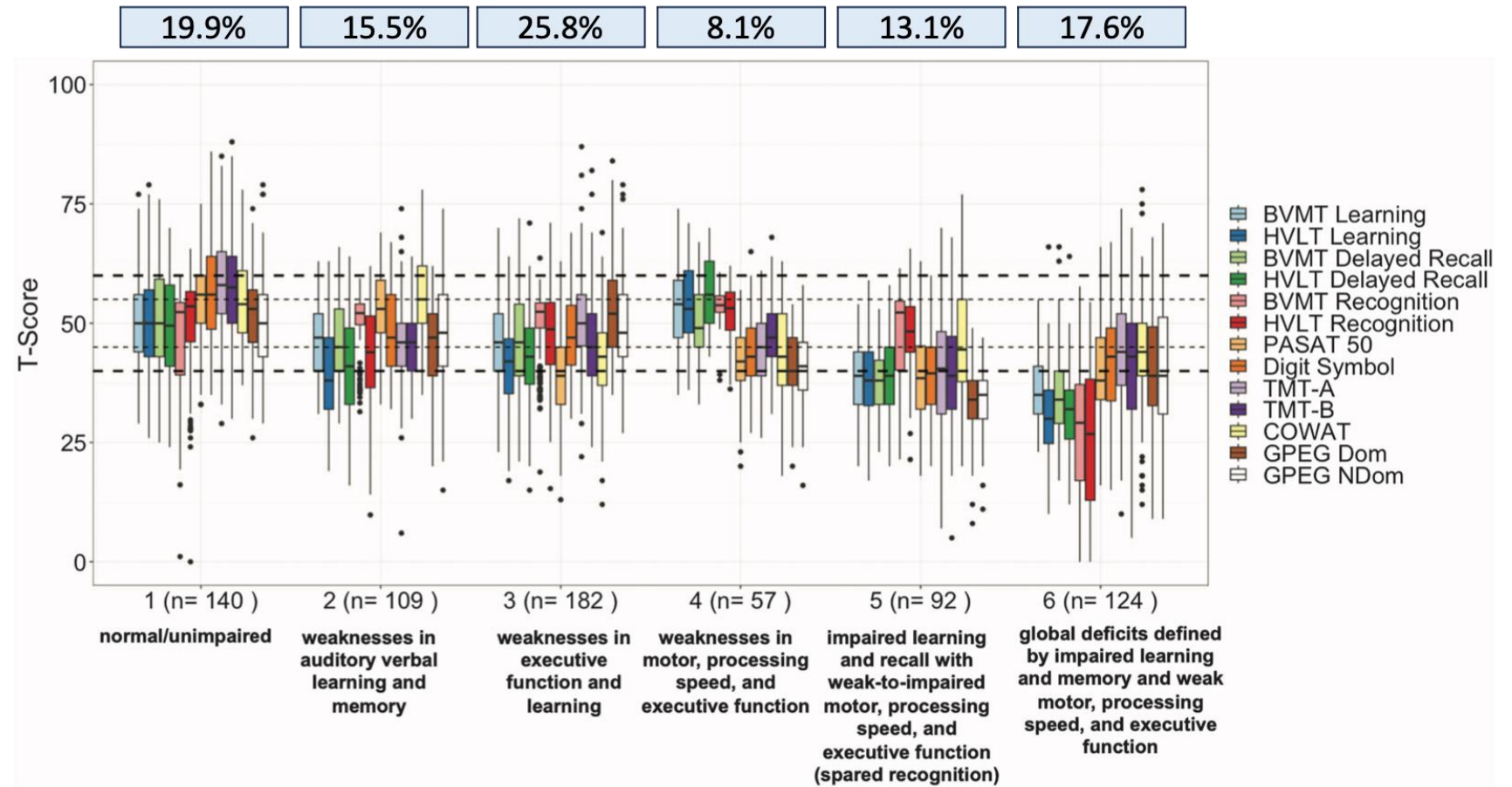
Can demonstrate

- HIV replication in the CSF
- RAMs in the CSF
- The presence of other microorganisms
- Intrathecal inflammation and immune activation
- Neuronal damage, abnormal proteins, BBB status

Can't measure

- HIV replication in the brain parenchyma
- PK of drugs in the the brain parenchyma
- Cognitive function (and reserve)

CNS phenotypes



How to measure brain health status?

Screening tests

- Poor sensitivity for MCI
- Ethnic/cultural differences

Full NC tests

- Time consuming
- Cognitive reserve seldom measured
- Great heterogeneity in tests used
- Redundancy
- Learning effect
- Validation often in specific populations and years ago (70-80)
- Correction for sex and education not available for all tests

MRI+

- Cortical and hippocampal thickness
- Brain Age Gap
- MRI-spectroscopy

What CSF can/can't tell us

Can demonstrate

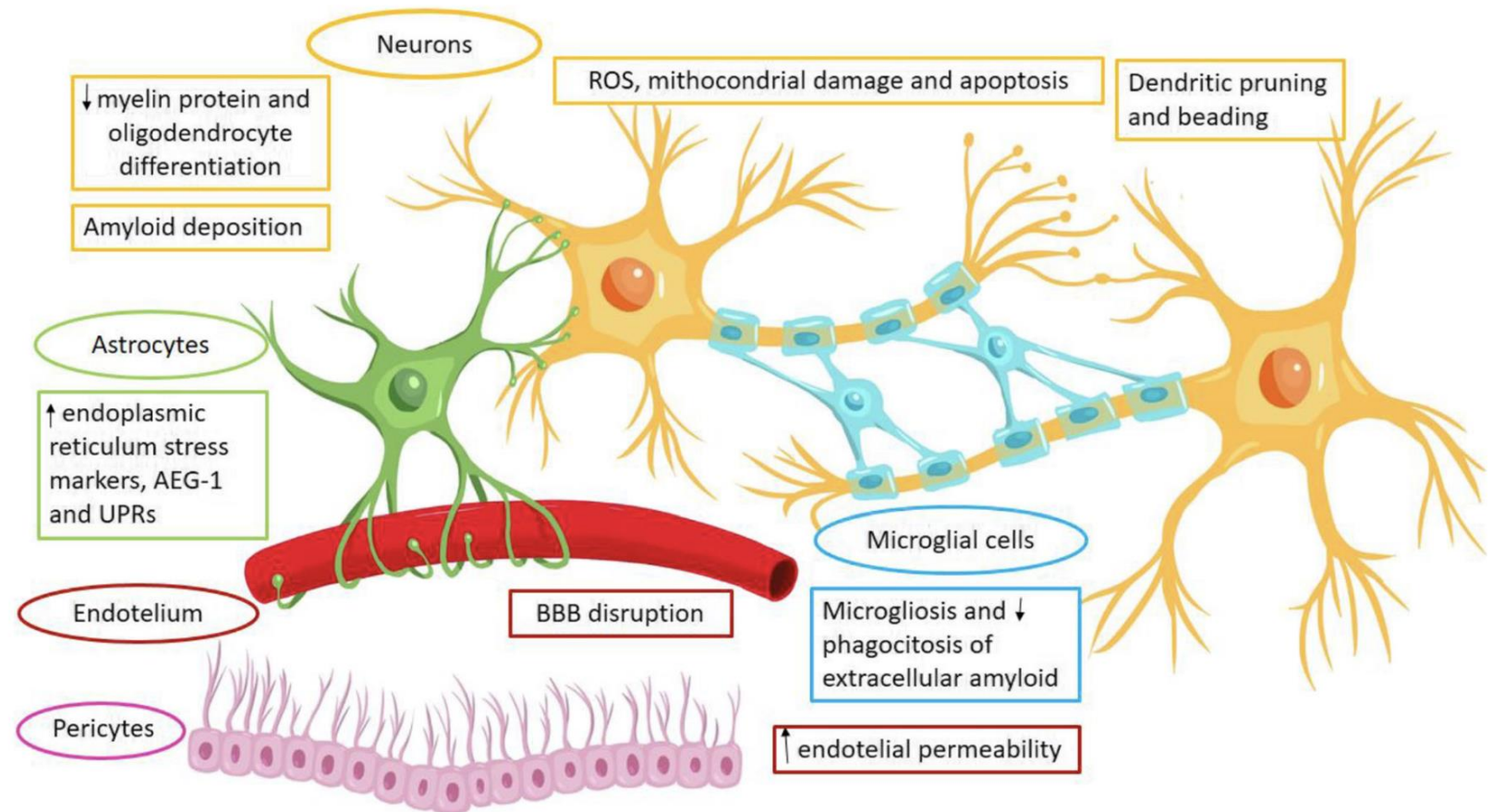
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Can't measure

- HIV replication in the brain parenchyma
- PK of drugs in the the brain parenchyma
- Cognitive function (and reserve)
- Neurotoxicity of ARVs and concomitant drugs

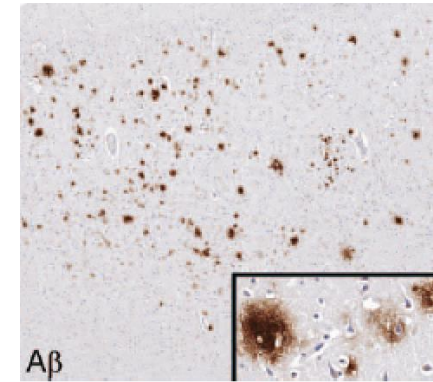
Evidence of neurotoxicity of ARVs

- Several potential mechanisms studied *in vitro* and *in vivo*

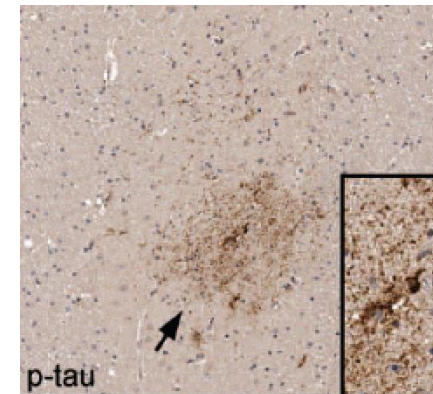


Alzheimer's biomarkers in PLWH

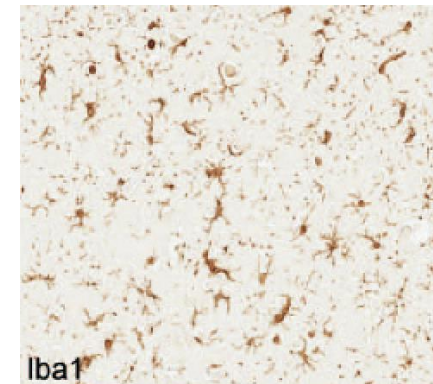
	HAD	AD
t-tau	+++	+++
p-tau	=	++
AB ₄₂	-	-
sAPP α	---	=
sAPP β	---	=
NFL	+++	+



Tenofovir use prior to death associated with lower odds of amyloid β plaque deposition (OR 0.13, $p=0.012$)

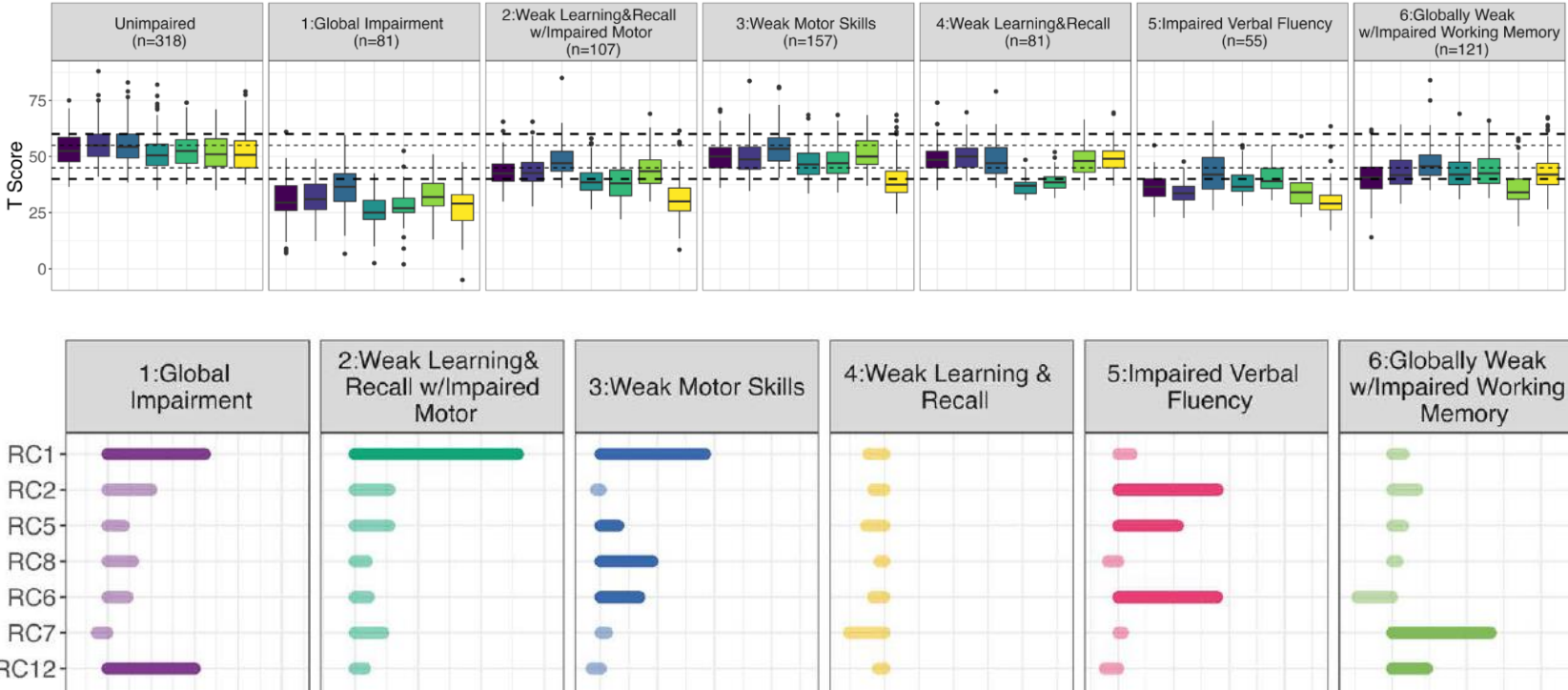


Darunavir use prior to death associated with higher odds of phospho-tau deposition in neurons (OR 15.3, $p=0.0005$)



Ritonavir use prior to death associated with higher odds of microgliosis (OR 4.96, $p=0.023$)

Concomitant drugs and NCI



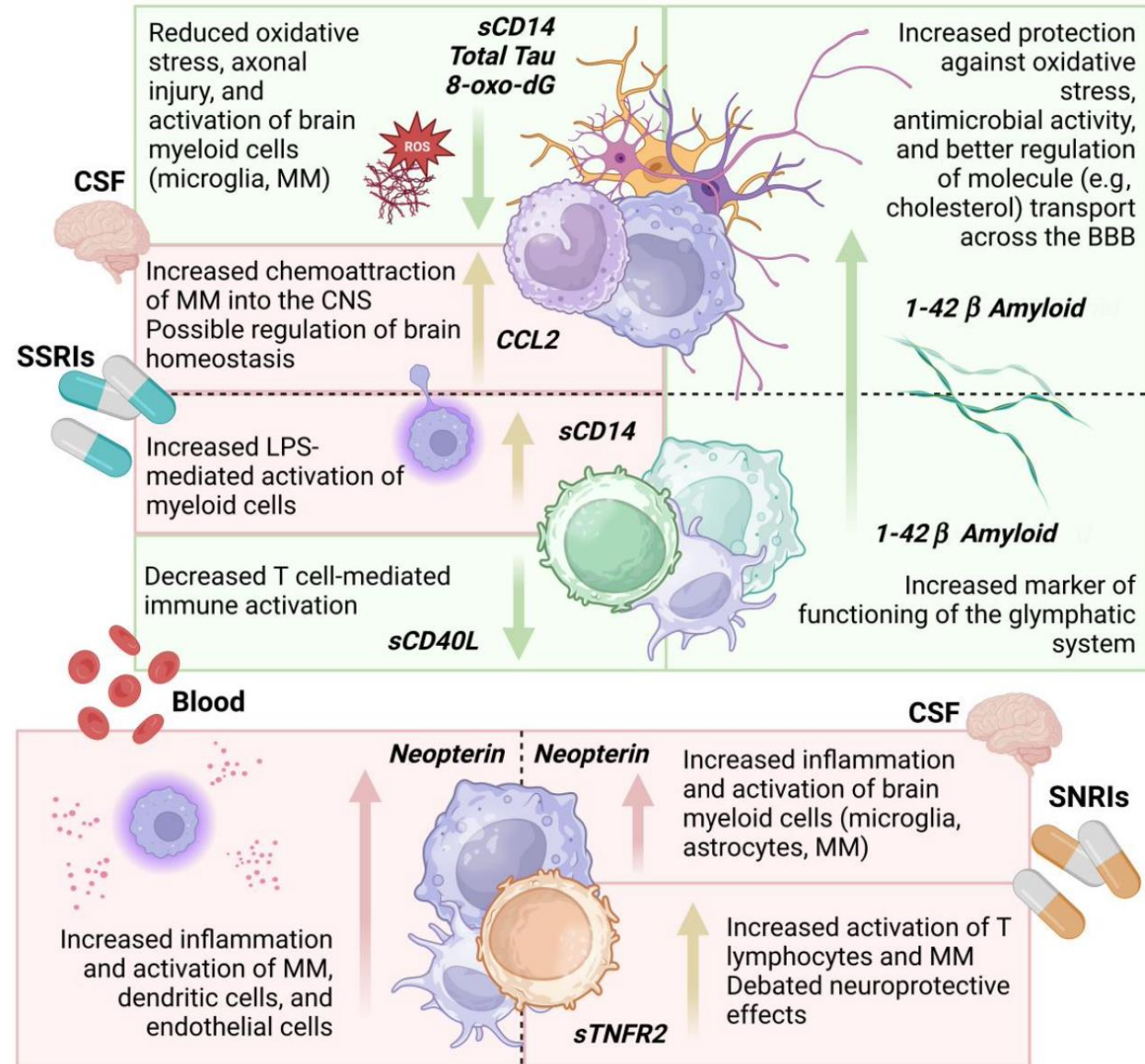
RC 1	
CNS Drugs	Gastrointestinal Drugs
Psychotherapeutic Drugs	Nonsteroidal Anti-inflammatory
Anxiolytics, Sedative, Hypnotics	Antipsychotics
Analgesics, Antipyretics	Benzodiazepines
Antidepressants	Opiate Agonists

RC 12
Autonomic Drugs
Skeletal Muscle Relaxants
Anorexigenic Agents & Stimulants

Dastgheyb et al, CROI 2022, Abstract 1305

Slide courtesy of Prof. Letendre

Positive effect of SSRIs in PLWH



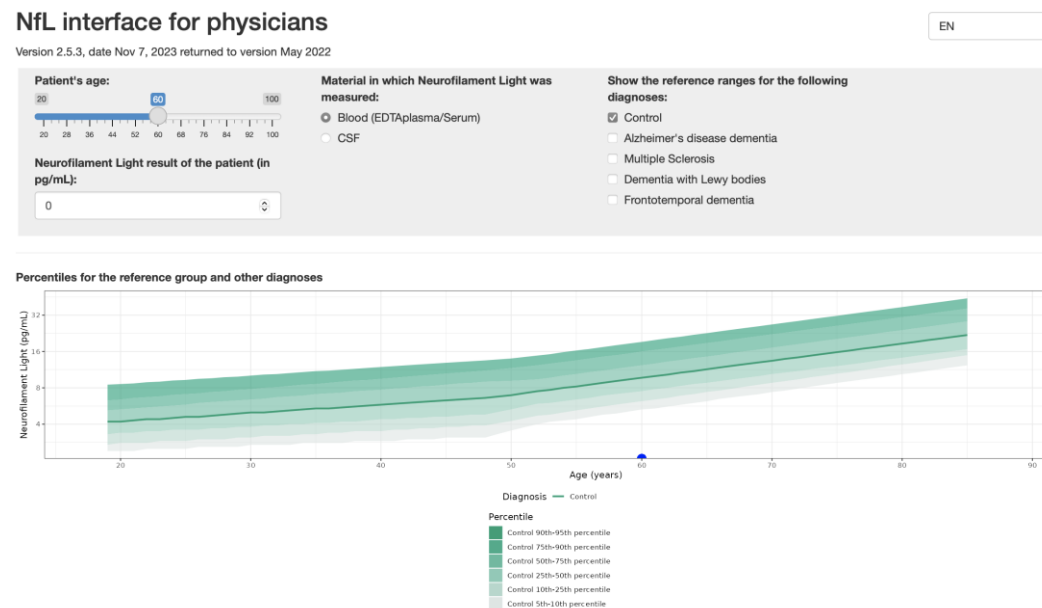
What about serum
biomarkers?

Serum biomarkers

Item	Use in other fields	Validation in PWH	Outcomes									Setting	Feasibility
			Survival	AIDS events	Non-AIDS events	CVD	Metabolic	Neurological	Cancers	CD4 recovery	HIV control		
CD4/CD8	Y	H										Clin Ext	H
T cell activation	Y	M/H										Res/Clin Ext	M/H
NK	Res	M										Res	M
MAIT/NKT INNATE LYPH CELLS	Res	L										Res	M/L
Monocyte activation	Y	M										Res	M
Blood dendritic cells	Y	M										Res	M
sCD14	Y	L										Res	M/L
sCD163	Y	M										Res/ Limited	L
Gut barrier dysfunction (LPS rDNA, IFABP, zonulin Kynurenina/triptofano- KT)	Y	L										Res	M/L
TMAO	Y	M										Target	M
C-reactive protein	Y	H										Clin Ext	H
IL-6	Y	H										Clin Ext	M
D-DIMER	Y	H										Clin Ext	H
ICAM-VCAM	Y	M										Target	M
sCD27	Y	L										Target	M
sCD4oL	Y	L										Target	M
PD-1/antiPD1	Y											Target	L
TNF system	Y	M										Res	L
IP-10 (CXCL-10)	Y	L										Res	H
MMPs (1, 2, 9)	Y	L										Res	L
IgG CMV	Y	M										Clin Ext/Res	H
IFN type 1	Y	L										Res	M
Neopterin	Y	H										Target	H
Beta-2-Microglobuline	Y	H										Target/Res	H
Chemokines (MCP-1/CCL2, MIP-1a/CCL3, MIP-1b/CCL4, RANTES/CCL5)	Y	M/L										Res	M

Serum Biomarkers of CNS involvement

- Technological development with Digital ELISA – Single Molecule Arrays (SIMOA)
- In the field of neurology: tau₁₈₁ and B-Amyloid₁₋₄₂ correlating with CSF levels, MRI, Amyloid-PET and future development of MCI
 - Normative data for serum NFL (<https://mybiomarkers.shinyapps.io/Neurofilament>)

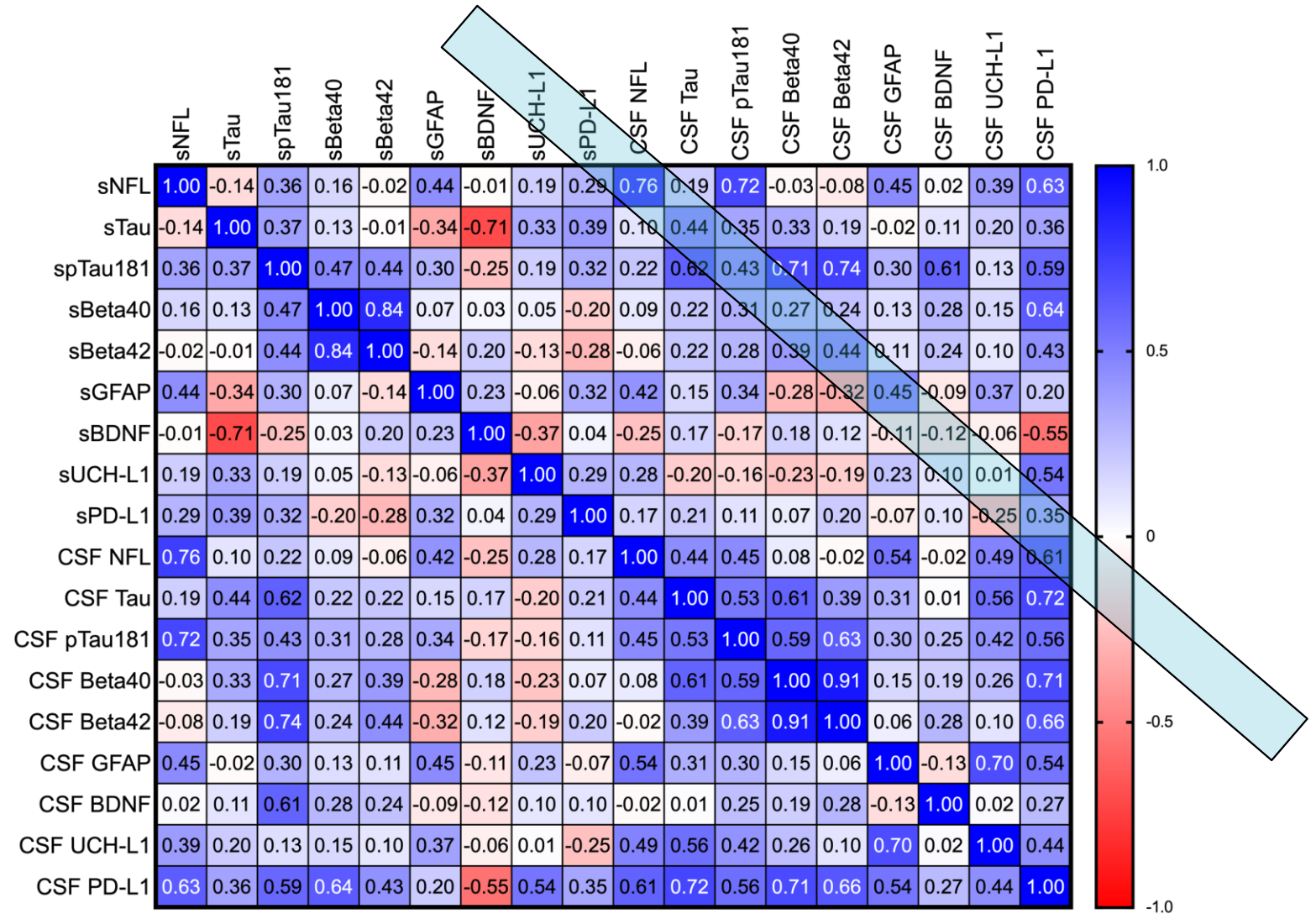


Karikari T, et al. Lancet Neurol 2020; Moscoso A, et al. JAMA Neurol 2021; Gisslen M, et al. EBioMedicine 2015; Ripamonti E, et al. JIM 2022; Alagaratnam J, et al. JNV 2022; Trunfio M, et al. CROI 2022

Serum Biomarkers of CNS involvement

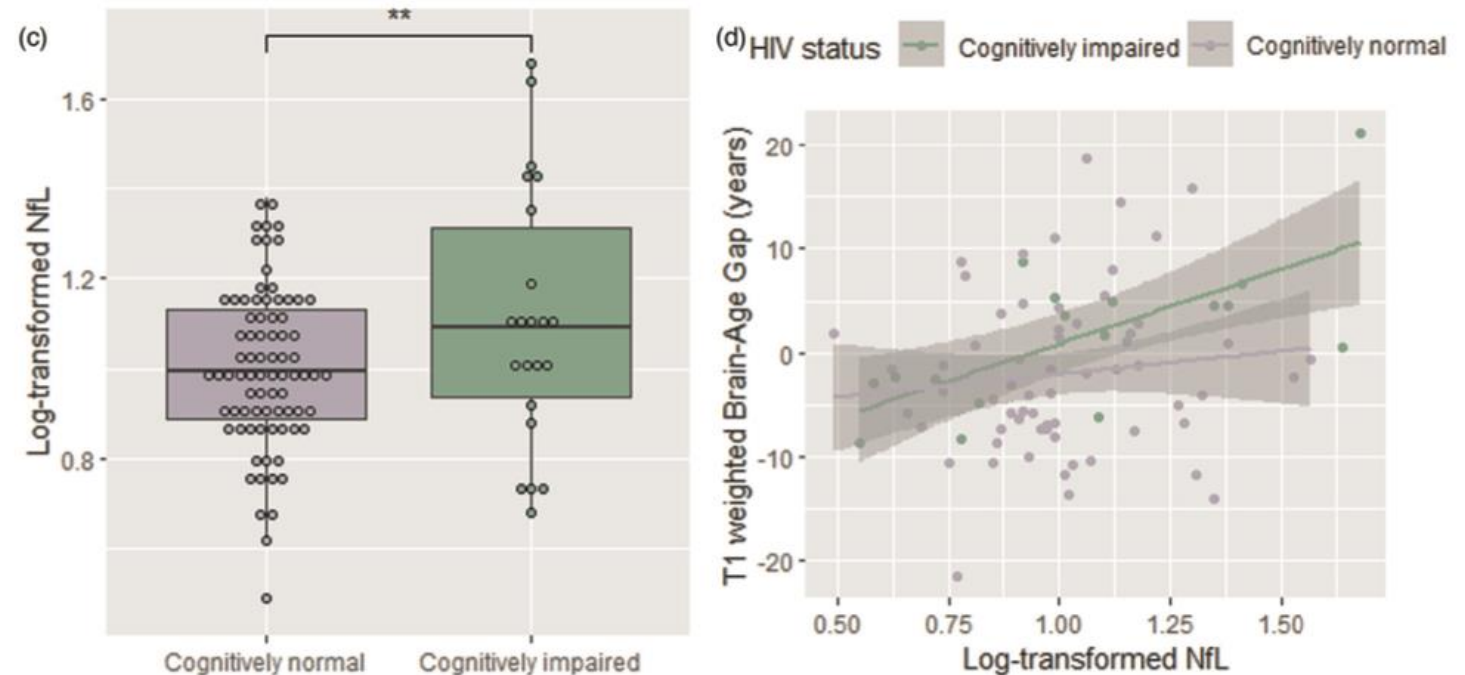
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 - Normative data for serum NFL (<https://mybiomarkers.shinyapps.io/Neurofilament>)
- In PLWH mostly on serum NFL
 - average/high correlation with CSF NFL
 - higher levels in patients with low CD4 and HAD
 - decline after antiretroviral initiation
 - preliminary data on correlation with cognitive function

Serum to CSF correlations in PLWH



Serum NFL and cognition in PWH

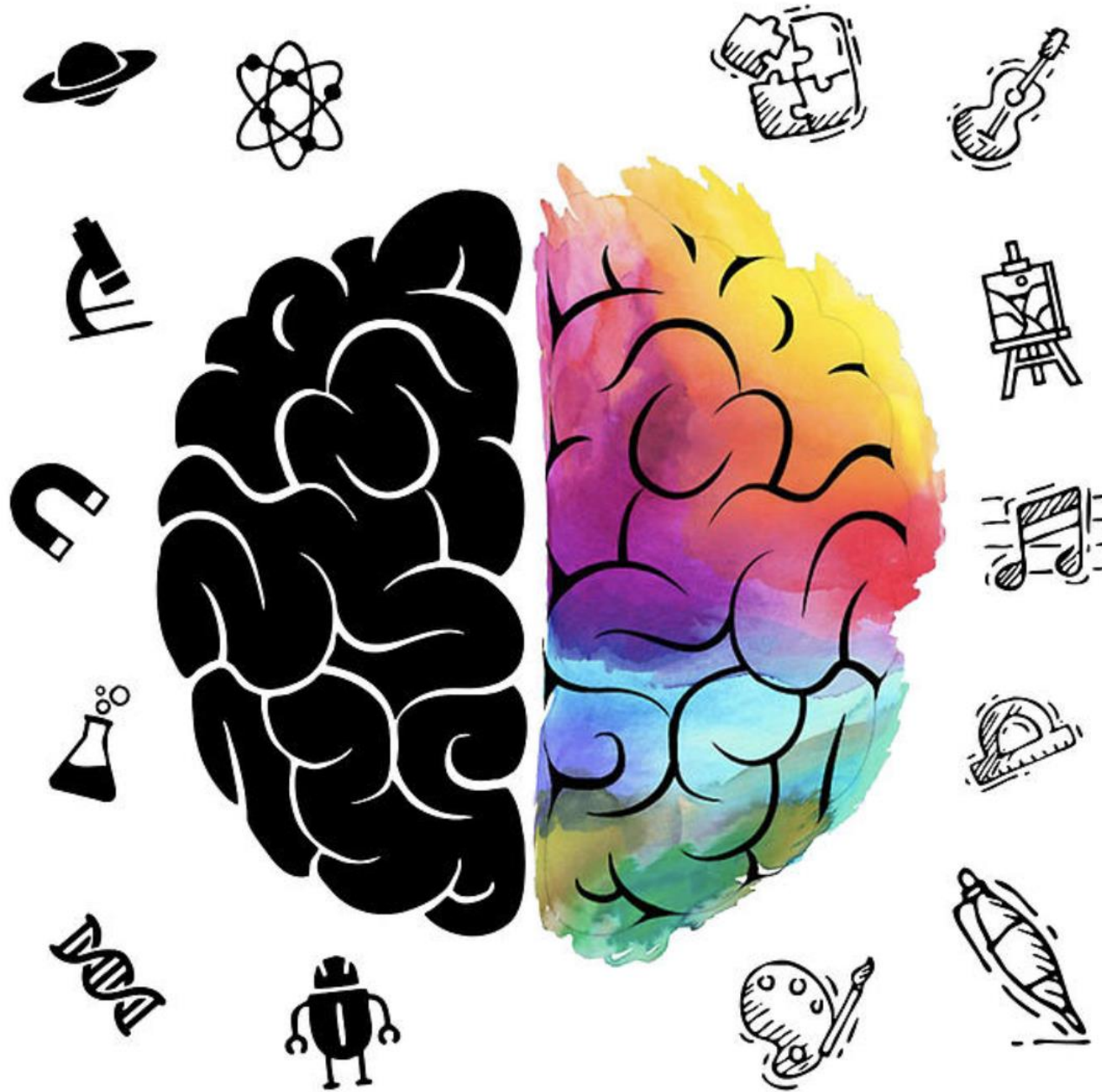
- 104 PWH (HIV RNA <50, CD 610) and 42 PWH
- No difference in NfL, higher with older age
- No association with typical HIV variables
- **Within PWH, NfL was higher with higher cardiovascular risk, cognitive impairment and a greater BAG.**



Conclusions

- CSF is very informative for
 - Quantifying the replication of HIV and looking at RAMs
 - Studying the reservoir (HIV DNA and intact DNA)
 - Looking for other microorganisms
 - Establishing the presence of intrathecal Ig synthesis and BBB status
- After many years of research, the role of the CSF is limited for:
 - Brain tissue PK
 - Brain tissue HIV replication/evolution
 - Predicting cognitive trajectory
- Serum biomarkers of CNS damage, AD pathology and inflammation/astrocytosis are now available

Thank you for your attention



Thanks to

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Mattia Trunfio

Jessica Cusato

Miriam Antonucci

Daniele Imperiale

Daniela Vai

Cristiana Atzori

Elisa Vuaran

Scott Letendre

Qing Ma