How to control HIV in the brain?
Searching the right target

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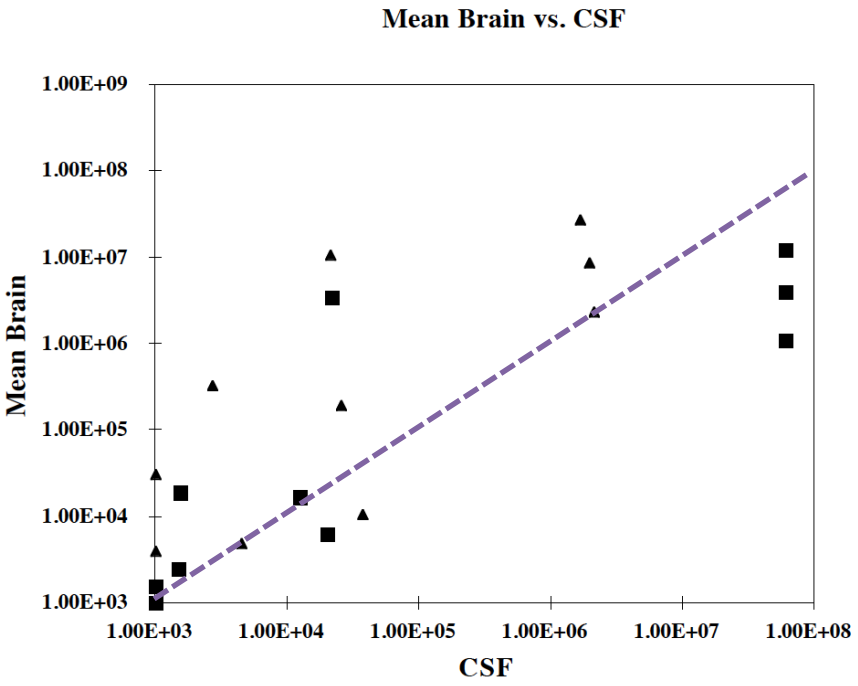
Targets of ARV THERAPY in the CNS

1. undetectable brain HIV RNA (?)/ CSF HIV RNA
2. normalize brain/CSF markers
3. normalize brain/CSF markers – MRI Spectroscopy
4. normalize MRI
5. normalize NPS tests

Brain HIV RNA



bRNA vs. CSFRNA
little correlation when the CSF levels were below 105 copies per mL

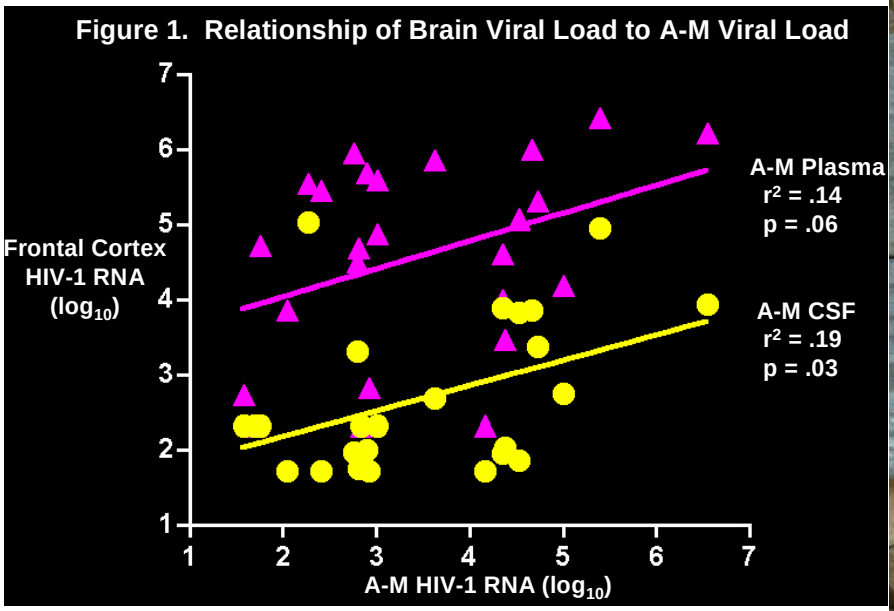
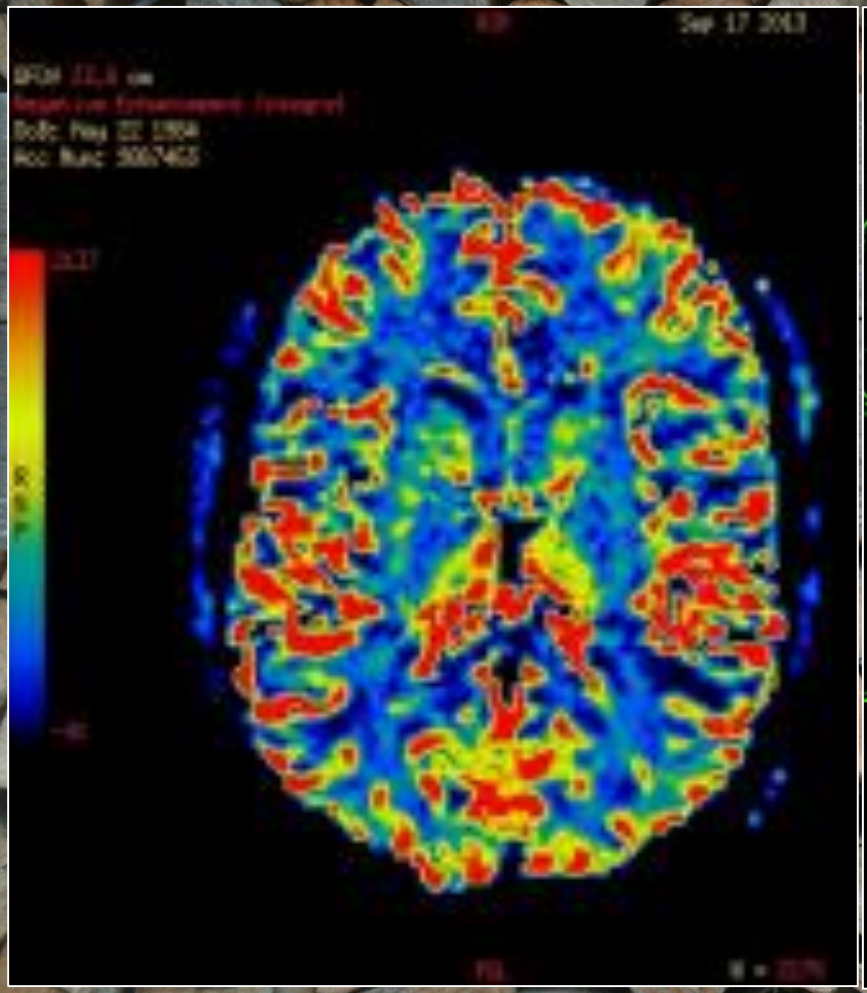


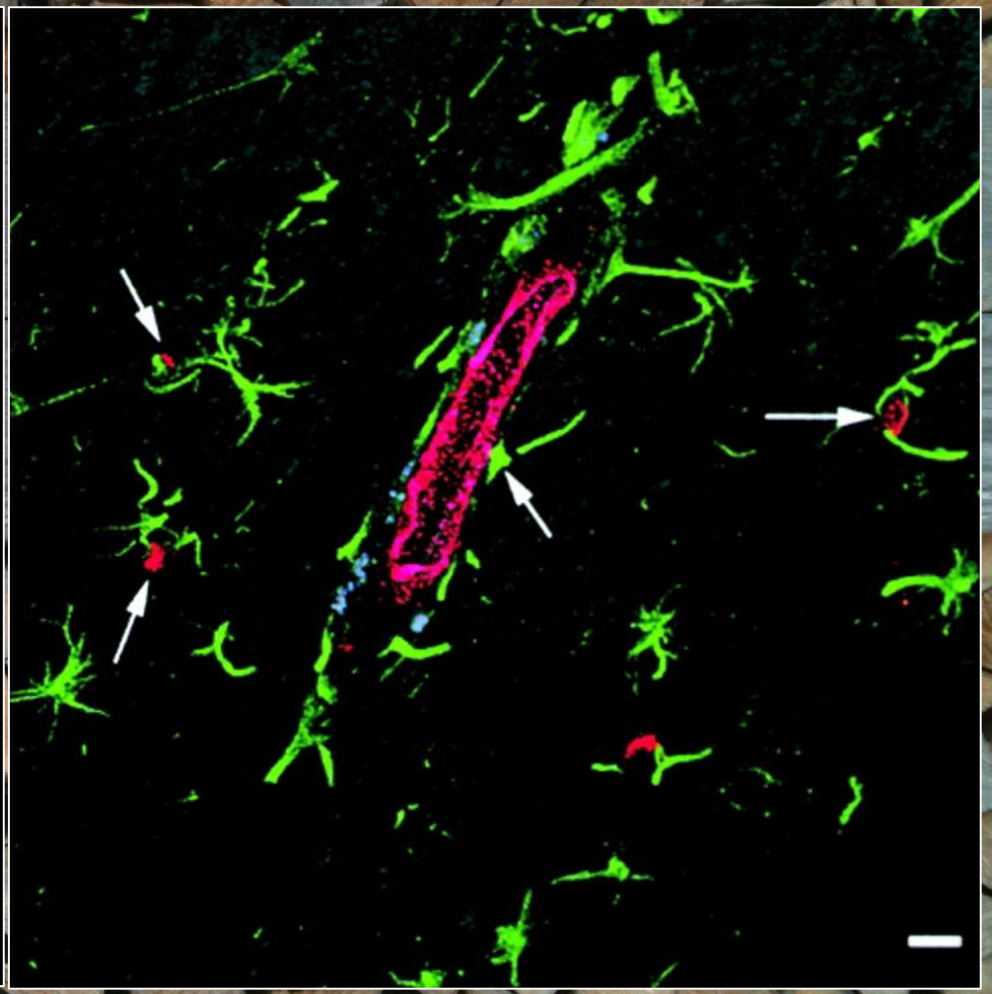
Table 2. Predictors of frontal cortex HIV viral load.

Predictors	F	R^2	ΔR^2	P
CSF HIV RNA	5.5	0.19	--	0.03
Plasma HIV RNA	3.8	0.14	--	0.06
CSF + Plasma HIV RNA	3.3	0.23	0.04	0.05

Wiley CA, et al. Brain Pathol, 1998; Ellis RJ, et al. CROI 2005

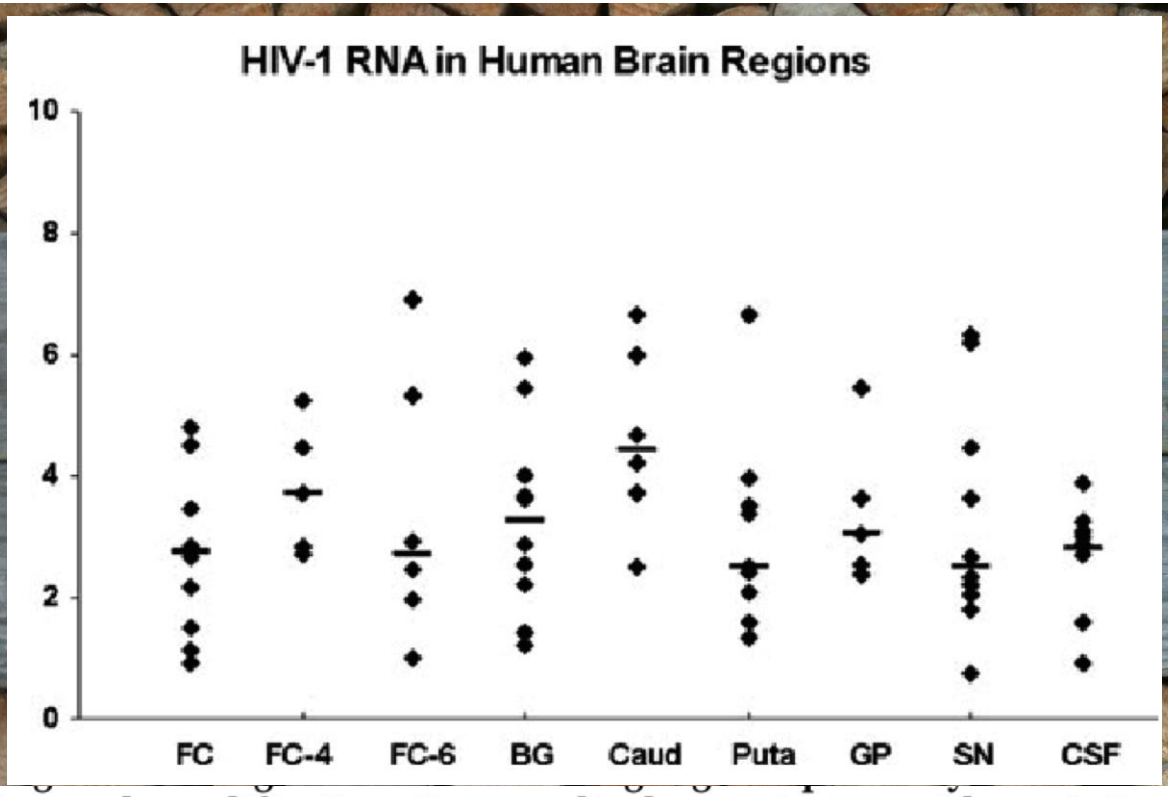
Brain HIV RNA - areas





1. www.istockphoto.com 2. Williams K C et al. J Exp Med 2001.

Brain HIV RNA - areas

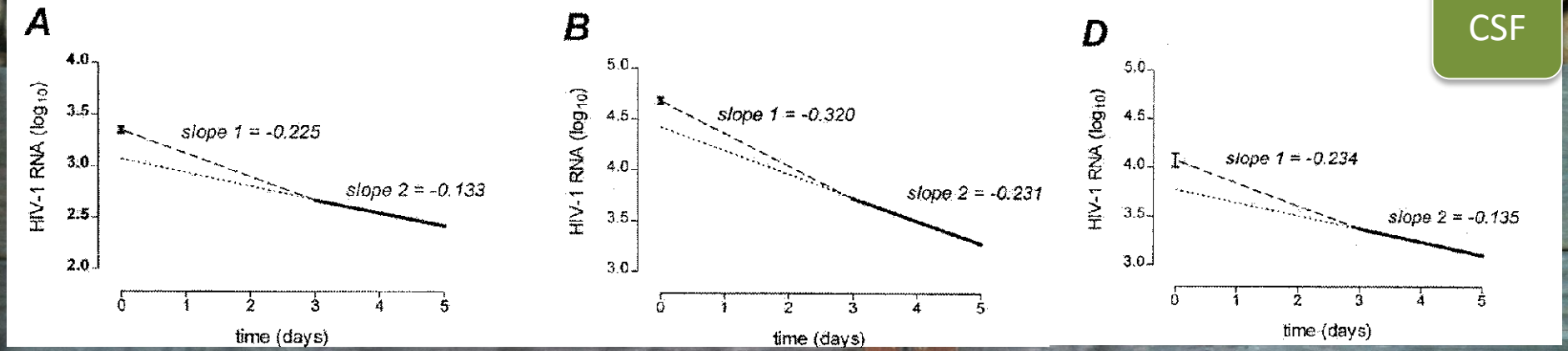


detection and amplification. Our results demonstrate a wide variation in the concentration of HIV-1 RNA in different brain regions (5.51 and 8,144,073; $\log_{10}$ 0.74 and 6.91 copies/g tissue), and despite the high specificity and sensitivity of this method, viral RNA was not detected in 50% of all the samples, and in 30% to 64% of samples of each region of HIV-1+ individuals. However, the highest concentration of viral RNA was found in the caudate nucleus and the lowest concentration in the frontal cortex and cerebrospinal fluid. The viral RNA was undetectable in all samples of HIV-negative individuals. *Journal of NeuroVirology* (2007) **13**, 210–224.

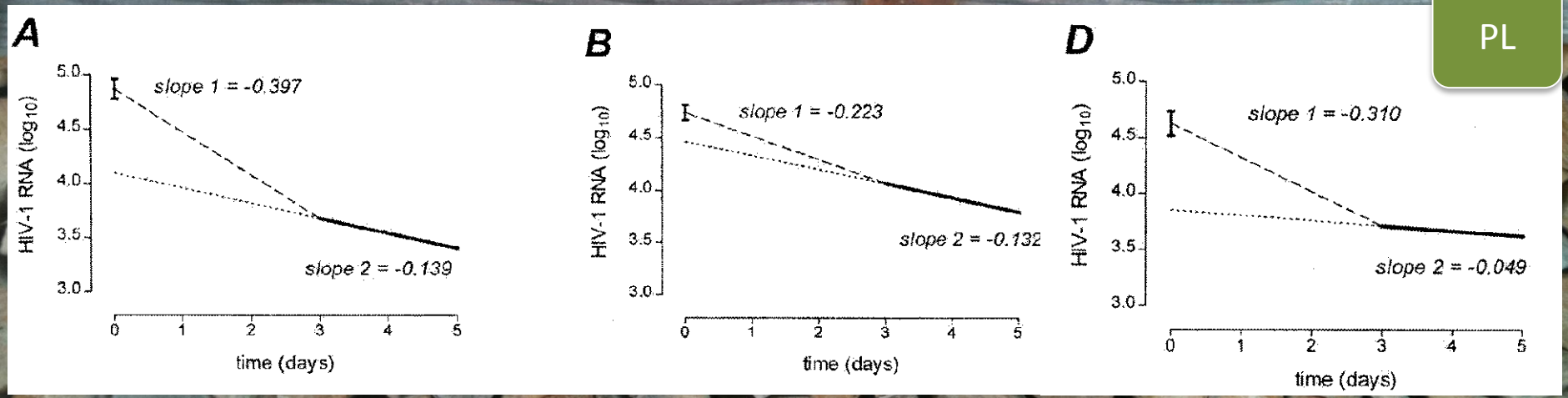
Kumar AM et al. JNV 2007

CSF HIV RNA decay

CSF

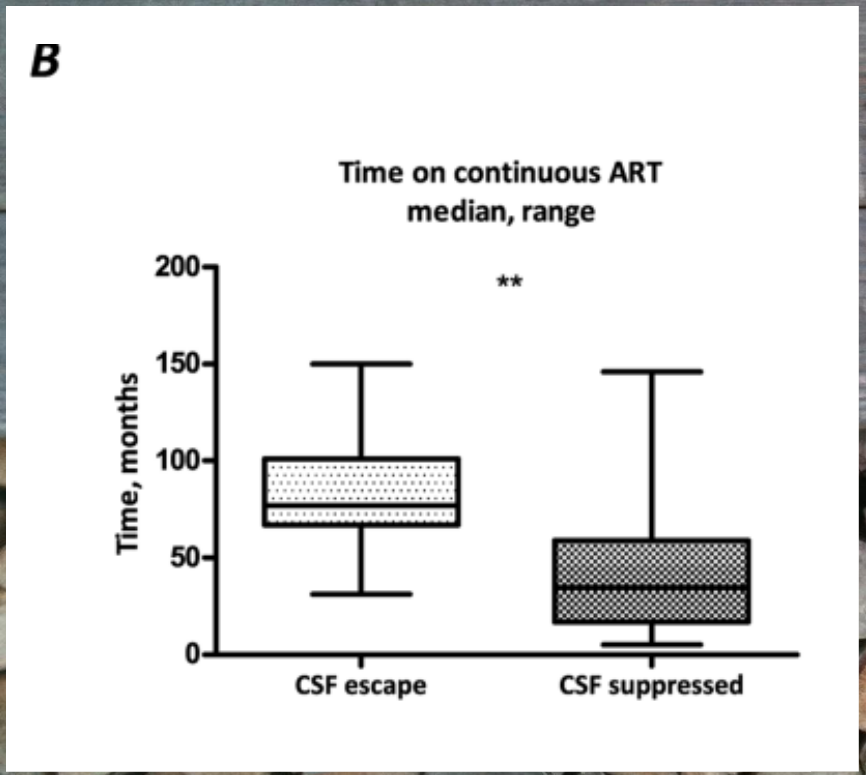


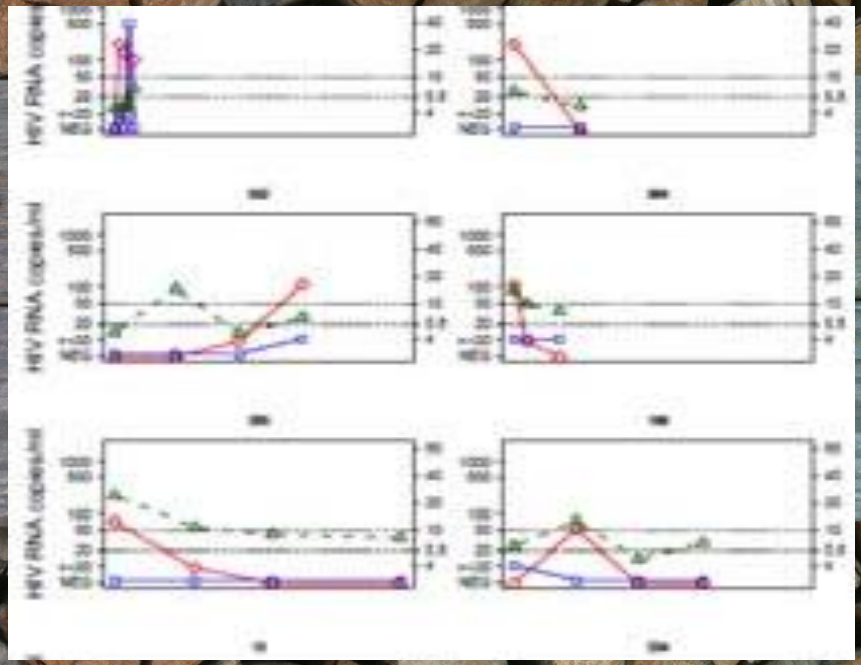
PL



Haas D, et al. Neurology 2003

CSF HIV RNA

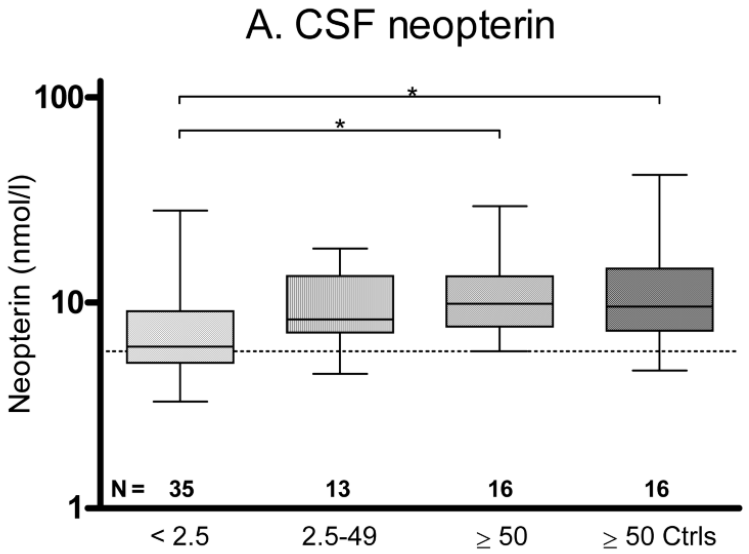


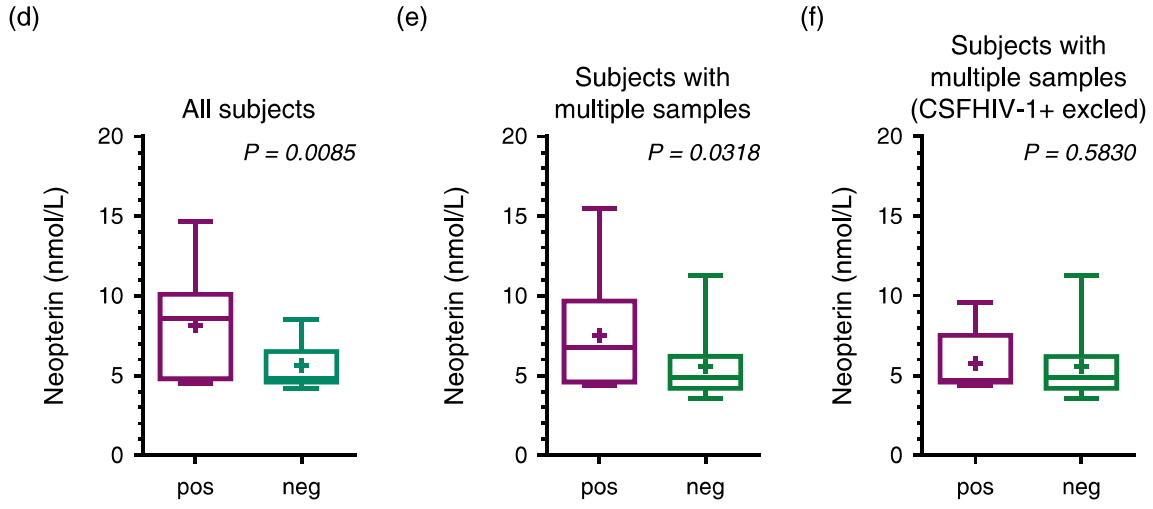


- 25% of neurologically stable patients on effective antiretroviral therapy had CSF HIV1 RNA >50 copies/ml on one or more occasions

Eden A, et al JID 2011; Eden A, et al. CROI 2012

usHIV RNA



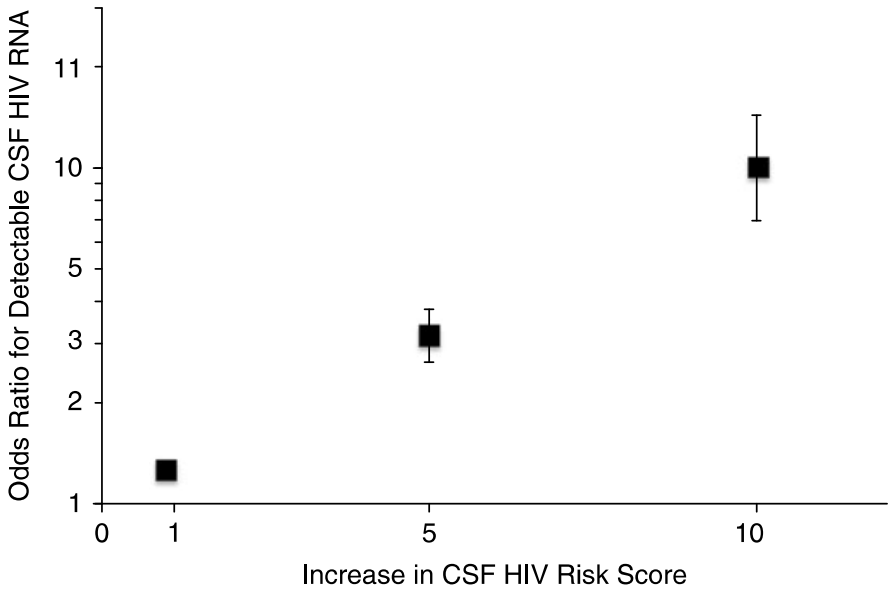


Yilmaz A, et al. JAIDS 2008, Dahl V, et al. AIDS 2014

Can we predict CNS efficacy?

Table 3. Predicted Probabilities of Detectable CSF HIV RNA (at >50 copies/mL) by the CSF HIV Risk Score in 811 Persons Receiving Combination Antiretroviral Therapy at Study Entry, CHARTER Study Cohort, 2004–2007

CSF HIV Risk Score	Predicted Probability, %	95% CI
0	0.24	0.11, 0.53
5	0.75	0.40, 1.4
10	2.32	1.5, 3.7
15	7.0	5.1, 9.5
20	19.2	15.7, 23.2
25	42.9	34.6, 49.6
30	70.3	61.5, 77.9
35	88.2	81.2, 92.9
39	95.0	90.5, 97.4



Hammond ER, et al. American journal of Epidemiology 2014

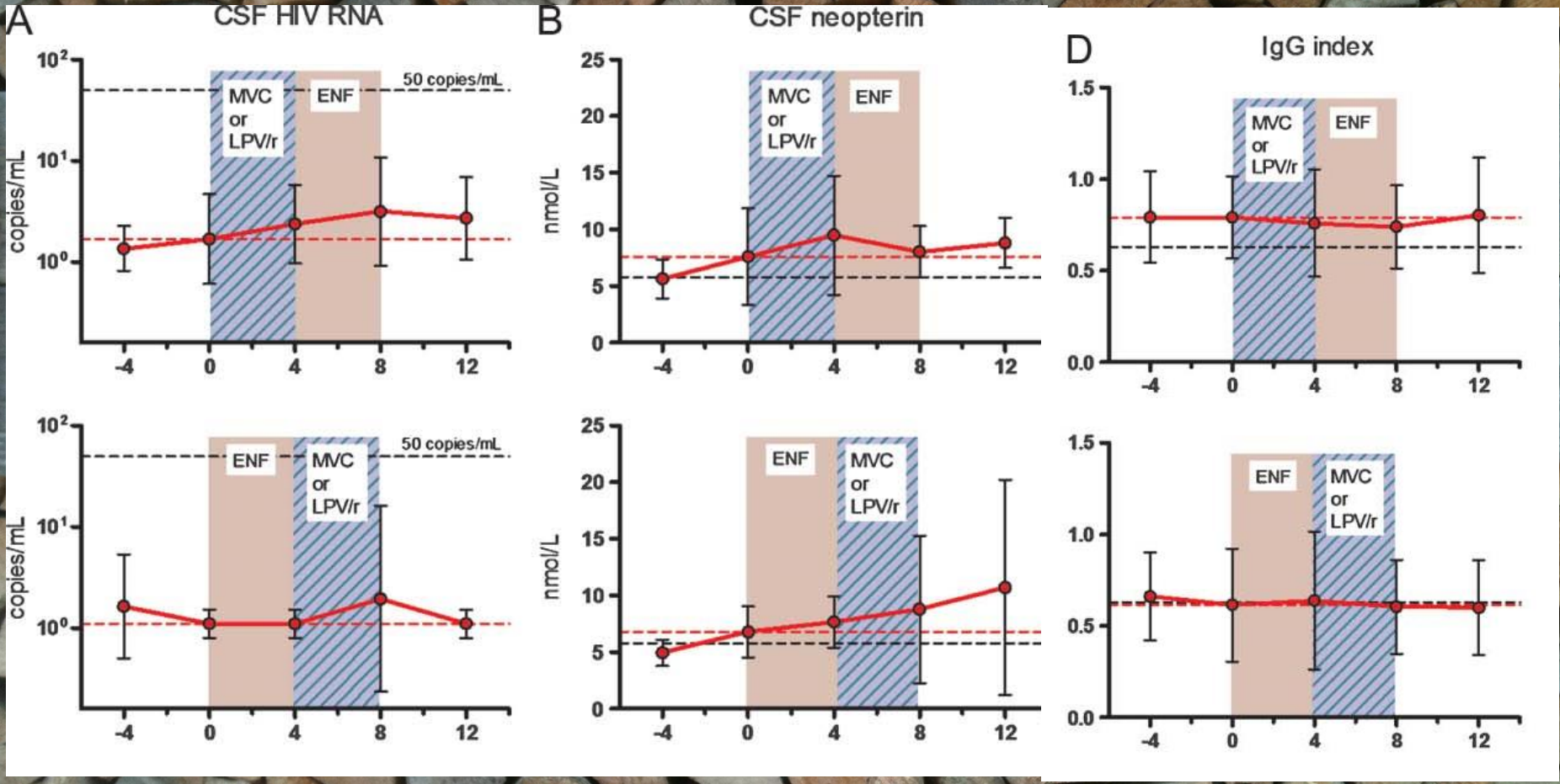
How many drugs are needed?

Detectable CSF HIV RNA according to		
Triple HAART	**Dual ART**	**Mono ART**
0-24%	?	0-15%
Varies according to inclusion criteria. Average 10%?	Very few reports Mostly PI+RAL or PI+MVC or PI+3TC	ATV/r monotherapy dismissed

ATV + RAL
7 CSF available
100% CSF HIV RNA<50 copies/mL

Carey D, et al. JAIDS 2012

How many drugs are needed? (2)



Yilmaz A, et al. JAIDS 2011

How much drug is needed?

- In Meningitis:
  - Concentrations above the MIC (cephalosporines and fluoroquinolones x10 MIC→ bactericidal activity)
  - For the whole dosing interval! (time-dependant)
- BBB permeability
- Low Protein binding

Practical implications for the interpretation of minimum plasma concentration/inhibitory concentration ratios

- C_{min} variability
- ICs variability:
  - various tests (measuring different markers of HIV replication)
  - cell types (T-cell or monocyte cell lines, or primary human cells)
  - types of infection (acute, chronic, or latent)
  - viral strains (wild-type, modestly resistant, highly resistant)
  - Combinations of agents can also show synergy when coadministered

Montaner J, et al Lancet 2001

Acosta's ICs

We derived serum protein binding correction factors (PBCF) for protease inhibitors, nonnucleoside reverse transcriptase inhibitors, and an integrase inhibitor by measuring the effect of a range of human serum concentra- tions on *in vitro* drug susceptibility measured with the PhenoSense HIV assay.

					WT IC[e]				PBIC (ng/ml)[g]			
					nM[f]		ng/ml					
Drug[a]	Dose (mg)[b]	Molar mass (g/mol)	C_{trough} (ng/ml)[c]	PBCF[d]	50%	95%	50%	95%	50%	95%	DHHS target (ng/ml)[h]	IQ_{95} (C_{trough}/$PBIC_{95}$)
APV	700/100 b.i.d.	505	2,120	11	10	62	5.3	31	60	358	400	5.9
ATV	300/100 q.d.	705	800	9.3	2.4	9.2	1.7	6.5	15.9	60	150	13
DRV	600/100 b.i.d.	548	3,500	14	0.71	3.4	0.4	1.9	5.3	25	NA	138
IDV	800/100 b.i.d.	614	1,300	3.5	7.0	34	4.3	21	15.2	73	100	18
LPV	400/100 b.i.d.	629	5,500	10	5.0	27	3.1	17	31	168	1,000	33
NFV	1,250 b.i.d.	664	1,000	77	16	75	11	50	819	3,865	800	0.26
SQV	1,000/100 b.i.d.	767	400	17	4.7	18	3.6	14	60	234	100–250	1.7
TPV	500/200 b.i.d.	603	15,670	15	88	433	53	261	796	3,902	NA	4.0
EFV	600 q.d.	316	1,800	27	4.2	15	1.3	4.7	36	126	1,000	14
ETR	200 b.i.d.	435	297	33	2.2	8.2	0.9	3.5	31	116	NA	2.6
NVP	400 q.d.	266	4,500	1.4	120	952	32	253	46	366	3,000	12
RAL	400 b.i.d.	444	114	2.1	8.0	100	3.6	44	7.5	94	NA	1.2

Acosta EP, et al. AAC 2012

CSF – inhibitory quotients (IQs)

$$IQ_{50} = \frac{\text{CSF PK}}{IC_{50}} \qquad IQ_{95} = \frac{\text{CSF PK}}{IC_{95}}$$

1. **Comparing different ARVs**
2. **Measurement of patients' CSF levels**

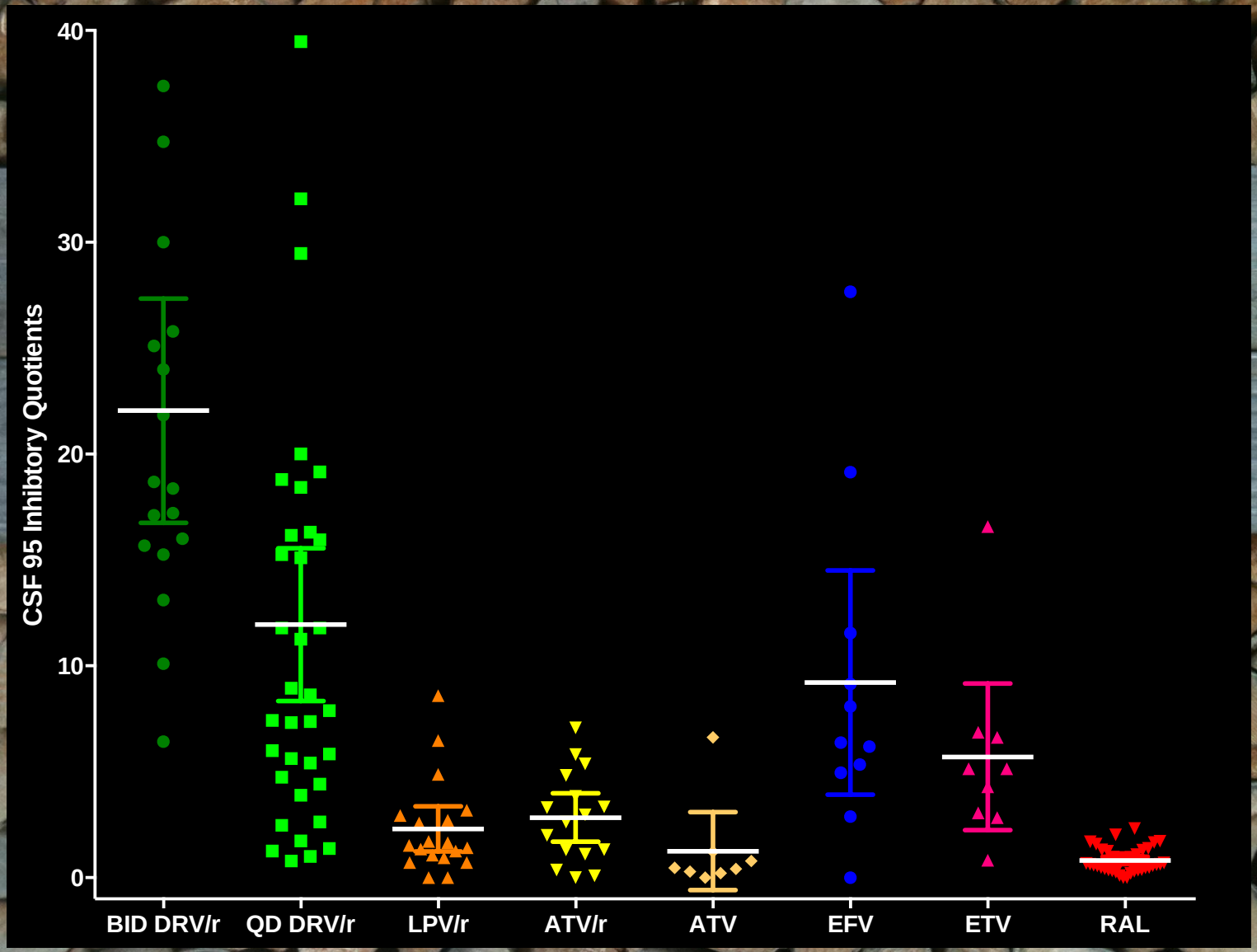
Mat & Meth

- Adults on combination antiretroviral therapy
- undergoing a lumbar puncture for either clinical reasons or specific research protocols
- prospectively included
- Exclusion criteria:
  - the presence of opportunistic infections or neoplasias,
  - the presence of plasma or cerebrospinal fluid of major resistance-associated mutations,
  - the concomitant use of drugs known to cause significant drug to drug interactions (rifampicin, proton pump inhibitors with atazanavir, etc.).

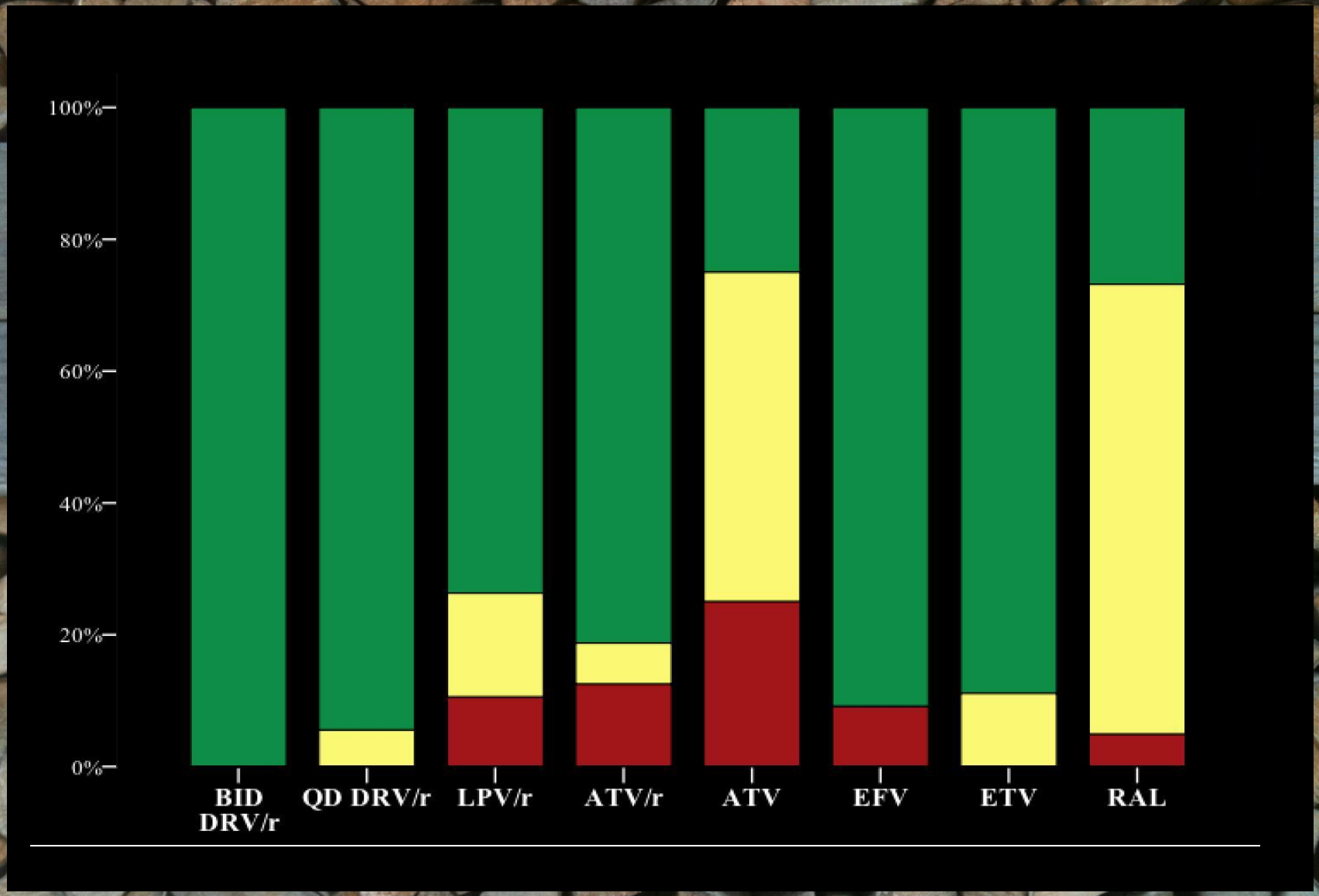
Results

- 174 samples from 124 patients
- male 71.7%, Caucasian 73.2%
- Median age 46 years (40.5–54.5 years) and BMI 22.2 kg/m2 (20–25.2 kg/m2).
- HCV 26%, HBV 11%, 10.2% liver cirrhosis.
- Lumbar punctures were performed for:
  - asymptomatic patients (either in the context of longitudinal studies or asymptomatic patients with <100 CD4 cells/µL) 49.6%
  - HAND 23.6%
  - Other neurological conditions 17.3:
    - epilepsy 3.9%
    - neuropathies 3.9%
    - myelopathies 3.9%
    - JC virus–negative leukoence-phalopathy 9.4%

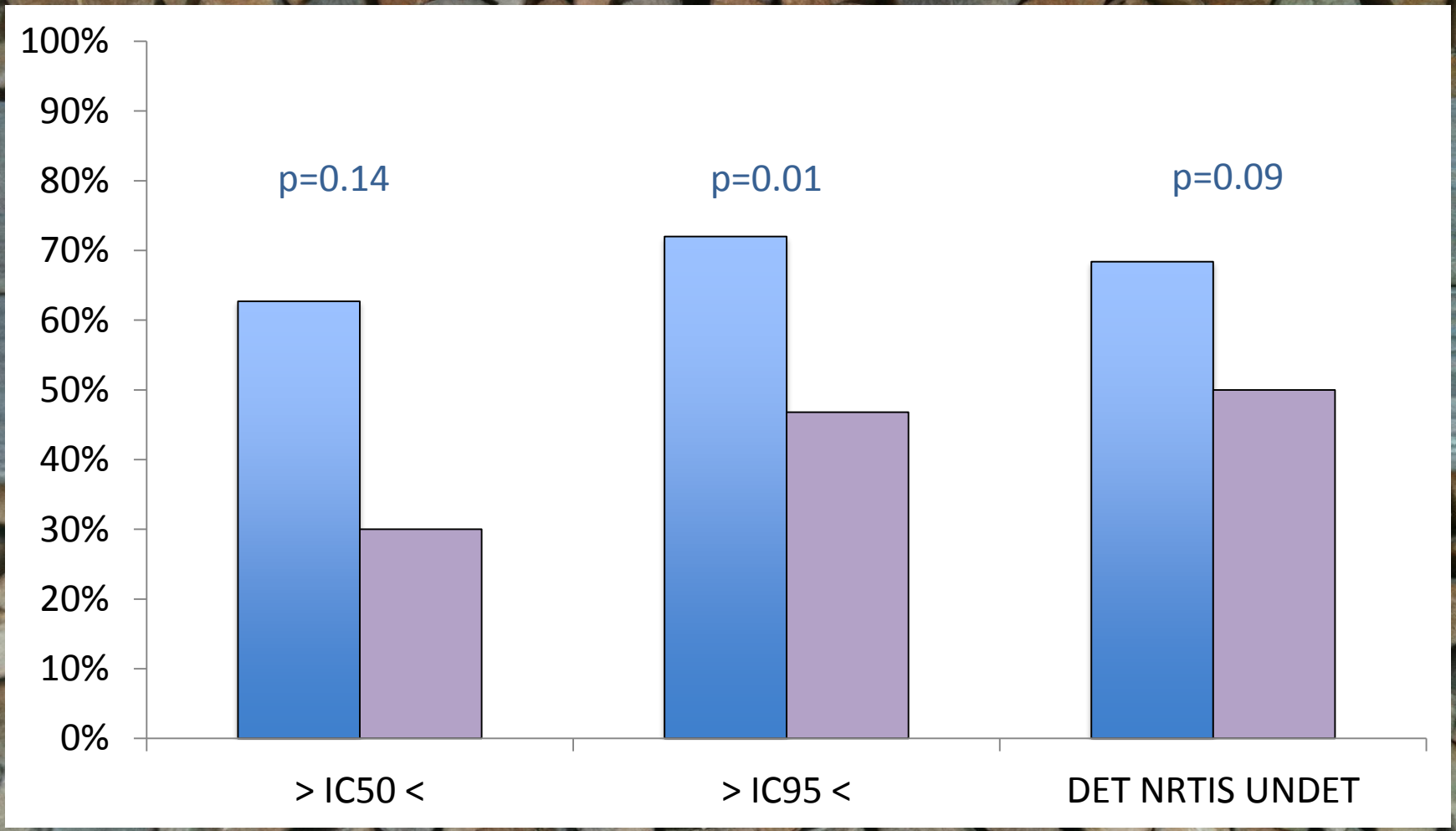
CSF IQs


CSF 95 Inhibtory Quotients
40
30
20
10
0
BID DRV/r
QD DRV/r
LPV/r
ATV/r
ATV
EFV
ETV
RAL

CSF Iqs (2)

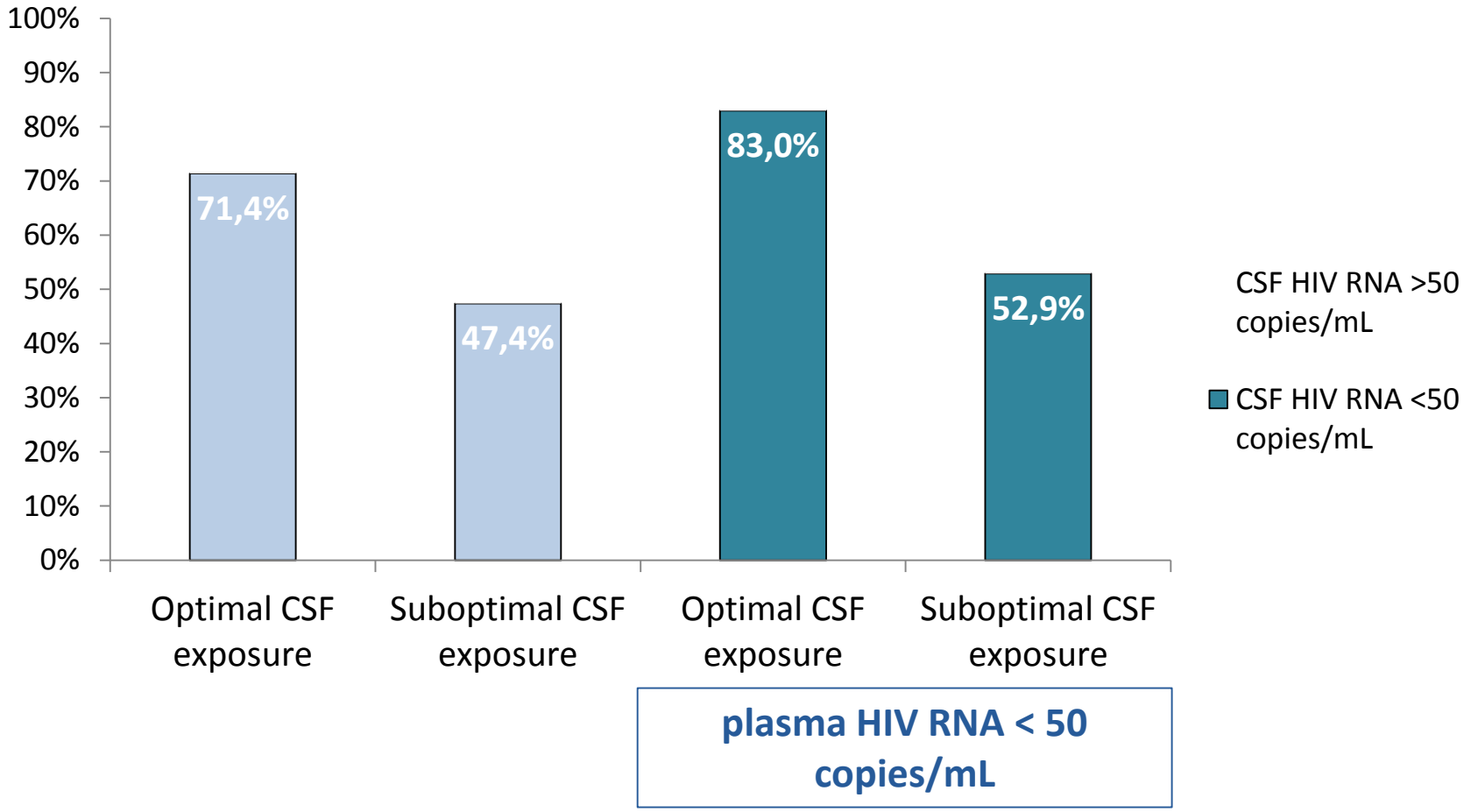


IQ_{50} vs. IQ_{95}


100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
p=0.14
p=0.01
p=0.09
> IC50 <
> IC95 <
DET NRTIS UNDET

CSF PK and CSF HIV RNA

Optimal CSF regimen exposure was defined as IQ95 >1 and detectability of CSF concentrations of all drugs contained in the regimen

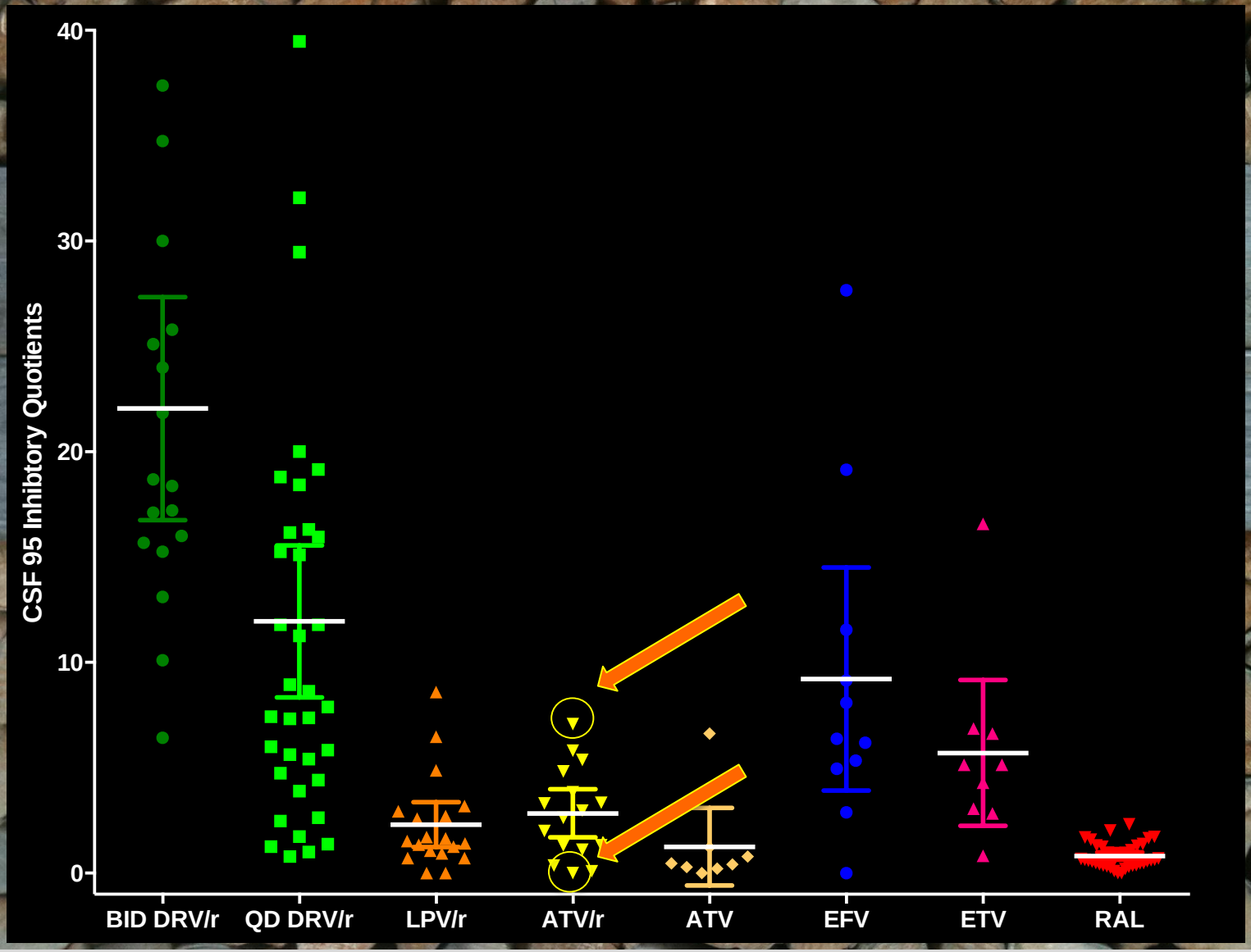


CSF PK and CSF HIV RNA (2)

Optimal CSF regimen exposure was defined as IQ95 >1 and detectability of CSF concentrations of all drugs contained in the regimen

	CSF HIV RNA <50 Copies/mL(n = 127)					CSF Escape in Patients With Plasma VL <50 Copies/mL (n = 84)				
	Univariate		Multivariate			Univariate		Multivariate		
Variable	*P* Value	OR	*P* Value	AOR	95% CI	*P* Value	OR	*P* Value	AOR	95% CI
Male sex	.94	. . .	. . .	. . .	. . .	.61	. . .	. . .	. . .	. . .
Age (10 y increase)	.80	. . .	. . .	. . .	. . .	.48	. . .	. . .	. . .	. . .
HCV Ab positivity	.21	. . .	. . .	. . .	. . .	.70	. . .	. . .	. . .	. . .
HBsAg positivity	.04	0.26	.10	. . .	. . .	.06	5.3	.66	. . .	. . .
Previous syphilis	.22	. . .	. . .	. . .	. . .	.15	. . .	. . .	. . .	. . .
CPE score (per 1-point increase)	.31	. . .	. . .	. . .	. . .	.44	. . .	. . .	. . .	. . .
Nadir CD4 count (per 100 cells/μL increase)	.09	. . .	**.01**	**2.04**	**1.18–3.54**	.35	. . .	. . .	. . .	. . .
Current CD4 count (per 100 cells/μL increase)	.01	. . .	.29	. . .	. . .	.32	. . .	. . .	. . .	. . .
Plasma VL <50 copies/mL	<.01	6.1	**<.01**	**8.14**	**2.67–24.76**	NA	NA	NA	NA	NA
Duration of VL <50 copies/mL (per 6-mo increase)	.56	. . .	. . .	. . .	. . .	.52	. . .	. . .	. . .	. . .
CSF Conc >IC_{95}	.01	2.55				.01	0.28			
Detectable CSF NRTIs or MVC	.09	2.36				.16	. . .			
Optimal CSF regimen exposure	<.01	2.78	.09	. . .	. . .	.01	0.28	**.01**	**0.28**	**.11–.76**

CSF TDM?


CSF 95 Inhibtory Quotients
40
30
20
10
0
BID DRV/r
QD DRV/r
LPV/r
ATV/r
ATV
EFV
ETV
RAL

HAND e HIV DNA

J Neuropsychiatry Clin Neurosci . 2009 ; 21(1): 68–74. doi:10.1176/appi.neuropsych.21.1.68.

Amount of HIV DNA in Peripheral Blood Mononuclear Cells is Proportional to the Severity of HIV-1-Associated Neurocognitive Disorders

Bruce Shiramizu, **Andrew E. Williams**, **Cecilia Shikuma**, and **Victor Valcour**
Drs. Shiramizu, Shikuma, and Valcour are affiliated with the Hawaii AIDS Clinical Research Program, University of Hawaii, Honolulu, HI; Dr. Williams is affiliated with Kaiser Foundation Hospitals, Center for Health Research, Honolulu, HI

HIV DNA in $CD14^+$ reservoirs is associated with regional brain atrophy in patients naive to combination antiretroviral therapy

Kalpana J. Kallianpur[a,*], Victor G. Valcour[b,*], Sukalaya Lerdlum[c], Edgar Busovaca[b], Melissa Agsalda[a], Pasiri Sithinamsuwan[d], Thep Chalermchai[e], James L.K. Fletcher[e], Somporn Tipsuk[e], Cecilia M. Shikuma[a], Bruce T. Shiramizu[a], Jintanat Ananworanich[c,e,f], on behalf of the SEARCH 011 study group

Shiramizu B, et al. J Neuropsychiatry Clin Neurosci. 2009; Kallianpur KJ, et al. AIDS 2014.

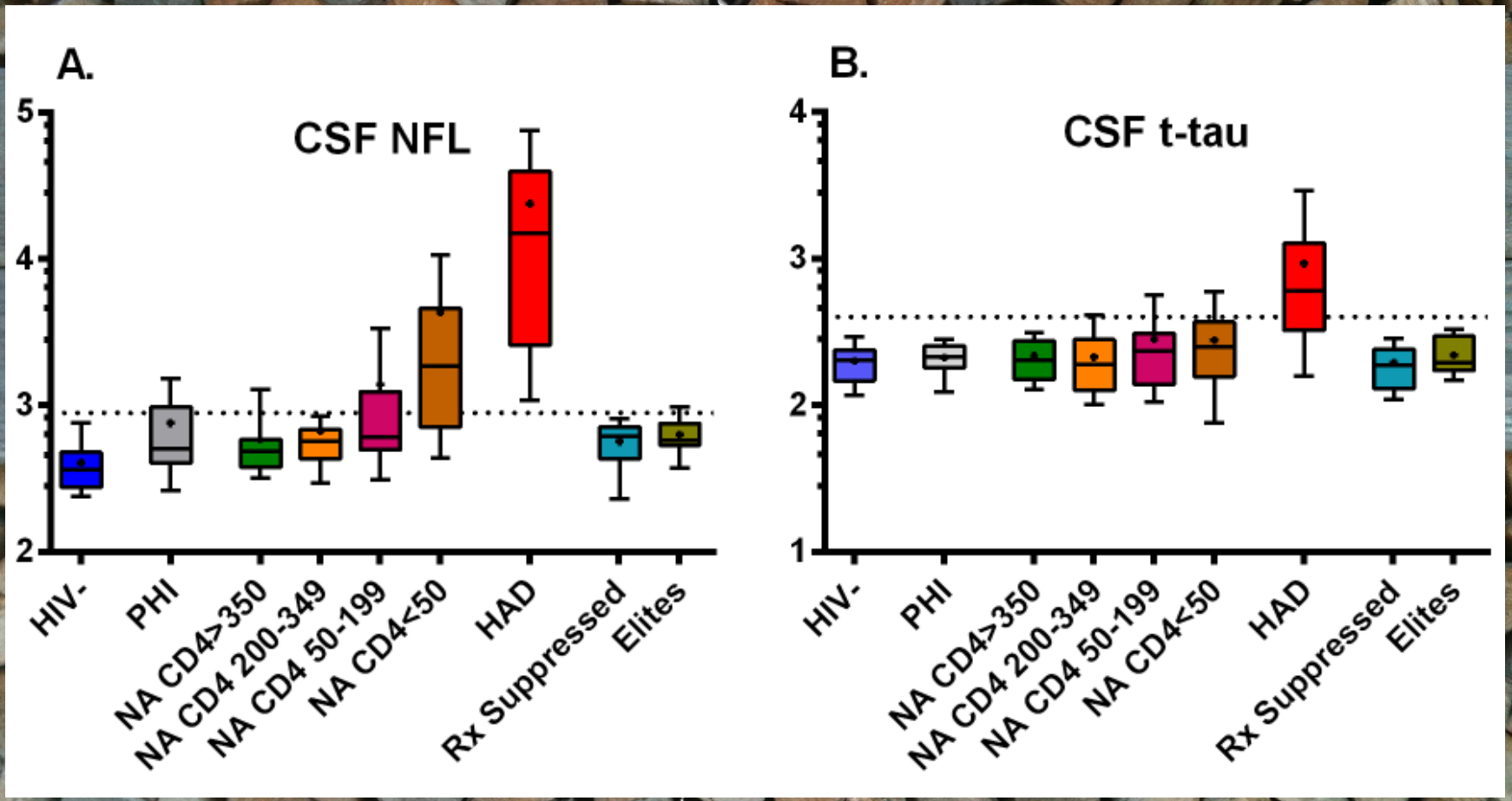
HIV DNA/NADIR CD4+

- CD4 nadir is a strong predictor of HAND

HIV DNA/NADIR CD4+

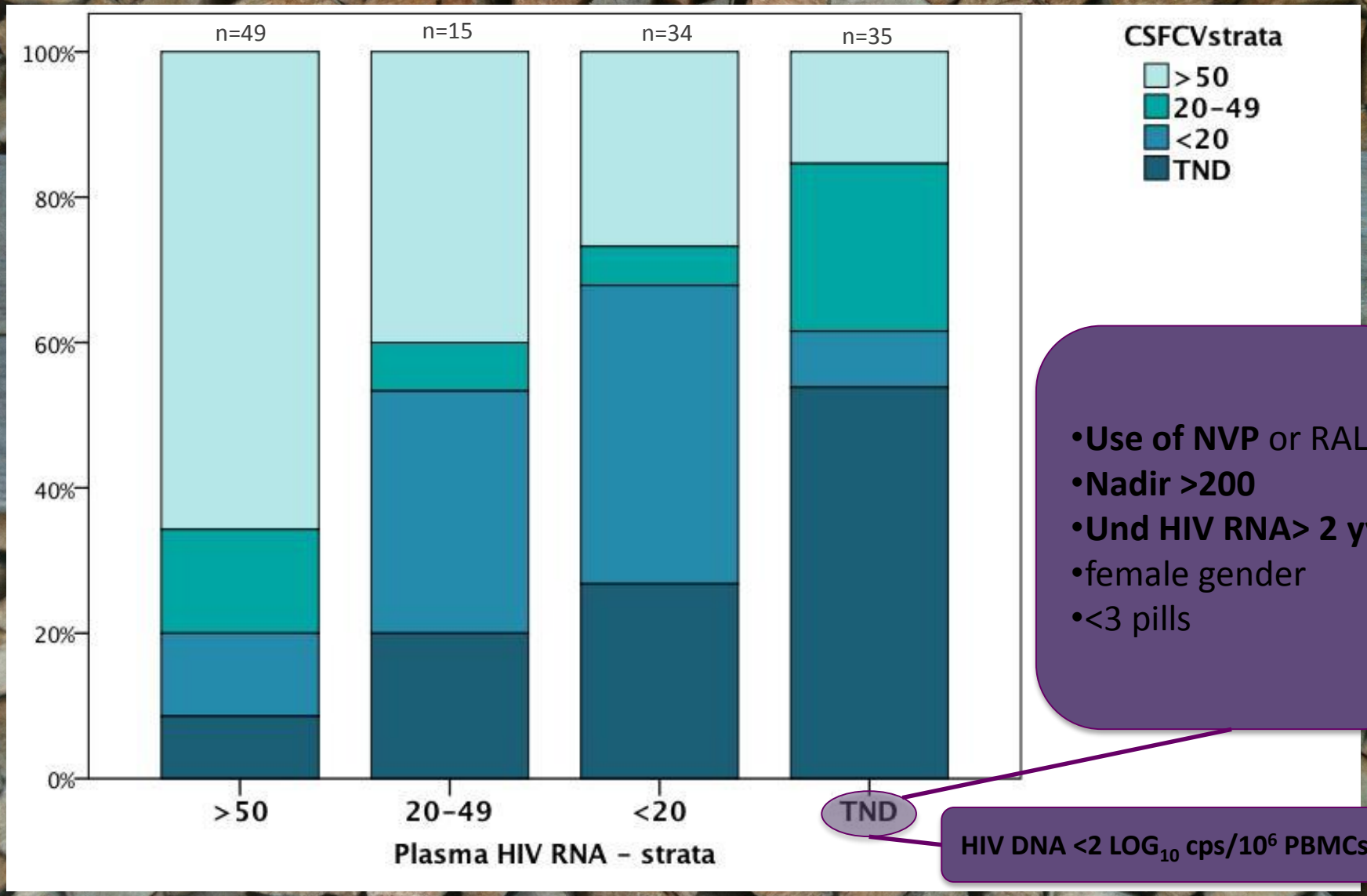
- CD4 nadir is a very strong predictor of HAND
- CD4 nadir is associated with failure to PI monotherapy
- HIV DNA is associated with failure to darunavir/ritonavir monotherapy
- Low CD4 nadir (17 and 166 cells/mm3) were associated with two cases of rise in CSF HIV RNA in the PROTEA study (one symptomatic)

Irreversible damage vs. full reservoires
DAMAGE MARKERS



Peterson J, et al. PlosOne 2014

Plasma TND?

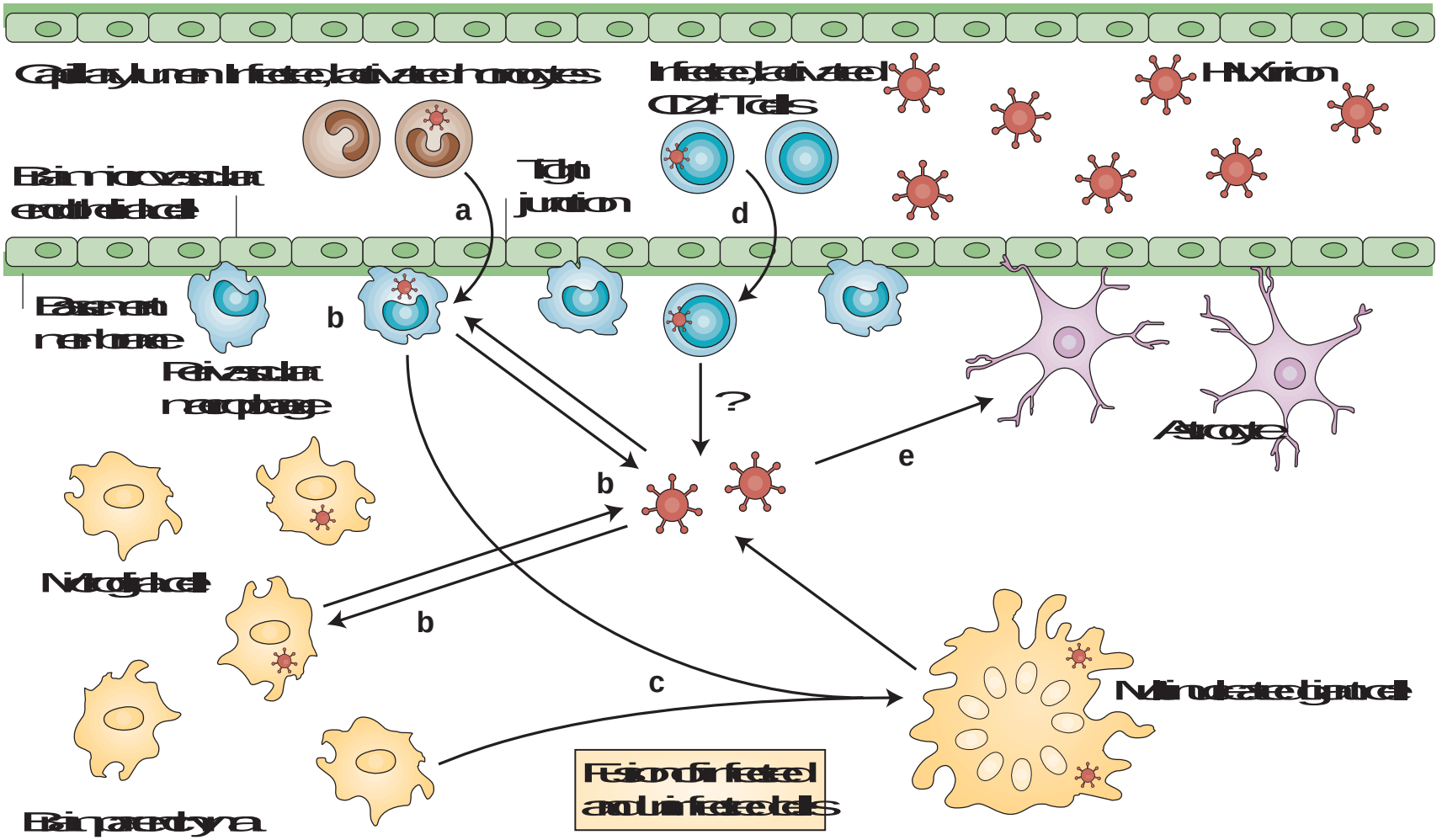


- **Use of NVP** or RAL
- **Nadir >200**
- **Und HIV RNA> 2 yy**
- female gender
- <3 pills

HIV DNA <2 LOG_{10} cps/10^6 PBMCs

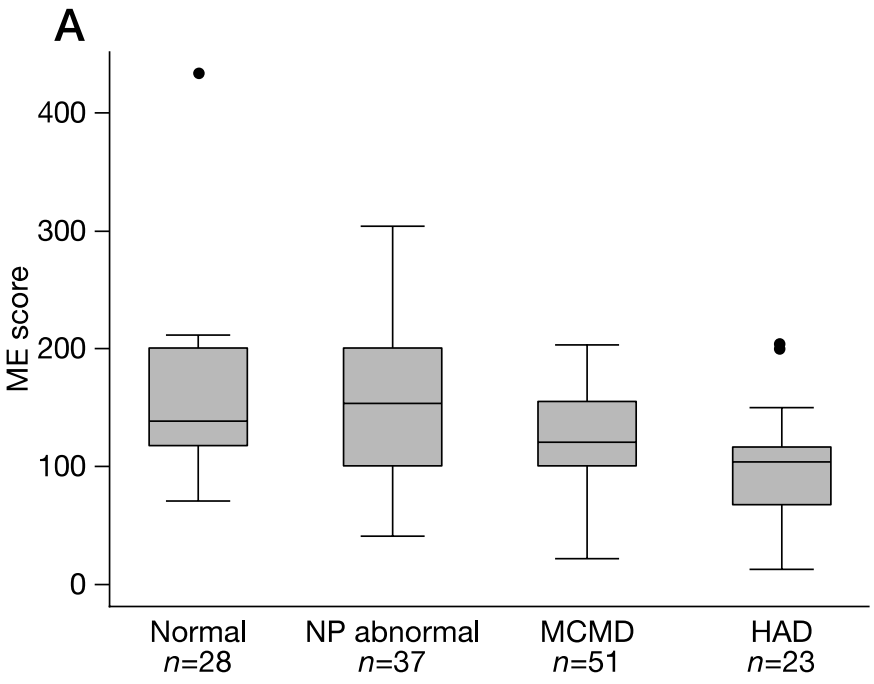
Calcagno A, et al. Personal Communication and Submitted

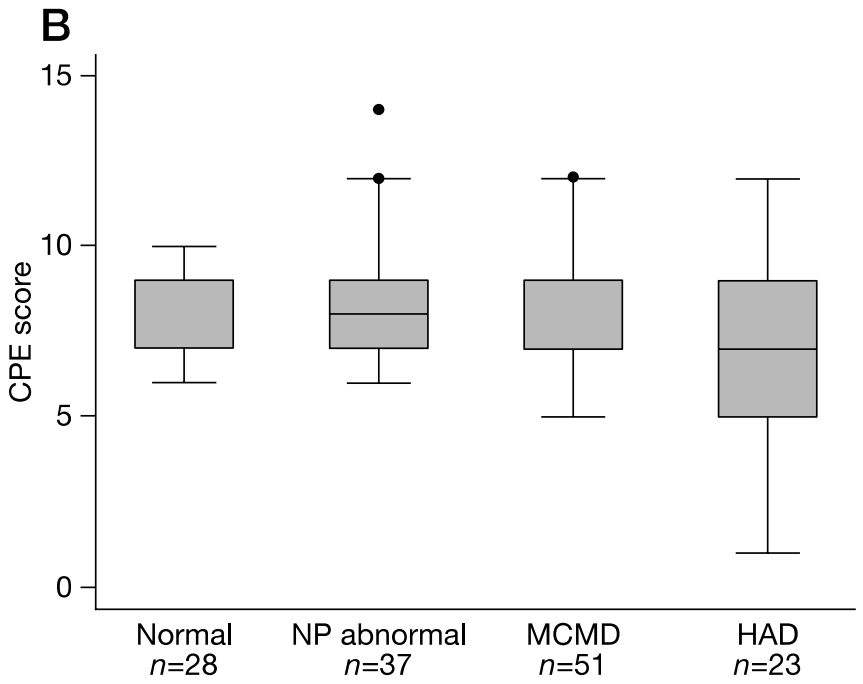
Monocyte-derived cells



Gonzalez-Scarano F, Nat Rev Immunol 2005

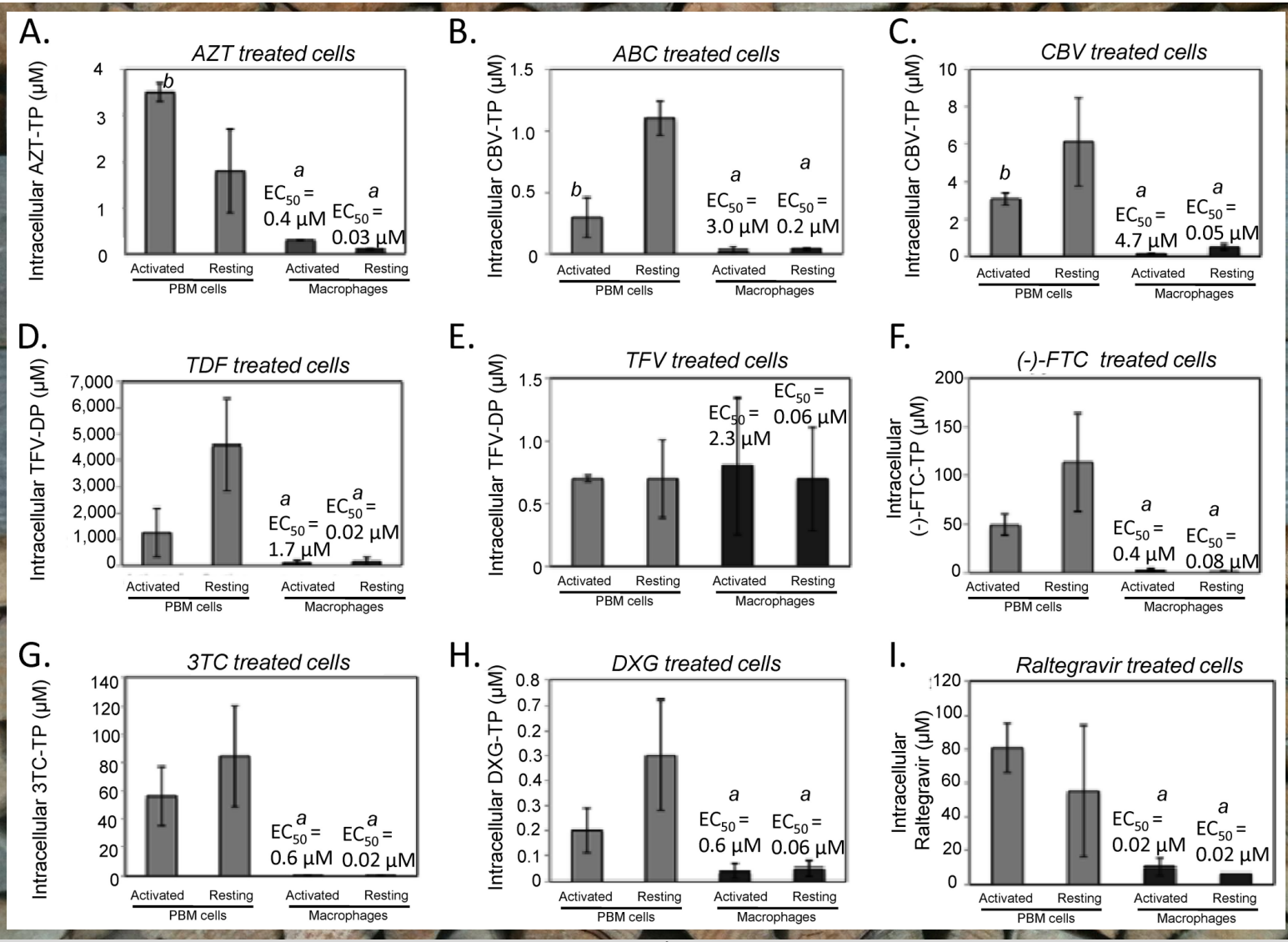
Monocyte efficacy





Shikuma C, et al. Antiviral Therapy 2012

Monocyte intracellular levels



Gavegnano C, et al. AAC 2013

Monocyte efficacy

Drug tested	State of cells	Active metabolite measured	Intracellular NTP or drug in PBM cells (μM) (±SD)	Intracellular NTP or drug in PBM cells (pmol/10^6 cells) (±SD)	EC_{50} in acutely infected PBM cells (μM) (±SD)	Intracellular NTP or drug in Mϕ (μM) (±SD)	Intracellular NTP or drug in Mϕ (pmol/10^6 cells) (±SD)	EC_{50} in acutely infected Mϕ (μM) (±SD)	EC_{50} in chronically infected Mϕ (μM)
AZT	Activated	AZT-TP	3.5 ± 0.9[b]	1.1 ± 0.3	0.004 ± 0.0022	0.3 ± 0.3[c]	0.8 ± 0.8	0.4 ± 0.04[d]	>50
	Resting	AZT-TP	1.8 ± 0.4	0.6 ± 0.1	NA	0.1 ± 0.1[c]	0.3 ± 0.3[c]	0.03 ± 0.007	>50
ABC	Activated	CBV-TP	0.3 ± 0.2[b]	0.1 ± 0.06	0.3 ± 0.2	0.03 ± 0.03[c]	0.08 ± 0.08[c]	3.0 ± 1.1[d]	>50
	Resting	CBV-TP	1.1 ± 0.1	0.4 ± 0.03	NA	0.04 ± 0.01[c]	0.1 ± 0.02[c]	0.2 ± 0.3	>50
CBV	Activated	CBV-TP	3.1 ± 0.3[b]	1.0 ± 0.1	0.08 ± 0.08	0.1 ± 0.1[c]	0.3 ± 0.3[c]	4.7 ± 3.2[d]	>50
	Resting	CBV-TP	7.7 ± 3.7	2.5 ± 1.2	NA	0.7 ± 0.3[c]	1.9 ± 0.8	0.05 ± 0.02	>50
TDF	Activated	TFV-DP	1.252 ± 896	400.6 ± 286	0.01 ± 0.01	160 ± 123[c]	425.6 ± 327	1.7 ± 1.2[d]	>50
	Resting	TFV-DP	4,589 ± 1,764	1,468.0 ± 564	NA	107 ± 144[c]	284.6 ± 383[c]	0.02 ± 0.02	>50
TFV	Activated	TFV-DP	0.7 ± 0.3	0.2 ± 0.1	1.6 ± 1.2	0.7 ± 0.6	1.9 ± 1.1	2.3 ± 0.9[d]	>50
	Resting	TFV-DP	0.7 ± 0.3	0.2 ± 0.1	NA	0.8 ± 0.4	2.1 ± 1.5	0.06 ± 0.03	>50
(−)-FTC	Activated	(−)-FTC-TP	49.2 ± 41.8	15.7 ± 3.4	0.008 ± 0.007	2.2 ± 2.0[c]	5.9 ± 5.0[c]	0.4 ± 0.2[d]	>50
	Resting	(−)-FTC-TP	113 ± 18.8	36.2 ± 6.0	NA	1.0 ± 0.6[c]	2.7 ± 1.6[c]	0.08 ± 0.02	>50
3TC	Activated	3TC-TP	56 ± 21	17.9 ± 6.7	0.06 ± 0.04	0.6 ± 0.4[c]	1.6 ± 1.0[c]	0.6 ± 0.3[d]	>50
	Resting	3TC-TP	84.5 ± 36	27.0 ± 11.5	NA	0.8 ± 0.3[c]	2.1 ± 0.8[c]	0.02 ± 0.01	>50
DXG	Activated	DXG-TP	0.2 ± 0.1	0.06 ± 0.03	0.3 ± 0.2	0.05 ± 0.04[c]	0.1 ± 0.1	0.6 ± 0.2[d]	>50
	Resting	DXG-TP	0.5 ± 0.2	0.2 ± 0.06	NA	0.04 ± 0.03[c]	0.1 ± 0.08[c]	0.06 ± 0.02	>50
RAL	Activated	Raltegravir	80.9 ± 14.7	25.9 ± 4.7	0.001 ± 0.002	10.5 ± 4.1[c]	27.9 ± 11.0	0.02 ± 0.02	>50
	Resting	Raltegravir	55.3 ± 24.1	17.7 ± 7.7	NA	7.0 ± 0.2[c]	18.6 ± 0.5[c]	0.02 ± 0.03	>50
ATV[e]	Activated	Atazanavir	ND	ND	0.007 ± 0.002	ND	ND	0.03 ± 0.03	0.09
	Resting	Atazanavir	ND	ND	NA	ND	ND	0.03 ± 0.04	0.06

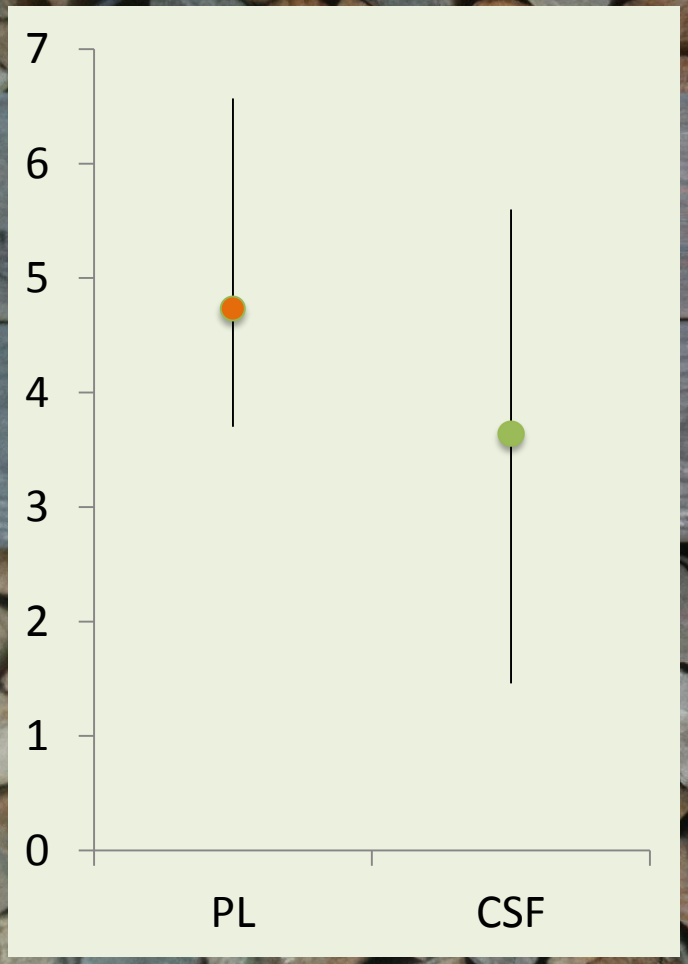
Gavegnano C, et al. AAC 2013

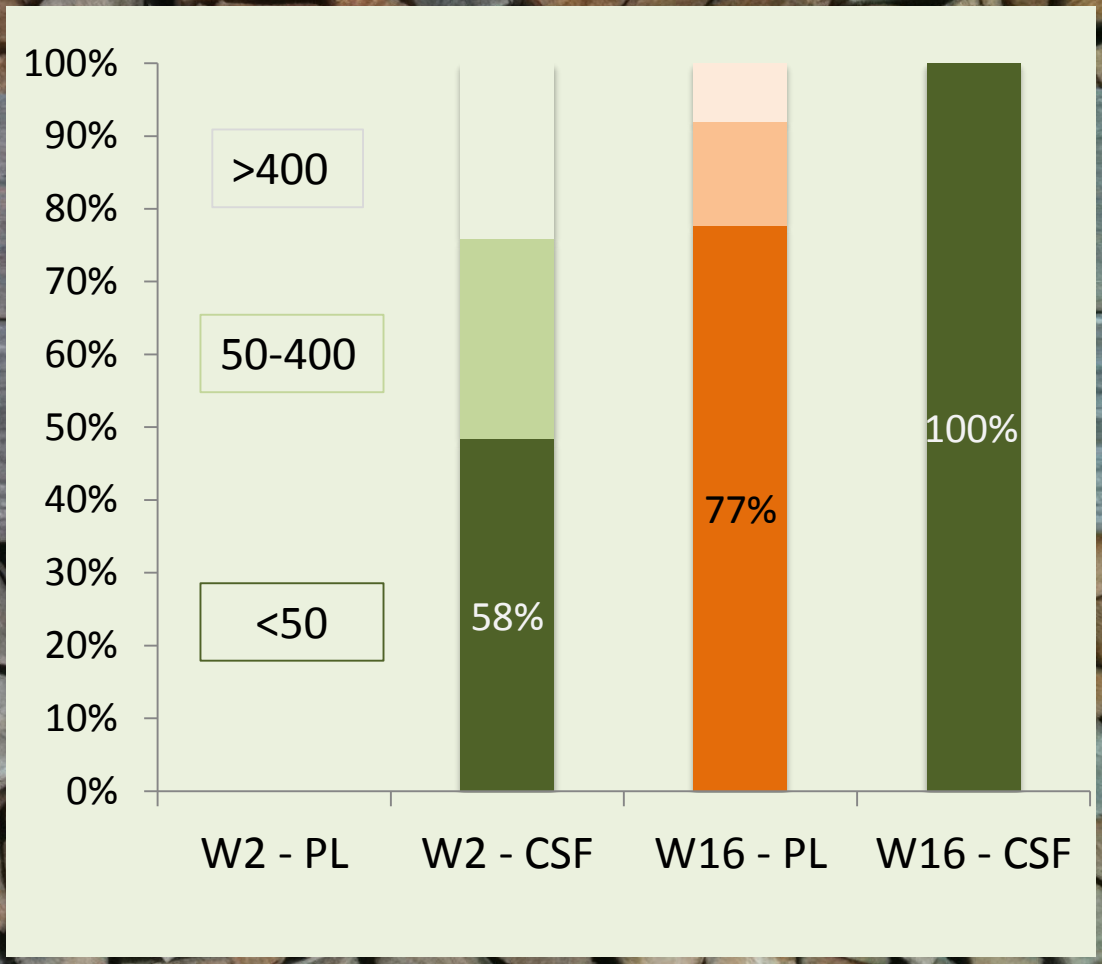
INSTI and Monocyte-derived cells

(nM)	EC_{50} PBMCs	EC_{90} PBMCs	EC_{50} MDM	EC_{90} MDM
RAL	1	14.5	0.3	6.9
DGV	2.7	14.8	1.1	5.5

Scopelliti F, et al. Antivr Res 2011, Pollicita M, et al. JAC 2014

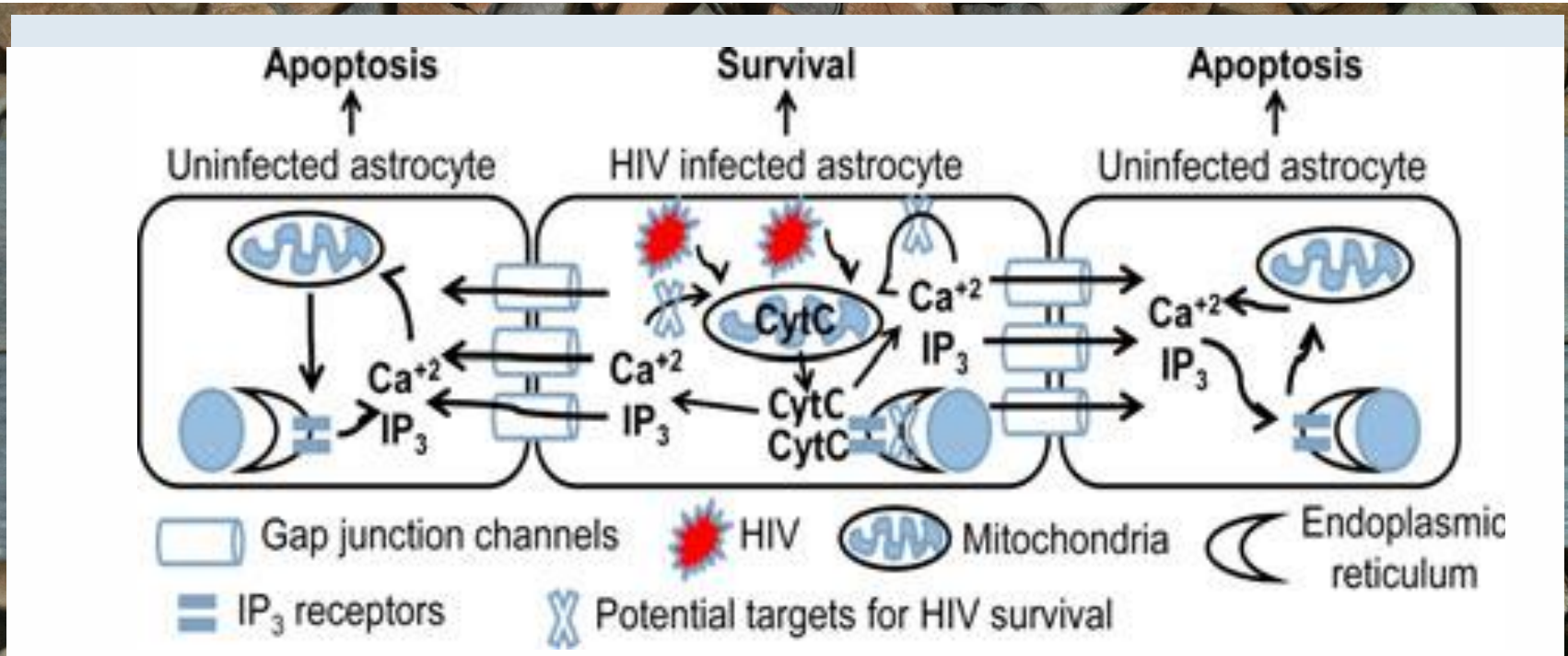
CSF decay and KVX + DGV

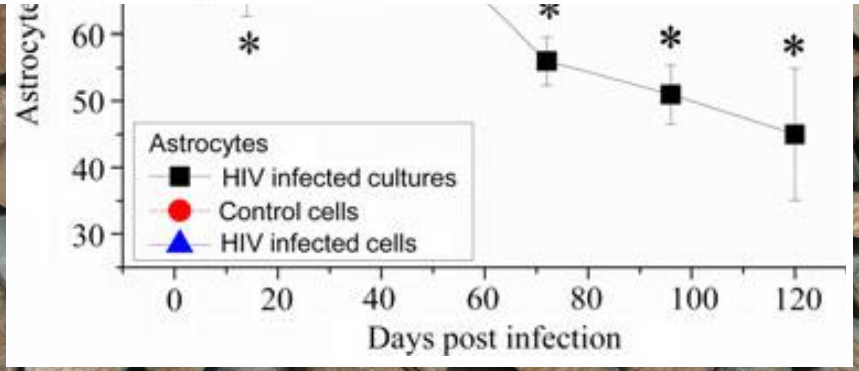




Letendre S, et al. CID 2014

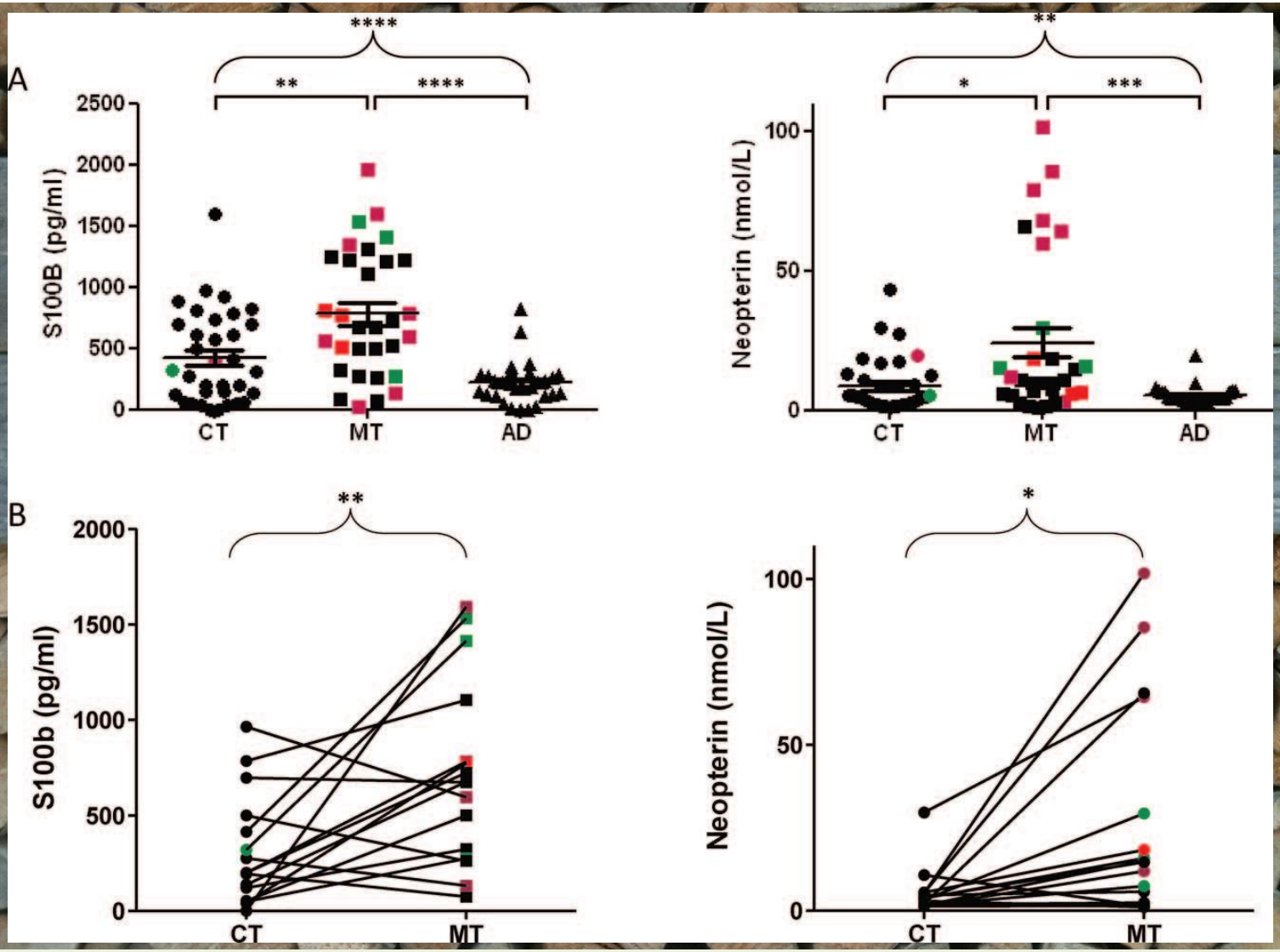
Astrocytes and HIV





Eugenin EA, J Neurochemistry 2013

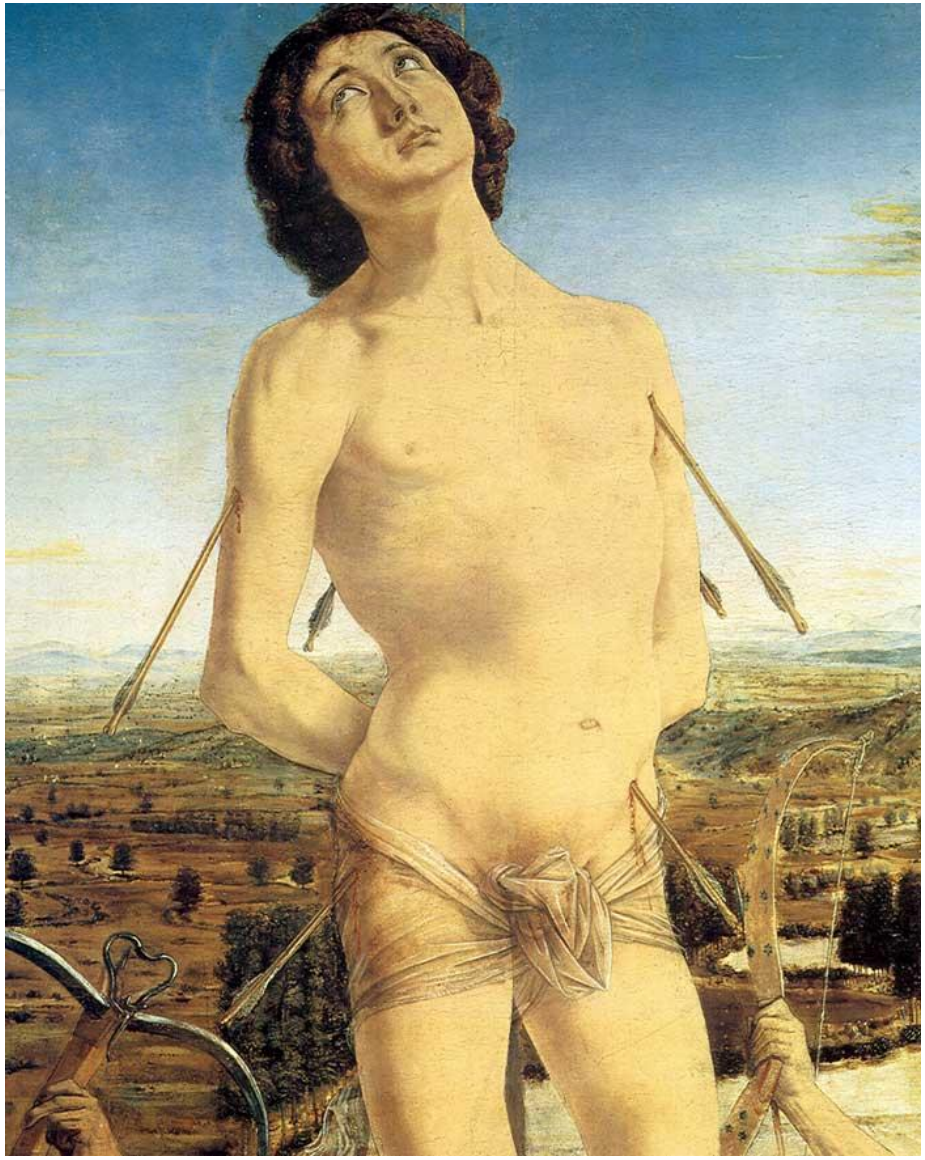
removing NRTIs → S100beta



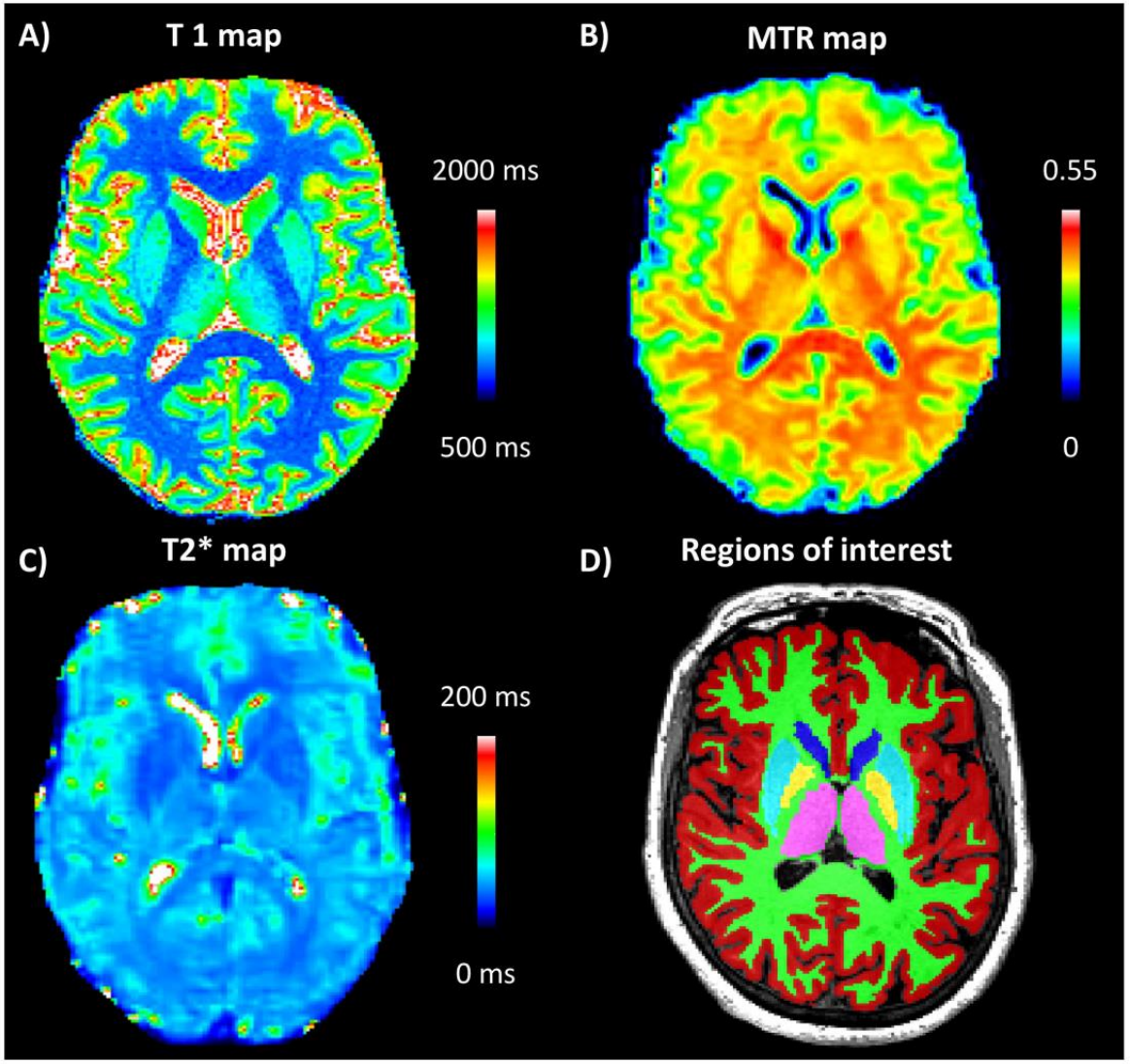
du Pasquier R, et al. JAIDS 2012


OTHER TARGETS?

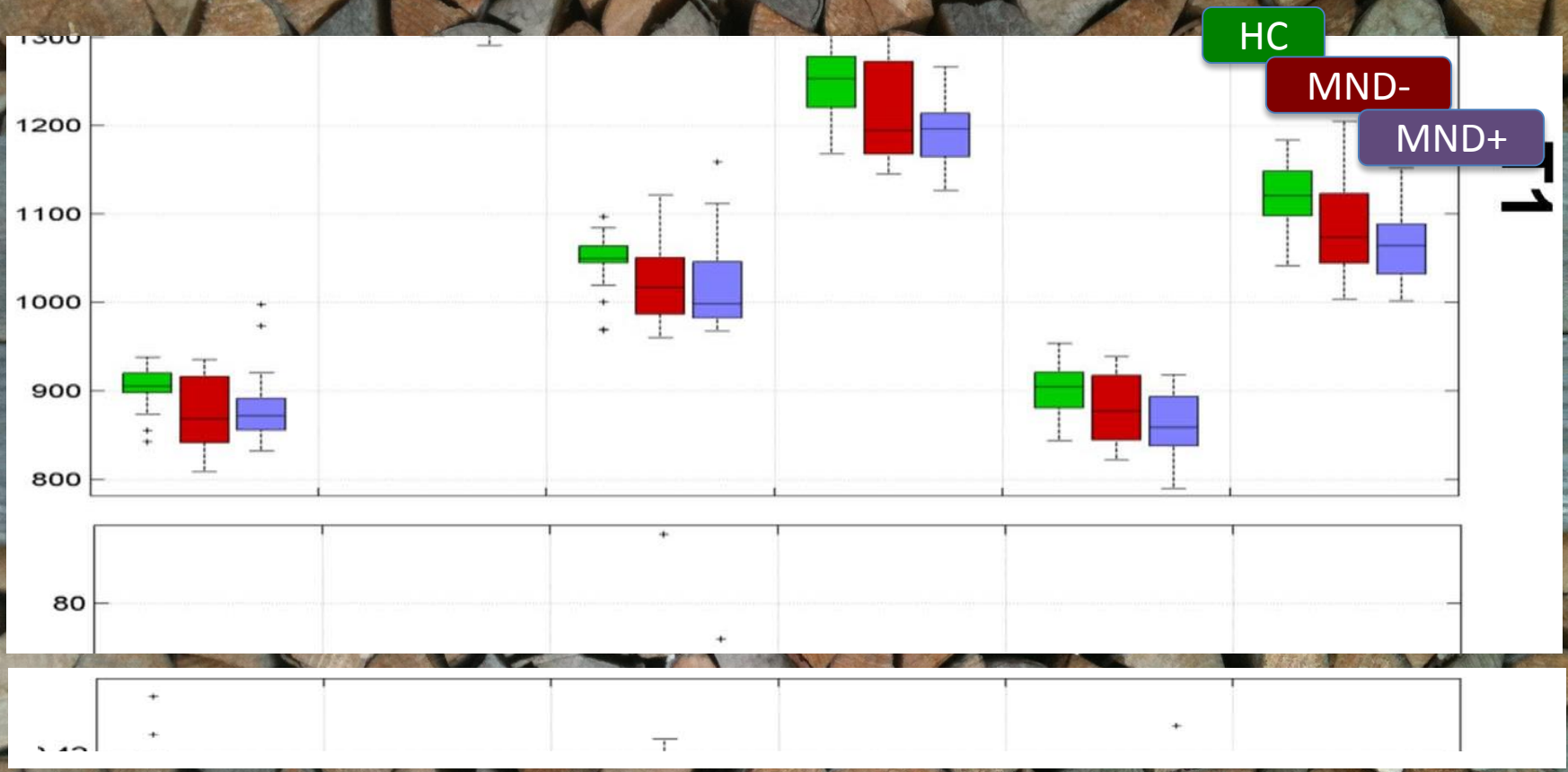

shutterstock



Advanced MRI – T3 and Spectro



Advanced MRI – T3 and Spectro



Multiple regression analysis showed a significant influence of sub-cortical nuclei alterations on the executive index of MND+ patients ($p = 0.04$ and $R^2 = 95.2$).

Granziera G, Plos One 2014

MR - PERFUSION

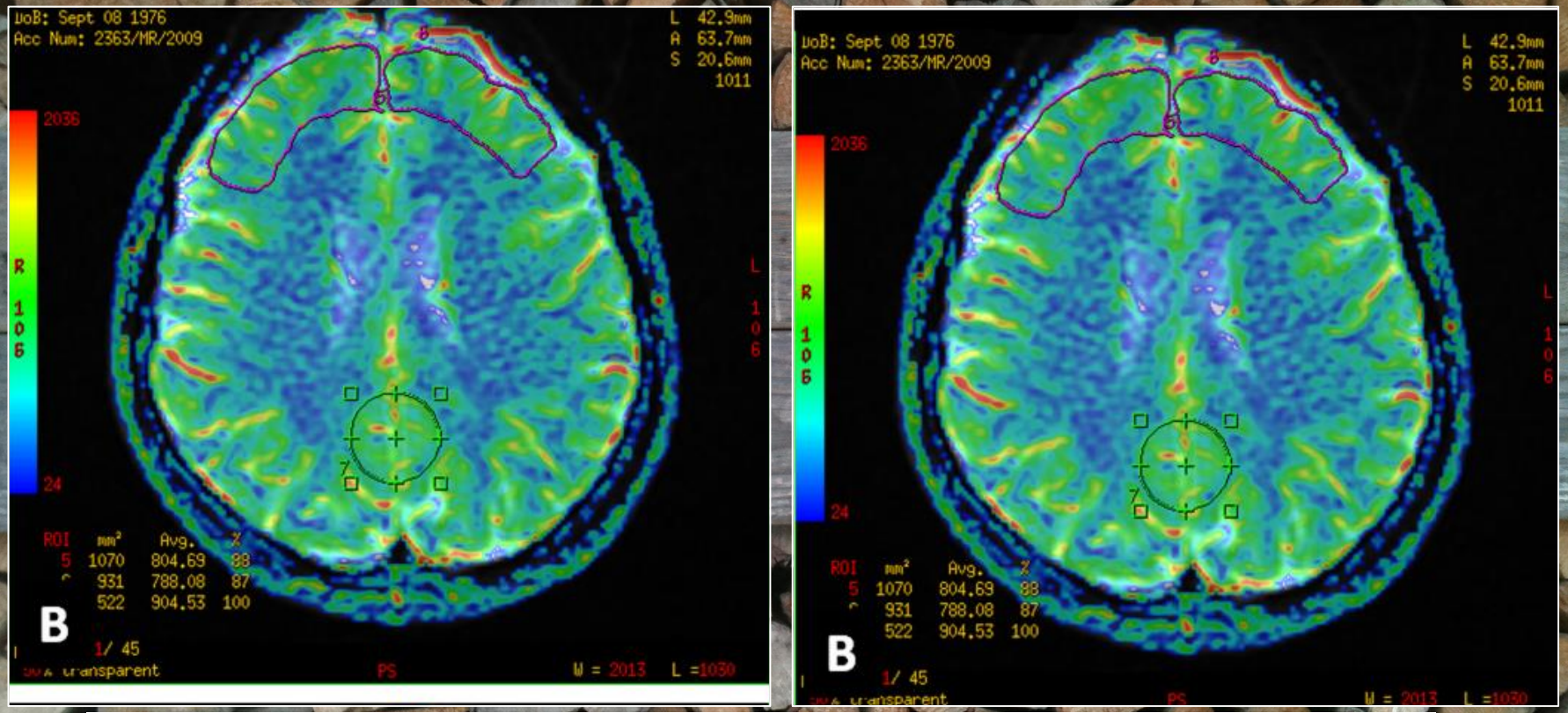


Table 3. Comparisons of rCBV measurements in the basal ganglia regions among HIV-1 naive, HIV-1 cART treated, HIV-1/HCV and HCV naive patients with the results of the post hoc Tukey LSD test (p values).

Location	G1 vs G2	G1 vs G3	G1 vs G4	G2 vs G3	G2 vs G4	G3 vs G4
Right basal ganglia (BG)	0.844	0.976	**<0.0001**[a,b]	0.857	**<0.0001**[a,b]	**0.0003**[a,b]
Left basal ganglia (BG)	0.218	0.537	**<0.0001**[a,b]	0.752	**<0.0001**[a,b]	**<0.0001**[a,b]

Bladowska J, et al. PlosOne 2014

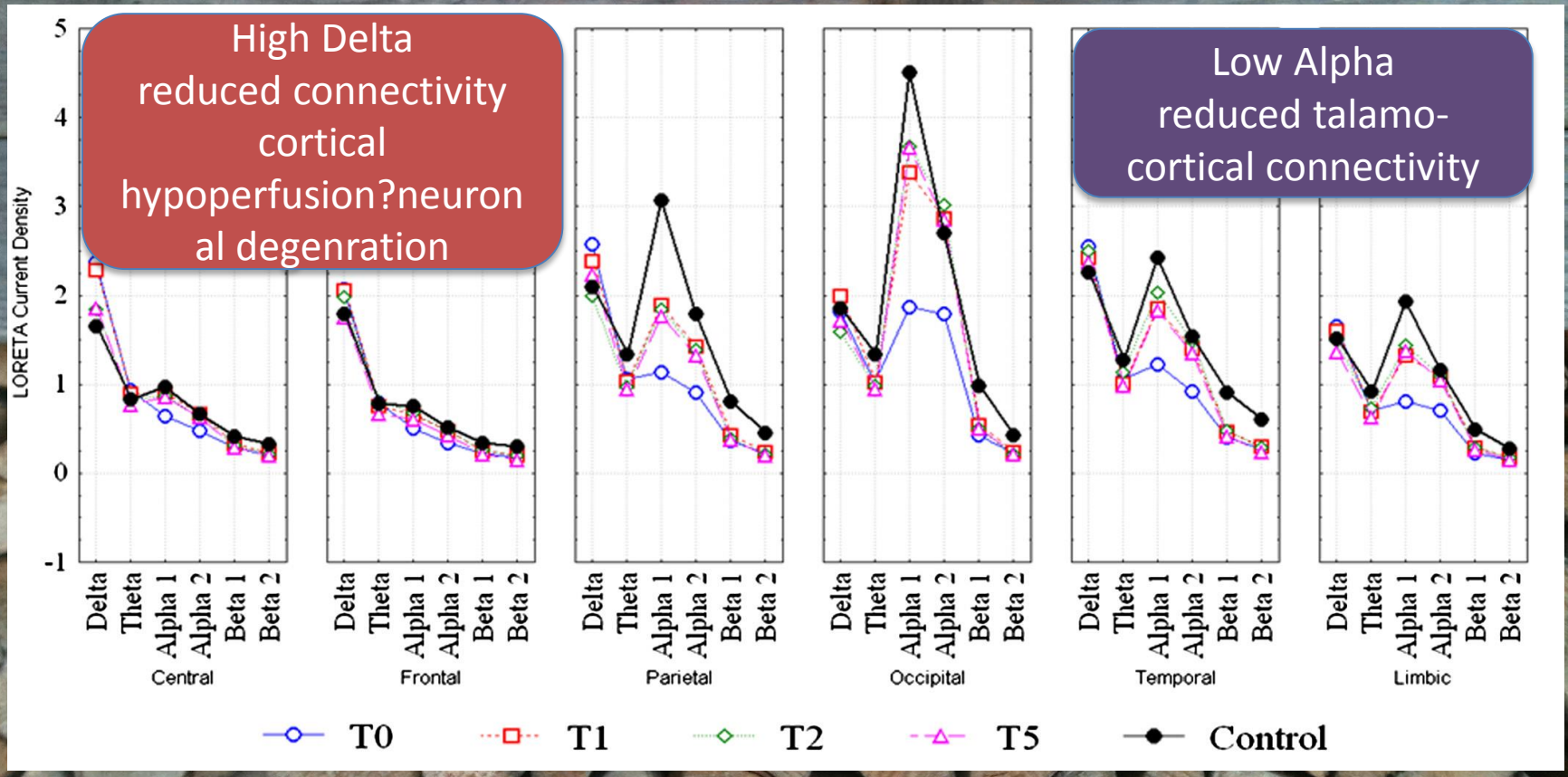
Advanced MRI for monitoring

- MARAVIROC intensification (based on CSF tropism, macaques data):
  - slight increase in NAA/Cr (neuronal integrity), Cho/Cr (membrane integrity) and mI/Cr (glial integrity) in Basal Ganglia
  - **increase in Naa/Cr directily proportional to MVC plasma concentrations**
  - reduction in CSF IP-10
  - reduction in CD16+ cells (monocytes) and HIV DNA content in monocytes

Garvey L, et al. JAC 2012; Vera JH, et al. HIV Clin Trials 2012; Ndhlovu LC, et al. JNV 2014

EEG for monitoring?

resting state scalp EEG rhythms through the mathematical estimation of the cortical sources of these rhythms by
low resolution brain electromagnetic tomography (LORETA)



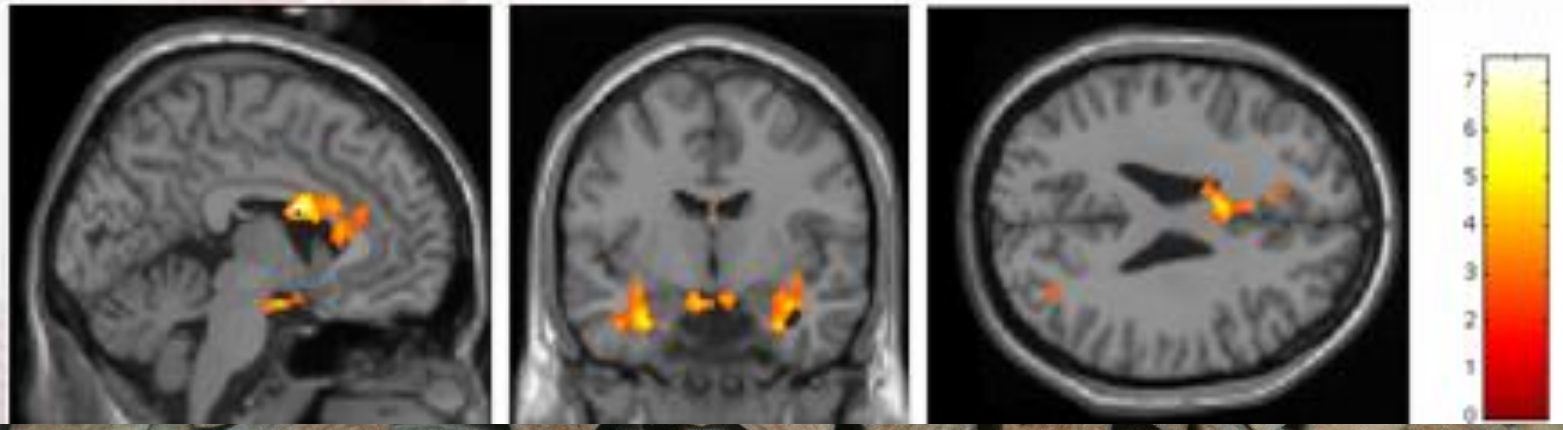
Babiloni C, et al. Clinical Neurophysiology 2014

PET imaging – PK11195

Subjects:
- HIV infected on cART
- Truly neuroasymptomatic

Increase binding PK11195 neuroasymptomatic HIV infection compared to HIV uninfected controls (n=7, 9)

Location of cluster	Z-score	MNI coordinates x	y	z	P-value
L corpus callosum	4.61	-4	4	22	0.001
R anterior cingulate	3.28	6	12	10	0.001
R temporal lobe	3.60	26	0	-32	0.001
Posterior corpus callosum/ L posterior cingulate	3.90	-4	-56	2	0.008
L temporal lobe	3.83	-40	-2	-28	0.026
L frontal lobe	3.82	-12	4	-16	0.038



Garvey L, et al. AIDS 2012

CONCLUSIONS

- Antiretroviral concentrations in the CNS must be challenged with PD targets;
- From a pharmacodynamic point of view IC_{95} may be useful for comparing different drugs;
- Intracellular (intra macrophages) levels and activity may differ from what observed in PBMCs;
- Other PD markers may be useful but need to be prospectively validated (MRI, EEG, NPS tests, etc).

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