HOW TO ASSESS NEUROTOXICITY?

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DISCLOSURES

I have read and understood ICMJE policy on declaration of interest and I declare that I have no conflicting interest

In the past five years I received:

- research grants from Gilead, Viiv and BMS;
- speaker's honoraria from Abbvie, BMS, Gilead, Janssen-Cilag, MSD, Viiv.

EDITORIAL REVIEW

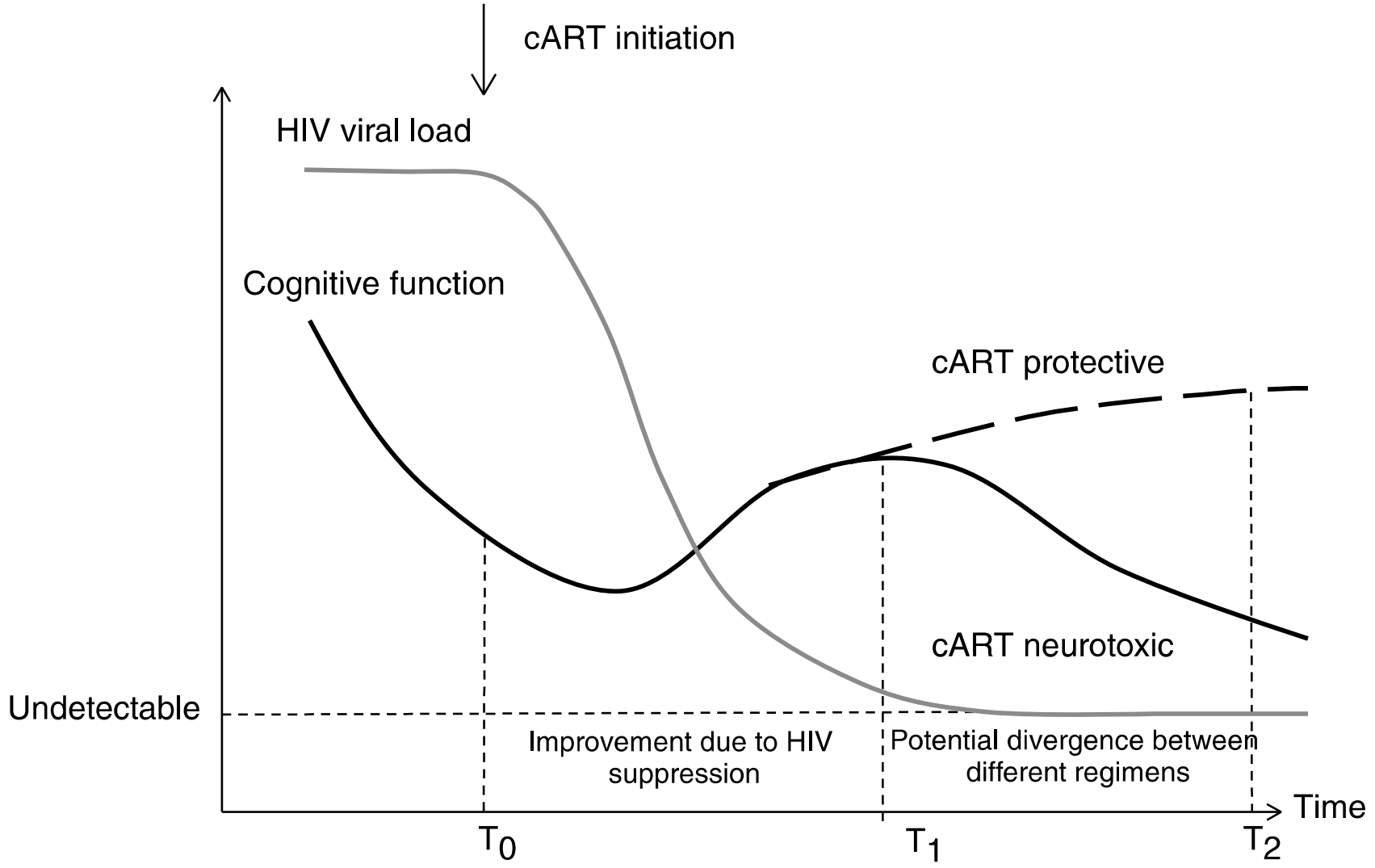
Could antiretroviral neurotoxicity play a role in the pathogenesis of cognitive impairment in treated HIV disease?

Jonathan Underwood[a], Kevin R. Robertson[b] and Alan Winston[a]

Whilst effective antiretroviral therapy is protective against the more severe forms of HIV-associated brain disease, there remains a large burden of clinically symptomatic cognitive impairment in the modern era. Although several potential pathogenic mechanisms have been proposed, the underlying pathology remains elusive. In this review, we summarize the evidence describing neuronal toxicity of antiretroviral agents themselves in both preclinical and clinical situations, as well as the potential pathological mechanisms underlying this toxicity. We also consider the implications for future practice and clinical research in which case determining optimal antiretroviral combinations that effectively suppress HIV replication whilst minimizing neurotoxic effects on the central nervous system may become paramount.

AIDS 2015, **29**:253–261

NEUROTOXICITY – A POTENTIAL MODEL



Underwood J, et al. AIDS 2015

THE EFAVIRENZ PARADIGM

- It took approximately 15 years to recognize efavirenz *in vivo (*despite earlier evidence *of in vitro* neuronal toxicity*)*
  - Detrimental effect on neurocognition
    - Full neurocognitive tests
    - Spectroscopic MRI
  - Association with suicidal ideation
    - Questionnaires - PROs

Tovar-y-Romo LB, et al. J Pharmacol Exp Ther, 2012; Robertson K, et al. Neurology 2010; Ciccarelli N, et al. Neurology 2011; Winston A, et al. Plos One 2015

MECHANISMS OF ANTIRETROVIRALS' CNS TOXICITY

① Direct Neuronal toxicity
- *In vitro*
- in macaques

② **Beta amyloid** metabolism interference

③ **Astrocytes** and blood brain barrier

④ **Olygodendrocytes** and myelin

⑤ Indirect effect on cerebral **blood vessels**

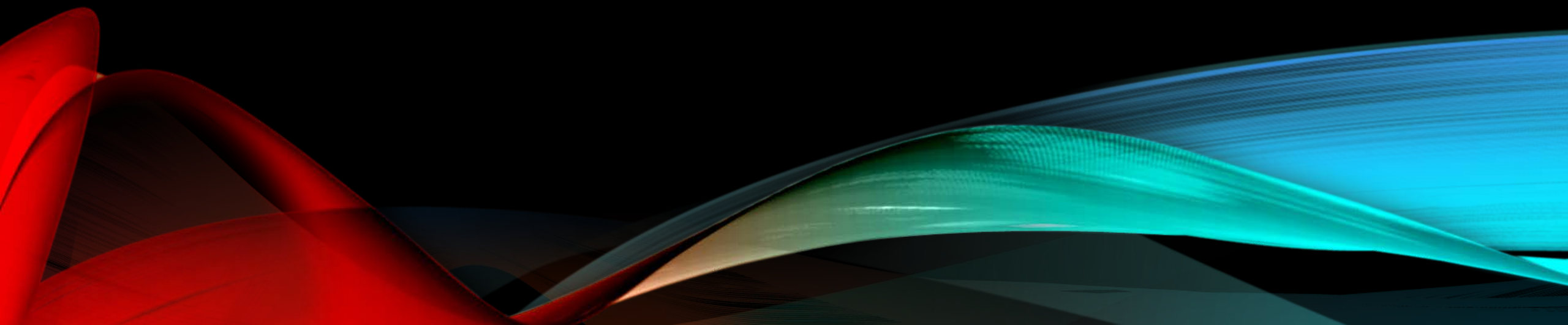
⑥ Efavirenz (and 8-08-EFV)

⑦ Impairment in mitochondrial function

⑧ Interference with neurotransmitters

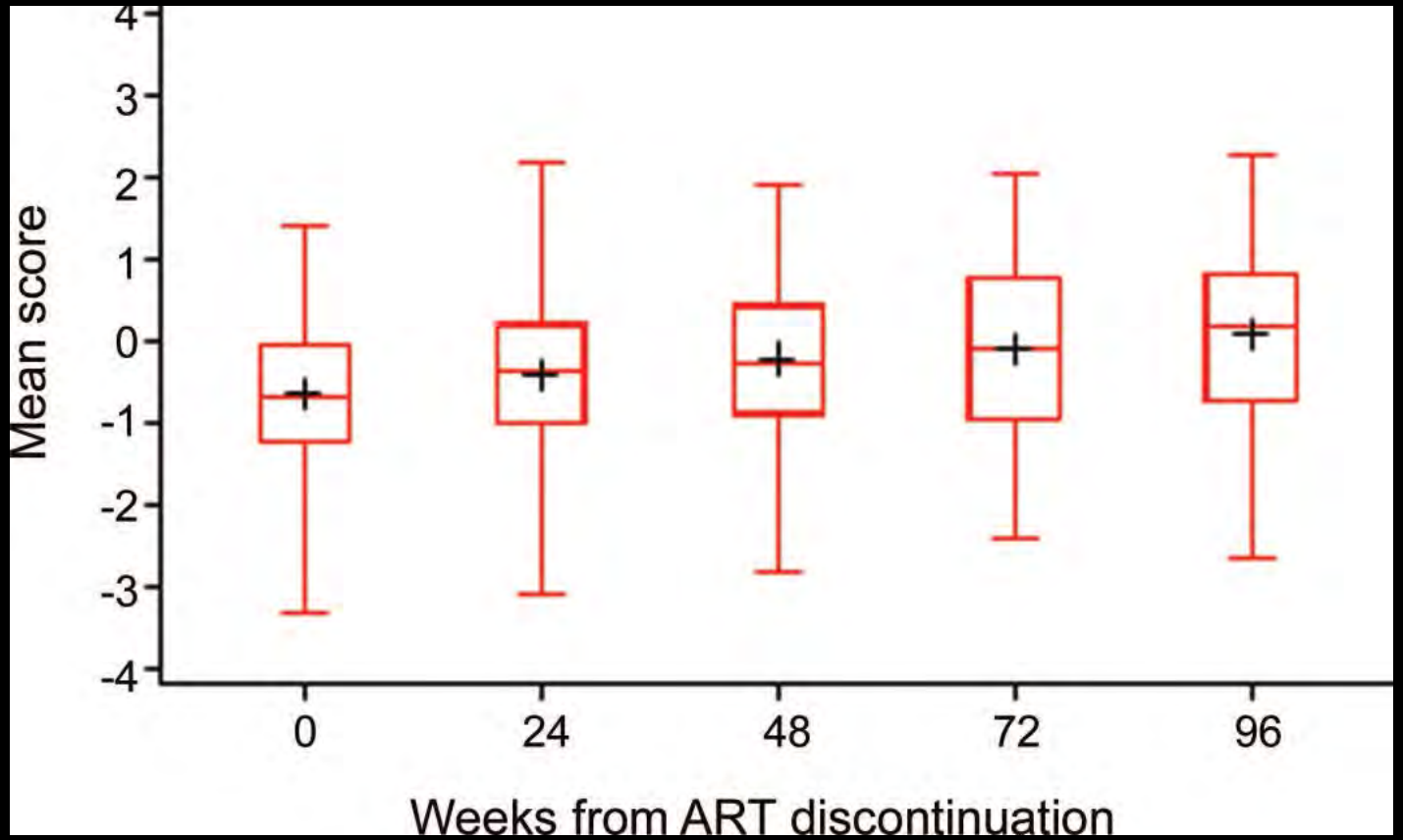
Tovar-y-Romo LB, et al. J Pharmacol Exp Ther, 2012; Robertson K, et al. JNV 2012; Akay C, et al. JNV 2014; Giunta B, et al. Mol Brain 2011; Robertson K, et al. Neurology 2010; Achim CL, et al. J Neuroimmune Pharmacol 2009; Ortega M and Ances BM, J Neuroimmune Pharmacol 2014; Ciccarelli N, et al. Neurology 2011; Vivithanaporn P, et al. AIDS 2016

1. FULL NEUROCOGNITIVE TESTS



NP IMPROVEMENT IN TREATMENT DISCONTINUATION

- 167 pts with CD4>350, HIV RNA <55000 copies/mL
- Elected to discontinue HAART
- Trail Making (A/B) and Digit Symbol mostly
- Greater benefit in those stopping EFV



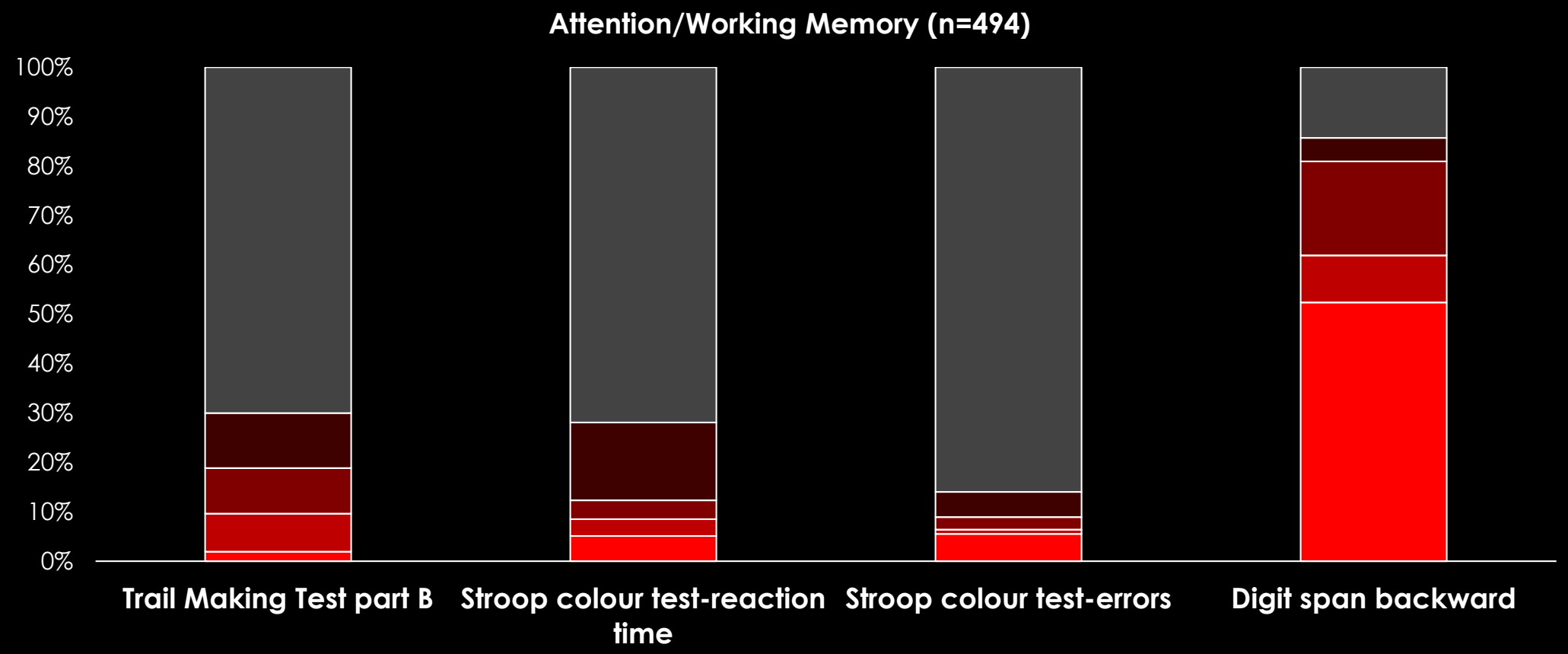
Robertson K, et al. Neurology 2010

EFV AND SPECIFIC AREAS?

	Patients (n = 146)	Controls (n = 30)	p^a
Mini-Mental State Examination	28.00 (1.88)	29.10 (0.88)	<0.001[b]
Immediate recall of Rey's words	37.90 (8.56)	42.3 (8.01)	0.010
Delayed recall of Rey's words	7.66 (2.78)	8.77 (2.62)	0.046
Digit span (forward)	5.61 (1.05)	5.77 (1.19)	0.488
Digit span (backward)	4.19 (1.26)	4.47 (1.33)	0.283
Spatial span (forward)	4.77 (0.92)	5.43 (0.90)	<0.001[b]
Spatial span (backward)	3.90 (1.00)	4.10 (1.18)	0.315
Spatial supraspan (% pathologic performance)	55/124 (44.4)	10/30 (33.3)	0.373
Constructional task (Rey's figure)	31.72 (3.91)	32.71 (2.64)	0.187
Delayed recall of Rey's figure	13.24 (6.07)	15.09 (5.35)	0.136
Stroop test (errors)	1.25 (1.86)	0.60 (0.87)	0.004
Stroop test (time)	19.19 (10.01)	21.65 (12.37)	0.242
Trail-Making Test B (time)	147.30 (62.28)	130.14 (48.39)	0.170
Trail-Making Test B (errors)	0.88 (1.13)	0.78 (0.92)	0.669
Drawings	4.71 (1.80)	5.17 (1.44)	0.191
Raven's matrices	29.11 (4.89)	30.78 (3.97)	0.083
Letter fluency	33.84 (12.23)	37.47 (11.47)	0.138
Wais Digit Symbol	8.62 (2.44)	9.57 (2.58)	0.057
Double barrage	0.95 (0.095)	0.98 (0.018)	0.087
Number of tasks with score below the cutoff	2.84 (2.60)	1.40 (0.97)	<0.001[b]

Ciccarelli N, et al. Neurology 2011

SPECIFIC TESTS?



Unpublished

2. MRI, MRS AND FMRI

MECHANISMS OF ANTIRETROVIRALS' CNS TOXICITY

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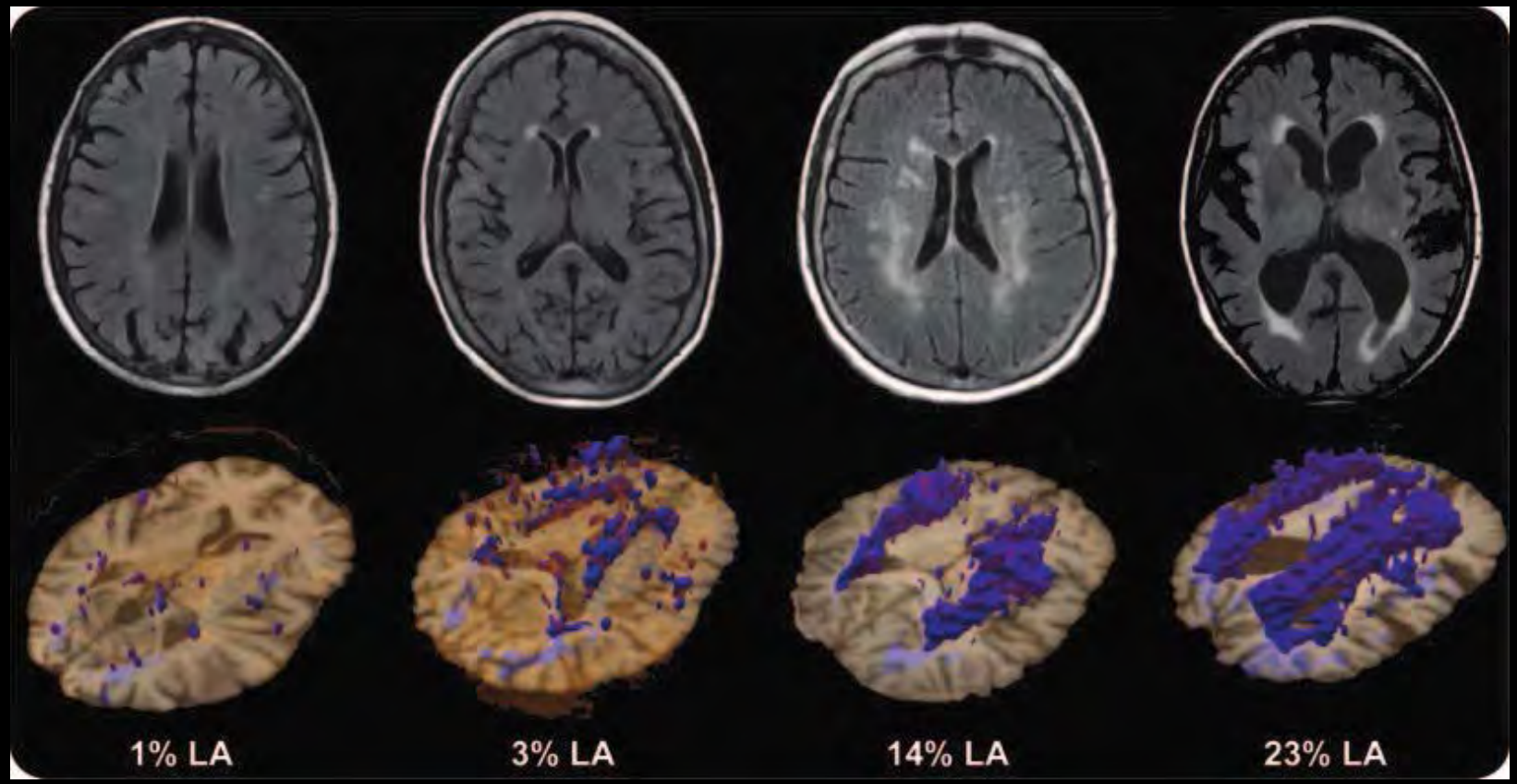
MRS

MRI, fMRI

Tovar-y-Romo LB, et al. J Pharmacol Exp Ther, 2012; Robertson K, et al. JNV 2012; Akay C, et al. JNV 2014; Giunta B, et al. Mol Brain 2011; Robertson K, et al. Neurology 2010; Achim CL, et al. J Neuroimmune Pharmacol 2009; Ortega M and Ances BM, J Neuroimmune Pharmacol 2014; Ciccarelli N, et al. Neurology 2011; Vivithanaporn P, et al. AIDS 2016

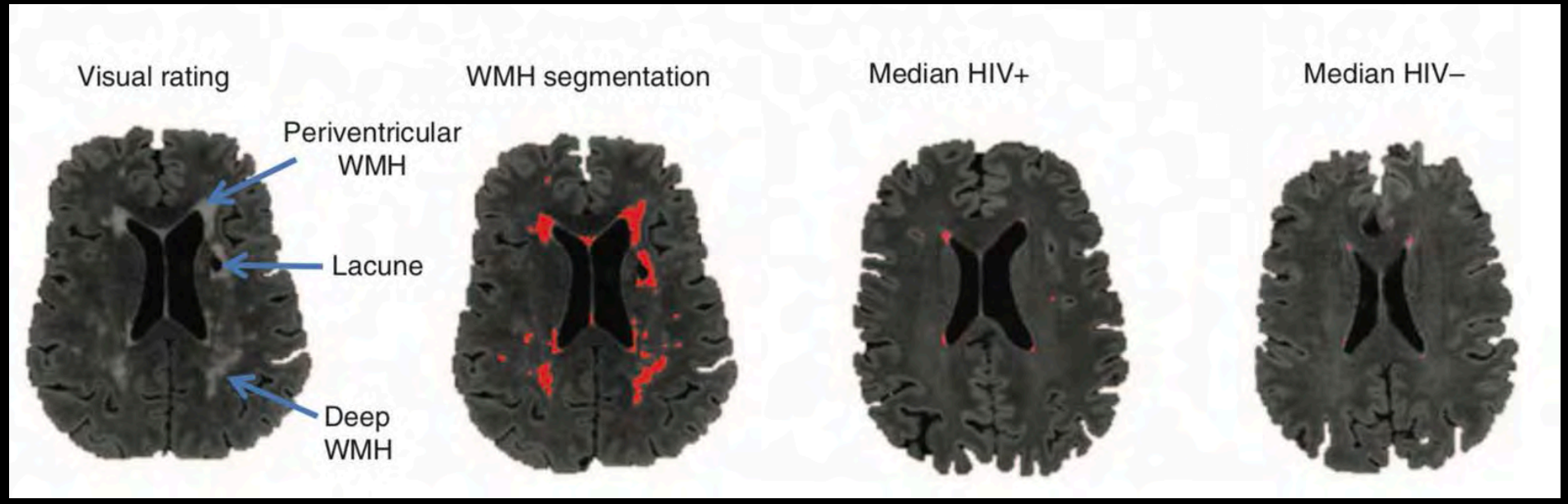
WHITE MATTER HYPERINTENSITIES **HIV-**

Heterogeneous abnormalities in White Matter as lacunar infarcts, micro-bleeding, alterations in perivascular spaces and linked to **small vessel cerebral disease**



Price CC, et al. Neurology 2012; Huijts M, et al. Front Ageing Neurosci 2013

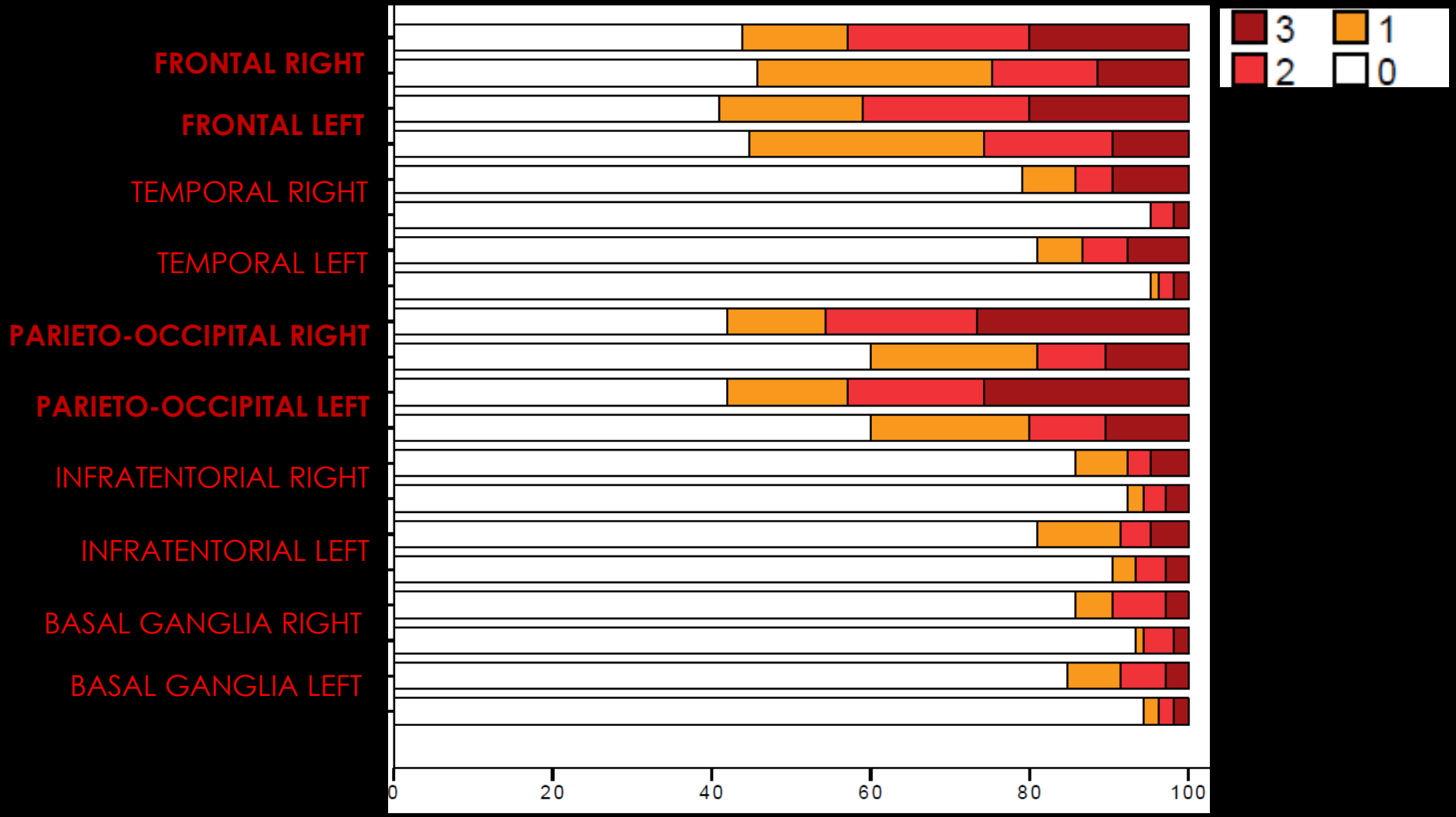
WHITE MATTER HYPERINTENSITIES PLWH



	Outcome measure: cognitive function[a]								
	Model 1			Model 2*			Model 3#		
	ß (95% CI)	*P*	η^2	ß (95% CI)	*P*	η^2	ß (95% CI)	*P*	η^2
HIV serostatus (0/1)[b]	−0.29 (−0.55–−0.03)	0.03	0.03	−0.29 (−0.55–−0.04)	**0.03**	0.03	−0.23 (−0.49–0.02)	**0.07**	0.02
Diabetes mellitus (0/1)[c]	–	–	–	−0.60 (−1.17–−0.03)	**0.04**	0.02	−0.56 (−1.13–0.01)	**0.05**	0.02
Age (years)	–	–	–	−0.011 (−0.028–0.006)	0.20	0.01	0.00 (−0.020–0.020)	0.99	0.0001
D-dimer (mg/l)	–	–	–	−0.43 (−1.04–0.19)	0.17	0.01	−0.28 (−0.90–0.34)	0.37	0.005
DBP (mmHg)	–	–	–	0.004 (−0.010–0.017)	0.60	0.002	0.006 (−0.007–0.020)	0.35	0.005
Total WMH load[d]	–	–	–	–	–	–	−0.33 (−0.64–−0.02)	**0.04**	0.03

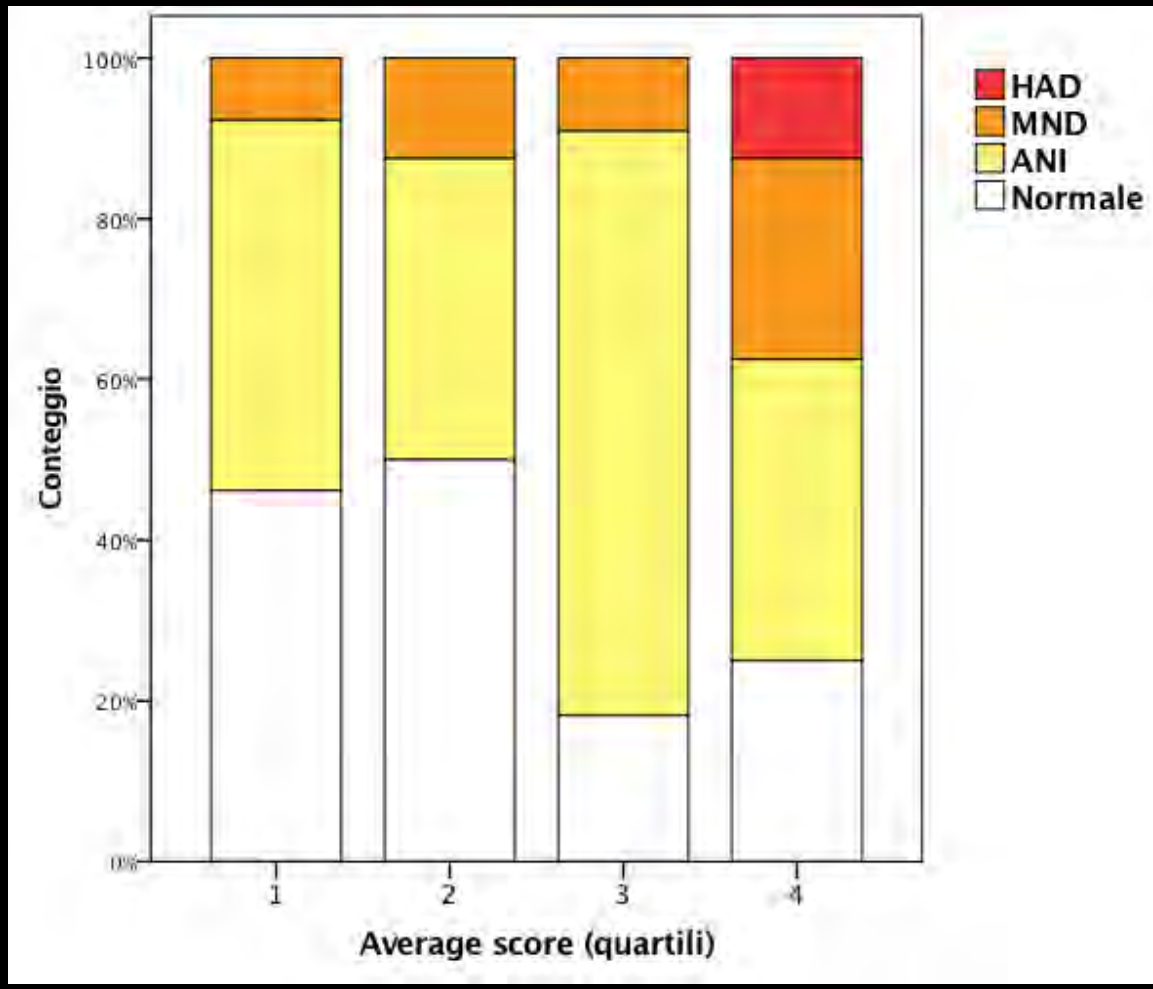
Su T, et al AIDS 2016

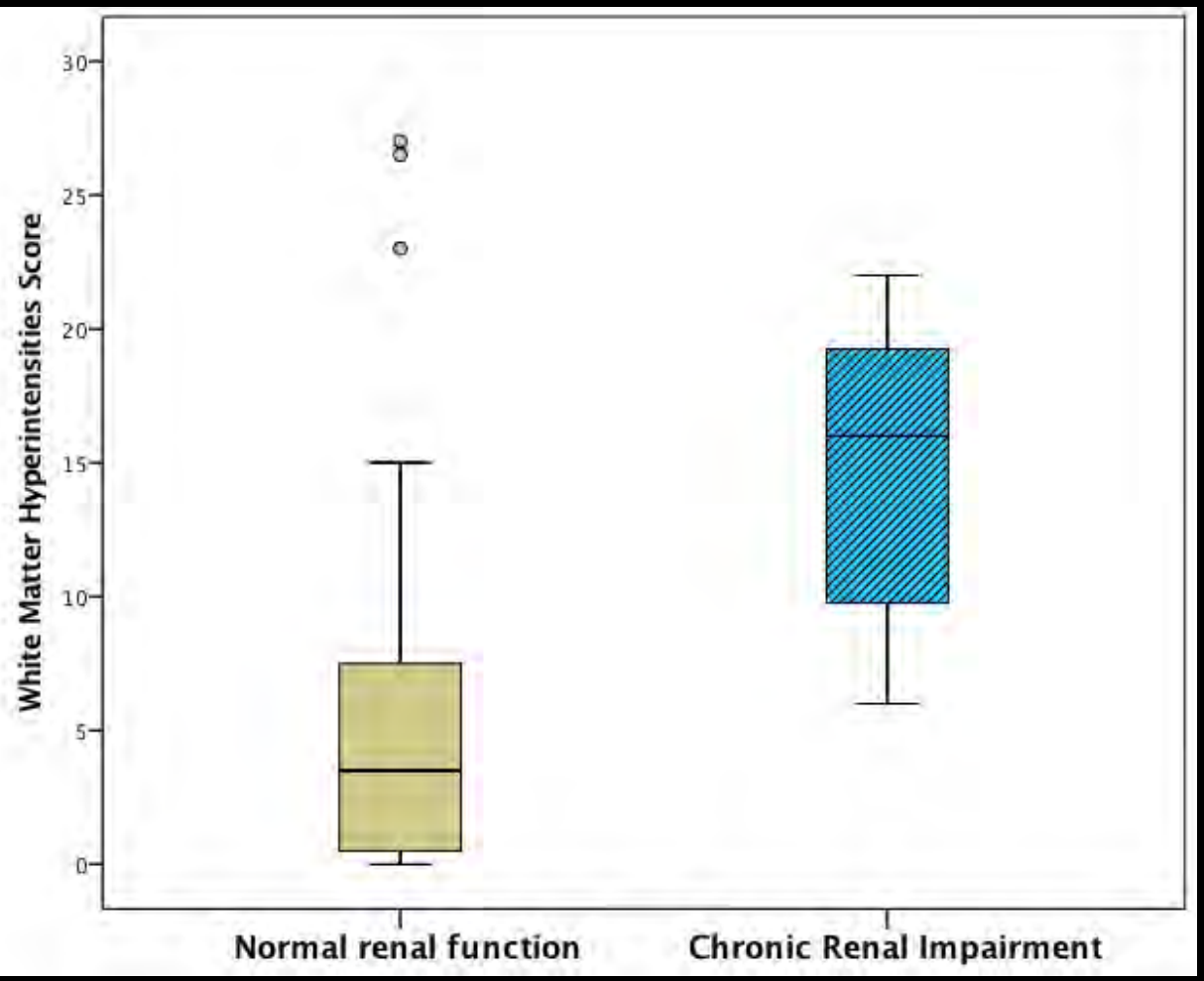
WMH (VISUAL SCALE) IN TORINO (N=107)



Trentalange A, et al. Brain Imaging and Behaviour 2018

WMH IN TORINO

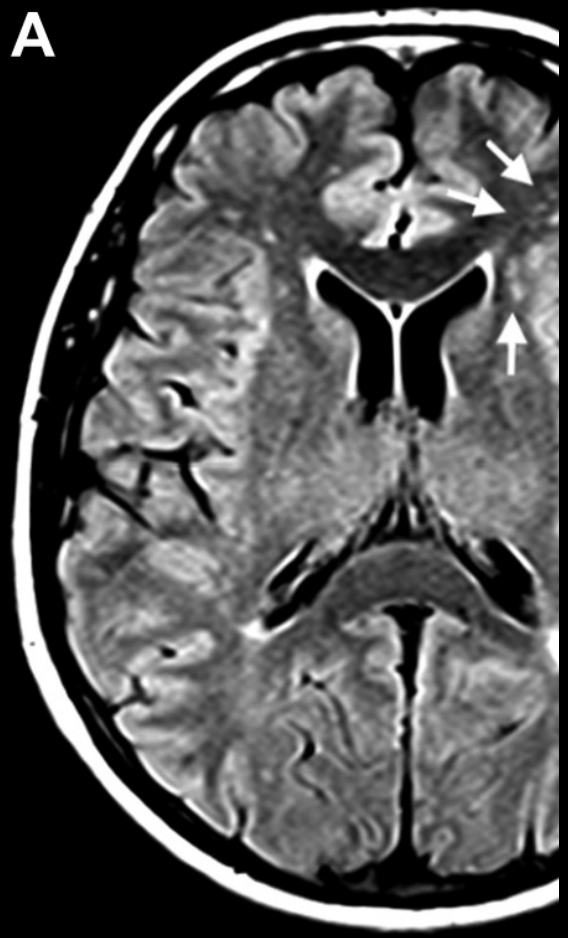


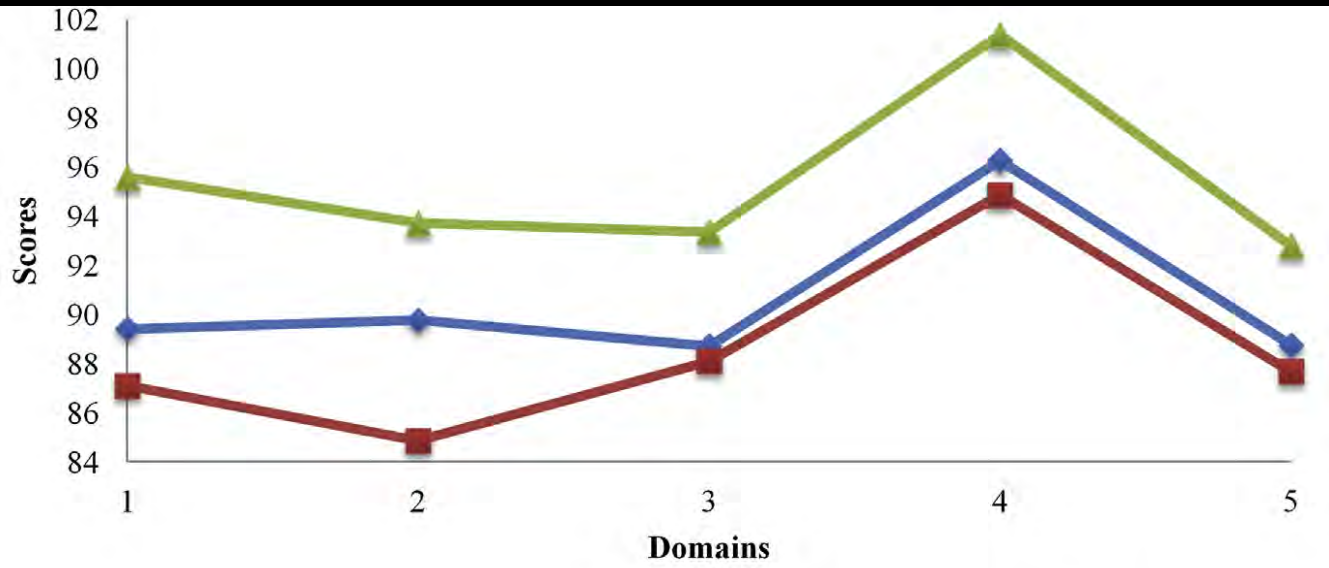


Trentalange A, et al. Brain Imaging and Behaviour 2018

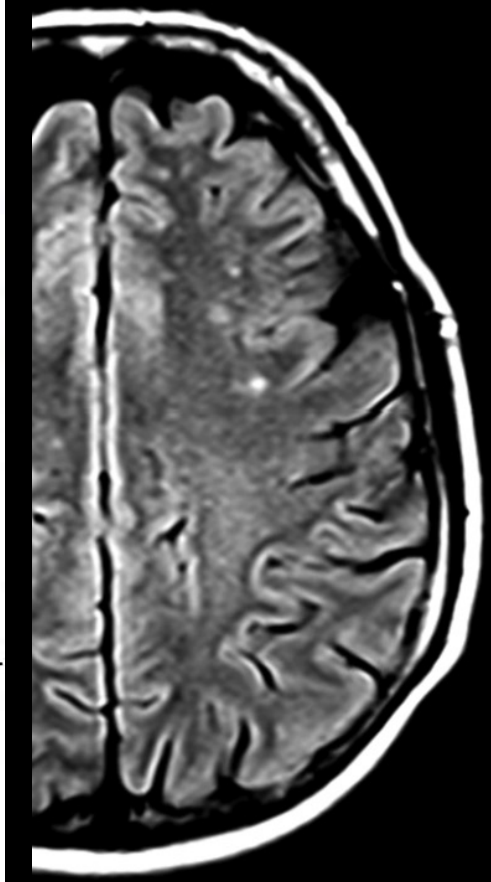
WMH AND ATRIAL FIBRILLATION

A



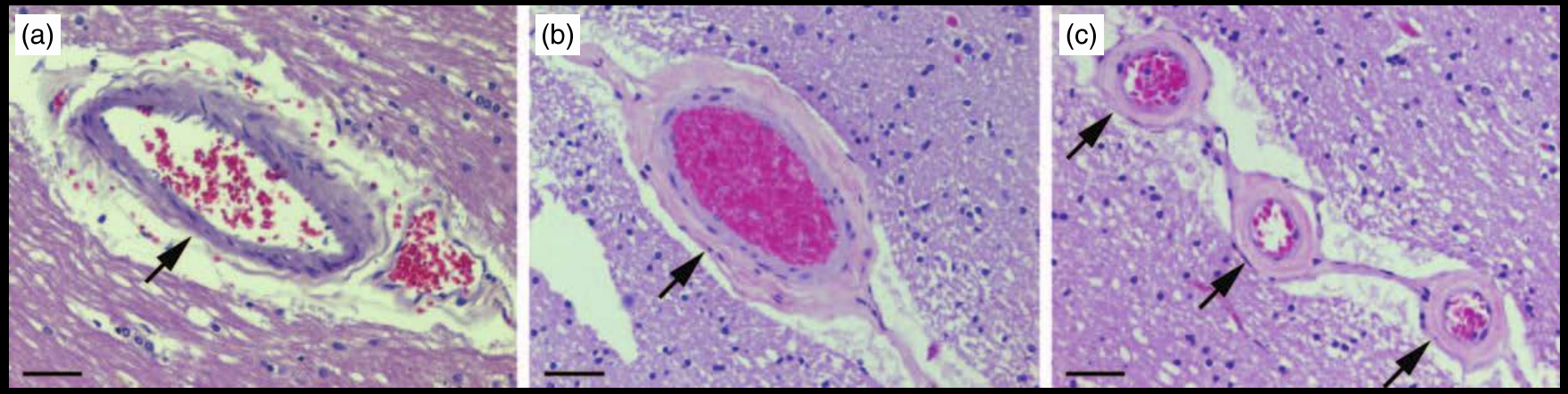


	Controls (N = 90)	PRX AF (N = 90)	PER AF (N = 90)	p PRX / controls	p PER / controls	p PRX/ PER
Domains	92.4 ± 15.4	86.2 ± 13.8	82.9 ± 11.5	< 0.01	< 0.01	0.08
1-Immediate Memory	95.6 ± 17.5	89.9 ± 14.7	87.1 ± 16.9	0.02	< 0.01	0.24
2-Visuo-spatial abilities	93.8 ± 16.7	89.9 ± 18.2	84.8 ± 14.8	0.14	< 0.01	0.04
3-Language	92.9 ± 11.4	88.8 ± 9.1	88.1 ± 8.7	< 0.01	< 0.01	0.59
4-Attention	101.4 ± 21.2	96.6 ± 16.6	94.9 ± 15.6	0.09	0.02	0.47
5-Delayed memory	93.5 ± 11.7	88.7 ± 14.7	87.7 ± 14	0.02	< 0.01	0.64



Gaita F, et al. JACC 2013

CEREBRAL SMALL VESSEL DISEASE



(137 autopsies, 1999-2011)

Mild CSVD **24.8%** - moderate/severe CSVD **47.4%**

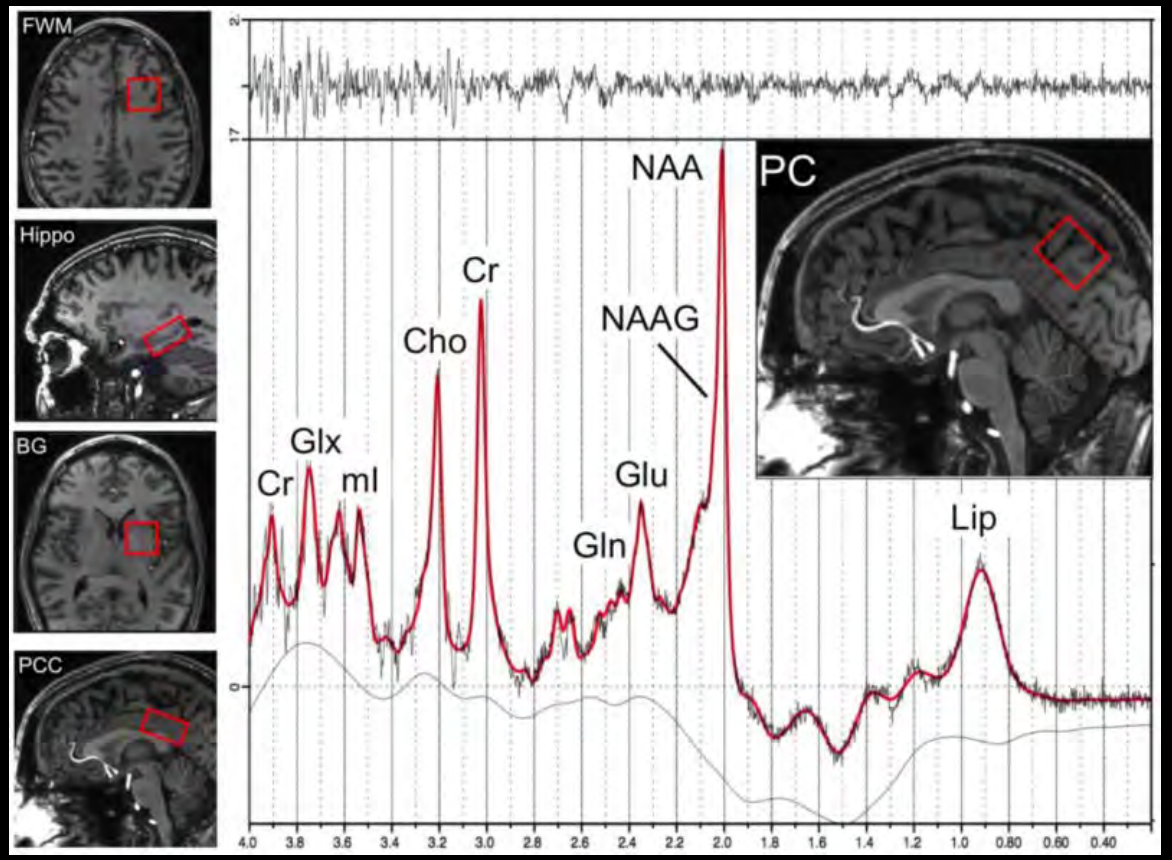
- associated with **PI-based HAARTs** and diabetes
- HAND associated with mild CSVD

Soontornniyomkij V, et al. AIDS 2014

N-ACETYL ASPARTATE AT MRS

- Localize to neurons (axons)
- Marker of neuronal integrity
- Marker of mitochondrial integrity
  - Reduced following ATP inhibition and impaired oxygen consumption

❖ Reduced Naa with "old" drugs and in patients with NCI



Schweinsburg BC, et al. JNV 2005; Mohamed M, et al. AJNR 2018

ALTAIR – WEEK 144

- 22 neuroasymptomatic naïve patients
- MRS and NCT at BL, and week 48 and 144
- Improvement at week 48 but decline between 48 and 144 week
- Reductions in NAA/Cr ratio (week 48–144) in RBG were associated with an increase in composite speed score change (week 48–144)

	Details		Mean absolute change				Mean absolute change	
		number	week 0–48	SD	*P-value*	week 48–144	SD	*P-value*
Cerebral Metabolite Ratio								
Anatomical area	Ratio							
Frontal Grey	*NAA/Cr*	22	0.31	0.66	0.36	0.13	0.91	1.00
	Cho/Cr	22	0.02	0.19	1.00	0.09	0.28	1.00
	ml/Cr	21	-0.27	1.35	1.00	1.13	1.71	0.06
Frontal White	*NAA/Cr*	22	0.04	0.74	1.00	0.14	0.77	1.00
	Cho/Cr	22	-0.08	0.30	1.00	0.14	0.24	0.09
	ml/Cr	21	-0.50	1.54	1.00	1.49	1.49	**0.002**
Right Basal Ganglia	*NAA/Cr*	20	0.64	1.20	0.27	-0.61	1.13	0.27
	Cho/Cr	20	-0.09	0.76	1.00	-0.17	0.33	0.27
	ml/Cr	20	-0.03	1.05	1.00	0.71	1.46	0.36
Cognitive test parameter								
Composite speed score	*decline in score represents improvement*	21	-0.186	0.486	0.10	-0.027	0.452	0.79
Composite accuracy score	*increase in score represents improvement*	21	0.220	0.497	0.06	-0.305	0.499	**0.01**
Executive function score	*decline in score represents improvement*	21	-0.222	0.858	0.25	0.351	1.20	0.19
Global composite score	*increase in score represents improvement*	21	0.627	1.16	**0.02**	-0.629	1.41	0.06

Winston A, et al. Plos One 2015

SWITCH EFV TO INSTI

20 ASYMPTOMATIC PTS ON EFV SWITCHED TO RAL OR EVG

Description of neuropsychological measures / **Neuropsychological changes with EFV switch**

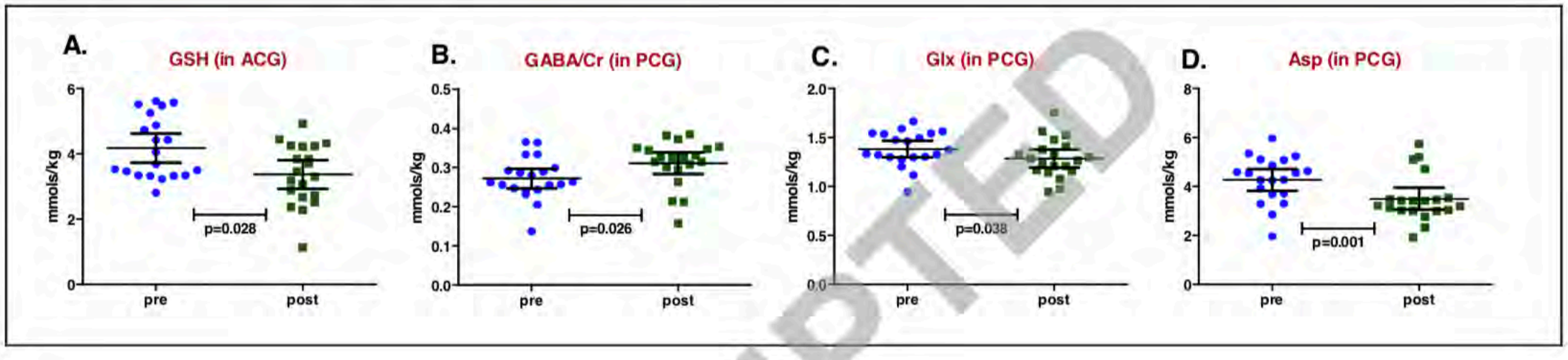
	Measures	Domains	Scale Range	Pre-Switch (SD)	Post-Switch (SD)	Change after EFV switch	P-value	Clinical interpretation
WAIS R-DSST	Weschler Adult Intelligence Scale	Cognitive test to assess executive function	0-100 Lower score = more cognitive dysfunction	48.7 (11.8)	[illegible]3.5 ([illegible])	+4.75	<0.001	Improved executive function
Trail Making	Trail Making test Parts A and B	Tests speed of information processing, attention and task switch to detect cognitive impairment	Time (log transformed, sec) Slower speed = greater cognitive impairment	3.9[illegible]	[illegible]	-0.036	0.19	Part A: faster information processing
				[illegible]4.52	4.67	0.146	0.31	Part B: unchanged executive function
HAMD	Hamilton Rating Scale for Depression	Standard clinical measure of severity of depression	0-7 is normal >20 = moderate [illegible]ere depression	5.1 (3.6)	2.8 (2.6)	-2.30	0.003	less depressive symptoms
DASS-21	Depression Anxiety Stress Scale	Self-reported symptoms over the past week for depression, anxiety and stress	[illegible] Higher score = increased [illegible] symptoms	*Depression*				
				7.0 (9.0)	3.4 (4.2)	-3.60	0.074	less reported depression
				Anxiety				
				4.3 (5.3)	2.5 (2.2)	-1.80	0.067	less self-reported anxiety
				Stress				
				6.9 (4.6)	4.4 (4.2)	-2.50	0.013	less self-reported stress level
FRSBE	Frontal Systems Behavioral Scale	Self-reported assessment for 3 frontal system: apathy, disinhibition and executive dysfunction	37-186 Higher score = greater behavioral impairment	78.4 (11.4)	71.4 (11.3)	-7.00	0.004	less self-reported behavioral symptoms
STAI	Spielberger state trait anxiety inventory	Assess specifically [illegible] and severity of anxiety	20-80 Higher score = more anxiety	29.1 (5.5)	25.7 (5.2)	-3.40	0.003	decreased anxiety
PSQI	Pittsburgh Sleep Quality Index	Subjective sleep quality to assess duration, sleep efficiency and disturbances	0-21 Higher score = greater sleep disturbances	5.1 (2.6)	3.5 (1.8)	-1.60	<0.001	improved sleep quality

Asundi A, et al. JAIDS 2019

SWITCH EFV TO INSTI

20 ASYMPTOMATIC PTS ON EFV SWITCHED TO RAL OR EVG

Figure 1a: Comparison of pre- and post-EFV switch neurometabolite levels measured by MRS in the anterior cingulate gyrus (ACG) or the posterior cingulate gyrus (PCG). Statistical comparison done by two-tailed paired t-test. A) GSH = glutathione, B) GABA/Cr = gamma-aminobutyric acid creatine ratio, C) Glx = glutamate + glutamine, D) Asp = aspartate.



Asundi A, et al. JAIDS 2019

3. PLASMA AND CSF BIOMARKERS

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Plasma and CSF NFL (t-tau?)

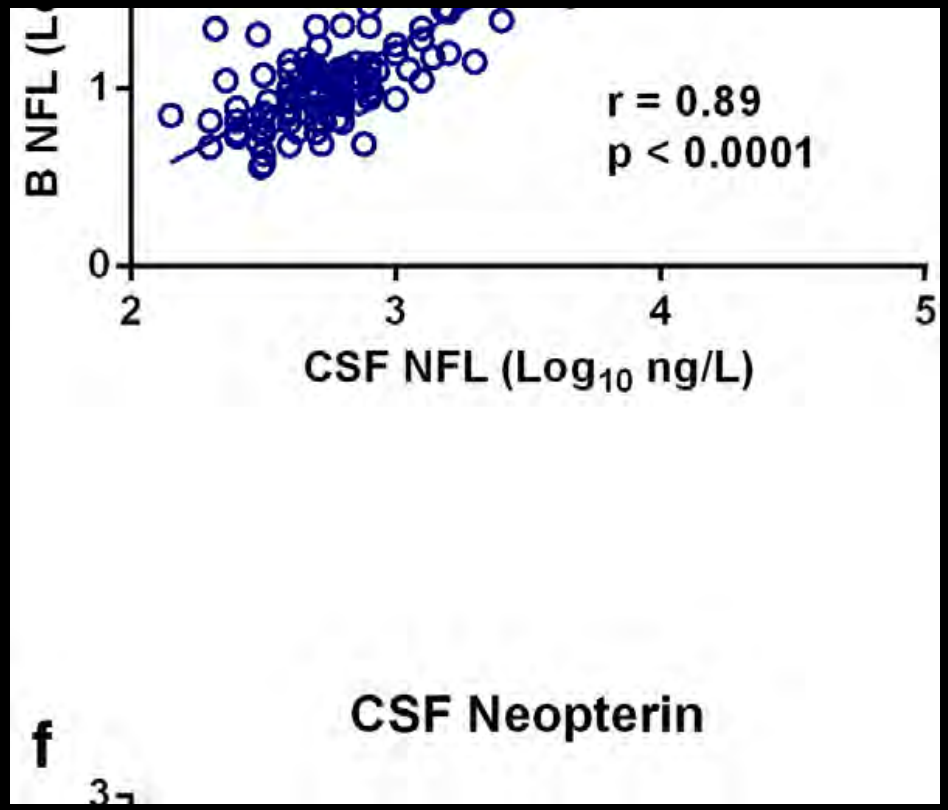
CSF Beta$^{1-42}$

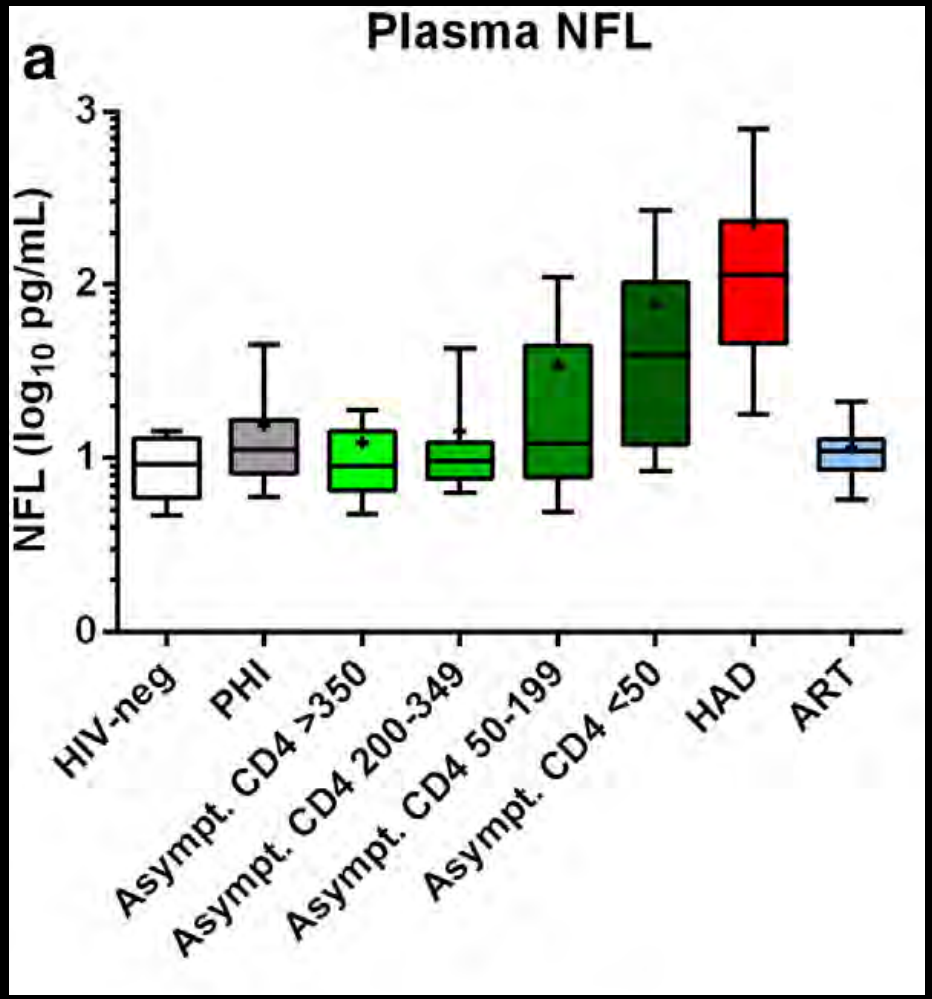
CSF S100beta?
CSAR

CSF mtDNA?

Tovar-y-Romo LB, et al. J Pharmacol Exp Ther, 2012; Robertson K, et al. JNV 2012; Akay C, et al. JNV 2014; Giunta B, et al. Mol Brain 2011; Robertson K, et al. Neurology 2010; Achim CL, et al. J Neuroimmune Pharmacol 2009; Ortega M and Ances BM, J Neuroimmune Pharmacol 2014; Ciccarelli N, et al. Neurology 2011; Vivithanaporn P, et al. AIDS 2016

PLASMA NFL





Gisslen M, et al. EBioMedicine 2015

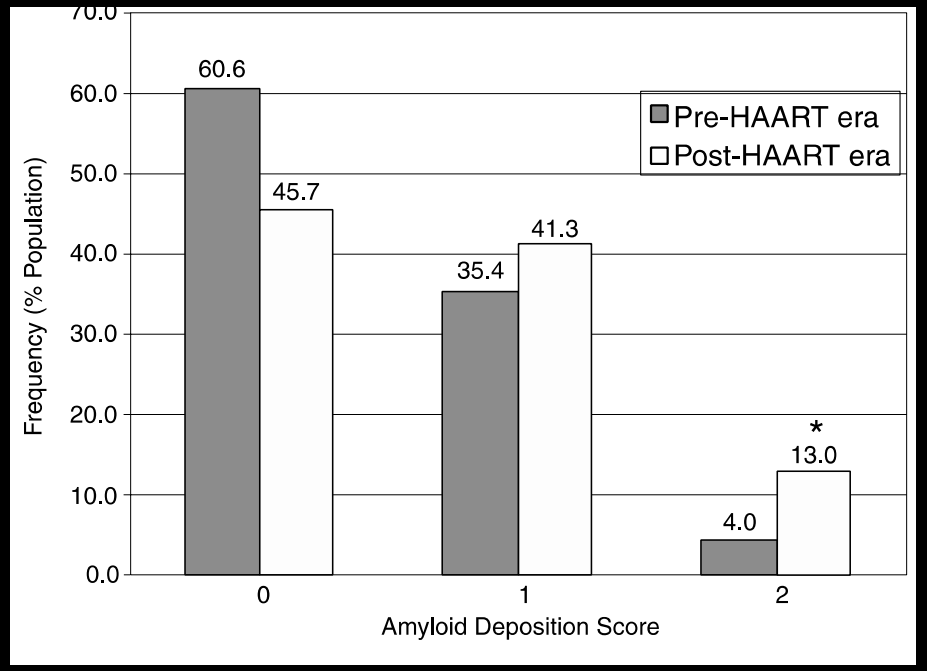
CSF ALZHEIMER'S BIOMARKERS IN PLWH

Different amyloid deposition
No premature amyloid increase
AD's patterns only in 12% of HAD patients
5 cases of possible AD in PLWH (3 biopsy/autopsy)

	HAD	AD
t-tau	++	+++
p-tau	=	++
AB42	-	-
sAPP□	---	=
sAPP□	---	=
NFL	+++	+

Green DA, et al. AIDS 2005; Cysique LA, et al. BMC Neurol 2015; Gisslen M, CROI 2016

HAART and amyloid deposition



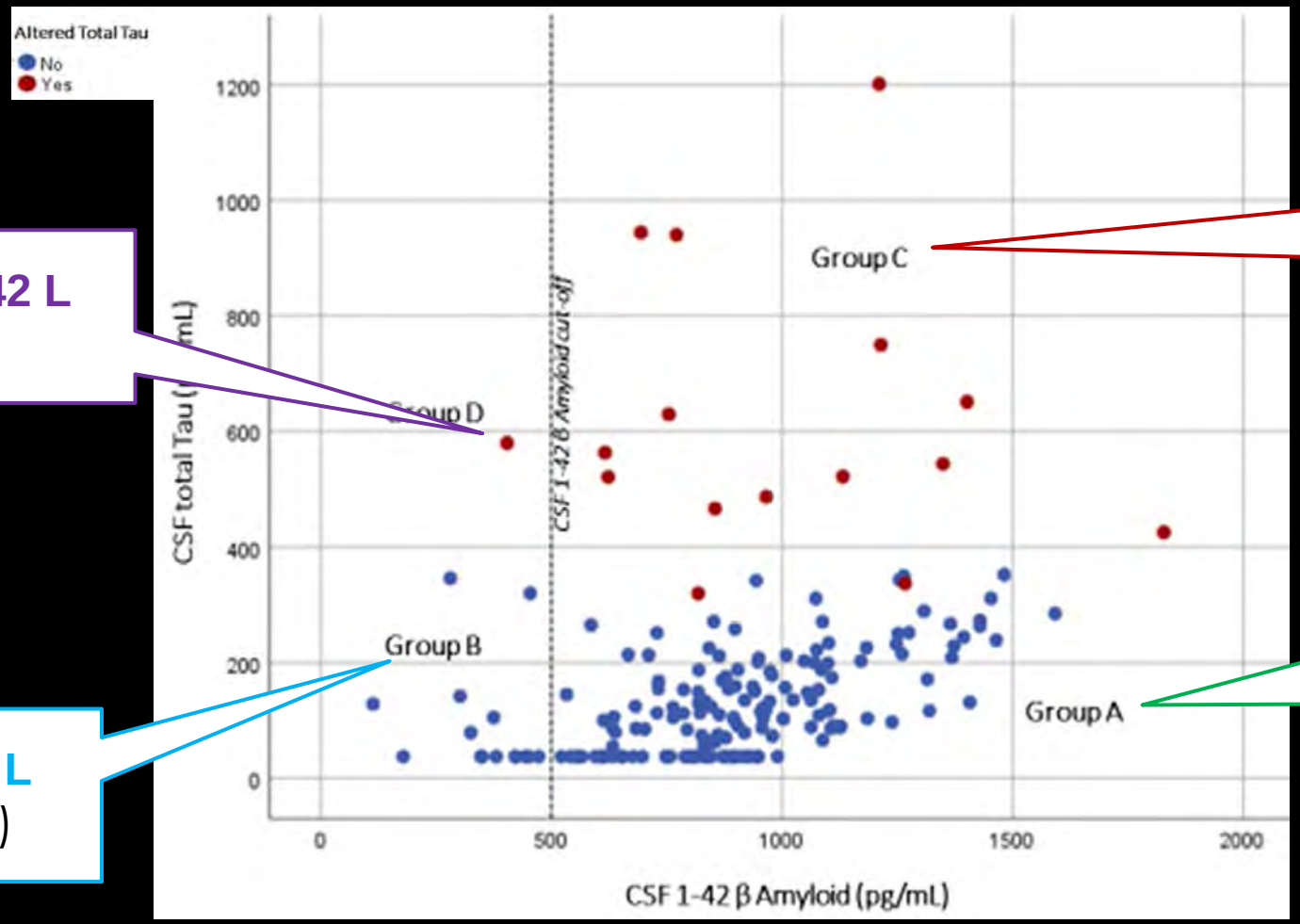
In patients with access to HAART, there is a clear trend towards decreasing prevalence of Grade 0, and an overall increase in Grades 1 and 2.

Green DA, et al. AIDS 2005

ARVs increase Aβ generation (50-200%) and markedly inhibit microglial phagocytosis of **Aβ1**-42 peptides in murine microglia. The most significant amyloidogenic effects were observed with combined ART.

Giunta B, et al. Molecular Brain 2011

ALZHEIMER'S DEMENTIA BIOMARKERS IN 181 PLWH



tTau H and Beta42 L
1 patient

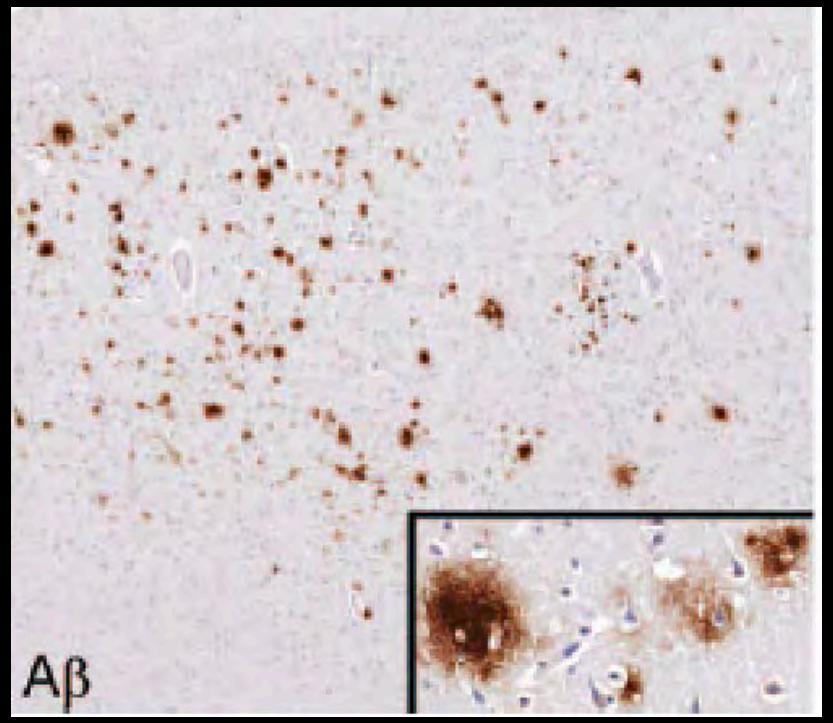
tTau H and Beta42 n
15 patients (8.3%)

tTau H and **Beta42 L**
15 patients (8.3%)

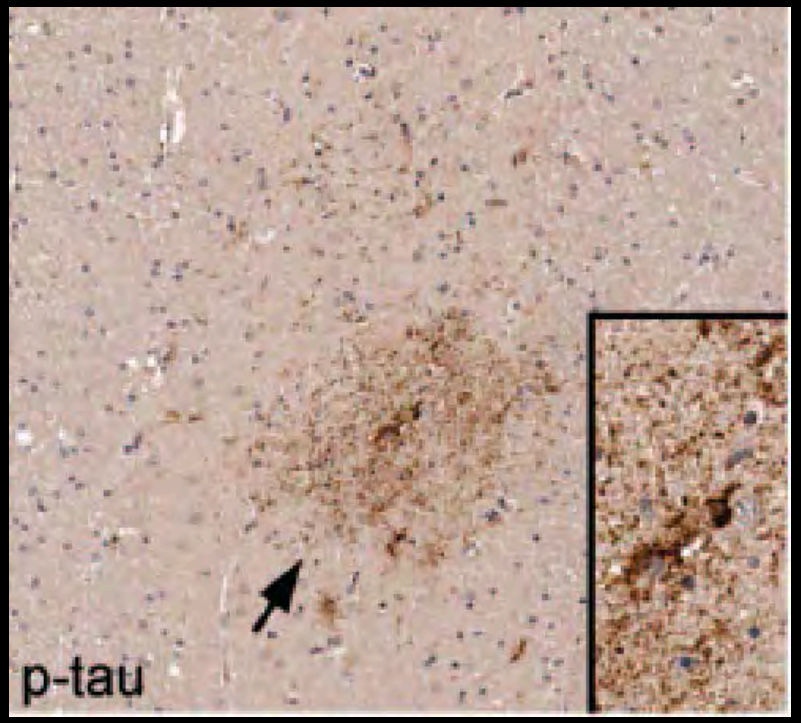
tTau n and Beta42 n
150 patients (82.9%)

Trunfio M, et al. CROI 2019

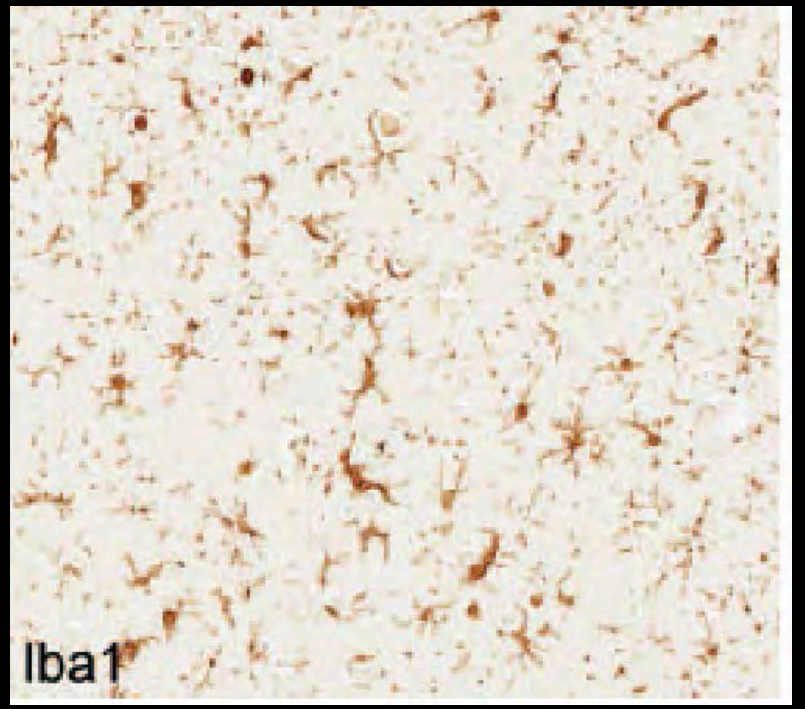
AMYLOID AND PHOSPHO-TAU NEUROPATHOLOGY MAY BE INFLUENCED BY ANTIRETROVIRALS



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



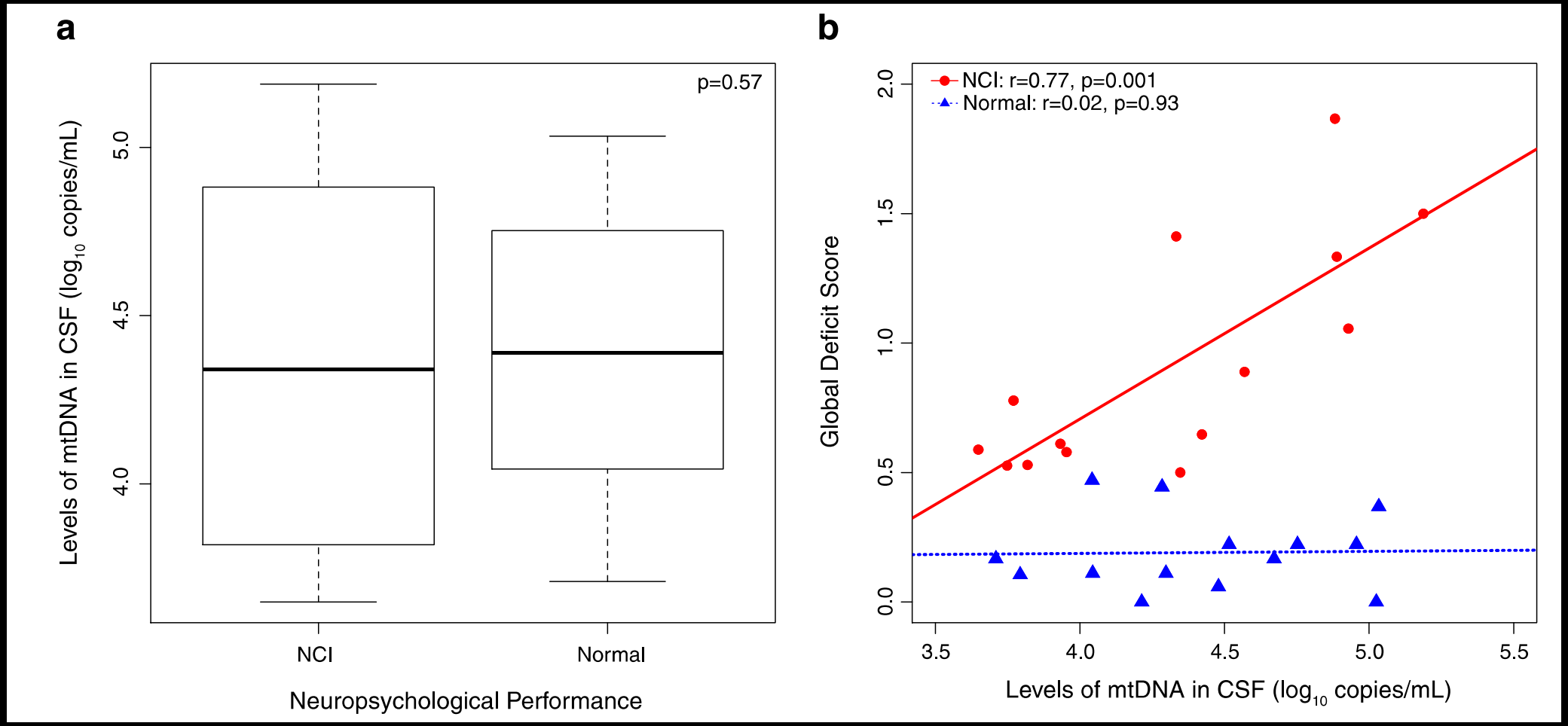
Darunavir use prior to death associated with higher odds of phopho-tau deposition in neurons
(OR 15.3, p=0.0005)



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

Soontornniyomkij et al, AIDS 2018

CSF MITOCHONDRIAL DNA AND NCI



Perez-Santiago J, et al. JNV 2015

4. FMRI AND EEG?

MECHANISMS OF ANTIRETROVIRALS' CNS TOXICITY

① Direct Neuronal toxicity
- *In vitro*
- in macaques

② **Beta amyloid** metabolism interference

③ **Astrocytes** and blood brain barrier

④ **Olygodendrocytes** and myelin

⑤ Indirect effect on cerebral **blood vessels**

⑥ Efavirenz (and 8-08-EFV)

⑦ Impairment in mitochondrial function

⑧ Interference with neurotransmitters

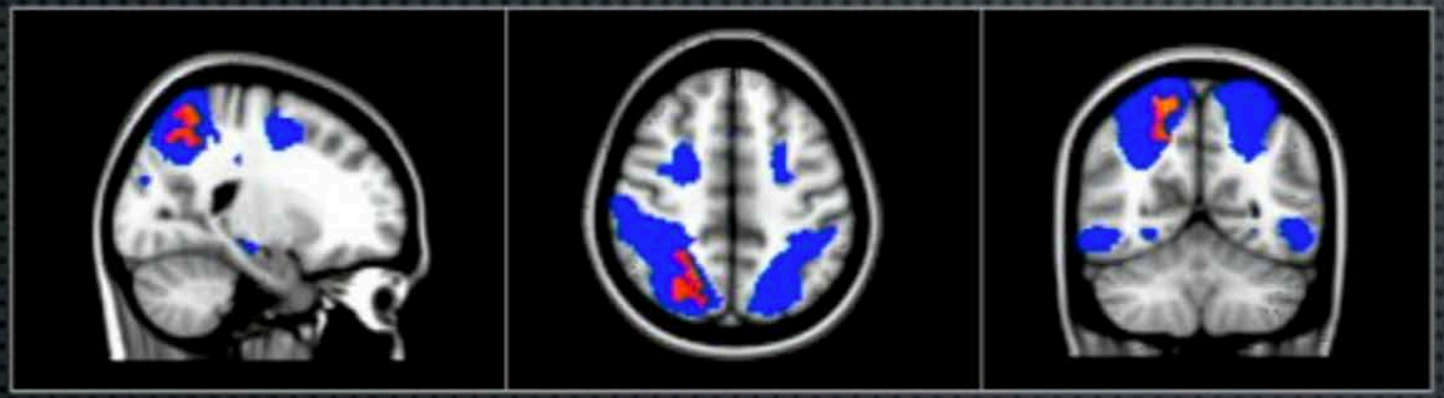
fMRI

fMRI? EEG?

Tovar-y-Romo LB, et al. J Pharmacol Exp Ther, 2012; Robertson K, et al. JNV 2012; Akay C, et al. JNV 2014; Giunta B, et al. Mol Brain 2011; Robertson K, et al. Neurology 2010; Achim CL, et al. J Neuroimmune Pharmacol 2009; Ortega M and Ances BM, J Neuroimmune Pharmacol 2014; Ciccarelli N, et al. Neurology 2011; Vivithanaporn P, et al. AIDS 2016

RESULTS: STUDY 2 SWITCHING EFAVIREZ TO RILPIVIRINE

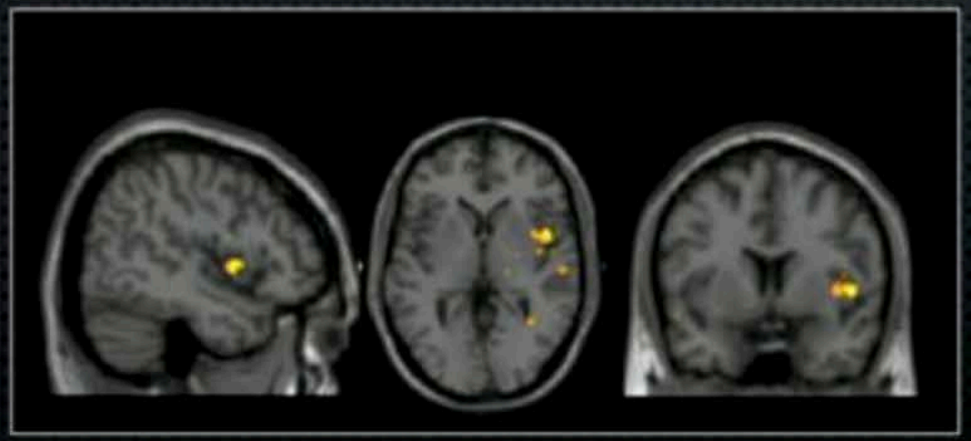
Resting state fMRI: effect of switching efavirenz to rilpivirine on RSN



Switching efavirenz to rilpivirine increased FC in the Default Attention Network (spatial attention); (P=0.005)

Increased FC in DAN network was associated with improvements in cognitive scores for attention ($p=0.020$, $\eta^2 = 0.47$)

SSRT task fMRI: effect of switching efavirenz to rilpivirine on inhibitory control (executive function)



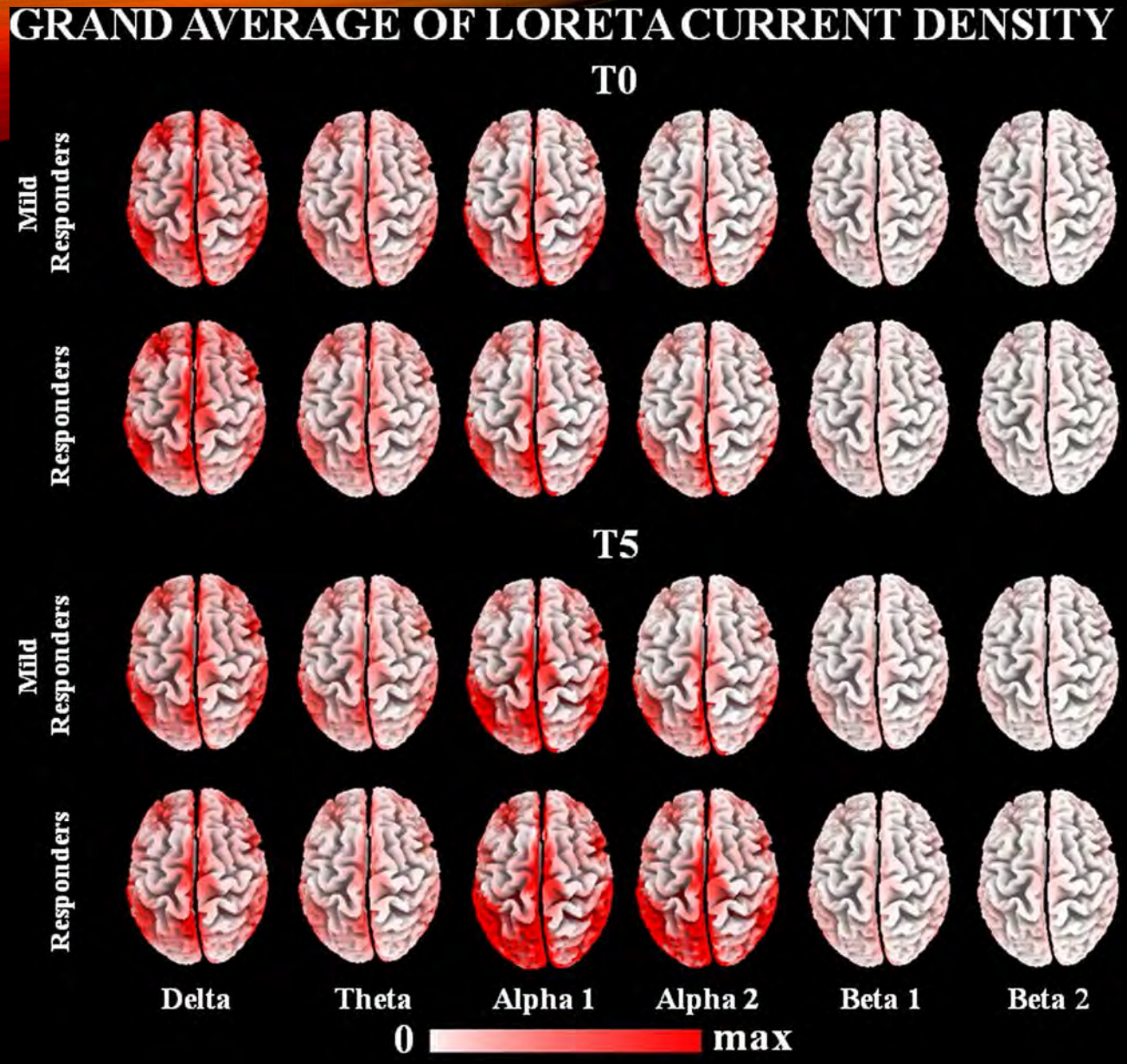
A reduction in brain activation after switching (less effort)

A reduction in stop-signal reaction times was observed after switching efavirenz to rilpivirine ($p=0.025$)

Increase stop-signal reaction time was associated with longer exposure to efavirenz ($p=0.020$, $\eta^2 = 0.875$), median exposure 5.2 years

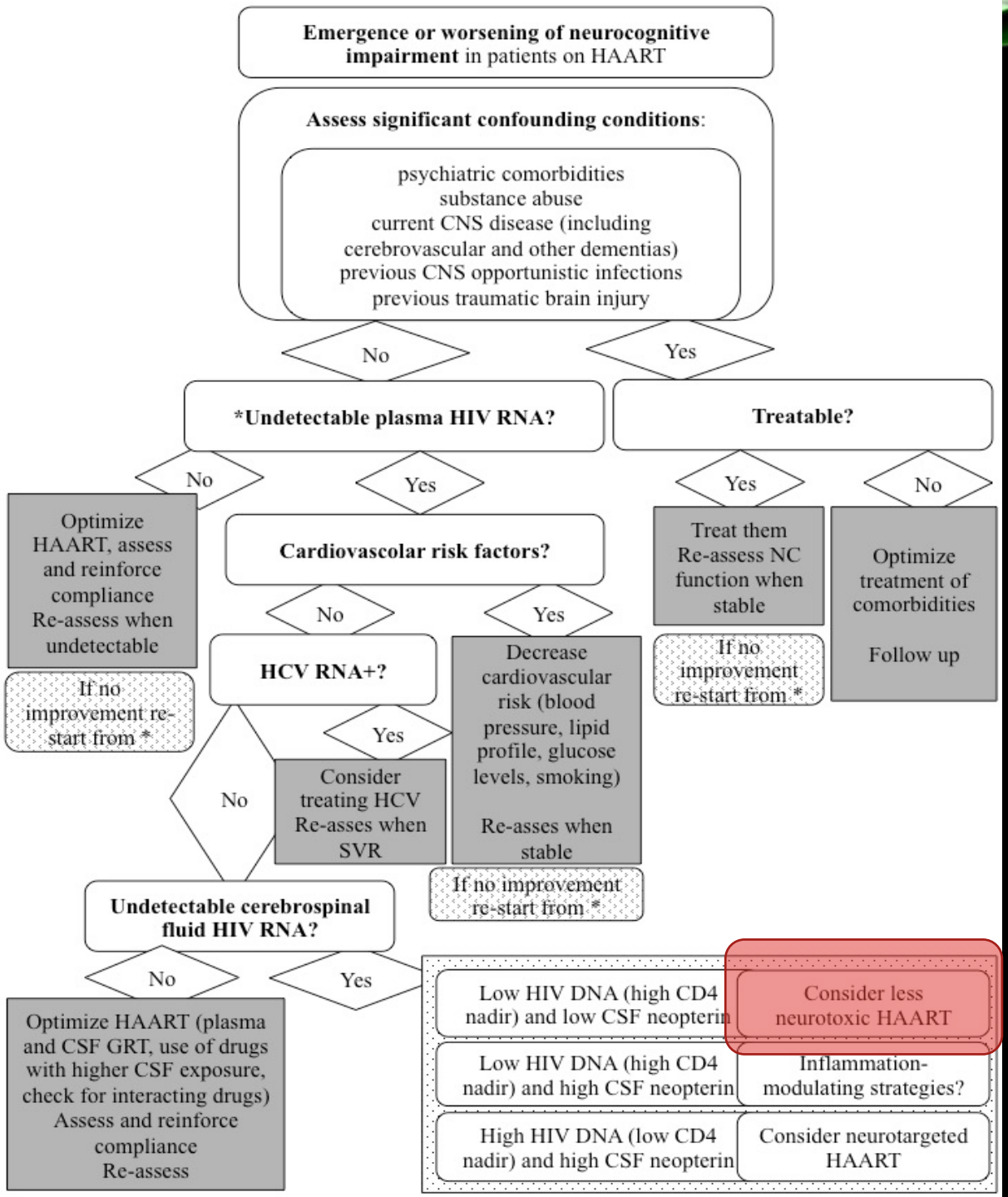
Vera J, et al. CROI 2019

EEG LORETA

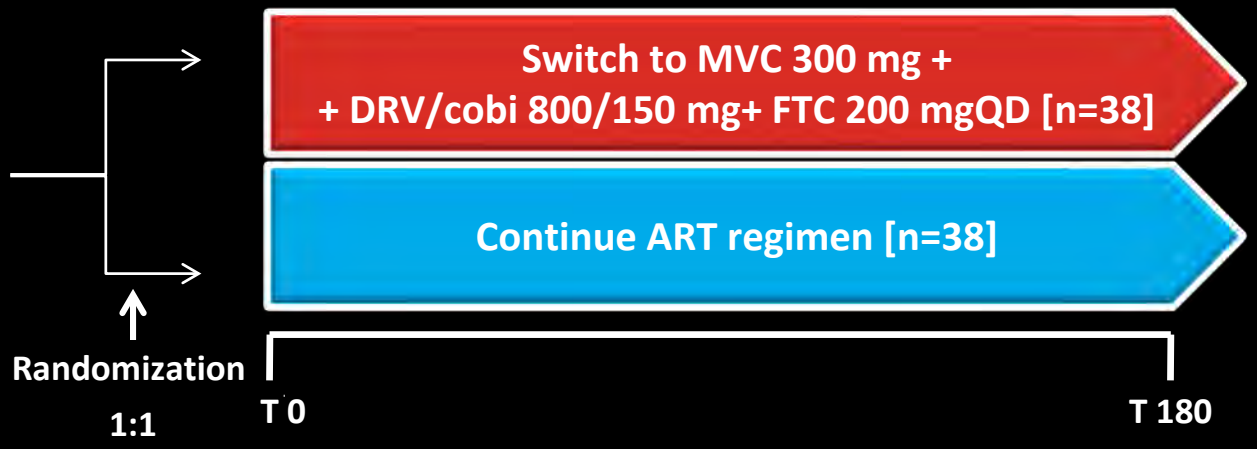


Babiloni C, et al. Clin Neurophysiology 2014, 2015 and 2017

5. OUR FLOWCHART



MARAND-X (NCT03163277)



Calcagno A, et al. Drugs 2017

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