Brain perfusion, regional volumes and cognitive function in HIV positive patients treated with protease inhibitor monotherapy

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Running title:

Neuroimaging after PI monotherapy in HIV

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Summary:

In virologically suppressed HIV+ patients, protease inhibitor monotherapy did not appear to confer any additional risk of regional cerebral hypoperfusion or brain volume loss, although some of these neuroimaging biomarkers were associated with impaired fine motor function regardless of regimen.

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Abstract

Background. Protease inhibitor monotherapy (PIM) for HIV may exert suboptimal viral control in the central nervous system. We determined whether cerebral blood flow (CBF) and regional brain volumes were associated with PIM, and whether specific cognitive domains were associated with imaging biomarkers.

Methods. Cognitive assessment and brain MRI were performed after the final visit of a randomized HIV treatment strategy trial. Participants were virologically suppressed on triple therapy at trial entry and followed for 3-5 years. Thirty-seven patients randomized to ongoing triple therapy and thirty-nine randomized to PIM were studied. Resting CBF and normalized volumes were calculated for brain regions of interest, and correlated with treatment strategy and neuropsychological performance.

Results. Mean age was 48.1 years (standard deviation 8.6 years), 63 were male (83%) and 64 were white (84%). Participants had median 8.1 years (interquartile range 6.4, 10.8) of antiretroviral therapy experience and $CD4^+$ counts of median 640 cells/mm^3 (interquartile range 490, 780). We found no difference between the two treatment arms in CBF or regional volumes. Regardless of treatment arm, poorer fine motor performance correlated with lower CBF in the caudate nucleus (p=0.01), thalamus (p=0.04), frontal cortex (p=0.01), occipital cortex (p=0.004) and cingulate cortex (p=0.02), and was associated with smaller supratentorial white matter volume (decrease of 0.16 in Z-score per -1% of total intracranial volume, 95% confidence interval 0.02-0.29, p=0.023).

Conclusions. PIM does not confer additional risk of neurological injury compared with triple therapy. There were correlations between fine motor impairment, grey matter hypoperfusion and white matter volume loss.

Key words:

HIV; magnetic resonance imaging; cerebral blood flow; cognitive function; white matter.

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Text

The prevalence of cognitive impairment has been reported in recent studies as 19-69% of HIV-positive (HIV+) patients in Europe and the United States [1-3]. One possible explanation is ongoing injury during antiretroviral therapy (ART) due to inadequate penetration of antiretroviral drugs into the central nervous system (CNS) [4, 5].

It has been hypothesized that treatment with a ritonavir-boosted protease inhibitor alone (protease inhibitor monotherapy [PIM]) may allow persistent viral replication within the CNS and increase neurological complications compared to triple ART [6]. Therefore, it is reassuring that the Protease Inhibitor Versus Ongoing Triple therapy (PIVOT) and Plasma RNA On Current Treatment (PROTEA) trials reported no differences in cognitive function between patients randomized to protease inhibitor monotherapy (PIM) or to triple therapy over at least 96 weeks of follow-up [7, 8]. In our published analysis of the PIVOT Neurological Substudy, we found no difference between treatment strategy arms in gross brain atrophy, cerebral small vessel disease, or single voxel magnetic resonance spectra associated with neurodegeneration [9]. This does not preclude other quantitative MRI modalities from detecting differences in the neuroprotective or neuropathological effects of PIM.

Several quantitative neuroimaging markers of HIV-related CNS disease have been proposed, including changes in regional brain volumes and cerebral blood flow (CBF). Quantitative volumetric analysis provides robust measurements that are reproducible and can be automated, and there is an extensive literature on brain volume changes in HIV+ individuals [10, 11]. Some studies of arterial spin labelling (ASL) have demonstrated CBF to have promise as a biomarker for HIV neuropathology [12-14]. Study findings of these two imaging modalities in HIV have been somewhat inconsistent, but they remain state of the art as HIV biomarkers in a rapidly changing field. Pseudo-continuous arterial spin labelling (pCASL) has recently been recommended as the labelling method of choice for non-invasive perfusion imaging [15] due to its ease of implementation and high signal-to-noise ratio. ASL has been used in the assessment of patients with dementia, where the patterns of hypoperfusion closely match the patterns of hypometabolism on fluorine 18 fluoro-deoxyglucose positron emission tomography (FDG-PET) [16]. The other important MR sequence in HIV neuroimaging is diffusion tensor imaging; a recent meta-analysis of the technique supported its role but highlighted a substantial variation in results from ostensibly similar study designs [17].

The first aim of this study was to evaluate whether differences in CBF, assessed with pCASL, or regional brain volumes could be detected between patients randomized to OTT or PIM after several years of virological suppression. The second aim was to determine if dysfunction in specific cognitive domains was associated with these imaging markers.

METHODS

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Experimental design

The study was approved by the Cambridgeshire 4 Research Ethics Committee and participants' consent was obtained according to the Declaration of Helsinki. This was a cross-sectional sub-study of PIVOT, a non-inferiority, randomized parallel-group trial (ISRCTN-04857074), in which 587 virologically suppressed HIV-positive adults on triple ART (two nucleoside reverse transcriptase inhibitors and one non-nucleoside reverse transcriptase inhibitor or protease inhibitor) were randomized 1:1 to ongoing triple therapy (OTT) or switch to PIM. Participants had plasma viral load <50 copies/ml for minimum 24 weeks at trial entry and were required to have been the same combination regimen for minimum 12 weeks. In the PIM arm, all licensed protease inhibitors were allowed, but ritonavir-boosted darunavir and lopinavir were recommended. At the time of exiting the main trial, 3-5 years after randomization, all PIVOT participants at five large sites were invited to perform multimodal brain MRI and a detailed cognitive evaluation.

MRI acquisition and analysis

All brain MRI investigations were carried out on a single 3 Tesla Philips Achieva system (Best, Netherlands) in a dedicated research facility. Non-invasive cerebral perfusion was assessed with pCASL with echo-planar imaging readouts (field of view [FOV] 240×240mm, 20 slices, slice thickness 5 mm, voxel area 3.75×3.75 mm, relaxation time [TR] / echo time [TE] 4300 ms/15 ms, SENSE factor: 2.3, labelling duration/post-labelling delay 1650 ms/1800 ms, 30 pairs of control-label volumes). A set of proton density-weighted images (M0) with identical readout but TR of 9000 ms and no labelling was also acquired for quantification. 3D structural imaging with T1-weighted MP-RAGE (magnetization prepared rapid gradient-echo) sequence was also performed (FOV 256×256×180 mm, voxel size 1×1×1 mm, flip angle 8°, TR/TE 6.8 ms/3 ms, shot interval 3000 ms, TFE factor 230, SENSE factor 2, effective TI 825 ms).

CBF was quantified based on the Buxton model [18] in MATLAB (The MathWorks Inc., Natick, Massachusetts, USA, 2000) using a recommended parameter set [15]. Regions of interest (ROI) were segmented on T1-weighted images using an automated algorithm, previously used in many clinical trials and observational studies [19]. The segmentation was used to compute the volume of each ROI. In order to avoid directionality bias caused by large differences in resolution between T1 and CBF data, the T1 tissue segmentations were aligned to the lower-resolution motion-corrected CBF maps using a robust symmetric block-matching rigid registration [20] and resampled using point spread function matching [21]. Finally, CBF maps were corrected for partial volume effects using in-house implementation of an existing method [22]. Caudate and lenticular nuclei, thalamus, frontal lobe, occipital lobe, cingulate cortex, supratentorial cortical grey matter and supratentorial white matter were chosen as ROI (Fig. 1). Region selection was guided by previous work using ASL in HIV [12-14, 23]. In addition, hippocampal, ventricular cerebrospinal fluid (CSF) and non-ventricular CSF volumes were measured. Left and right CBF estimates were averaged, whereas left and right volumes were summed and normalized as a proportion of total intracranial volume (TICV).

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Cognitive assessment

Cognitive function was assessed across five domains: executive function (Color Trails Test Part 2 and Stroop Color-Word Test), fine motor skills (Grooved Pegboard Test and Finger-Tapping Test with dominant and non-dominant hands), attention and working memory (Color Trails Test Part 1 and digit-symbol test), verbal memory (Hopkins Verbal Learning Test – Revised) and non-verbal memory (Rey Complex Figure Test). Raw scores for each cognitive test were transformed into Z-scores as previously described using normative data stratified for age (all tests) and years of education (Color Trail Test, Stroop Color-Word interference Test) [9]. A global score (referred to as NPZ) was calculated by averaging all neuropsychological Z-scores. Information about cognitive symptoms (using question 10 of the Medical Outcomes Study HIV questionnaire [MOS-HIV] [24]), alcohol consumption (using the Alcohol Use Disorders Identification Test consumption questions[AUDIT-C] [25]) and recreational drugs was also collected. Patients were classified as having symptomatic cognitive impairment if they had two or more cognitive domain Z-scores < -1 and reported at least one of four cognitive symptoms from the MOS-HIV questionnaire at least "a good bit of the time".

Statistical analysis

Statistical analyses were performed using STATA version 14 (StataCorp, Texas, United States). Single variable comparisons between the two randomly allocated treatment arms were made using the t-test (between-group comparison of age, blood pressure and cholesterol), Mann-Whitney test (years since HIV diagnosis and ART initiation, CD4 count, education, alcohol consumption score, hemoglobin and body mass index), Fisher's exact test (gender, ethnic group, history of AIDS and recreational drug use), or Chi-square test (smoking history). Patients with plasma VL >400 copies/ml at the time of MRI were omitted from the analysis.

We conducted a series of regression analyses. First, the two treatment arms were compared using multivariable linear regression models with CBF and volume in each ROI as the dependent variables, and treatment arm, age, sex, ethnic group and either alcohol consumption (for volume) or hemoglobin (hematocrit having an effect on the ASL signal) as the independent variables. Linear regression assumptions were checked and, where residuals were not normally distributed, the variable in question was log-transformed before repeating the analysis. Potential confounding effects of cardiovascular risk factors (blood pressure, smoking, total cholesterol, and body mass index), recreational drug and alcohol use, and clinical variables (current and nadir CD4 count, history of AIDS, years since HIV diagnosis, years of ART, duration of trial follow-up, and years between diagnosis and treatment initiation) were explored by adding these to the models, if there was evidence of association in bivariate models (p<0.10). We also explored duration of trial follow-up as a possible effect modifier on the impact of treatment arm. In a sensitivity analysis, treatment groups were compared after excluding patients who had switched to the opposite treatment strategy arm at any time during the trial.

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Second, CBF of each ROI was plotted against each cognitive domain's Z-score, and linear regression was performed, again after checking assumptions and adjusting for the same covariates as in the analysis of treatment effects. To allow presentation of results on the same y-axis, CBF measurements were normalized using the mean and standard deviation in the whole patient sample in each region of interest. Third, volumes of cortical and subcortical grey matter, supratentorial white matter, ventricular CSF and non-ventricular CSF were similarly plotted against each cognitive domain score and regression analyses were again performed.

Primarily, we used 0.05 as the p-value threshold, but we calculated corrected p-value thresholds, using the Benjamini-Liu method for multiple hypothesis testing [26], considering each of the four sets of regression models separately. Outlier analysis was performed on models where there was evidence to reject the null hypothesis. Outliers were identified by visual inspection of scatterplots, by an outlier nomination algorithm [27], or by removal of participants with severe impairment ($Z<-2$) of the neuropsychological domain under consideration, and the models were re-analyzed.

RESULTS

Patient characteristics

Of 219 PIVOT participants at participating sites, 92 agreed to the study, although three had contraindications to MRI and 11 did not attend, leaving 78 who completed the study. Analysis of neuropsychological, volumetric and spectroscopic data has been published, along with comparisons of participating and non-participating individuals [9] (see Supplementary Table). Participants were mainly white and male, with high levels of education, and a median of 8 years of ART at the time of image acquisition. There were 39 patients in each arm at baseline, but two patients in the OTT arm had VL >1000 copies/ml and were excluded. There were no differences in demographic variables, HIV history, cardiovascular risk factors or drug and alcohol use between arms (Table 1). Sixty four patients were on their original allocated treatment strategy (30 PIM and 34 OTT) and had VL <400 copies/ml. In the trial as a whole, 35% of PIM patients and 3% of OTT patients had experienced viral rebound.

Comparison between treatment arms

The mean global neuropsychological Z-score (NPZ) was -0.24 (standard deviation [SD] 0.69). In the sample overall there was evidence of impaired fine motor skills, relative to expected norms (mean Z -0.84, SD 1.00). Symptomatic cognitive impairment, as defined above, affected 13/78 patients (16.7%). As previously reported, there was no difference in cognitive function between treatment arms [9].

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Mean resting CBF measurements for all ROIs were compared between allocated treatment arms (upper part of Table 2). There were no differences in the perfusion of any region studied between patients treated with PIM or OTT. Similarly, when regional volumes were compared between treatment arms, no differences were seen (lower part of Table 2). The sensitivity analyses comparing only patients who remained on their randomly allocated strategy at the end of the trial showed no significant differences between treatments in regional resting CBF or volumes (all adjusted p values >0.05; Table 3).

Association between cognitive function and neuroimaging markers

Sample images of participants at each end of the range of values are shown in Fig. 2. Analysis of cognitive domain scores and CBF (Fig. 3) showed an association between resting CBF and fine motor skills scores in total cerebral grey matter (correlation coefficient [β] 3.8 ml/100 ml/min decrease in CBF per -1 change in Z-score, 95% confidence interval [CI] 1.0-6.6, p=0.008) and in most grey matter ROI (caudate nucleus, β 2.6, CI 0.7-4.6, p=0.01; thalamus, β 2.7, 95% CI 0.2-5.2, p=0.04; frontal cortex, β 4.4, 95% CI 1.1-7.7, p=0.01; occipital cortex, β 4.3, 95% CI 1.4-7.1, p=0.004; cingulate cortex, β 3.5, 95% CI 0.7-6.3, p=0.02). The corrected critical p-value threshold was 0.0013, therefore all null hypotheses were credible under this more stringent criterion. There was no evidence for an association between fine motor skills and cerebral white matter CBF (β 0.6, 95% CI -0.1-1.2, p=0.09). No other cognitive domain showed any association with resting CBF in any of the regions measured.

In the analysis of neuropsychological scores and normalized regional volumes (Fig. 4), there was a significant association between poorer fine motor skills and smaller cerebral white matter volume, independent of treatment (decrease of 0.16 in Z-score per -1% of TICV, 95% CI 0.02-0.29, p=0.023). The corrected critical p-value threshold was 0.0015, lower than the test statistic observed here. No other cognitive domain showed any association with the volume of any of the regions measured.

Where there were significant associations between neuropsychological scores and MRI biomarkers, outlier analysis did not change the overall findings.

DISCUSSION

In this sample of 76 HIV+ patients with plasma VL <400 copies/ml on treatment, we found that patients who had received PIM did not differ in regional brain perfusion or volumes from those who had received OTT. We also observed that grey matter hypoperfusion and white matter atrophy were associated with poorer fine motor skills, independently of treatment regimen. Our study supports other PIVOT analyses, that PIM carries no additional risk of neurological harm as a long-term treatment option for HIV [7, 9].

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This is the first study to compare CBF between different antiretroviral drug strategies and to correlate perfusion with specific cognitive domains in HIV+ patients. Previous work using ASL has reported lower CBF in HIV+ patients than in seronegative controls: one study reported lower CBF in the caudate nuclei only in HIV+ patients with cognitive impairment [12], while the other found lower CBF in the lenticular nuclei and visual cortex in both impaired and unimpaired patients [13]. In a third study by the same group [14], there was weak evidence (p=0.07) for reduced CBF in HIV+ patients. A recent study from the Netherlands reported lower grey matter CBF in virologically suppressed HIV+ men, of whom 17% were classified as cognitively impaired [28]. By contrast, two other studies of virologically suppressed patients found no difference in CBF between HIV+ and seronegative individuals [23, 29]. It may be that in asymptomatic, virologically-suppressed HIV+ patients, grey matter hypoperfusion is specifically associated only with fine motor impairment.

The mechanism by which HIV might affect brain perfusion and its association with one particular cognitive domain cannot be fully explained at present. It is curious that there is no association between grey matter hypoperfusion and cognitive domains such as executive function and learning and memory. One hypothesis is that tests of higher-order cognitive domains are more variable, therefore a larger sample size is required to see similar correlations. Another potential explanation is that patients who are relatively neurocognitively intact can develop compensatory strategies to maintain their performance in higher-level tasks but not in basic motor skills.

It also remains unclear whether brain hypoperfusion in HIV leads to functional impairment and parenchymal loss, or whether it is a consequence of brain dysfunction brought about by other mechanisms. Regulation of CBF is a complex process, but the two cell types central to the process are astrocytes and vascular endothelial cells [30] and both are vulnerable to invasion and activation by HIV [31].

The association between white matter volume loss and fine motor impairment complements previous work in which HIV status and impaired neuropsychological function were associated with smaller white matter volumes [32, 33]. To our knowledge, this is the first time that this structural change in white matter has been correlated with a specific cognitive domain in HIV. Impaired fine motor skills, slower finger tapping speed and deterioration in handwriting have long been recognized as features of HIV-associated brain disease. In a systematic review of MRI studies prior to 2000, subcortical atrophy was consistently associated with cognitive impairment in three studies of HIV+ patients [11]. In more recent studies, with more advanced HIV treatment, neuroimaging and post-processing techniques, HIV has been associated with small decreases in thalamic, caudate and frontal cortical volumes [34, 35] in patients with virological suppression, and total brain volume has been associated with current or previous immunodeficiency [36, 37]. In the Comorbidity in Relation to AIDS (COBRA) cohort, virologically-suppressed HIV+ patients aged over 45 had smaller brain volumes than HIV-negative controls in the baseline comparison [38] but there was no difference between the two groups in the rate of decline over 2 years of follow-up [29].

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Our study had some limitations. First, the sample size was relatively small and the number of hypotheses tested was large, therefore our findings may be influenced by chance variations or the effect of outliers. We have presented additional analyses of multiple hypothesis testing and outlier removal, with the intention that attempts are made to replicate our findings in further work. Second, we only evaluated HIV+ participants, limiting our ability to explore potential HIV-related effects on neuroimaging markers. Third, we cannot exclude temporary effects of PI monotherapy in this cross-sectional study, because there was an appreciable rate of switching away from the PIM strategy early on in the trial, largely due to its higher early virological failure rate [7]. However, we did conduct sensitivity analyses, comparing patients by their final treatment at trial exit as well as by initial treatment allocation.

Our findings have clinical importance for management of HIV+ patients. These results provide further evidence that in virologically suppressed HIV+ patients, PIM does not confer additional risk of brain injury over a period of 3-5 years. The long-term significance of brain hypoperfusion and volume loss and their relationship with impaired motor function in virologically suppressed HIV seropositive patients require further exploration. There are bedside screening tools available for assessing motor and psychomotor function, which may be useful for identifying HIV-associated neurodegeneration in treated patients, especially if supported by quantitation of white matter volume and cerebral perfusion. Cerebral perfusion imaging using ASL has also been shown useful in the pre-dementia stages of Alzheimer's disease, with a linear relationship between declining cortical CBF and advancing stages of pre-dementia and Alzheimer's dementia [39]. As quantitative neuroradiology becomes translated to clinical settings, novel techniques will have the potential for monitoring HIV-related neurodegeneration and for use in drug trials.

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Notes

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Tables

Table 1. Study participants with viral load <400 copies/ml at the time of brain imaging, compared by randomly allocated treatment group

Variable	Ongoing triple ART (n=37) [a]	Protease inhibitor monotherapy (n=39)	All patients	*P* value [b]
Age, mean years (SD)	47.1 (7.8)	49.0 (9.3)	48.1 (8.6)	0.34
White ethnicity, n (%)	33 (89)	31 (79)	64 (84)	0.35
Male, n (%)	31 (84)	32 (82)	63 (83)	1.0
Years since HIV diagnosis, median (IQR)	10.8 (6.7, 13.8)	11.2 (8.7, 16.3)	11.0 (8.3, 14.6)	0.26
Years of ART, median (IQR)	8.0 (6.4, 10.0)	8.1 (6.4, 11.1)	8.1 (6.4, 10.8)	0.78
Years of trial follow-up, median (IQR)	3.9 (3.7, 4.4)	3.9 (3.7, 4.3)	3.9 (3.7, 4.3)	0.73
Nadir CD4 count, median cells/mm^3 (IQR)	190 (70, 270)	150 (70, 240)	170 (70, 250)	0.20
Current CD4 count, median cells/mm^3 (IQR)	652 (550, 830)	590 (476, 760)	640 (490, 780)	0.13
Previous AIDS, n (%)	11 (30)	6 (15)	17 (22)	0.17
Alcohol use, median AUDIT-C score (IQR)	8 (4, 12)	5 (2, 12)	6 (3, 12)	0.12
Recreational drug use, n (%)				
None	9 (24)	8 (21)	17 (22)	0.95
>3 months ago	15 (41)	15 (38)	30 (39)	
Within past 3 months	12 (32)	15 (38)	27 (36)	
No response	1 (3)	1 (3)	2 (3)	

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Specific drug use, n (%)				
Cannabis	6 (16)	3 (8)	9 (12)	0.30
GBL / GHB	3 (8)	2 (5)	5 (7)	0.67
Cocaine	5 (14)	2 (5)	7 (9)	0.26
MDMA	4 (11)	1 (3)	5 (7)	0.19
Ketamine	2 (5)	2 (5)	4 (5)	1.0
Crystal meth	1 (3)	2 (5)	3 (4)	1.0
Mephedrone	2 (5)	1 (3)	3 (4)	0.61
Smoking, n (%)				
Past smoker	12 (32)	14 (36)	26 (34)	
Current smoker	12 (32)	8 (21)	20 (26)	0.49
BP, mean mm Hg (SD)				
Systolic	120 (12)	126 (20)	123 (17)	0.12
Diastolic	77 (10)	79 (12)	78 (11)	0.43
Body mass index, median kg/m^2 (IQR)	25.0 (23.4, 27.1)	25.5 (23.1, 30.2)	25.0 (23.2, 27.7)	0.76
Total fasting cholesterol, mean mmol/L (SD) [c]	5.2 (0.9)	5.4 (1.1)	5.3 (1.0)	0.48
Hemoglobin, median g/dL (IQR)	14.9 (13.6, 15.3)	14.6 (13.6, 15.3)	14.7 (13.6, 15.3)	0.51

Abbreviations: ART, antiretroviral therapy; AUDIT-C, Alcohol Use Disorders Identification Test consumption questions; BP, blood pressure; GBL, gamma-butyrolactone; GHB, gamma-hydroxybutyric acid; IQR, inter-quartile range; MDMA, 3,4-methylenedioxy-methamphetamine; SD, standard deviation.

[a] Excludes 2 patients who were failing or discontinued therapy (VL >400 copies/ml) at the time of brain imaging.

[b] P value for comparison between ongoing triple ART and protease inhibitor monotherapy groups. Statistical tests were the t-test (between-group comparison of age, blood pressure and cholesterol), Mann-Whitney test (years since HIV diagnosis and ART initiation, years in trial, CD4 count, education, hemoglobin, alcohol consumption score and body mass index), Fisher's exact test (gender, ethnic group, previous AIDS and recreational drug use), or Chi-square test (smoking history).

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^c Conversion factor for total cholesterol concentration: 1 mmol/L = 38.7 mg/dL.

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Table 2. Comparison of cerebral blood flow and regional volumes between treatment strategies, as allocated at baseline trial visit

Region of interest [a]	Ongoing triple ART (n=37)	Protease inhibitor monotherapy (n=39)	*P* value (adjusted) [b]
Resting cerebral blood flow, mean (SD) in mL 100g^{-1} min^{-1}			
Caudate nucleus	37.5 (8.5)	37.2 (7.9)	0.74
Lentiform nucleus	35.3 (6.1)	34.4 (6.3)	0.90
Thalamus	43.8 (10.6)	43.6 (8.4)	0.81
Frontal cortex	80.1 (13.2)	77.3 (13.3)	0.71
Occipital cortex	67.9 (12.6)	70.8 (10.9)	0.08
Cingulate cortex	64.9 (9.9)	63.8 (12.6)	0.87
Cerebral white matter	13.7 (2.9)	13.0 (2.4)	0.43
Cerebral cortical GM	69.8 (11.4)	69.1 (11.4)	0.70
Regional volume, mean (SD) in mL			
Caudate nucleus	7.5 (1.0)	7.2 (0.9)	0.72
Lentiform nucleus	13.2 (1.3)	12.7 (1.4)	0.16
Thalamus	13.3 (1.3)	12.6 (1.4)	0.09
Hippocampus	9.5 (1.0)	9.2 (1.0)	0.60
Frontal cortex	171.1 (15.0)	165.9 (17.6)	0.47
Occipital cortex	79.9 (8.4)	75.4 (8.1)	0.07
Cingulate cortex	26.4 (2.6)	25.3 (3.0)	0.50
Cerebral white matter	432.4 (51.0)	422.3 (45.1)	0.63
Cerebral cortical GM	496.2 (39.7)	479.4 (47.0)	0.15
Non-ventricular CSF	318.8 (24.8)	316.4 (27.7)	0.36
Ventricular CSF [c]	27.0 (1.4)	31.8 (1.5)	0.047

Abbreviations: ART, antiretroviral therapy; GM, grey matter; SD, standard deviation.

[a] Cerebral blood flow was averaged between left and right sides; regional volumes are totals of left and right.

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[b] Linear regression models adjusted for age, sex and ethnic group, and alcohol use (regional volumes) or hemoglobin (cerebral blood flow).

[c] Ventricular CSF volume was log-transformed before analysis, and back-transformed to display the geometric mean and SD.

Table 3. Comparison of regional volumes and cerebral blood flow by treatment strategy, in participants who remained on their allocated treatment arm throughout the trial

Region of interest [a]	Ongoing triple ART (n=34)	PI monotherapy (n=30)	P value (adjusted) [b]
Cerebral blood flow, mean (SD) in mL 100g^{-1} min^{-1}			
Caudate nucleus	37.3 (9.0)	36.4 (8.2)	0.87
Lentiform nucleus	35.5 (6.3)	34.3 (6.3)	0.58
Thalamus	43.8 (11.3)	44.3 (8.8)	0.70
Frontal cortex	80.2 (13.8)	77.5 (13.9)	0.73
Occipital cortex	67.8 (12.9)	71.3 (11.5)	0.12
Cingulate cortex	65.4 (10.3)	63.9 (13.2)	0.86
Cerebral white matter	13.8 (3.1)	13.3 (2.3)	0.66
Cerebral cortical GM	70.0 (11.9)	69.3 (11.8)	0.79
Regional volume, mean (SD) in mL			
Caudate nucleus	7.4 (0.9)	7.2 (0.9)	0.93
Lentiform nucleus	13.2 (1.4)	12.8 (1.3)	0.37
Thalamus	13.1 (1.3)	12.7 (1.4)	0.18
Hippocampus	9.4 (1.0)	9.3 (0.8)	0.86
Frontal cortex	170.5 (15.4)	165.3 (17.6)	0.30
Occipital cortex	79.5 (8.6)	76.0 (7.8)	0.21
Cingulate cortex	26.4 (2.7)	25.3 (3.2)	0.14
Cerebral white matter	429.5 (51.6)	426.4 (39.1)	0.95
Cerebral cortical GM	494.5 (40.9)	480.9 (45.0)	0.16
Non-ventricular CSF	317.6 (25.4)	322.2 (25.4)	0.22
Ventricular CSF [c]	27.8 (1.4)	32.6 (1.4)	0.11

Abbreviations: ART, antiretroviral therapy; CSF, cerebrospinal fluid; GM, grey matter; PI, protease inhibitor; SD, standard deviation.

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a Cerebral blood flow was averaged between left and right sides; regional volumes are totals of left and right

b Linear regression models were adjusted for age, sex and race, and alcohol use (regional volumes) or haemoglobin (cerebral blood flow)

c Ventricular CSF volume was log-transformed before analysis, and back-transformed to display the geometric mean and SD

Figure legends

Figure 1: Illustration of segmentation of regions of interest.

A: Sample of T1-weighted image slices after skull stripping. B: Color highlight applied to the same T1-weighted image showing caudate nucleus (deep pink), lenticular nucleus (light purple), thalamus (light pink), frontal lobe (green), occipital lobe (brown), cingulate cortex (deep purple), other cortical grey matter (light blue) and supratentorial white matter (yellow).

Figure 2: Example images of volumetric and arterial spin labelling (ASL) sequences showing examples of opposite ends of the spectrum of neuropathology

A, B: Volumetric T1-weighted and ASL sequences (respectively) from a participant with normal fine motor function, higher ratio of white matter volume to total intracranial volume (TICV), and normal cerebral blood flow (CBF). C, D: Volumetric T1-weighted and ASL sequences (respectively) from a participant with impaired fine motor function, lower ratio of white matter volume to TICV, and lower CBF.

Figure 3: Association between normalized regional cerebral blood flow and neuropsychological domain Z-scores

Abbreviations: WM, working memory.

From left to right, the eight spikes within each cognitive domain correspond to strength of association between Z-score and cerebral blood flow in the following regions: caudate nucleus, lentiform nucleus, thalamus (all subcortical grey matter in red open circles), frontal cortex, occipital cortex, cingulate cortex, cerebral cortical grey matter (all cortical grey matter in blue triangles) and cerebral white matter (dark grey diamonds). To allow presentation of results on the same y-axis, cerebral blood flow measurements were normalized using the mean and standard deviation in the whole patient sample in each region of interest. Regression coefficients were adjusted for age, sex, ethnicity and hemoglobin. Cognitive tests were of executive function (Color Trails Test Part 2 and Stroop Color-Word Test), fine motor skills (Grooved Pegboard Test and Finger-Tapping Test with dominant and non-dominant hands), attention and working memory (Color Trails Test Part 1 and digit-symbol test), verbal memory (Hopkins Verbal Learning Test – Revised) and non-verbal memory (Rey Complex Figure Test).

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Figure 4: Association between normalized brain volumes and neuropsychological domain Z-scores

Abbreviations: CSF, cerebrospinal fluid; WM, working memory.

From left to right, the seven points in each cognitive domain correspond to strength of association between Z-score and volume of the following regions: caudate nucleus, lentiform nucleus, thalamus (all subcortical grey matter in red open circles), cerebral cortical grey matter (blue triangles), cerebral white matter (dark gray diamonds), ventricular and non-ventricular CSF (green open squares). To allow presentation of results on the same y-axis, volume measurements were normalized using the mean and standard deviation in the whole patient sample in each region of interest. Regression coefficients were adjusted for age, sex, education, ethnicity and alcohol consumption. Ventricular CSF volume was log-transformed to ensure linear regression assumptions were met. Cognitive tests were of executive function (Color Trails Test Part 2 and Stroop Color-Word Test), fine motor skills (Grooved Pegboard Test and Finger-Tapping Test with dominant and non-dominant hands), attention and working memory (Color Trails Test Part 1 and digit-symbol test), verbal memory (Hopkins Verbal Learning Test – Revised) and non-verbal memory (Rey Complex Figure Test).
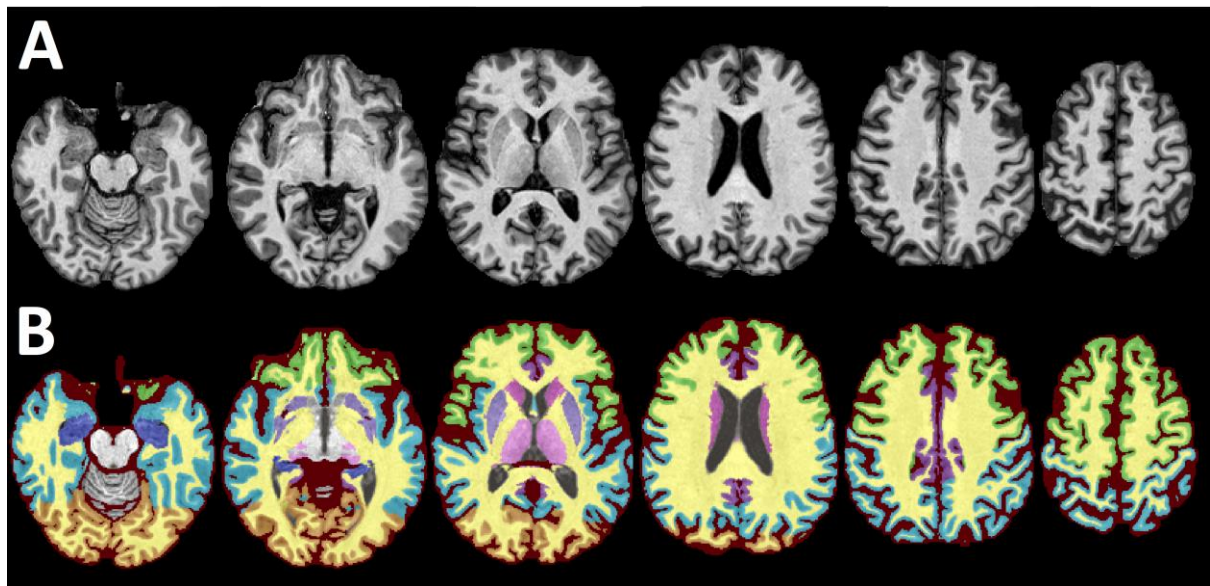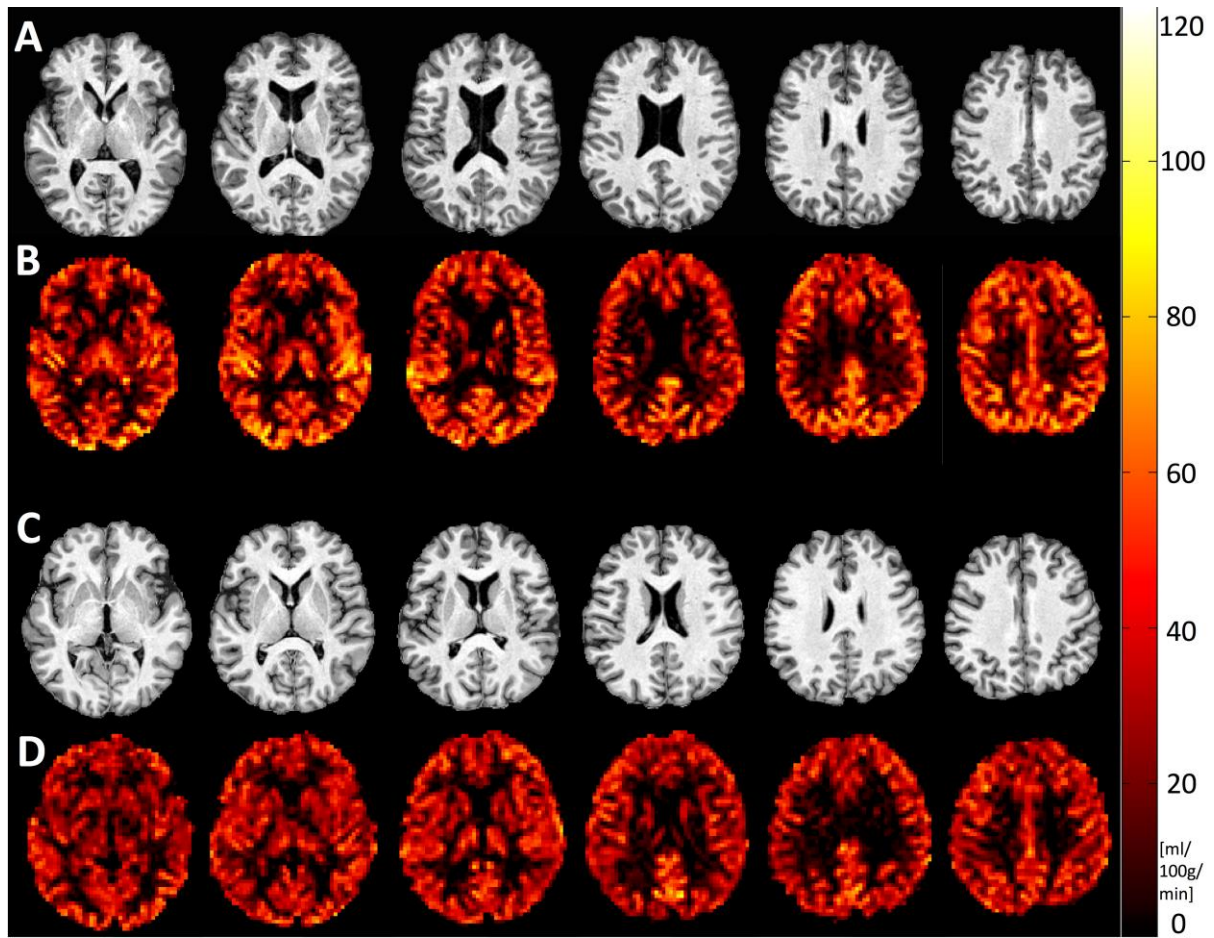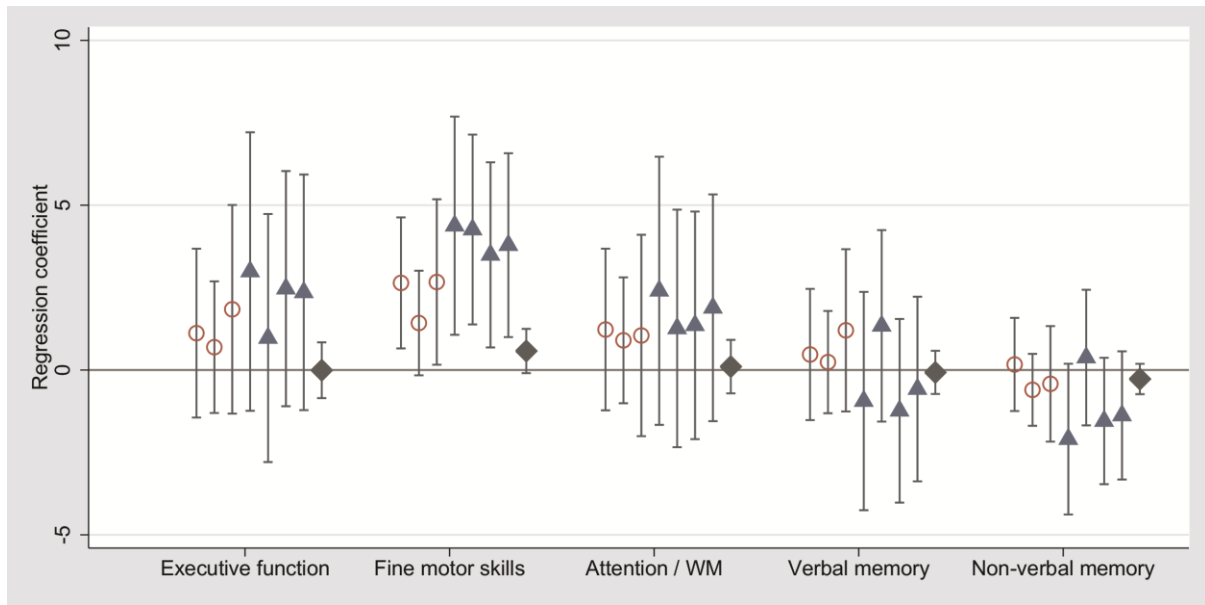
Figure 1

Figure 2

Figure 3

Figure 4