Title: Choice of combination antiretroviral therapy (cART) alters changes in cerebral function testing after 48 weeks in treatment-naïve, HIV-1 infected subjects commencing cART: a randomised controlled study.

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In a prospective, randomised study, we describe different changes in cerebral-function testing parameters, including neurocognitive function, in therapy naive HIV-infected subjects commencing different combination antiretroviral regimens.

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Abstract:

Background: Neurocognitive (NC) impairment remains prevalent, despite combination antiretroviral therapy (cART). Differences between changes in cerebral-function and alternative cARTs have not been prospectively assessed.

Methods: HIV-infected therapy-naïve individuals, randomly allocated to commence cART (TDF/FTC plus either EFV(*arm1*), ATV/RTV(*arm2*) or AZT/ABC(*arm3*)) were eligible. Cerebral-function tests included NC testing (CogState™) and assessment of cerebral metabolites using proton-MRS in several anatomical voxels including right frontal white matter (FWM) and basal ganglia (RBG), at baseline and after 48 weeks. N-acetyl-aspartate/creatine (NAA/Cr) ratios were calculated. Differences between changes in NC function and NAA/Cr ratios over 48 weeks and study arms (*arm 1v2 and 1v3*) were assessed.

Results: Thirty subjects completed study procedures (9, 9, 12 subjects in *arms 1,2,3* respectively). Mean CD4+ counts(SD, cells/μL) were 218(87) and 342(145) at baseline and week 48, respectively. Plasma HIV RNA was <50 copies/mL in 28/30 subjects at week 48. Over 48 weeks, greater improvements in identification reaction time (IRT, p=0.04) and executive function (p=0.02) were observed in *arm 3v1* (+0.03, -0.30, -0.50 $\log_{10}$ msec change IRT, in *arms 1,2,3* respectively). Increases in NAA/Cr were observed in all voxels (maximum 38% in RBG) with greater increases observed in *arm 1v2* (p=0.03) in FWM (30%, -7%, 0% change in NAA/Cr, in *arms 1,2,3* respectively).

Conclusions: This is the first study to prospectively describe different changes in cerebral-function testing parameters between different cARTs. Greater improvements in neuronal recovery (NAA/Cr ratio) were observed in recipients of TDF/FTC plus EFV (*arm1*) and greater improvements in NC function testing observed in recipients of TDF/FTC plus ABC/AZT (arm3).

Keywords: HIV; antiretroviral therapy; central nervous system; cognitive; magnetic resonance spectroscopy.

Manuscript

Introduction

In recent years, the development of combination antiretroviral therapy (cART) for the treatment of human-immune-deficiency virus-1 (HIV) has been associated with extraordinary improvements in prognosis for persons living with chronic HIV infection. Life expectancy has increased dramatically [1]. Despite effective therapies, challenges remain in the management of chronic HIV infection. One such challenges is ongoing HIV associated cerebral impairment [2, 3]. Although severe HIV related cerebral impairment, termed HIV-associated-dementia, is now less frequently observed [4], less fulminant forms of neurocognitive (NC) impairment are increasingly being recognised [2, 5, 6]. Impairment in NC function in HIV-infected subjects in the cART era has been associated with poor compliance with cART [7], reduced quality of life [8] and increased mortality [9].

Reported factors associated with the development of NC function impairment in HIV disease and risks associated with progression of such impairment include degree of immune suppression related to HIV infection [10], other chronic viral infections, such as chronic hepatitis C co-infection [11] and age [12].

Furthermore, specific antiretroviral regimens may have different effects on cerebral function. In general, NC function improves after commencing cART [13], but the effects of different antiretroviral therapies on these changes have not been prospectively assessed. The penetration of different antiretroviral agents into the central nervous system (CNS) differ [14]. Agents with better CNS penetration may offer improved HIV viral suppression in the CNS compartment and may therefore be associated with greater improvements in NC function. However, cerebral toxicities may ensue, thus limiting these potential benefits [15].

The aim of this study was to assess changes in cerebral function testing in antiretroviral therapy-naïve HIV-infected individuals commencing three different combination treatment regimens within a prospective, randomised study. Assessment of cerebral function included NC function testing and measurement of cerebral metabolite ratios using magnetic resonance spectroscopy (MRS).

Methods

Patient selection and study procedures

Patients attending 4 sites (St. Mary's Hospital, London, UK; Queen Elizabeth Hospital, Kowloon, Hong Kong; HIVNAT, Bangkok, Thailand; Southern Alberta HIV clinic, Calgary, Canada) entering the ALTAIR study (A Randomised, Open-Label, 96-Week Study Comparing the Safety and Efficacy of Three Different Combination Antiretroviral Regimens as Initial Therapy for HIV Infection) [16] were eligible to enter this 48 week sub-study.

Study subjects were randomly allocated to commence cART comprising tenofovir/emtricitabine 300/200 mg once daily with either efavirenz 600 mg once daily (*arm1*), atazanavir/ritonavir 300/100 mg once daily (*arm2*), or zidovudine/abacavir 250 or 300 mg twice daily/600 mg once daily (*arm3*).

Eligible subjects were HIV antibody positive and naïve to antiretroviral therapy. Specific exclusion criteria for this sub-study included current or recent use of antidepressant or antipsychotic therapies, current or recent history of alcohol or recreational drug dependence, recent significant head injury, established dementia, active opportunistic infections, untreated early syphilis, hepatitis C infection (hepatitis C antibody positive) or evidence of established chronic liver disease, cirrhosis or hepatic encephalopathy (recent defined as the previous 12 weeks). Furthermore, in the 48 hour period prior to study investigations being performed, consumption of alcohol or caffeine was not permitted.

Specific study procedures to assess cerebral function involved patients attending for NC testing at baseline and week 48, and cerebral MRS at baseline and week 48 as detailed below. Subjects also attended after 1 month of therapy and thereafter every 3 months for assessment of safety laboratory parameters, CD4+ lymphocyte count and plasma HIV RNA (all performed by local laboratories).

Cognitive Testing

A computerised cognitive test battery was undertaken (CogState™) which has previously been described in detail and validated in HIV-infected subjects [17]. The computerised

assessment was presented on a desktop computer. All tasks within the battery are adaptations of standard neuropsychological and experimental psychological tests, which assess a range of cognitive functions. The following domains were assessed: detection, identification, learning (matching learning and associate learning), monitoring, working memory and executive function. The computerised battery requires approximately 15 – 20 minutes for completion. The battery consists of tasks in the form of card games, therefore subjects need only have an understanding of playing cards, thereby minimising language and cultural differences between study subjects. All study participants completed one full practice test prior to undertaking the study examination to obtain optimal performance at baseline [18].

Cerebral ^{1}H Magnetic Resonance Spectroscopy

Proton (^{1}H) MRS was performed on an Achieva™ 1.5 Tesla scanner (St. Mary's Site, London, UK), a Siemens Avanto™ 3.0 Tesla scanner (Queen Elizabeth Hospital Site, Kowloon, Hong Kong), a 1.5 Tesla Signa General Electric scanner (HIVNAT site, Bangkok, Thailand) and a 3.0 Tesla Signa Excite^{TM} scanner (South Alberta HIV clinic, Calgary, Canada). Examinations began with sagittal, coronal and axial T_1-weighted MR images of the brain to enable accurate positioning of the voxels and T_2-weighted axial double spin echo images to exclude any visible cerebral pathology.

^{1}H MRS was performed in three voxel locations: right frontal white matter (FWM), mid-frontal grey matter (FGM) and the right basal ganglia (RBG) (*Figure 1*). These anatomical voxels were selected based on previous imaging studies describing cerebral metabolite abnormalities patterns in such voxels in HIV-infected individuals [19, 20]. MRS data were acquired by single voxel examination in the three areas, using a double spin echo point resolved spectroscopy (PRESS) sequence with the following settings: echo time (TE) 36 ms, repetition time (TR) 3000 ms, 2048 data points, spectral width of 2500 Hz and 128 data acquisitions. Spectra were post-processed using the MRI machine manufacturer's software for automated water signal suppression and water shimming. Each examination lasted approximately 35 minutes. A study MRS operations manual was developed to ensure all MRS examinations at each study site were undertaken using identical operational settings.

All spectra were analysed and quantified by one observer (AW) using a java-based version of the Magnetic Resonance User Interface package (jMRUI Version Number: 3.0)[21], incorporating the AMARES algorithm [22]. Metabolites assessed were N-acetyl-aspartate (NAA), creatine (Cr), choline (Cho) and myo-Inositol (MI). To adjust for different MRI scanners across sites, all metabolites were expressed as ratios with respect to cerebral Cr.

Statistical Methods

Statistical analyses were conducted with SAS version 9.13 (SAS, Cary, NC, USA) and Stata version 10.1 (Statacorp, College Station, TX, USA). Linear regression modelling was used to estimate the effect size for absolute differences in metabolite ratio for individual subjects from baseline to week 48, in pairwise comparisons between *arm1* and *arms 2* and *3*. A Wilcoxon rank sum test was also used to confirm the *p*-values derived from regression models. Other baseline covariates were also tested in univariate regression models, and those with $p \leq 0.15$ were entered into multivariate models. For the cognitive testing results, analysis was conducted according to Cogstate™ recommendations. Reaction times were $\log_{10}$ transformed due to a positive skew of the distribution, and accuracy measures were transformed using arcsine-root transformation. Change scores were calculated for each subject, and these scores standardised according to the within-subject standard deviation (SD). Changes in performance for *arms2* and *3* compared to *arm1* were standardised with a pooled SD, and this used as the outcome variable in linear regression models to calculate an overall effect size for the difference between treatment groups. Composite changes from baseline scores were calculated on the average of standardised reaction time and accuracy scores.

Results

Subject characteristics

30 subjects were enrolled and all completed study procedures (9, 9, 12 subjects in *arms 1,2,3* respectively). Mean CD4+ lymphocyte count (SD, cells/uL) was 218 (87) and 342 (145)

at baseline and week 48, respectively (*Table 1*). At week 48, all subjects remained on randomised study therapy and HIV RNA was less than <50 copies/mL in 28/30 subjects. In the remaining two subjects, HIV RNA was 490 copies/mL and 113 988 copies/mL. This was considered a HIV RNA viral blip in the first subject (study *arm* 2; with no changes to antiretroviral therapy undertaken and subsequent HIV RNA was below detection) and virological failure in the second subject (*arm3*), in whom antiretroviral therapy was modified.

Cognitive testing results

NC testing results, including changes over 48 weeks are shown on *Table 2*. Overall, improvements in all NC testing parameters were observed over the study period. In domains where speed of task was the primary measure (detection, identification, monitoring and matched learning), mean speed improved (reduction in time) over the study period. For example, mean monitoring reaction time changed from 2.61 to 2.58 $\log_{10}$milliseconds at baseline and week 48. In the cognitive domains where accuracy was the primary measurement (one card learning, working memory and associate learning), where an increase in accuracy represents improved function, improvements were also observed over the study period. Lastly, the domain of executive function was assessed via total errors made on testing, where a reduction in score represented improvement in performance. Again improvements were observed over the study period (total mean number of errors on executive function tests reduced from 49.64 errors at baseline to 44.82 errors at week 48).

Statistically significantly greater improvements in study treatment *arm3* versus *arm1* were observed in speed of identification task (p=0.04), number of errors in executive function task (p=0.02) and in overall composite speed performance (p=0.02). A box-plot showing changes in speed performance tasks between study treatment arms is shown in *Figure 2*. No other statistically significant differences between changes in NC testing results and study treatment arms were observed and none of the described associations differed when excluding subjects with detectable plasma HIV RNA at week 48 or correcting for age in a sensitivity analysis.

Magnetic resonance spectroscopy results

Over 48 weeks, increases in NAA/Cr ratios were observed in all anatomical voxels (mean percentage increase 5, 17 and 38% in FWM, FGM and RBG respectively). Smaller changes in Cho/Cr and MI/Cr ratios were observed over 48 weeks (mean percentage change 3, 4 and -12% for Cho/Cr ratio and 14, 5 and 15% for MI/Cr ratio in FWM, FGM and RBG respectively).

Absolute changes in metabolite ratios between study treatment arms in a univariate model are shown on *Table 3*. A greater increases in NAA/Cr ratio in FWM was observed in *arm1*, compared to *arm2* (29% increase versus 18% increase for *arm1* versus *arm2*, respectively: p=0.041) and compared to *arm3* (29% increase versus 9% increase for *arm1* versus *arm3*, respectively: p=0.054). A box-plot of absolute NAA/Cr ratio changes between study treatment arms is shown in *Figure 3*.

A trend towards a greater increase in MI/Cr ratio over 48 weeks in *arm3* versus *arm1* was observed in FWM (mean percentage change 39 versus -24% in *arm3* versus *arm1*: p=0.097). No other significant changes between individual metabolite ratios and study treatment arms were observed.

In a multivariate model (*Table 4*), absolute change in NAA/Cr ratio over 48 weeks was statistically significantly greater in *arm1* versus *arm2* (p=0.03). No other factors, including ethnicity, age or detectable plasma HIV RNA at week 48 were associated with these changes (p>0.15 for all comparisons).

Lastly, no significant associations were observed between changes in cerebral metabolite ratios and NC testing results.

Discussion

In this prospective, randomised study, we observed improvement in overall NC function and in cerebral metabolite ratios over the 48 week study period, with significant differences between study treatment groups.

Several previous reports have described a reduction in NAA and NAA/Cr ratio, particularly in frontal cerebral areas, in subjects with HIV-associated dementia [19, 23-27] and associations between the reduction of NAA/Cr ratios and the degree of cognitive decline have been reported [28]. Improvements in MRS-measured NAA values have been described after commencing antiretroviral therapy, and such improvements associated with the degree of cognitive recovery [29, 30]. However, to our knowledge, differences in such improvements between different cART regimens have not been described before. We have observed significantly greater increases in subjects on study *arm1*, compared to the other study treatment groups. A possible explanation for this finding is a greater effect on CNS HIV viral replication associated with the cART regimen in study *arm1*, thereby facilitating greater neuronal recovery. This treatment arm contained the non-nucleoside-reverse-transcriptase-inhibitor, efavirenz. In general the non-nucleoside-reverse-transcriptase-inhibitor have been described to penetrate into the cerebro-spinal-fluid (CSF) [14] and the reported CNS penetration of efavirenz greater than the drug concentration required to suppress HIV viral replication [31]. This characteristic may have greater effects on HIV viral suppression in the cerebral compartment, hence explaining the greater rise in neuronal markers we have observed.

As NAA is a marker of neuronal integrity, decreased NAA/Cr is assumed either to be due to neuronal loss, or perhaps, neuronal dysfunction. Of importance to this study, we used metabolite ratios to obviate differences in field strength and manufacturer operator-dependent differences between MR scanners across study sites (something which has hampered the execution of multi-centre MRS studies until now).

MI is an intracellular osmolyte, governing cell size regulation and is present in all brain cells, including microglia. Elevations in this metabolite are thought to reflect microglial activation in the context of HIV disease [32].. Elevations in MI/Cr [33, 34] ratios have been described in HIV-infected subjects with cognitive decline. Previous groups have described improvement in these elevations in HIV-infected subjects after commencing antiretroviral therapy [30]. In this study, we observed a rise in MI/Cr ratio, rather than a reduction (MI/Cr ratio rise of 14, 5 and 15% in FWM, FGM and RBG respectively). Our study differs from previous MRS studies in HIV-infected individuals as subjects recruited had no prior symptoms of NC function disturbance at study entry, whereas other studies have focused on investigating subjects

with HIV-associated-dementia. These differences in natural history of study populations could explain the differences in MI/Cr ratio that we have observed, compared to the published literature. Commencement of cART in neurologically asymptomatic individuals with little evidence of cerebral microglial activation may be associated with a drug-induced inflammatory response, rather than the improvements as previously described.

Previous MRS studies also have suggested that some antiretroviral agents with good CNS penetration are associated with specific CNS drug-induced toxicity. Cerebral metabolites have been compared in HIV-infected subjects receiving cART, containing either the nucleoside-analogues didanosine or stavudine, compared to subjects receiving zidovudine and lamivudine [35]. As didanosine and stavudine have been associated with greater peripheral mitochondrial toxicities [36], the authors wished to assess if evidence of CNS mitochondrial toxicities were present. Results from this study reported NAA concentration in the FGM 11% lower, compared to healthy control data, in subjects receiving stavudine or didanosine, whereas NAA concentrations in the zidovudine and lamivudine group were intermediate supporting this theory. Interestingly, in our study, temporal differences in MI/Cr ratio in FWM were observed with treatment: a rise of 39% was observed in study *arm3* versus a reduction of 24% in *arm1* ($p=0.097$ and 0.080 in univariate and multivariate models, respectively). Although study *arm3* did not contain either stavudine or didanosine, it did comprise a 4 nucleoside-analogue cART regimen (tenofovir, emtricitabine, zidovudine and abacavir). If such mitochondrial toxicities are a class effect of the nucleoside-analogues, the rise in MI/Cr ratio we have observed may be secondary to these effects.

Despite the propensity to cause mitochondrial toxicities, the nucleoside-analogues have in general been described to have beneficial CNS penetration [14] and have be associated with improvements in cerebral function. Zidovudine was the first antiretroviral agent available and in both randomised [37] and observational [38, 39] clinical studies improvement in NC function were observed in HIV-infected individuals treated with this agent. Rather disappointing results were described with the advent of abacavir with regards to improvements in NC function, but the lack of effect observed in a randomised study may have been related to HIV viral resistance to this agent in heavily drug-treatment experienced HIV-infected subjects [40]. We have observed greater improvement in overall NC performance in subjects randomised to study *arm3*. This may be related to several

factors. First, a quadruple nucleoside-analogue regimen may confer optimal CNS antiretroviral drug penetration and the suppression of HIV viraemia in the CNS. Second, clinical toxicities secondary to other treatment arms in the study may have effects on NC testing. For instance efavirenz, a component of *arm1*, is associated with several neuropsychiatric side effects [41, 42], such as abnormal dreams, lethargy and depression.. These effects may have blunted improvement on serial NC testing in *arm1* in this study.

Other groups have reported discrepancies between antiretroviral drug penetration into the CNS and clinical NC responses. In a recently reported prospective cohort, HIV-infected subjects underwent serial NC function testing and CSF HIV RNA quantification [15]. This series included subjects on differing cART regimens. As expected, subjects receiving cART with greater CNS penetration scores [14], had lower CSF HIV RNA on lumbar puncture examination. However poorer NC performance was also observed in the group with greater cART CNS penetration scores. These data suggest some antiviral agents with good CNS penetration may have added CNS toxicities and thereby impair NC function testing results. Unfortunately the study was not powered to assess the effects of specific antiretroviral agents on NC testing results.

It may be asked how our results relate to clinical practice. We studied a small number of patients and hence the interpretation of the findings should be made with some degree of caution. However, we have observed significant differences in cerebral effects between different antiretroviral regimens. A quadruple nucleoside-analogue regimen displayed greater benefits after 48 weeks of therapy as measured by NC testing results. These data could be used to power future studies assessing changes in NC function between different regimens. Novel HIV treatment studies are underway assessing nucleoside-analogue-sparing cART in order to avoid the mitochondrial toxicities associated with this class of agents. Such studies are justified and timely, but investigators should also consider cerebral function testing as part of these programmes to ensure NC impairment does not develop when utilising nucleoside-analogue-sparing treatment options. Lastly, we have observed the greatest improvement in neuronal cerebral metabolite recovery (NAA/Cr ratios) within the efavirenz arm (*arm1*) and observed increased MI/Cr ratios (probably representing cerebral microglial activation) with a quadruple nucleoside-analogue regimen (*arm3*). These data add to the literature of evidence suggesting the CNS penetration of antiretroviral therapy

may have both therapeutic benefits and potential toxicities and may assist in the design of future non-invasive imaging studies assessing the effects of different antiretroviral treatment options on the CNS.

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Table 1

Baseline and follow up patient characteristics

	Overall	**Arm 1** efavirenz + tenofovir/emtricitabine	**Arm 2** atazanavir/ritonavir + tenofovir/emtricitabine	**Arm 3** zidovudine/abacavir + tenofovir/emtricitabine
Baseline Characteristics:				
number subjects	**30**	**9**	**9**	**12**
baseline CD4+, cell/µL (SD)	218 (87)	235 (56)	194 (84)	222 (109)
baseline CD4+ %, % (SD)	13.6 (4.4)	14.3 (2.7)	13.7 (6)	13.08 (4.4)
nadir CD4+, cell/µL (SD)	192 (83)	170 (50)	171 (67)	207 (112)
HIV RNA, log10 copies/mL (SD)	4.64 (0.68)	4.47 (0.76)	4.86 (0.63)	4.59 (0.67)
age, years (SD[†])	35 (10)	35 (11)	37 (10)	33 (19)
ethnicity, (n)				
Asian	18	4	7	7
Caucasian	11	4	2	5
Afro Caribbean	1	1	0	0
Follow up results				
week 24 CD4+, cell/µL (SD)	337 (121)	332 (96)	346 (120)	333 (147)
week 24 HIV RNA < 50 copies/mL (n of total)	27 of 30	8 of 9	8 of 9	11 of 12
List of HIV RNA loads where detectable at week 24, (copies/mL)		64	200	1287
week 48 CD4+, cell/uL (SD)	342 (145)	299 (135)	400 (160)	331 (138)
week 48 HIV RNA < 50 copies/mL (n of total)	28 of 30	9 of 9	8 of 9	11 of 12
List of HIV RNA loads where detectable at week 48, (copies/mL)			490	113988
Changes to randomised therapy, (n)	1	0	0	1 †

(*Table 1* legend:

Abbreviation – SD = standard deviation.

† switched to boosted protease inhibitor after week 48 visit)

Table 2:

Neurocognitive testing parameters and changes over 48 weeks

	Overall			Arm 1			Arm 2				Arm 3			
Cognitive domain	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**	***p*-value arm 2 v 1**	**N**	**Mean**	**SD**	***p*-value arm 3 v 1**
Detection†														
Baseline	28	2.55	0.13	9	2.51	0.13	8	2.56	0.16		11	2.57	0.11	
Week 48	29	2.54	0.13	9	2.55	0.18	8	2.55	0.10		12	2.54	0.13	
Change versus arm1 (95% CI)*								-0.513	(-1.501 to 0.475)	0.30		*-0.717*	(-1.631 to 0.197)	0.12
Identification†														
Baseline	28	2.74	0.09	9	2.72	0.12	8	2.76	0.08		11	2.75	0.07	
Week 48	29	2.72	0.09	9	2.75	0.14	8	2.73	0.06		12	2.70	0.05	
Change versus arm1 (95% CI)*								-0.681	(-1.635 to 0.273)	0.15		-0.908	(-1.791 to -0.026)	**0.04**
Monitoring†														
Baseline	28	2.61	0.10	9	2.58	0.10	8	2.66	0.10		11	2.60	0.10	
Week 48	29	2.58	0.09	9	2.57	0.11	8	2.60	0.11		12	2.58	0.07	
Change versus arm1 (95% CI)*								-0.809	(-1.793 to 0.175)	0.10		-0.288	(-1.198 to 0.623)	0.51
Learning (matched)†														
Baseline	28	2.83	0.06	9	2.82	0.09	8	2.83	0.04		11	2.83	0.05	
Week 48	29	2.82	0.09	9	2.83	0.15	8	2.83	0.05		12	2.80	0.06	
Change versus arm1 (95% CI)*								-0.290	(-1.288 to 0.708)	0.56		-0.652	(-1.576 to 0.271)	0.27
One card learning††														
Baseline	28	2.61	0.10	9	2.58	0.10	8	2.66	0.10		11	2.60	0.10	
Week 48	29	2.58	0.09	9	2.57	0.11	8	2.60	0.11		12	2.58	0.07	
Change versus arm1 (95% CI)*								-0.046	(-1.060 to 0.969)	0.93		0.383	(-0.538 to 1.304)	0.40
Working memory††														
Baseline	28	1.11	0.35	9	1.08	0.36	8	1.17	0.21		11	1.09	0.44	
Week 48	29	1.22	0.20	9	1.18	0.30	8	1.25	0.15		12	1.22	0.14	

Change versus arm1 (95% CI)*								-0.057	(-1.094 to 0.981)	0.91		0.105	(-0.854 to 1.065)	0.82
Associate learning††														
Baseline	28	0.88	0.20	9	0.82	0.26	8	0.99	0.17		11	0.86	0.16	
Week 48	29	0.91	0.22	9	0.81	0.24	8	1.03	0.13		12	0.89	0.23	
Change versus arm1 (95% CI)*								0.240	(-0.793 to 1.274)	0.64		0.229	(-0.727 to 1.185)	0.63
Executive function†††														
Baseline	28	49.64	25.22	9	43.44	27.86	8	47.38	18.55		11	56.36	27.69	
Week 48	28	44.82	20.96	9	48.44	21.83	8	48.63	18.28		11	39.09	22.61	
Change versus arm1 (95% CI)*								-0.259	(-1.652 to 1.134)	0.71		-1.539	(-2.828 to -0.251)	**0.02**
Composite speed score														
Baseline	28	2.68	0.08	9	2.66	0.10	8	2.70	0.08		11	2.69	0.07	
Week 48	29	2.67	0.09	9	2.68	0.08	8	2.68	0.07		12	2.65	0.06	
Change versus arm1 (95% CI)*								-0.785	(-1.729 to 0.158)	0.10		-0.939	(-1.812 to -0.066)	**0.04**
Composite accuracy score														
Baseline	28	0.93	0.22	9	0.88	0.23	8	1.02	0.15		11	0.91	0.24	
Week 48	29	0.99	0.14	9	0.92	0.18	8	1.06	0.15		12	0.99	0.12	
Change versus arm1 (95% CI)*								0.055	(-0.974 to 1.084)	0.91		0.362	(-0635 to 1.268)	0.50

(*Table 2* legend: abbreviations – CI = confidence interval, SD = standard deviation.

* Changes assessed using methodology recommended by Cogstate™. Briefly, change standardised scores were weighted by the pooled standard deviation, and entered into a linear regression model with arm as a categorical covariate. Change coefficient represents the mean difference for each treatment group compared to *arm1*, and *p*-values are the pair-wise comparative significance tests.

† Values $\log_{10}$ milliseconds (speed) – lower score represents improved response

†† Values arcsine correct responses (accuracy response) – higher score represents improved response

††† Total number of errors – lower score represents improved response)

Table 3:

Cerebral metabolite ratios and changes over 48 weeks.

	Overall			Arm 1			Arm 2			Arm 3			
Frontal White	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**	***p*-value arm 2 v 1 ††**	**N**	**Mean**	**SD**	***p*-value arm 3 v 1 ††**
NAA/Cr baseline	28	1.879	0.344	7	1.860	0.280	9	1.834	0.269		12	1.924	0.436	
NAA/Cr 48 weeks	28	1.956	0.746	7	2.481	1.115	9	1.677	0.174		12	1.859	0.646	
Change versus arm1 (95% CI) †								-0.777	(-1.519 to -0.036)	***0.041***		-0.686	(-1.385 to 0.014)	**0.054**
Cho/Cr baseline	28	1.182	0.314	7	1.107	0.168	9	1.159	0.283		12	1.243	0.400	
Cho/Cr 48 weeks	28	1.162	0.173	7	1.168	0.183	9	1.105	0.133		12	1.201	0.195	
Change versus arm1 (95% CI) †								-0.116	(-0.450 to 0.219)	0.483		-0.103	(-0.419 to 0.213)	0.508
MI/Cr baseline	28	3.849	1.632	7	3.854	1.761	9	3.803	1.092		12	3.881	1.994	
MI/Cr 48 weeks	27	3.711	1.471	6	2.595	1.581	9	3.729	0.770		12	4.255	1.596	
Change versus arm1 (95% CI) †								1.065	(-0.842 to 2.972)	0.261		1.513	(-0.297 to 3.322)	**0.097**
Frontal Grey														
NAA/Cr baseline	29	1.586	0.250	8	1.561	0.286	9	1.539	0.166		12	1.637	0.286	
NAA/Cr 48 weeks	30	1.815	0.573	9	1.919	0.357	9	1.814	0.953		12	1.737	0.312	
Change versus arm1 (95% CI) †								-0.120	(-0.758 to 0.517)	0.701		-0.295	(-0.894 to 0.303)	0.320
Cho/Cr baseline	29	0.687	0.150	8	0.714	0.146	9	0.705	0.179		12	0.657	0.137	
Cho/Cr 48 weeks	30	0.693	0.155	9	0.688	0.161	9	0.724	0.171		12	0.674	0.149	
Change versus arm1 (95% CI) †								0.047	(-0.130 to 0.225)	0.587		0.045	(-0.121 to 0.212)	0.580
MI/Cr baseline	27	3.041	1.161	6	3.268	1.804	9	3.247	0.857		12	2.774	1.017	
MI/Cr 48 weeks	28	2.850	1.439	8	2.997	1.662	9	2.970	1.422		11	2.646	1.400	
Change versus arm1 (95% CI) †								-0.253	(-1.754 to 1.249)	0.731		-0.160	(-1.606 to 1.285)	0.821
Right Basal Ganglia														
NAA/Cr baseline	27	2.022	0.613	7	1.908	0.431	8	2.274	0.976		12	1.921	0.340	
NAA/Cr 48 weeks	27	2.689	1.096	8	2.723	1.477	7	2.782	0.824		12	2.612	1.032	

Change versus arm1 (95% CI) †							-0.427	(-1.893 to 1.038)	0.552		-0.150	(-1.467 to 1.167)	0.815
Cho/Cr baseline	27	1.013	0.618	7	0.974	0.183	8	1.225	1.121	12	0.893	0.186	
Cho/Cr 48 weeks	27	0.930	0.294	8	0.910	0.235	7	0.875	0.188	12	0.976	0.381	
Change versus arm1 (95% CI) †							-0.347	(-1.121 to 0.427)	0.363		0.139	(-0.557 to 0.835)	0.683
MI/Cr baseline	27	3.041	1.161	6	3.268	1.804	9	3.247	0.857	12	2.774	1.017	
MI/Cr 48 weeks	25	2.887	1.007	7	3.219	1.452	7	3.001	0.907	11	2.604	0.708	
Change versus arm1 (95% CI) †							-0.016	(-1.446 to 1.414)	0.982		0.099	(-1.218 to 1.416)	0.877

(*Table 3* Legend:

† Coefficient of change represents the difference in mean changes from week 0 to week 48 between treatment groups in a linear regression model.

†† *p*-values below 0.15 shown in bold, values below 0.05 shown in bold-italic.

Abbreviations – NAA = N-acetyl aspartate, MI = myoinositol, Cho= choline, Cr = creatine, CI = confidence interval, SD = standard deviation.)

Table 5:

Univariate and multivariate models assessing factors associated with cerebral metabolite ratio changes over 48 weeks.

	Univariate Model				Multivariate Model			
	Coef.	**LCI**	**UCI**	***p*-value**	**Coef.**	**LCI**	**UCI**	***p*-value** [*]
NAA/Cr ratio change in frontal white matter								
Baseline CD4 cell count (cells/µL)	-0.002	-0.005	0.002	0.36				
Ethnicity (Caucasian vs Asian)	0.105	-0.520	0.731	0.73				
Log HIV RNA at baseline	0.033	-0.428	0.494	0.88				
Age (years)	-0.012	-0.042	0.017	0.39				
Nadir CD4 count (cells/µL)	-0.003	-0.006	0.001	0.15	-0.002	-0.006	0.001	0.15
Detectable HIV RNA at week 48 [**]	0.277	-0.884	1.439	0.63				
CD4 change from baseline to week 48 (cells/µL)	0.000	-0.002	0.002	0.93				
Treatment Arm								
Arm 2 (vs Arm 1)	-0.777	-1.519	-0.036	0.04	-0.789	-1.516	-0.063	**0.03**
Arm 3 (vs Arm 1)	-0.686	-1.385	0.014	0.05	-0.610	-1.303	0.084	0.08
MI/Cr ratio change in frontal white matter								
Baseline CD4 cell count (cells/ µL)	-0.006	-0.014	0.002	0.125	-0.003	-0.012	0.005	0.443
Ethnicity (Caucasian vs Asian)	-0.162	-1.655	1.332	0.825				
Log HIV RNA at baseline	0.612	-0.576	1.799	0.299				
Age (years)	0.022	-0.049	0.092	0.532				
Nadir CD4 count (cells µL)	-0.002	-0.010	0.007	0.675				
Detectable HIV RNA at week 48 [**]	0.559	-2.188	3.306	0.679				
CD4 change from baseline to week 48 (cells/µL)	0.002	-0.004	0.008	0.462				
Treatment Arm								
Arm 2 (vs Arm 1)	1.065	-0.842	2.972	0.261	1.083	-0.845	3.011	0.257
Arm 3 (vs Arm 1)	1.513	-0.297	3.322	0.097	1.651	-0.214	3.516	0.080

(*Table 5* legend:

Abbreviations – Coef = correlation coefficient, LCI = lower confidence interval, UCI = upper confidence interval, NAA = N-acetyl aspartate, MI = myoinositol, Cho= choline, Cr = creatine.

* *p*-values below 0.05 shown in bold, **assay detection limit to 50 copies/mL).

Figure 1:
Volumes of interest

Figure 1a: Right frontal white matter (FWM) and mid-frontal grey matter (FGM)

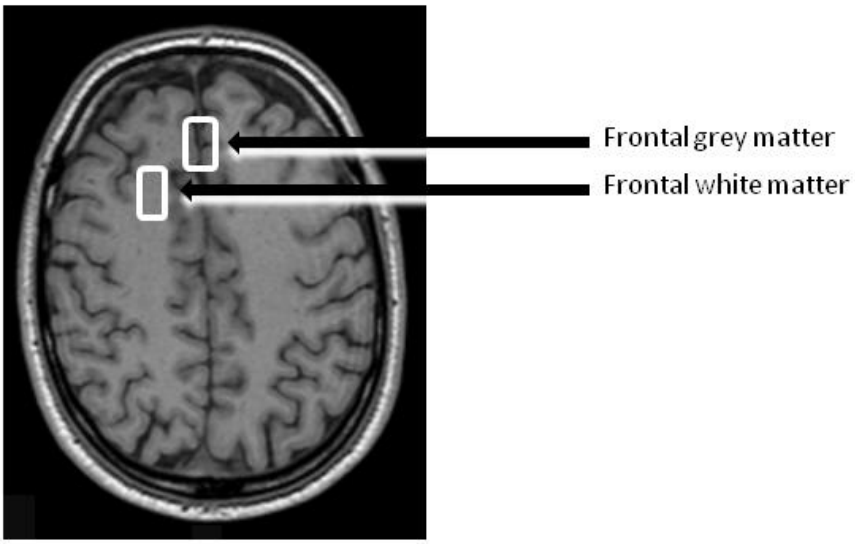
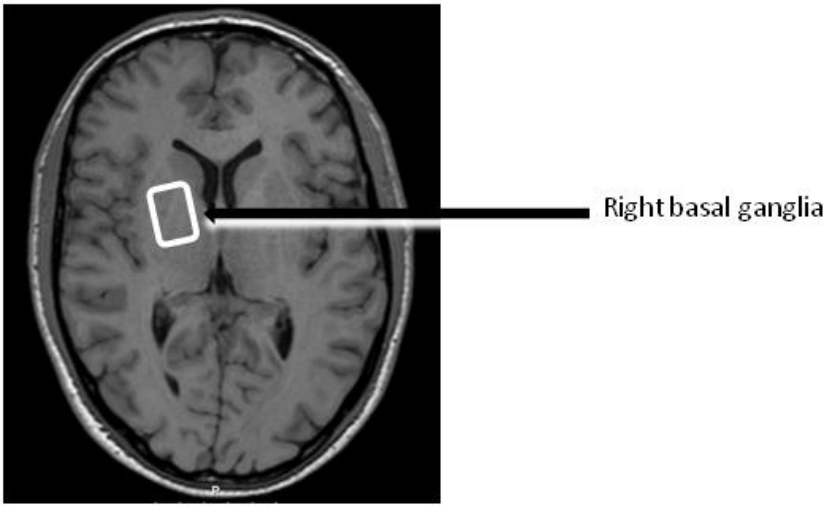


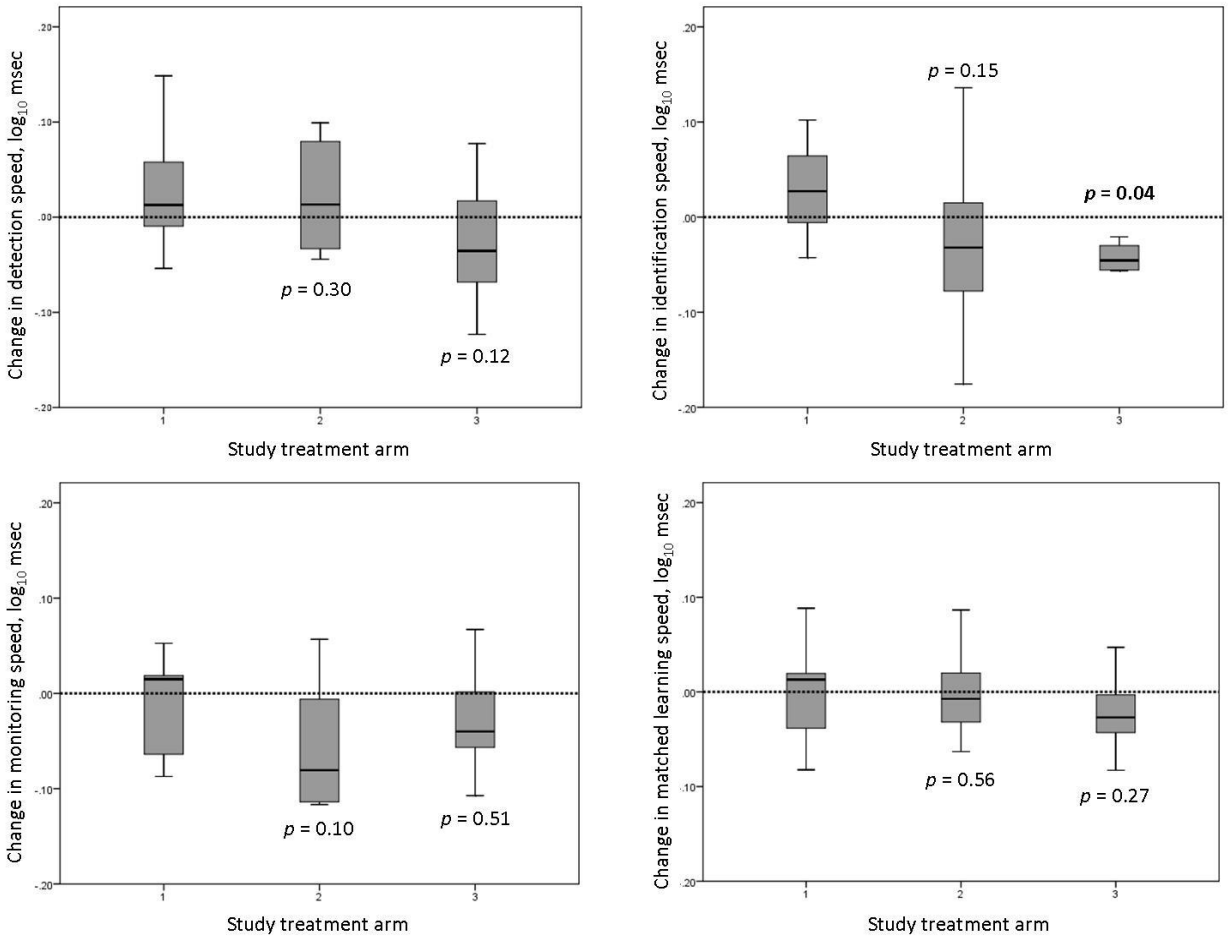
Figure 1b: Right basal ganglia (RBG)



(Figure 1 legend: Plane of section - axial)

Figure 2:

Box plots showing changes in neurocognitive speed domain parameters over 48 weeks:



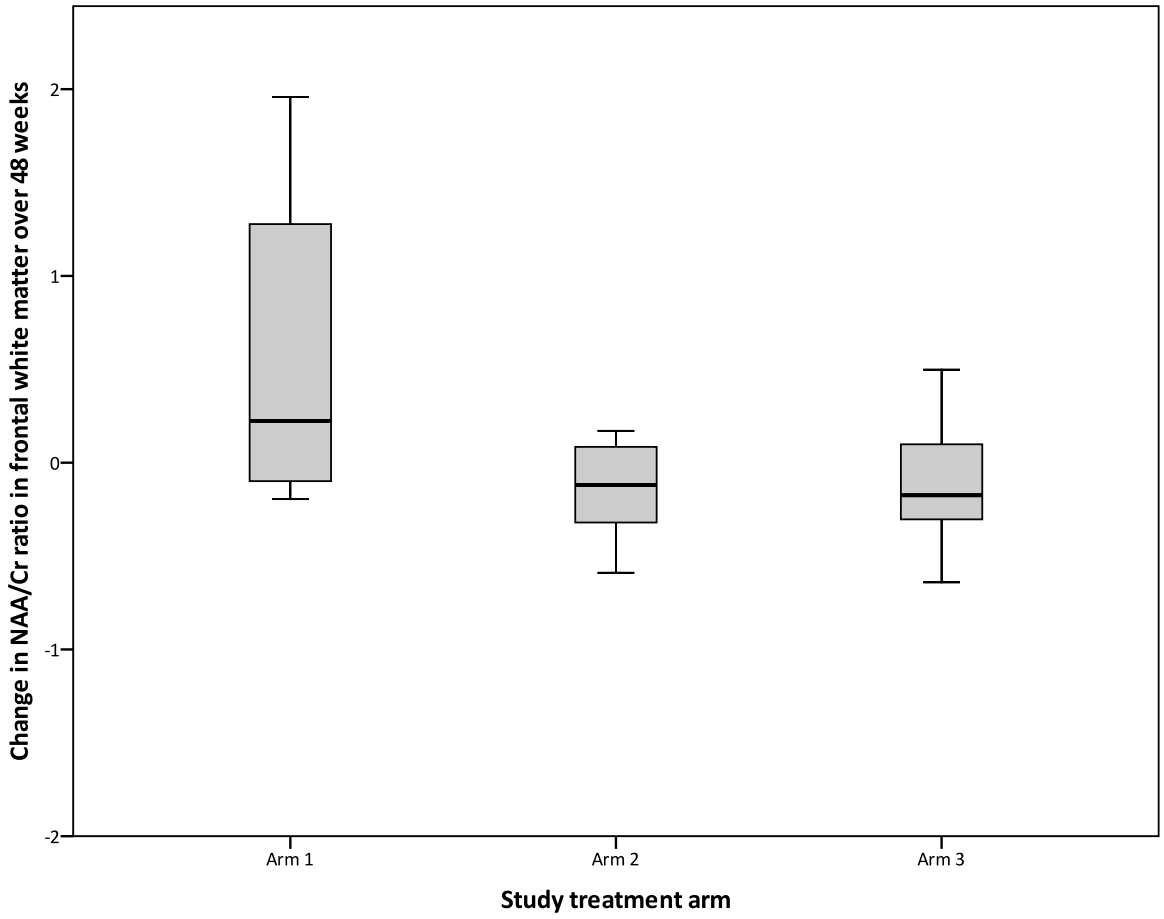
(*Figure 2* legend:

Box plot showing median absolute change – horizontal black line; boxes – interquartile ranges; tails – upper and lower adjacent values.

p-values shown are mean difference in treatment group compared to *arm1*).

Figure 3:

Box plot showing absolute changes in NAA/Cr ratio in frontal white matter over 48 weeks.



(*Figure 3* legend:

Box plot showing median absolute change – horizontal black line; boxes – interquartile ranges; tails – upper and lower adjacent values

Abbreviations – NAA = N-acetyl aspartate, Cr = creatine).