Maraviroc-intensified combined antiretroviral therapy improves cognition in virally suppressed HIV-associated neurocognitive disorder

Thomas M. Gates[a], Lucette A. Cysique[a,b,c], Krista J. Siefried[a,b], Joga Chaganti[d], Kirsten J. Moffat[d] and Bruce J. Brew[a,b,e]

Objective: To investigate whether intensification of combined antiretroviral therapy (cART) with the CC chemokine receptor type 5 (CCR5) entry inhibitor maraviroc leads to improvement in global neurocognitive functioning in virally suppressed men with HIV-associated neurocognitive disorder (HAND).

Design: Prospective, double observer-blinded, open-label pilot randomized-controlled trial. Participants were randomized to remain on their existing cART regimen (control arm; $n = 8$) or receive maraviroc-intensification (maraviroc arm; $n = 9$).

Methods: Participants completed a five-domain neuropsychological battery at baseline, 6- and 12-month visits. Raw scores were transformed into age-corrected *z*-scores and averaged into a global *z*-score. Single voxel (^{1}H)-magnetic resonance spectroscopy (MRS) major cerebral metabolite concentrations were collected at baseline and 12 months in the basal ganglia and frontal white matter and quantified using jMRUI. Neuroinflammatory biomarkers cerebrospinal fluid neopterin and β_2-microglobulin were also measured.

Results: Fourteen of the 17 participants completed the study: nine maraviroc arm and five control. We found medium to large effect sizes in favour of improved global neurocognitive performance in the maraviroc arm over time {arm*time interaction: $P < 0.05$; 6 month: [$\beta = -0.10$, standard error (SE) $= 0.04$, 90% confidence interval (90%CI) $= -0.18,.03$; $P < 0.03$] yielding a large effect-size $d = 0.77$ (90%CI $= -0.19,1.71$); 12 month: [$\beta = -0.01$; SE $= 0.05$; 90%CI $= -0.09$, 0.06; $P < 0.77$] yielding a moderate effect-size $d = 0.55$ (90%CI $= -0.47,1.55$)}. No treatment-related changes were detected for ^{1}H-MRS metabolites or cerebrospinal fluid biomarkers.

Conclusion: This pilot study provides feasibility, tolerability, proof-of-concept and preliminary evidence for clinically relevant neurocognitive improvement in cART enhancement with maraviroc in virally suppressed HAND patients. Lack of concomitant brain metabolite and biomarker change may be related to complex dynamics of brain repair.

AIDS 2016, **30**:591–600

Keywords: CCR5 receptor, cognition, HIV-associated neurocognitive disorders, magnetic resonance spectroscopy, maraviroc

[a]Departments of Neurology and HIV Medicine, St Vincent's Hospital and Peter Duncan Neurosciences Unit, St Vincent's Centre for Applied Medical Research, [b]Faculty of Medicine, The University of New South Wales, [c]Neuroscience Research Australia, [d]Department of Medical Imaging, St Vincent's Hospital and [e]School of Medicine, The University of Notre Dame, Sydney, Australia.

Correspondence to Professor Bruce J. Brew, Department of Neurology, Level 4 Xavier Building, St Vincent's Hospital Sydney, 390 Victoria Street, Darlinghurst, NSW 2010, Australia.

E-mail: b.brew@unsw.edu.au
Received: 17 June 2015; revised: 16 October 2015; accepted: 16 October 2015.

DOI:10.1097/QAD.0000000000000951

ISSN 0269-9370

Introduction

HIV affects the brain in 20–50% of infected patients leading to varying degrees of cognitive impairment, predominantly affecting psychomotor speed, attention/working memory, new learning/memory, and executive functioning [1–4]. Collectively, these neurological manifestations of HIV are termed HIV-associated neurocognitive disorders (HAND) [5]. Despite the introduction of combined antiretroviral therapy (cART), milder forms of HAND such as asymptomatic neurocognitive disorder (ANI) and mild neurocognitive disorder (MND) remain common [1,3,4,6,7]. Both ANI and MND are functionally significant, predicting increased rate of mortality [8], progression to more severe impairment [9], unemployment [10,11], and poor medication adherence [12].

The continued presence of HAND in cART-treated patients may reflect ongoing low-level viral replication and/or chronic inflammation in the brain despite undetectable plasma and cerebrospinal fluid (CSF) HIV RNA. This may relate to differences in antiretroviral drugs' ability to cross the blood–brain barrier [13,14] and failure to inhibit post-integration transcription [15,16]. The concept of high central nervous system (CNS)-penetrating cART (neuro-cART) remains controversial. However, the majority of rigorous prospective studies to date support a beneficial effect in terms of improved neurocognition and reduced CSF HIV RNA [15,17]. Although a recent randomized-controlled trial (RCT) did not show a differential benefit of neuro-cART [18], there were several notable methodological issues affecting interpretation including inadequate power [19], relatively brief (16-week) follow-up period to observe neurocognitive benefits, possible antiretroviral instability prior to study entry (participants were cART-naive or stable for minimum 8 weeks) leading to imbalances in viral suppression between study arms, and high rate of hepatitis C co-infection in neuro-cART arm potentially impacting the likelihood of improvement at follow-up.

Neurovirological and neuropathological studies of HAND have shown that HIV replication within CNS target cells almost exclusively depends on interactions with the CCR5 chemokine co-receptor [20–22]. Therefore, the CCR5 antagonist maraviroc would be a suitable candidate for cART intensification. Maraviroc has a good resistance barrier rendering archived resistance unlikely [23], good CSF penetration [24,25], antineuroinflammatory properties, and inhibits CNS viral replication as demonstrated in simian immunodeficiency virus (SIV)-infected Macaque models [26] and humans [27]. The mechanism of maraviroc action in blocking HIV entry into uninfected cells not only inhibits cellular replication (as all antiretroviral drugs do), but indirectly inhibits postintegrase transcription, unlike protease inhibitors which act at the postintegration step of viral replication [16]. Preliminary data supporting a potential neurocognitive benefit of maraviroc-intensified cART was reported in a recent single-arm, open-label pilot study evaluating the impact of maraviroc on monocyte activation phenotypes. In this study, analyses of a subset ($n = 6$) of HIV-infected participants with some degree of cognitive impairment (global z-score < -0.5, derived from six cognitive domains) revealed moderate improvement over 24 weeks [28]. However, neuropsychological scores at follow-up were not corrected for practice effects, which is highly problematic especially in the absence of a control-comparison arm [17]. It was also unclear whether participants met current Frascati criteria for HAND [5], leaving open the possibility of larger treatment effect sizes in patients with greater initial cognitive impairment [29].

We conducted a pilot RCT to investigate the efficacy of cART intensification with maraviroc on neurocognition in virally suppressed (blood and CSF) HIV+ participants on stable cART with HAND. We hypothesized maraviroc intensification would result in improved neurocognitive performance over 12 months relative to continuation on background therapy, along with reversal of HIV-mediated neuropathology as assessed using single voxel (^{1}H)-magnetic resonance spectroscopy (MRS) and CSF inflammatory biomarker panel.

Methods

Trial design

The study was a 12-month prospective, interventional, observer-blinded, open-label Phase-IV RCT conducted at St Vincent's Hospital (SVH), Sydney, Australia (ClinicalTrials.gov Identifier: NCT01449006). Local ethics approval was obtained from SVH Human Research Ethics Committee and all participants provided written informed consent prior to enrolment. This article followed CONSORT statement for reporting clinical trials.

Participants

Participants were maraviroc-naive on stable cART with plasma HIV RNA below 50 cpml for minimum 12 months, CSF HIV RNA below 50 cpml at study entry and diagnosed with HAND with symptom progression within the last 6 months noted by their primary physician. Figure 1 details the two-step process used to confirm HAND [5] at screening/baseline. Specifically, if demographically adjusted (age, education, sex, and ethnicity) scores on the brief study neurocognitive research battery were not impaired enough to support HAND, participants subsequently completed a larger battery of standardized neuropsychological tests assessing a more diverse selection of cognitive domains to more precisely quantify degree of impairment (see Table, Supplemental Digital Content 1, for tests, http://links.lww.com/QAD/A822). Participants

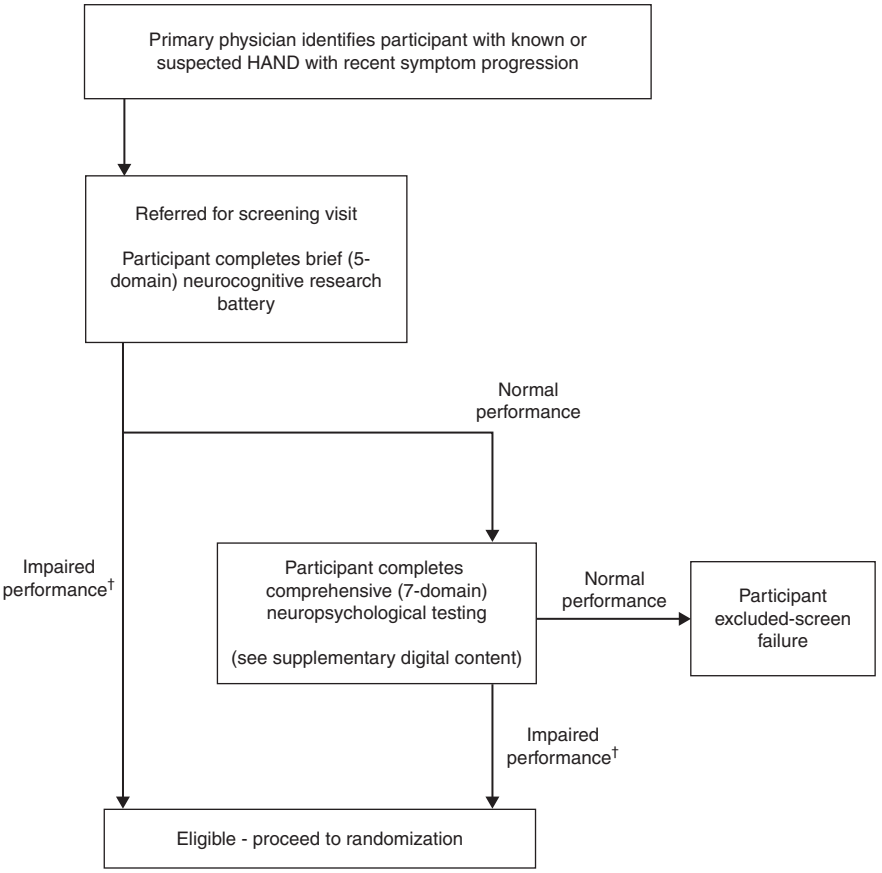

Fig. 1. Two-step neurocognitive testing procedure during screening/baseline visit to confirm HIV-associated neurocognitive disorder (HAND). †To be diagnosed with HAND, participants must perform at least 1 SD below the mean on demographically corrected norms (based on age, education, sex, and ethnicity) in at least *two* cognitive domains as per Frascati criteria [5].

were excluded as screen failures if current HAND could not be established from this more comprehensive assessment.

Other exclusion criteria included current non-HIV-related neurological disorders or active CNS-opportunistic infections, history of psychotic disorder, current untreated major depression, current/previous 12-month untreated substance abuse disorder, active hepatitis C serology, history of loss of consciousness more than 1 h, nonproficiency in English, currently taking medications known to pharmacologically interact with antiretroviral drugs, history of taking an entry inhibitor, and pregnancy.

Randomization

Participants were 1 : 1 randomly allocated to remain on their existing cART regimen (control arm) or receive maraviroc 150 mg/300 mg/600 mg *bd* according to background therapy (maraviroc arm). Given the small sample size, covariate adaptive randomization based on a minimization method was employed to diminish potential baseline biases between study arms by balancing nadir $CD4^+$ cell count and cardiovascular risk (measured using Framingham risk score), two factors associated with neurocognitive decline in HIV [30–33]. The randomization program was custom-generated at The Kirby Institute, UNSW, Australia, using SAS v9.3.

Intervention

Participants assigned to maraviroc arm received their first dose of study drug (i.e. oral maraviroc 150 mg/300 mg/600 mg *bd*) on the day of randomization. They were instructed to self-administer maraviroc tablets according to standard instructions alongside background cART and provided with a 3-month supply. Further supplies were dispensed by SVH Clinical Trials Pharmacy during the study period. Participants assigned to control arm were required to remain on their current cART regimen for the entire study duration.

Study procedure and measures

There were three study visits in total – a screening/baseline visit confirming eligibility and follow-up visits at 6 months and 12 months. Screening consisted of brief neurocognitive testing (primary outcome measure), MRI/^{1}H-MRS scan, neurological examination, blood work, and CSF lumbar puncture (secondary outcome/safety measures). After eligibility was confirmed participants proceeded to randomization. Screening neurocognitive and MRI results were used as baseline data for

longitudinal analyses. Screening bloods and CSF obtained within 2 weeks of neurocognitive testing were also used. Neurocognitive testing was repeated at 6 months, whereas all baseline procedures were repeated at 12 months to permit longitudinal analysis.

Neurocognitive testing

A relatively short neurocognitive research battery was adopted to derive a composite global neurocognitive *z*-score (primary endpoint). The battery was administered by a qualified neuropsychologist or neuropsychologist-in-training who remained blind to treatment allocation. Completion time was around 1 h. It consisted of a brief computerized battery, CogState, supplemented with a small selection of standardized pencil-and-paper neuropsychological tests to provide greater coverage of other cognitive domains (motor coordination and psychomotor speed). CogState is sensitive to HAND [34] and in this study we selected an updated version that included a new measure of verbal episodic memory and improved existing attention/working memory measures (see www.cogstate.com). In addition to having adequate validity for assessing HAND, tests were selected based on their optimal psychometric properties for RCTs [i.e. high test–retest reliability ($rs > 0.70$), small practice effects ($ds < 0.30$) and wide performance range] [35,36].

Overall, the battery assessed the following five cognitive domains affected in HAND [5]: 'speed of information processing' – CogState Detection and Identification, Trail-Making Test A&B, WAIS-III Digit-Symbol Coding; 'attention/working memory' – CogState One-Back and Two-Back; 'motor coordination' – Grooved Pegboard Test; 'verbal learning' – CogState International Shopping List Task-Learning; and 'verbal memory' – CogState International Shopping List Task-Delayed Recall. Additionally, premorbid intellectual functioning was estimated using National Adult Reading Test (NART) or a demographic-based regression equation when NART could not be validly administered [37]. Psychological measures included depression, anxiety, and stress scales (DASS-21) and mini international psychiatric interview (MINI v5.0.). Functional decline was measured via a standard Independence of activities of daily living (IADL) questionnaire [38].

Single voxel (^{1}H)-magnetic resonance spectroscopy

Quantitative ^{1}H-MRS was conducted to measure change in cerebral metabolite concentrations in frontal white matter (FWM) and basal ganglia-caudate nucleus [32,39–41]. Spectra were acquired on Philips Achieva 3T-MRI scanner (Philips, Best, Netherlands) with an 8-channel head coil, using point-resolved spectroscopy (PRESS) sequence with short echo time (TE) [TE32/repetition time 2000 ms]; number of acquisitions: FWM = 128/basal ganglia = 64; 2.0 kHz bandwidth; voxel size: FWM = 20 mm^3/basal ganglia = 15 mm^3 (see Figures 1 and 2, Supplemental Digital Content 2, http://links.lww.com/QAD/A824, for voxel positioning and spectra images). Quantitative analysis of spectra was accomplished using jMRUI v3.0/AMARES, described previously [32]. In total, 14 signals were fitted in FWM and five in basal ganglia. Metabolite ratios were calculated in both regions for *N*-acetyl aspartate (NAA), choline (Cho), creatine (Cr), and *myo*-inositol (mIo) in relation to internal H_2O as standard [32], while glutamate/glutamine (Glx)/H_2O was also calculated for FWM.

Cerebrospinal fluid neuroinflammatory biomarkers

CSF specimens were analyzed for levels of a neuroinflammatory biomarker panel consisting of neopterin (HPLC assay; detectable range > 8 nmol/l) and β_2-microglobulin (reference interval: 0.1–2.4 mg/l) using commercially available test kits (BN ProSpec; Siemens, Germany). Both markers are elevated in CSF of HIV+ participants and have been associated with neurocognitive impairment in HAND [2,42,43]. Specimens were stored in 0.5 and 1 ml aliquots.

Primary outcome

The primary endpoint was change in cognitive functioning across study time-points (baseline, 6 months, 12 months) as measured by a global neurocognitive *z*-score. To derive this score, CogState raw data were converted to age-corrected *z*-scores using CogState normative data, whereas raw scores on standardized neuropsychological tests were first converted to age-corrected scaled scores ($M = 10$, $SD = 3$) using published normative data [44], then transformed to *z*-scores. The set of individual subtest *z*-scores were then averaged to derive a single composite *z*-score.

Secondary outcomes

Although the primary outcome was specified in the original protocol, given the exploratory nature of this study we later obtained ethical approval to evaluate the following secondary outcomes to provide supportive biomarker-based evidence of attenuated HAND-mediated neuropathology following maraviroc intensification:

Change in major brain metabolites in FWM and basal ganglia between baseline and 12 months (measured by ^{1}H-MRS).

Change in levels of CSF neuroinflammatory markers neopterin and β_2-microglobulin from baseline to 12 months.

Statistical analyses

Data were analyzed on a modified intention-to-treat basis (see Participant Enrolment section). Between-group demographic and clinical differences at baseline were assessed using independent-samples *t*-tests or Wilcoxon

sum-rank tests for continuous data and χ^2 tests of independence for categorical data. The primary outcome, change in global neurocognitive *z*-score over time, was analyzed using a mixed-effects regression model with arm and time as fixed linear effects, arm*time interaction as a nonlinear fixed effect and participant as a random effect to account for attrition. Participants with only baseline data available were logically not included in analyses ($n = 2$). In total, 14 participants (maraviroc: $n = 9$, control: $n = 5$) yielding 41 data-points (maraviroc: $n = 27$, control: $n = 14$) were included. Coefficients of variation (CVs) were calculated per Hopkins [45] as comparative indicators of effort across repeated test sessions between study arms. For secondary outcomes, changes in ^{1}H-MRS cerebral metabolite ratios (n pairs = 13) and CSF biomarker levels (n pairs = 11) were analyzed using repeated-measures analysis of variance with the same fixed effects as primary analyses.

This pilot study was conducted to generate effect sizes for a potential larger investigation. Therefore, by design, the small sample size had limited power to detect a statistically significant effect at P below 0.05 and no P-value threshold was strictly set. However, we reported available unstandardized (e.g. raw regression coefficient B) and standardized effect sizes (e.g. Cohen's d, standardized β coefficient) along with 90% confidence intervals (90%CIs) to assess the clinical relevance of observed effects. Statistical analyses were performed using JMP v11.0.

Results

Participant enrolment and completion

Participants were recruited from January 2012 to November 2013; the last follow-up visit occurred in September 2014. Figure 2 displays participant flow throughout the study. In total, $n = 9$ were randomized to maraviroc arm and $n = 8$ to control arm. There were two notable protocol deviations in the control arm. First, one participant switched antiretroviral drugs during the study period; he remained in the modified intention-to-treat analysis as the overall CNS-penetration rating was unaffected during the changeover. Second, during monitoring it emerged one participant was mistakenly randomized without more extensive neuropsychological evidence of cognitive impairment confirming his eligibility; he completed study but the data were excluded

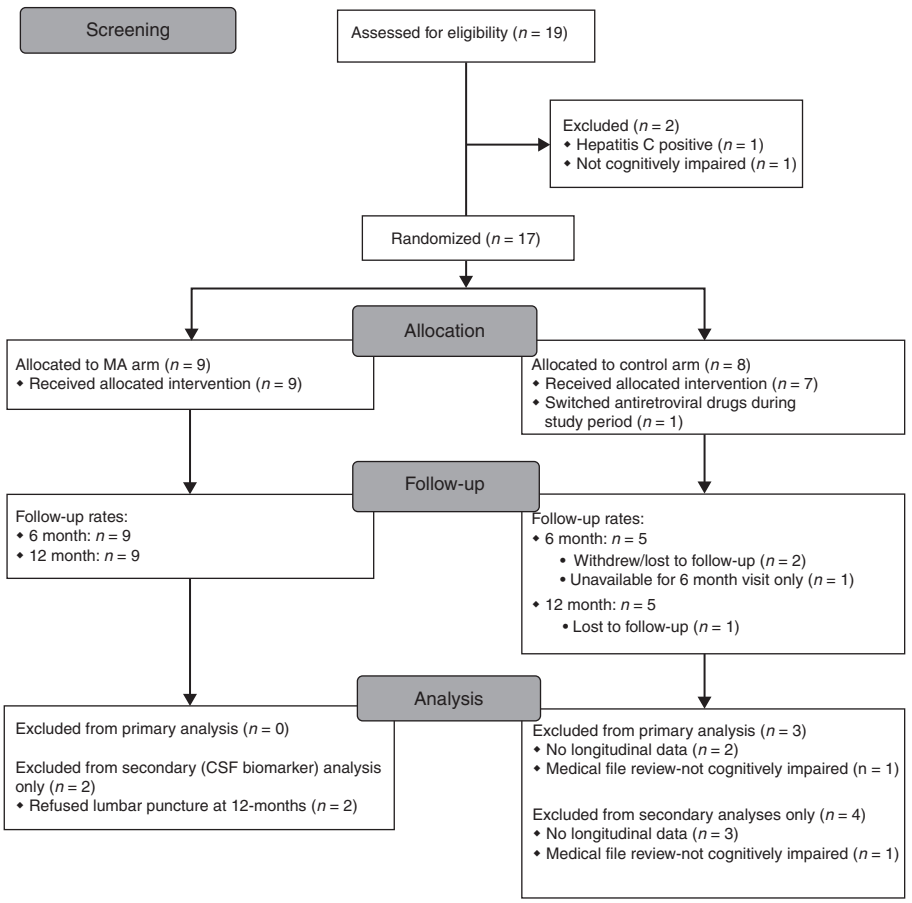

Fig. 2. Participant flow diagram with details of exclusions from primary and secondary analyses.

Table 1. Demographic and clinical characteristics of study sample included in primary analyses.

	Maraviroc arm ($n=9$)	Control arm ($n=5$)	P
Age (years)	52.2 (3.7)	60.0 (9.4)	0.14
Sex (M:F)	9:0	5:0	1.0
Ethnicity (White:Other)	9:0	5:0	1.0
Education (years)	12.3 (2.8)	11.6 (2.3)	0.61
NART[a]	102.2 (16.3)	104.4 (18.9)	0.83
Nadir CD4[b]	150 (220)	310 (339)	0.35
Current CD4[b]			
Baseline	499 (489.5)	980 (493)	0.06
12 month[c]	484 (270.5)	829 (574.5)	0.14
Baseline HAND Status[d]			0.09
ANI	1	1	
MND	8	2	
HAD	0	2	

ANI, asymptomatic neurocognitive disorder; HAD, HIV-associated dementia; MND, mild neurocognitive disorder NART, National Adult Reading Test. Continuous data presented as mean (SD) unless otherwise specified. Cell counts provided for categorical data. P values reflect independent samples t-test unless otherwise specified.
[a]Predicted WAIS-III full-scale IQ (FSIQ) based on NART error score except for $n=2$ maraviroc arm with lifetime learning disability – verbal IQ estimated for these participants using regression formula from Sullivan *et al.* [37]: 85.54 + 5.0 (Educ) + 0.2 (Age)–2.87 (Sex).
[b]Data presented as median (IQR) and analyzed using Wilcoxon rank-sum test due to non-normal distribution.
[c]Maraviroc arm $n=9$; control arm $n=4$.
[d]Categorical data analyzed using χ^2 test of independence. In maraviroc arm, $n=4$ participants were borderline-impaired on brief study neurocognitive battery and subsequently completed a larger neuropsychological battery to confirm HAND diagnosis. Results indicated: $n=1$ ANI, $n=3$ MND.

from analysis. A further two controls were lost to follow-up for personal reasons prior to 6 months and thus could not be included in longitudinal analyses. However, importantly, their baseline global neurocognitive performance was in line with the rest of the sample and ranged within the mild-to-moderate levels of impairment [case 1: $z=-1.10$ (MND); case 2: $z=-0.67$ (ANI), versus *Med* $z=-0.75$; *IQR* (interquartile) $=0.94$ (*Min* $=-1.91$; *Max* $=-0.03$) for remaining sample]. Moreover, one control did not attend his final 12-month visit. His completed neurocognitive data were included in primary analyses but removed from secondary analyses. Lastly, baseline CSF biomarker data were excluded in two maraviroc participants who refused lumbar punctures at 12 months.

Study sample demographic and clinical characteristics

Demographic and clinical information for the sample are presented in Table 1. Overall, the sample primarily comprised middle-aged ($M=55$ years) White males with average education level ($M=12.1$ years) and premorbid intelligence (FSIQ: $M=103$). Nadir $CD4^+$ cell count was less than 200 cells/μl in eight of 14 (57%) participants. Current $CD4^+$ cell count was above 200 cells/μl in all participants with eight of 14 (57%) $CD4^+$ cell count below 500 cells/μl at baseline and seven of 13 (54%) at 12 months. No significant differences were found between study arms on any of these variables, although trends were observed for higher baseline $CD4^+$ cell counts in controls ($P<0.06$), whereas maraviroc participants were almost exclusively MND (89%) compared with a larger spread of HAND severity in controls ($P<0.09$). Two maraviroc and one control had confirmed CXCR4-tropic virus in blood; one control had confirmed CCR5-tropic virus. HIV-tropism status was unavailable for other participants as their HIV RNA fell below the lower limit of assay detection and could not be amplified by PCR for V3-loop DNA. All participants had undetectable HIV RNA (<50 cpml) at baseline per inclusion criteria. However, at 12 months one maraviroc had detectable plasma and CSF HIV RNA whereas one control had mildly elevated plasma HIV RNA with undetectable CSF HIV RNA. Both had CXCR4-tropic virus and the maraviroc participant self-reported antiretroviral nonadherence in the week preceding the study visit.

Primary endpoint

Figure 3 displays mean change in global neurocognitive functioning in the study arms at each time-point. There were no baseline differences between the two arms

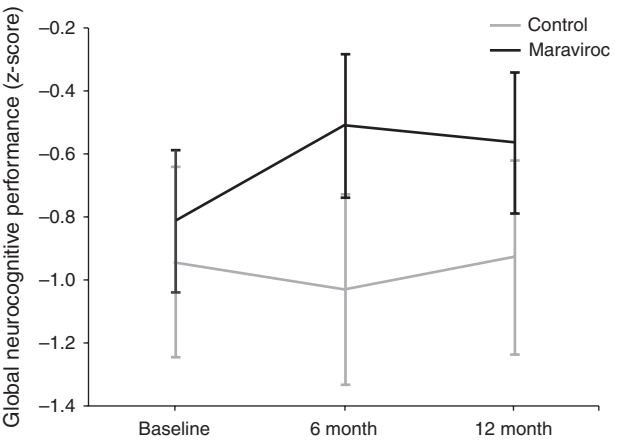

Fig. 3. Mean (±standard error) global cognitive performance over time based on model least mean squares and adjusted for attrition (treatment arm∗time interaction $P<0.05$).

Table 2. Coefficients of variation of neurocognitive performance from baseline to each follow-up visit.

	Maraviroc arm ($n=9$)		Control arm ($n=5$)	
	CV	90% CI	CV	90% CI
6 months	4.7	3.4–8.2	8.4	5.4–21.1
12 months	11.7	8.3–20.8	7.2	4.4–22.6

CI, confidence interval; CV, coefficient of variation. CV is a measure of intra-individual variation [here determined from trial 1 (baseline) to trial 2 (follow-up)]. In this table it is expressed as a percentage of group mean change score. CV can be used as a surrogate marker of cognitive effort [55]. We therefore assessed if there was any difference in cognitive effort between the two study arms because the participants were not blind to treatment (though the examiner was). Note that CVs were generated according to the methods described in Hopkins [45]. To do this, global neurocognitive *z*-scores were first converted to scaled scores ($M=10$, $SD=3$) to permit log transformation. Next, the typical error (s) of the log-transformed variable was calculated: $s=SDdiff_{T2-T1}/\sqrt{2}$. Finally, the typical error was converted to %CV according to the formula: $CV=100(e^{s/100}-1)$.

($P<0.71$). Medium to large effect sizes were observed favouring improved global neurocognitive functioning in maraviroc arm over control arm over time [arm*time interaction: $P<0.05$; 6 months: $\beta=-0.10$, standard error (SE) $=0.04$, 90% CI $=-0.18$, 0.03; $P<0.03$; 12 months: $\beta=-0.01$, SE $=0.05$, 90% CI $=-0.09$, 0.06; $P<0.77$] yielding a large between-groups effect-size at 6 months: ($d=0.77$; 90% CI $=-0.19$,1.71) and medium effect-size at 12 months ($d=0.55$; 90% CI:$-$0.47, 1.55). CVs comparing baseline to follow-up visits did not differ between study arms (Table 2).

Secondary endpoint I

^{1}H-MRS analysis with jMRUI did not reveal any treatment effects on metabolites of interest in each brain region ($P>0.30$ for all arm*time interaction terms).

Secondary endpoint II

CSF neopterin levels were below detectable range (i.e. <8 nmol/l) in five of 22 (23%) specimens analyzed. No relevant effects were detected for CSF neopterin (arm*time interaction: $P=0.82$) or β_2-microglobulin levels (arm*time interaction: $P=0.71$).

Discussion

cART intensification with maraviroc in this open-label prospective pilot RCT of virally suppressed (in blood and CSF) HAND participants led to clinically relevant improvements in neurocognition, with a large effect observed after 6 months ($d=0.77$) and moderate effect after 12 months ($d=0.55$). There were no concomitant changes in ^{1}H-MRS metabolites in FWM and basal ganglia or CSF neuroinflammatory biomarkers. The study provides informative new data regarding the ongoing debate over how to optimally manage HAND in the cART era. Currently, clinicians are generally unwilling to change the regimen of HAND patients if they are otherwise clinically stable with a controlled viral load. There are no guidelines on managing deterioration or what to do if a patient newly develops HAND. Conclusive evidence for cART intensification with a high CNS-penetrating antiretroviral drug is still lacking, but our study supports such a strategy. The detection of such effect sizes is encouraging, reinforcing the notion that some antiretrovirals are more beneficial for brain functioning and could even be neuroprotective for HAND, an increasingly important consideration given the effects of HIV on normal ageing processes remain relatively unknown [46].

Maraviroc has anti-inflammatory properties, good antiretroviral efficacy in cells including those of monocyte/macrophage lineage and good CNS penetration [26,27,41], highlighting its clear potential for therapeutic use. However, there is sparse evidence supporting its efficacy in reducing the severity of neurological conditions in HIV infection. To our knowledge, this study, albeit a pilot, provides the most convincing data supporting maraviroc intensification to date. By employing a randomized-controlled design, longer follow-up period, and optimal neuropsychological methods for longitudinal research, this study supports and extends recent observations of improved neurocognition in a small sample ($n=6$) of HIV-infected participants with some degree of cognitive impairment who underwent maraviroc intensification for 24 weeks [28]. That study also reported partial reversal of monocyte-mediated pathological changes previously associated with neurocognitive impairment, namely reducing the proportion of circling intermediate and nonclassical CD16-expressing monocytes, $CD14^{+}$ HIV DNA monocyte burden and pro-inflammatory biomarker sCD163 levels in plasma [47–50]. However, the authors did not directly examine whether changes observed in plasma also occurred in the CNS.

There are several possible reasons for the negative ^{1}H-MRS findings. First, further analyses combining the study arms and comparing them to age-matched HIV controls at baseline (data not shown) suggested that HAND was chronic (i.e. abnormally reduced Cho in the context of abnormally reduced NAA reflected chronic neuronal integrity loss and cell density loss, but no acute neuroinflammation). This differs from earlier studies reporting attenuation of neurochemical abnormalities, in particular neuroinflammatory markers (Cho/mIo), soon after cART initiation in treatment-naïve HAND [51,52]. However, other studies have shown that abnormalities persist even at relatively short intervals [53], underscoring that once HAND becomes chronic, changes in brain metabolites may have complex dynamics yet to be

properly delineated. This task becomes even harder in the context of chronic HIV infection where, depending on the metabolite and region of interest, the influences of other clinical and demographic factors also need to be considered [54]. Second, the timeline of our MRI/^{1}H-MRS scans may have failed to capture the period of peak neurocognitive improvement. In planning the study we decided against a 6-months scan primarily for feasibility and financial reasons, preferentially opting for the later scan based on prior evidence that neurocognitive improvement following cART initiation is sustained up to 12 months [29]. However, the timeline of a maraviroc-intensification effect (when added to an already virologically successful regimen) may be shorter considering the stronger effects on cognition we observed at 6 months compared with 12 months. This requires confirmation in a larger study with ^{1}H-MRS completed at all time-points if feasible. A third reason may be that maraviroc intensification is associated with neurochemical change in brain regions that were not examined (e.g. frontal gray matter). Although this is a possibility, basal ganglia and FWM are both well established sites of HIV-associated brain injury [32,39–41] and heavily recruited in the neurocognitive functions assessed in our study battery. Finally, improvements may have occurred in metabolites not well resolved with the short-echo PRESS sequence employed, such as glutamate.

We acknowledge several limitations pertaining to the study. First, the small pilot sample size, whereas producing clinically relevant effect sizes also led to large confidence intervals. Larger studies will be needed to confirm the exact size of a maraviroc-intensification effect, although our findings are encouraging. Secondly, the magnitude of neurocognitive improvement may have been somewhat overestimated due to practice effects arising from repeated testing. However, including a control-comparison arm with similar education level and baseline neurocognitive functioning affords us greater confidence that the additional improvement observed in maraviroc arm reflects a real treatment effect. Thirdly, the open-label design could have contributed to reduced effort on follow-up neurocognitive testing in control arm. However, the neuropsychologist examiner was blind to treatment status and there was no supportive clinical evidence. Additionally, when considering CVs as a surrogate of effort [55] from baseline to 6 months we found no significant difference between study arms. Moreover, although the open-label design may have contributed to loss-to-follow-up in the control arm, these participants were not significantly more or less impaired at baseline than most of the sample, suggesting that those remaining in the study had a similar neurocognitive profile. Furthermore, to control for attrition we used mixed-effects statistical models with participant as a random factor. Fourth, the modified intention-to-treat design potentially introduced bias into the analyses. Still, this was done for ethical considerations and to allow better characterization of treatment effects over time which is more appropriate for longitudinal research. Lastly, despite the study sample being fairly representative of the wider Australian HIV-infected community, the efficacy of maraviroc intensification in other international populations is not clear; for example, those with unsuppressed virus, more severe HAND, or commenced cART at a later stage in their disease history. Importantly, lack of timely cART-initiation and long-term adherence is associated with poorer socio-economic background, ethnic minority status, and women [56]: groups not represented here. Future research will need to include a more diverse HIV+ sample.

Conclusion

This pilot RCT provides feasibility, tolerability, proof-of-concept and preliminary evidence for efficacy of the CCR5 co-receptor antagonist maraviroc in the management of HAND by improving neurocognitive functioning. This finding, should it be replicated in a larger study, has important implications for the clinical management of HAND by supporting maraviroc intensification as a preferred option to cautious monitoring of participants over time.

Acknowledgements

The authors would also like to thank clinical trial coordinators Brett Sinclair, Fiona Kilkenny, Brendan Clifford, and Serena Knowles for their assistance in running the study, Margaret (Peggy) Bain for conducting comprehensive neuropsychological testing when required, Prof. Matthew Law for developing the randomization procedure used during the study, and Prof. Caroline Rae for her input into the MRS analyses.

Authors' contributions: B.B. conceptualized the study. B.B., L.C., J.C., K.S., and K.M. contributed to study design. B.B. neurologically assessed study participants. K.S. and T.G. coordinated participants' accrual and visits. L.C. designed and piloted neurocognitive assessment. T.G. conducted neurocognitive testing. L.C. conducted some neurocognitive testing and otherwise supervised evaluation, scoring and interpretation. T.G. conducted statistical analyses, which were supervised by L.C. J.C. oversaw MRI/^{1}H-MRS data acquisition and assisted with some of the ^{1}H-MRS data analyses. T.G. fitted ^{1}H-MRS data under supervision of L.C. T.G. compiled first draft of manuscript. All listed authors contributed to subsequent drafts and revision of manuscript.

Sources of funding: ViiV Healthcare Australia provided study drug (maraviroc) and financial support through an unrestricted Investigator-Initiated Study educational grant (Protocol #114560).

Conflicts of interest

T.G. has received conference sponsorship from ViiV Healthcare and partial salary support from St Vincent's Curran Foundation. L.C. currently holds a NHMRC Career Development Fellowship and received funding from an NHMRC project grant (Australia), UNSW postdoctoral Brain Science fellowship (2009–2012), and the Peter Duncan Neurosciences Unit (Head Prof. Bruce Brew). She also received partial salary support from Mercks Sharp & Dohme (MSD) in 2012. MSD had no direct participation in the current study design, data analyses and interpretation. She has also received speaker honoraria and research support from ViiV Healthcare and Abbvie. K.S. has received a research fellowship from Gilead Sciences and conference sponsorship from Gilead Sciences and ViiV Healthcare. B.B. has received speaker honoraria and research support from ViiV, Biogen Idec, Novartis and Boehringer Ingelheim. He has also received research grant support from NHMRC and NIH. For the remaining authors no conflicts of interest were declared.

A subset of study data were presented as a poster at CROI 2015, Seattle, Washington, USA, on 23–26 February 2015.

References

1. Cysique LA, Brew BJ. **Prevalence of nonconfounded HIV-associated neurocognitive impairment in the context of plasma HIV RNA suppression.** *J Neurovirol* 2011; **17**:176–183.
2. Brew BJ. **AIDS dementia complex..** In: Berger J, Portegies P,editors. *HIV/AIDS and the nervous system*. Handbook of Clinical Neurology, no. 85. Amsterdam, The Netherlands: Elsevier Press; 2007. pp. 79–92.
3. Robertson KR, Smurzynski M, Parsons TD, Wu K, Bosch RJ, Wu J, *et al*. **The prevalence and incidence of neurocognitive impairment in the HAART era.** *AIDS* 2007; **21**:1915–1921.
4. Heaton RK, Clifford DB, Franklin DR Jr, Woods SP, Ake C, Vaida F, *et al*. **HIV-associated neurocognitive disorders persist in the era of potent antiretroviral therapy: CHARTER study.** *Neurology* 2010; **75**:2087–2096.
5. Antinori A, Arendt G, Becker JT, Brew BJ, Byrd DA, Cherner M, *et al*. **Updated research nosology for HIV-associated neurocognitive disorders.** *Neurology* 2007; **69**:1789–1799.
6. Sevigny JJ, Albert SM, McDermott MP, McArthur JC, Sacktor N, Conant K, *et al*. **Evaluation of HIV RNA and markers of immune activation as predictors of HIV-associated dementia.** *Neurology* 2004; **63**:2084–2090.
7. Cysique LA, Heaton RK, Kamminga J, Lane T, Gates TM, Moore DM, *et al*. **HIV-associated neurocognitive disorder in Australia: a case of a high functioning and optimally treated cohort and implications for international neuroHIV research.** *J Neurovirol* 2014; **20**:258–268.
8. Wilkie FL, Goodkin K, Eisdorfer C, Feaster D, Morgan R, Fletcher MA, *et al*. **Mild cognitive impairment and risk of mortality in HIV-1 infection.** *J Neuropsychiatry Clin Neurosci* 1998; **10**:125–132.
9. Stern Y, McDermott MP, Albert S, Palumbo D, Selnes OA, McArthur J, *et al*. **Factors associated with incident human immunodeficiency virus-dementia.** *Arch Neurol* 2001; **58**:473–479.
10. Albert SM, Marder K, Dooneief G, Bell K, Sano M, Todak G, *et al*. **Neuropsychologic impairment in early HIV infection: a risk factor for work disability.** *Arch Neurol* 1995; **52**:525–530.
11. Marcotte TD, Heaton RK, Wolfson T, Taylor MJ, Alhassoon O, Arfaa K, *et al*. **The impact of HIV-related neuropsychological dysfunction on driving behavior. The HNRC Group.** *J Int Neuropsychol Soc* 1999; **5**:579–592.
12. Hinkin CH, Castellon SA, Durvasula RS, Hardy DJ, Lam MN, Mason KI, *et al*. **Medication adherence among HIV+ adults: effects of cognitive dysfunction and regimen complexity.** *Neurology* 2002; **59**:1944–1950.
13. Wynn HE, Brundage RC, Fletcher CV. **Clinical implications of CNS penetration of antiretroviral drugs.** *CNS Drugs* 2002; **16**:595–609.
14. McCutchan JA, Letendre SL. **Pharmacology of antiretroviral drugs in the central nervous system: pharmacokinetics, antiretroviral resistance, and pharmacodynamics.** In: Gendelman HE, Grant I, Everall IP, Lipton SA, Swindells S,editors. *The neurology of AIDS*. 2nd ed. New York, NY: Oxford University Press; 2005. pp. 729–734.
15. Mothobi NZ, Brew BJ. **Neurocognitive dysfunction in the highly active antiretroviral therapy era.** *Curr Opin Infect Dis* 2012; **25**:4–9.
16. Johnson TP, Patel K, Johnson KR, Maric D, Calabresi PA, Hasbun R, *et al*. **Induction of IL-17 and nonclassical T-cell activation by HIV-tat protein.** *Proc Natl Acad Sci USA* 2013; **110**:13588–13593.
17. Cysique LA, Waters EK, Brew BJ. **Central nervous system antiretroviral efficacy in HIV infection: a qualitative and quantitative review and implications for future research.** *BMC Neurol* 2011; **11**:148.
18. Ellis RJ, Letendre S, Vaida F, Haubrich R, Heaton RK, Sacktor N, *et al*. **Randomized trial of central nervous system-targeted antiretrovirals for HIV-associated neurocognitive disorder.** *Clin Infect Dis* 2014; **58**:1015–1022.
19. Cysique LA, Brew BJ. **Neuropsychological functioning and antiretroviral treatment in HIV/AIDS: a review.** *Neuropsychol Rev* 2009; **19**:169–185.
20. Roche M, Salimi H, Duncan R, Wilkinson BL, Chikere K, Moore MS, *et al*. **A common mechanism of clinical HIV-1 resistance to the CCR5 antagonist maraviroc despite divergent resistance levels and lack of common gp120 resistance mutations.** *Retrovirology* 2013; **10**:doi: 10.1186/1742-4690-10-43.
21. Gray L, Roche M, Churchill MJ, Sterjovski J, Ellett A, Poumbourios P, *et al*. **Tissue-specific sequence alterations in the human immunodeficiency virus type 1 envelope favoring CCR5 usage contribute to persistence of dual-tropic virus in the brain.** *J Virol* 2009; **83**:5430–5441.
22. Lisi L, Tramutola A, De Luca A, Navarra P, Dello Russo C. **Modulatory effects of the CCR5 antagonist maraviroc on microglial pro-inflammatory activation elicited by gp120.** *J Neurochem* 2012; **120**:106–114.
23. Seclén E, del Mar González M, Lapaz M, Rodríguez C, del Romero J, Aguilera A, *et al*. **Primary resistance to maraviroc in a large set of R5-V3 viral sequences from HIV-1-infected patients.** *J Antimicrob Chemother* 2010; **65**:2502–2504.
24. Yilmaz A, Watson V, Else L, Gisslèn M. **Cerebrospinal fluid maraviroc concentrations in HIV-1 infected subjects.** *AIDS* 2009; **23**:2537–2540.
25. Tiraboschi JM, Niubo J, Curto J, Podzamczer D. **Maraviroc concentrations in cerebrospinal fluid in HIV-infected subjects.** *J Acquir Immune Defic Syndr* 2010; **55**:606–609.
26. Kelly KM, Beck SE, Pate KA, Queen SE, Dorsey JL, Adams RJ, *et al*. **Neuroprotective maraviroc monotherapy in simian immunodeficiency virus-infected macaques: reduced replicating and latent SIV in the brain.** *AIDS* 2013; **27**:F21–28.
27. Melica G, Canestri A, Peytavin G, Lelivre J, Bouvier-Alias M, Clavel C, *et al*. **Maraviroc-containing regimen suppresses HIV replication in the cerebrospinal fluid of patients with neurological symptoms.** *AIDS* 2010; **24**:2130–2133.
28. Ndhlovu L, Umaki T, Chew G, Chow D, Agsalda M, Kalpana J, *et al*. **Treatment intensification with maraviroc (CCR5 antagonist) leads to declines in CD16-expressing monocytes in cART-suppressed chronic HIV-infected subjects and is associated with improvements in neurocognitive test performance: implications for HIV-associated neurocognitive disease (HAND).** *J Neurovirol* 2014; **20**:571–582.
29. Cysique LA, Vaida F, Letendre S, Gibson S, Cherner M, Woods SP, *et al*. **Dynamics of cognitive change in impaired HIV-positive patients initiating antiretroviral therapy.** *Neurology* 2009; **73**:342–348.
30. Gonzalez E, Rovin BH, Sen L, Cooke G, Dhanda R, Mummidi S, *et al*. **HIV-1 infection and AIDS dementia are influenced by a mutant MCP-1 allele linked to increased monocyte infiltration of tissues and MCP-1 levels.** *Proc Natl Acad Sci USA* 2002; **99**:13795–13800.

31. Bhaskaran K, Mussini C, Antinori A, Walker AS, Dorrucci M, Sabin C, *et al*. **Changes in the incidence and predictors of human immunodeficiency virus-associated dementia in the era of highly active antiretroviral therapy.** *Ann Neurol* 2008; **63**:213–221.
32. Cysique LA, Moffat K, Moore DM, Lane TA, Davies NWS, Carr A, *et al*. **HIV, vascular and aging injuries in the brain of clinically stable HIV-infected adults: a (1)H MRS study.** *PLoS One* 2013; **8**:e61738doi:10.1371/journal.pone.0061738.
33. Fabbiani M, Ciccarelli N, Tana M, Farina S, Baldonero E, Di Cristo V, *et al*. **Cardiovascular risk factors and carotid intima-media thickness are associated with lower cognitive performance in HIV-infected patients.** *HIV Med* 2013; **14**:136–144.
34. Cysique LA, Maruff P, Darby D, Brew BJ. **The assessment of cognitive function in advanced HIV-1 infection and AIDS dementia complex using a new computerized cognitive test battery.** *Arch Clin Neuropsychol* 2006; **21**:185–194.
35. Falleti MA, Maruff P, Collie A, Darby DG. **Practice effects associated with the repeated assessment of cognitive function using the CogState battery at 10-min, one week and one month test-retest intervals.** *J Clin Exp Neuropsychol* 2006; **28**:1095–1112.
36. Cysique LA, Franklin D Jr, Abramson I, Ellis R, Letendre S, Collier A, *et al*. **Normative data and validation of a regression model for assessing meaningful neuropsychological change.** *J Clin Exp Neuropsychol* 2011; **33**:505–522.
37. Sullivan R, Senior G, Hennessy M. Regression equations: Australian age-education and premorbid cognitive/intellectual estimates for the WAIS-III. Poster presented at 6th Annual Conference of the APS College of Clinical Neuropsychologists, NSW, Australia, 12–15 October 2000. Assessment Psychology Online. http://www.assessmentpsychology.com/iq-estimates-3.htm. [Accessed 22 January 2015]
38. Lane T, Moore D, Batchelor J, Brew B, Cysique L. **Facial emotional processing in HIV infection: relation to neurocognitive and neuropsychiatric status.** *Neurosychol* 2012; **26**:713–722.
39. Harezlak J, Butchthal S, Taylor M, Schifitto G, Zhong J, Daar E, *et al*. **Persistence of HIV-associated cognitive impairment, inflammation, and neuronal injury in era of highly active antiretroviral treatment.** *J Acquir Immune Defic Syndr* 2011; **25**:623–633.
40. Mohamed M, Barker P, Skolasky R, Selnes O, Moxley R, Pomper M, *et al*. **Brain metabolism and cognitive impairment in HIV infection: a 3-T magnetic resonance spectroscopy study.** *Magn Reson Imaging* 2010; **28**:1251–1257.
41. Vera JH, Garvey LJ, Allsop JM, Kaye S, McClure MO, Back D, *et al*. **Alterations in cerebrospinal fluid chemokines are associated with maraviroc exposure and in vivo metabolites measurable by magnetic resonance spectroscopy.** *HIV Clin Trials* 2012; **13**:222–227.
42. Hagberg L, Cinque P, Gisslen M, Brew B, Spudich S, Bestetti A, *et al*. **Cerebrospinal fluid neopterin: an informative biomarker of central nervous system immune activation in HIV-1 infection.** *AIDS Res Ther* 2010; **7**:doi: 10.1186/1742-6405-7-15.
43. Brew B, Letendre S. **Biomarkers of HIV related central nervous system disease.** *Int Rev Psych* 2008; **20**:73–88.
44. Heaton RK, Miller SW, Taylor MJ, Grant I. *Revised comprehensive norms for an expanded Halstead Reitan Battery: demographically adjusted neuropsychological norms for African American and Caucasian adults*. Lutz: Psychological Assessment Resources, Inc; 2004.
45. Hopkins WG. **Measures of reliability in sports medicine and science.** *Sports Med* 2000; **30**:1–15.
46. Cysique LA, Brew BJ. **The effects of HIV and ageing on brain functions: proposing a research framework and update on the last 3 years' findings.** *Curr Op HIV AIDS* 2014; **9**:355–364.
47. Pulliam L, Sun B, Rempel H. **Invasive chronic inflammatory monocyte phenotype in subjects with high HIV-1 viral load.** *J Neuroimmunol* 2004; **157**:93–98.
48. Shiramizu B, Ananworanich J, Chalermchai T, Siangphoe U, Troelstrup D, Shikuma C, *et al*. **Failure to clear intra-monocyte HIV infection linked to persistent neuropsychological testing impairment after first-line combined antiretroviral therapy.** *J Neurovirol* 2004; **18**:69–73.
49. Kusao I, Shiramizu B, Liang CY, Grove J, Agsalda M, Troelstrup D, *et al*. **Cognitive performance related to HIV-1-infected monocytes.** *J Neuropsychiatry Clin Neurosci* 2012; **24**:71–80.
50. Burdo TH, Weiffenbach A, Woods SP, Letendre S, Ellis RJ, Williams KC. **Elevated sCD163 in plasma but not cerebrospinal fluid is a marker of neurocognitive impairment in HIV infection.** *AIDS* 2013; **27**:1387–1395.
51. Chang L, Ernst T, Leonido-Yee M, Witt M, Speck O, Walot I, *et al*. **Highly active antiretroviral therapy reverses brain metabolite abnormalities in mild HIV dementia.** *Neurology* 1999; **53**:782–789.
52. Stankoff B, Tourbah A, Suarez S, Turell E, Stievenart JL, Payan C, *et al*. **Clinical and spectroscopic improvement in HIV-associated cognitive impairment.** *Neurology* 2001; **56**:112–115.
53. Chang L, Ernst T, Witt MD, Ames N, Walot I, Jovicich J, *et al*. **Persistent brain abnormalities in antiretroviral-naïve HIV patients 3 months after HAART.** *Antivir Ther* 2003; **8**:17–26.
54. Harezlak J, Cohen R, Gongvatana A, Taylor M, Buchthal S, Schifitto G, *et al*. **Predictors of CNS injury as measured by proton magnetic resonance spectroscopy in the setting of chronic HIV infection and CART.** *J Neurovirol* 2014; **20**:294–303.
55. Hill BD, Rohling MD, Boettcher AC, Meyers JE. **Cognitive intra-individual variability has a positive association with traumatic brain injury severity and suboptimal effort.** *Arch Clin Neuropsychol* 2013; **28**:640–648.
56. Maki PM, Rubin LH, Valcour V, Martin E, Crystal H, Young M, *et al*. **Cognitive function in women with HIV: findings from the Women's Interagency HIV study.** *Neurology* 2015; **84**:231–240.