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# Cerebrospinal and peripheral human immunodeficiency virus type 1 load in a multisite, randomized, double-blind, placebo-controlled trial of D-Ala$_1$-peptide T-amide for HIV-1-associated cognitive-motor impairment

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**D-Ala$_1$-peptide T-amide (DAPTA) has shown neuroprotection *in vitro* against gp120-induced loss of dendritic arborization and is promulgated as a CCR5 antagonist. A multisite, randomized, double-blind clinical trial of DAPTA versus placebo prior to combination antiretroviral therapy conducted with human immunodeficiency virus (HIV)-1 seropositive participants having cognitive impairment showed no overall cognitive effect, though subgroups with greater impairment and CD4 cell counts of 201 to 500 cells/mm$^3$ at baseline showed significant improvement. The objective of this study was to examine whether intranasal administration of DAPTA at a dose of 2 mg three times per day (tid) was associated with a reduction of cerebrospinal fluid (CSF) and peripheral (plasma and serum) viral load among a subgroup of participants completing 6 months of treatment. Baseline and 6-month CSF ($n = 92$) and peripheral (plasma $n = 33$; serum $n = 24$) viral load were measured by the Roche Ultrasensitive assay, version 1.5, with reflexive use of the AMPLICOR assay and preservation of the blind. A DAPTA treatment indicator variable was tested using generalized linear models on change in viral load. Peripheral load (combined plasma and serum) was significantly reduced in the DAPTA-treated group. No group differences in CSF viral load were found. This retrospective study on a limited subgroup of the original trial sample indicated that DAPTA treatment may reduce peripheral viral load without concomitant CSF effects. Future studies should be undertaken to confirm the existence of this result and the CSF-periphery dissociation observed with respect to HIV-1–associated cognitive-motor impairment.** *Journal of NeuroVirology* (2006) **12,** 178–189.

**Keywords:** CSF; clinical trial; cognition; DAPTA; HIV; peptide T; viral load

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## Introduction

D-$Ala_1$-peptide T-amide (DAPTA; "peptide T") is an octapeptide, Ala - Ser - Thr - Thr - Thr - Asn - Tyr - Thr, named for its high threonine content. It was derived by a database search assuming that a receptor binding epitope within gp120 would have a sequence homology with a known signaling peptide. DAPTA was originally described to bind to CD4 receptor (Pert *et al*, 1986), the only human immunodeficiency virus (HIV)-1 receptor then known, in the V2 region. However, the results documenting CD4 receptor binding were inconsistent (Sodroski *et al*, 1987; Walczak *et al*, 1991; Ramsdale *et al*, 1993). With the discovery of the chemokine coreceptors, investigation began of the interaction between DAPTA and the two major chemokine coreceptors for HIV-1, CXCR4 and CCR5. DAPTA inhibited $\beta$-chemokine–induced chemotaxis and was a partial inhibitor of $^{125}$I-MIP-1$\beta$ (macrophage inflammatory protein-1$\beta$) binding (Raychaudhuri *et al*, 1998; Redwine *et al*, 1999). Studies of brain-derived microglial cells showed that CCR5 and CCR3 permit entry of HIV-1 (He *et al*, 1997). As CCR5 is the major chemokine for monocyte-derived macrophages (MDMs) and as monocytes carry HIV-1 from periphery to brain, the implications of DAPTA treatment became clear for reduction of HIV-1 in brain, a known HIV-1 reservoir, in the combination antiretroviral therapy (CART) era. Further studies have shown that DAPTA inhibited infection of MDMs with BaL (a CCR5-tropic isolate) and of activated CD4+ lymphocytes with dual-tropic isolates (Ruff *et al*, 2001). DAPTA also blocked infection using HIV-1 virions pseudotyped with ADA envelope in the luciferase reporter assay (Ruff *et al*, 2001). Inhibition was much greater with MAGI cells expressing CCR5 than with those expressing CXCR4, potentially explaining the inconsistent results obtained with DAPTA prior to identification of the chemokine co-receptors.

Hill *et al* (1993) demonstrated that gp120 administration in nanogram quantities to neonatal rats delayed behavioral milestones and was associated with reduced dendritic arborization. Subsequently, Brenneman *et al* (1999) found that neuronal cell death in rat cerebral cortical cultures induced by five variants of gp120 was prevented by DAPTA. DAPTA has also been associated with reductions in tumor necrosis factor (TNF)-$\alpha$ (Phipps *et al*, 1996), interleukin (IL)-1, IL-6, and interferon (IFN)-$\gamma$ (Raychaudhuri *et al*, 1999) and with an increase in IL-4 (Ruff *et al*, 2003), suggesting induction of a neuroprotective milieu in brain (Goodkin *et al*, 2000; Heyes *et al*, 1992; Shapshak *et al*, 2004).

As part of a National Institute of Mental Health (NIMH)–sponsored, randomized clinical trial of DAPTA, cerebrospinal fluid (CSF) and peripheral blood samples were collected at study entry and post-treatment (after 6 months of participation). The purpose of this report is to retrospectively examine whether DAPTA treatment was associated with changes in CSF and/or peripheral viral load using ultrasensitive viral load assay methodology not available during the pre-CART trial period on samples taken from a subgroup of participants from the original clinical trial sample.

## Results

### *Characteristics of the subsamples with CSF and peripheral viral load data*

The samples used for these analyses were retrospectively defined by the availability of CSF and plasma or serum to analyze their specimens at baseline and at 6 months. As the availability may not have been random, we compared these two subsamples to the initially randomized clinical trial sample (Table 1). Comparing the CSF viral load completers to the originally randomized sample (effectively a comparison with noncompleters, data not shown), it can be noted (see test 1 $P$ values) that there was one significant difference (on presence of severe cognitive deficit) and two nonsignificant trends toward a difference (on CD4 cell count and on peripheral viral load). Comparing peripheral load completers similarly, it is apparent (see test 2 $P$ values) that there were two significant differences (on antiretroviral therapy use and length of such use) and two nonsignificant trends toward a difference (on presence of severe cognitive deficit and gender). Fewer participants with severe cognitive deficits were present among the CSF and peripheral viral load completers than among the noncompleters, as might be expected due to differential attrition. There was a higher CD4 cell count among the CSF viral load completers than noncompleters, reflecting a similar association. In accord with this interpretation as well, the peripheral viral load completers had higher frequencies of antiretroviral medication use and duration of use than their respective noncompleters. Peripheral viral load completers also reflected a somewhat higher frequency of males and a lower frequency of severe cognitive deficit. These analyses indicate that generalizations from the analytic samples herein to the randomized sample may be limited on the basis of a greater access to antiretroviral therapy and less severe levels of cognitive impairment in the analytic sample.

### *Characteristics of the subsamples with CSF and peripheral viral load data by treatment assignment*

The second methodological issue that must be addressed prior to analysis is the potential for failure of randomization across these two sub-samples by treatment assignment. In this respect, there were also significant differences noted (Table 2). Regarding CSF viral load completers in the DAPTA and placebo groups, there was a significant difference in the frequency of Caucasian Americans by Fisher's Exact Test ($P = .02$) (see test 1 $P$ values). The DAPTA group

**Table 1** Characteristics of randomization variables at screening: randomized sample and CSF and peripheral viral load completers

| | *Randomized sample* | | | | *CSF viral load completers*[a] | | | | *Test 1* | | *Peripheral viral load completers*[a] | | | | *Test 2* | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *N* | *%* | *Mean* | *SD* | *N* | *%* | *Mean* | *SD* | *P value* | *Note*[b] | *N* | *%* | *Mean* | *SD* | *P value* | *Note*[b] |
| Sample size | 215 | | | | 92 | | | | | | 57 | | | | | |
| Sex, male | 205 | 95.4 | — | — | 90 | 97.8 | — | — | 0.19 | 1 | 57 | 100 | — | — | 0.07 | 1 |
| Age, years | 215 | — | 39.3 | 7.5 | 92 | — | 39.8 | 7.2 | 0.45 | 2 | 57 | — | 39.9 | 7.0 | 0.50 | 2 |
| 18–39 years | 123 | 57.2 | — | — | 47 | 51.1 | — | — | 0.13 | 1 | 30 | 52.6 | — | — | 0.44 | 1 |
| Education, years | 215 | — | 15.0 | 2.2 | 92 | — | 15.2 | 2.2 | 0.20 | 2 | 57 | — | 14.9 | 2.2 | 0.52 | 2 |
| ≥16 years | 91 | 42.3 | — | — | 42 | 45.7 | — | — | 0.41 | 1 | 25 | 43.9 | — | — | 0.88 | 1 |
| Race/ethnicity | | | | | | | | | | | | | | | | |
| Caucasian | 177 | 82.3 | — | — | 78 | 84.8 | — | — | 0.47 | 1 | 45 | 79.0 | — | — | 0.43 | 1 |
| Hispanic | 25 | 11.6 | — | — | 10 | 10.9 | — | — | 0.83 | 1 | 8 | 14.0 | — | — | 0.48 | 1 |
| African American | 10 | 4.7 | — | — | 4 | 4.4 | — | — | 1.00 | 1 | 4 | 7.0 | — | — | 0.46 | 1 |
| CD4 cell count | 214 | — | 219.6 | 191.6 | 92 | — | 245.2 | 197.5 | 0.09 | 2 | 57 | — | 227.5 | 212.8 | 0.72 | 2 |
| Severe immunosuppression | | | | | | | | | | | | | | | | |
| CD4 cell ≤200 cells/mm[c] | 109 | 50.9 | — | — | 41 | 44.6 | — | — | 0.13 | 1 | 28 | 49.1 | — | — | 0.76 | 1 |
| History of substance abuse | 121 | 56.3 | — | — | 56 | 60.9 | — | — | 0.27 | 1 | 37 | 64.9 | — | — | 0.16 | 1 |
| Prior DAPTA use | 5 | 2.3 | — | — | 2 | 2.2 | — | — | 1.00 | 1 | 1 | 1.8 | — | — | 1.00 | 1 |
| Severe cognitive deficit | 141 | 65.6 | — | — | 53 | 57.6 | — | — | 0.04 | 1 | 32 | 56.1 | — | — | 0.10 | 1 |
| Current antiretroviral (ARV) use: zidovudine and other[c] | 141 | 65.6 | — | — | 65 | 70.7 | — | — | 0.19 | 1 | 44 | 77.2 | — | — | 0.03 | 1 |
| Length of ARV use >6 months | 101 | 71.6 | — | — | 51 | 78.5 | — | — | 0.13 | 1 | 37 | 84.1 | — | — | 0.03 | 1 |
| CSF viral load (log10)[d] | 180 | — | 2.83 | 0.94 | 92 | — | 2.87 | 0.94 | 0.38 | 3 | 56 | — | 2.77 | 1.00 | 0.46 | 3 |
| Peripheral viral load (log10)[d] | 112 | — | 4.11 | 0.90 | 65 | — | 3.98 | 0.86 | 0.09 | 2 | 57 | — | 4.02 | 0.83 | 0.34 | 2 |

[a]CSF viral load completers are defined as all participants who completed the placebo-controlled trial phase with baseline and 6-month CSF viral load measurements. Peripheral viral load completers are defined as all participants who completed the placebo-controlled trial phase with baseline and 6-month viral load measurements in plasma or in serum. Test 1: comparing CSF viral load completers and its noncompleters ($n = 92$ vs $n = 123$); Test 2: comparing peripheral viral load completers and noncompleters ($n = 57$ vs $n = 158$). Data are given as number (or percentage) unless otherwise indicated.

[b]1 = Fisher's Exact Test; 2 = *t*-tests for independent samples; 3 = Wilcoxon signed rank test.

[c]Among zidovudine monotherapy, other monotherapy, and nucleoside RT inhibitor combination ARV medication users: $n = 141$, 65, and 44 for randomized sample, CSF viral load completers, and peripheral viral load completers, respectively.

[d]Plasma samples = 55 in randomized sample, 37 in CSF viral load completers, and 33 in peripheral viral load completers. The median baseline CSF and peripheral viral loads (with their interquartile ranges) for the randomized sample were 2.70 (1.60) and 4.06 (1.26), respectively.

**Table 2** Characteristics of CSF and peripheral viral load completers at baseline by treatment group

| | *CSF viral load completers*[a] | | | | | | | | | | *Peripheral viral load completers*[a] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *DAPTA* | | | | *Placebo* | | | | *Test 1*[b] | | *DAPTA* | | | | *Placebo* | | | | *Test 2*[b] | |
| | *N* | % | *Mean* | *SD* | *N* | % | *Mean* | *SD* | *P value* | *Note*[c] | *N* | % | *Mean* | *SD* | *N* | % | *Mean* | *SD* | *P value* | *Note*[c] |
| Sample size | 44 | | | | 48 | | | | | | 29 | | | | 28 | | | | | |
| Sex, male | 42 | 95.5 | | | 48 | 100 | | | 1.00 | 1 | 29 | 100 | | | 28 | 100 | | | 1.00 | 1 |
| Age, year | 44 | | 39.4 | 7.3 | 48 | | 40.1 | 7.1 | 0.66 | 2 | 29 | | 40.0 | 7.7 | 28 | | 39.8 | 6.3 | 0.92 | 2 |
| 18–39 years | 24 | 54.6 | | | 23 | 47.9 | | | 0.54 | 1 | 16 | 55.2 | | | 14 | 50.0 | | | 0.79 | 1 |
| Education, years | 44 | | 15.0 | 2.2 | 48 | | 15.4 | 2.1 | 0.36 | 2 | 29 | | 14.7 | 2.2 | 28 | | 15.1 | 2.1 | 0.48 | 2 |
| ≥16 years | 19 | 43.2 | | | 23 | 47.9 | | | 0.68 | 1 | 11 | 37.9 | | | 14 | 50.0 | | | 0.43 | 1 |
| Race/Ethnicity | | | | | | | | | | | | | | | | | | | | |
| Caucasian | 33 | 75.0 | | | 45 | 93.8 | | | 0.02 | 1 | 19 | 65.5 | | | 26 | 92.9 | | | 0.02 | 1 |
| Hispanic | 9 | 20.5 | | | 1 | 2.1 | | | 1.00 | 1 | 8 | 27.6 | | | 0 | 0 | | | 1.00 | 1 |
| African American | 2 | 4.6 | | | 2 | 4.2 | | | 1.00 | 1 | 2 | 6.9 | | | 2 | 7.1 | | | 1.00 | 1 |
| CD4 cell count (cells/mm$^3$) | 44 | | 211.6 | 174.6 | 48 | | 275.9 | 213.6 | 0.12 | 2 | 29 | 173.6 | 156.4 | | 28 | | 283.3 | 249.4 | 0.05 | 2 |
| Severe immunosuppression: CD4 cell ≤200 | 22 | 50.0 | | | 19 | 39.6 | | | 0.40 | 1 | 17 | 58.6 | | | 11 | 39.3 | | | 0.19 | 1 |
| History of substance abuse | 29 | 65.9 | | | 27 | 56.3 | | | 0.40 | 1 | 22 | 75.9 | | | 15 | 53.6 | | | 0.10 | 1 |
| Prior DAPTA use | 0 | 0 | | | 2 | 4.2 | | | 1.00 | 1 | 0 | 0 | | | 1 | 3.6 | | | 1.00 | 1 |
| Severe cognitive deficit | 25 | 56.8 | | | 28 | 58.3 | | | 1.00 | 1 | 13 | 44.8 | | | 19 | 67.9 | | | 0.11 | 1 |
| Current antiretroviral (ARV) use: zidovudine and other[d] | 30 | 68.2 | | | 35 | 72.9 | | | 0.65 | 1 | 21 | 72.4 | | | 23 | 82.1 | | | 0.53 | 1 |
| Length of ARV use >6 months | 22 | 73.3 | | | 29 | 82.9 | | | 0.38 | 1 | 17 | 81.0 | | | 20 | 87.0 | | | 0.69 | 1 |
| CSF viral load (log10) | 44 | | 2.87 | 0.97 | 48 | | 2.87 | 0.91 | 0.99 | 2 | 29 | | 2.82 | 1.03 | 27 | | 2.70 | 0.98 | 0.66 | 2 |
| Peripheral viral load (log10) | 33 | | 3.93 | 0.96 | 32 | | 4.04 | 0.77 | 0.62 | 2 | 29 | | 4.13 | 0.94 | 28 | | 3.91 | 0.69 | 0.33 | 2 |

[a]CSF viral load completers are defined as all participants who completed the placebo-controlled trial phase with baseline and 6-month CSF viral load measurements. Peripheral viral load completers are defined as all participants who completed the placebo-controlled trial phase with baseline and 6-month viral load measurements in plasma or in serum.
[b]Test 1: comparing CSF viral load completers treated with DAPTA ($n = 44$) to those treated with placebo ($n = 48$); Test 2: comparing peripheral viral load completers treated with DAPTA ($n = 29$) to those treated with placebo ($n = 28$). Data are given as number (or percentage) unless otherwise indicated.
[c]1 = Fisher's Exact Test; 2 = *t*-tests for independent samples.
[d]Among zidovudine and nucleoside RT inhibitor combination ARV medication users: $n = 30$ and 35, for CSF viral load completers treated with DAPTA and placebo, respectively; $n = 21$ and 23 for peripheral viral load completers treated with DAPTA and placebo, respectively.

had a lower percentage (75.0%) than the placebo group (93.8%), driven by a higher percentage of Hispanic Americans.

Regarding peripheral viral load completers, there were two significant differences by treatment assignment. The DAPTA group again showed a lower frequency of Caucasians (65.5%) than the placebo group (92.9%) by Fisher's Exact Test ($P = .02$) (see test 2 $P$ values). In addition, a significant CD4 cell count difference was found between groups, with the DAPTA group having a lower baseline mean CD4 cell count (174 cells/mm$^3$) than the placebo group (283 cells/mm$^3$) by the student's $t$ test ($P = .05$). In addition, there was a nonsignificant trend toward a difference in history of substance abuse ($P = .10$), with a higher frequency in the DAPTA group (75.9%) than in the placebo group (53.6%). It might be concluded that both the CSF and peripheral viral load completers were quite similar by treatment assignment, overall. Of possible significance, the peripheral viral load completers treated with DAPTA had a lower CD4 cell count and a (potentially associated) higher frequency of substance abuse history, which could operate against detecting an effect on change in peripheral viral load.

*Viral load assay validation study*

A preliminary validation study of measurements in CSF and plasma was undertaken for the Ultra-Sensitive Roche HIV-1 MONITOR test, version 1.5. (This was not done for serum or for the Standard AMPLICOR HIV-1 MONITOR test). Twenty-four coded replicates were assayed, 12 of which consisted of HIV-1 spiked into plasma and 12 consisted of HIV-1 spiked into CSF at the same concentrations: 50 400, 4000, and 40,000 HIV-1 RNA copies/ml. HIV-1–negative specimens of plasma and CSF were also included.

No false positives were detected. All five HIV-1–negative samples yielded nondetectable results. Six (10%) of the 60 assays at the lower limit of assay sensitivity of 50 HIV-1 RNA copies/ml failed to generate a detectable result, whereas the expected rate based upon manufacturer's claims is 5%. Thus, further data analyses of this validation study excluded data at 50 copies/ml because of the increased variation observed at this concentration. Composite estimates of intra-assay standard deviation (SD) were, therefore, generated from the other HIV-1 RNA concentrations used: 400, 4000, and 40,000 copies/ml. These results showed that the SD, overall, was less than 0.15 logs for both CSF and plasma—i.e., within the limit set by the AIDS Clinical Trials Group (ACTG) Virology Quality Assessment program.

The panels were also analyzed by laboratory (coding for each of three). Two laboratories contributed four sets of values, and one laboratory contributed two sets. On 6 of 10 total assay runs, CSF results were lower than those in plasma with the same virus concentration. The results varied significantly by laboratory, ranging from 0.19 to 0.34 logs. Significant variation by HIV-1 RNA concentration occurred in 2 of 10 runs, with 1 showing a variation within 25%. It was concluded that the assay was valid and performed with the expected level of sensitivity and reproducibility in CSF and plasma. Averaged over the laboratories, the estimated copy numbers were higher in plasma than in CSF, though the discrepancy was relatively small. No evidence for assay inhibition by CSF was found. By runs, the differences between CSF and plasma estimated copy numbers were wide. This suggests the possibility that the estimated difference in copy number between CSF and plasma could be associated with interassay variability dependent upon specimen matrix (CSF versus plasma) rather than upon specimen matrix alone.

*The relationship of CSF and peripheral viral load measures*

Viral load was assayed in plasma or serum (depending upon study site) in this trial; thus, no data were available to examine a correlation directly between these two measures performed on the same participants. However, it was possible (and of interest) to compare their relationships to a third measure—CSF viral load. Due to the issue of skewness of the CSF, plasma, and serum viral load distributions related to inclusion of measures at the lower limit of detection, a Spearman's rank-order correlation was computed to assess these relationships. The $r_S$ between serum and CSF viral load and that between plasma and CSF viral load were not significantly different from 0. Thus, it was considered justifiable to collapse serum and plasma viral load data into a single category ("peripheral") for examining the potential therapeutic effects of DAPTA. This decision is also justified by the literature showing HIV-1 RNA polymerase chain reaction (PCR) to be equally sensitive in plasma or serum (Vandamme *et al*, 1995). The foregoing lack of a statistically significant difference does not imply that the relationships between serum and plasma viral loads will be the equivalent when they are entered into a generalized linear model (GLM) on CSF viral load with other predictors. The differential impact of plasma versus serum generates a $\beta$ weight ascribed to relationship to the outcome variable that is independent of all of the other predictors. Therefore, to conduct a conservative analysis and control for any potentially confounding residual influence of the relationship of plasma versus serum viral load to the CSF viral load outcome measure, GLMs were conducted that incorporated peripheral tissue source for the load assay in blood as a control predictor of the effect of treatment assignment on change in CSF viral load. Confirming our expectation, this control variable was not a significant contributor in any of those GLMs (data not shown).

*CSF viral load and DAPTA treatment*

The median CSF viral load for the DAPTA group at baseline was 2.65 (Q1: 0.92; Q3: 4.38), with a mean

**Table 3** Analytical models of the effects of DAPTA treatment on changes in central and peripheral viral load over 6 months

| | | *Central (CSF)* | | | | *Peripheral (plasma or serum)*[a] | | | |
|---|---|---|---|---|---|---|---|---|---|
| *Model* | *Treatment* | *No.* | *Mean* | *SE* | *P value*[b] | *No.* | *Mean* | *SE* | *P value*[b] |
| Adjusted GLM[c] | DAPTA | 44 | −0.02 | 0.11 | Model N.S. | 29 | −0.23 | 0.11 | .019 |
| | Placebo | 48 | 0.06 | 0.10 | | 28 | 0.15 | 0.11 | |
| Adjusted GLM[d] | DAPTA | 44 | −0.02 | 0.11 | Model N.S. | 29 | −0.23 | 0.11 | .019 |
| | Placebo | 48 | 0.06 | 0.10 | | 28 | 0.15 | 0.11 | |
| Adjusted GLM[e] | DAPTA | 14 | −0.15 | 0.21 | Model N.S. | — | — | — | — |
| | Placebo | 16 | 0.23 | 0.20 | | — | — | — | — |

[a]The plasma and serum subsamples are collapsed in this category. Separate values are not reported by plasma and serum because the resulting sample sizes generate insufficient statistical power.
[b]*P* value for the $\beta$ weight for the least square adjusted means on the categorical treatment indicator variable (DAPTA or placebo). This is not reported if the overall model was not statistically significant — "Model N.S."
[c]Adjusted for CD4 cell count.
[d]Adjusted for age, years of education, and CD4 cell count.
[e]Adjusted for age, years of education, CD4 cell count, and the baseline CSF monocyte count.

of 2.87 (SD = 0.97). The median CSF viral load for the placebo group at baseline was 2.80 (Q1: 1.44; Q3: 4.16), with the same mean as the DAPTA group of 2.87 (SD = 0.91). As a crude estimate of treatment efficacy, the student's *t* test was conducted on the change in CSF viral load by treatment group and was not statistically significant ($t = -0.57$, $P = .57$). Controlling for the CD4 cell count did not change this result (overall model $F_{(2,89)} = 0.22$, $P = .81$] (Table 3), nor did adding controls for age and years of education (overall model $F_{(4,87)} = 0.16$, $P = .96$), nor did adding a control for baseline CSF monocyte count (overall model $F_{(5,86)} = 0.34$, $P = .88$). A GLM controlling for baseline CDC clinical disease stage, CD4 cell count, antiretroviral medication use, and CSF monocyte count also yielded a nonsignificant overall model ($F_{(5,86)} = 0.83$, $P = .54$) (data not shown).

As confirmatory analyses, goodness-of-fit tests were done to ensure that the change in the viral load outcome measures (CSF and peripheral) met the requirements for a normal distribution assumed for the GLM analytic procedure. The Kolmogorov-Smirnov goodness-of-fit test on the CSF viral load outcome ($n = 92$) yielded a $P < .10$ ($D = 0.096$, $P = .038$), indicating that the change in CSF viral load outcome measure was not normally distributed. Thus, two additional analyses were conducted. The first analysis used a GLM to analyze the effect of DAPTA treatment assignment on change in CSF viral load as a categorical outcome (change from detectable to nondetectable versus either change from nondetectable to detectable or remaining detectable) ($\chi^2 = 1.38$, $P = .24$). Controlling for CD4 cell count yielded a significant overall model ($\chi^2 = 6.46$, $P = .04$), with no effect of the DAPTA predictor variable ($\beta = 0.65$, Wald's $\chi^2 = 0.71$, $P = .40$), although the CD4 cell count variable showed an effect ($\beta = -.006$, Wald's $\chi^2 = 3.76$, $P = .053$) such that changing to nondetectable was associated with a lower baseline CD4 cell count. Adding controls for age and educational level resulted in reversion to a nonsignificant overall model ($\chi^2 = 6.92$, $P = .14$). Controlling for two measures—CSF monocyte count and the influence of missing data on this measure—yielded a nonsignificant overall model as well ($\chi^2 = 2.56$, $P = .47$). The second set of analyses used a GLM model on the truncated outcome measure of change in CSF viral load when both baseline and 6-month values were detectable. The student's *t* test was not statistically significant ($t = -0.05$, $P = .97$). Adding controls for CD4 cell count as well as age and educational level resulted in similarly nonsignificant overall models, as did a separate model controlling for CSF monocyte count and the influence of missing data on the CSF monocyte count.

*CSF monocyte count and DAPTA treatment*

As planned, we also conducted a secondary analysis on the outcome of the CSF monocyte count. The median CSF monocyte count for the DAPTA group at baseline was 0.41 cells/mm$^3$ (Q1: 0; Q3: 0.95), with a mean of 0.80 cells/mm$^3$ (SD = 1.44). The median CSF monocyte count for the placebo group at baseline was comparable at 0.37 (Q1: 0; Q3: 0.99), with a mean of 0.97 cells/mm$^3$ (SD = 2.44). The GLM was set up with the control of peripheral white blood cell (WBC) count (excluding monocytes) as the first step to examine the hypothesized effect of DAPTA in reducing the CSF monocyte count. The analysis showed a significant overall model ($F_{(2,29)} = 3.54$, $P = .04$). The DAPTA predictor variable was not statistically significant ($\beta = 0.94$, $t = 1.54$, $P = .14$). The control for baseline peripheral WBCs excluding monocytes showed a nonsignificant trend in the expected direction toward an inverse relationship to change in CSF monocyte count ($\beta = -0.00046$, $t = -1.79$, $P = .09$). Addition of the predictor for CD4 cell count resulted in a nonsignificant trend for the overall model ($F_{(3,28)} = 2.32$, $P = .09$) in which the DAPTA predictor variable was not statistically significant ($\beta = 0.77$, $t = 1.28$, $P = .22$).

*Peripheral viral load and DAPTA treatment*

The median peripheral viral load for the DAPTA group at baseline was 3.88 (Q1: 2.58; Q3: 5.18), with a

mean of 3.93 (SD = 0.96). The median peripheral viral load for the placebo group at baseline was highly comparable at 3.97 (Q1: 3.04, Q3: 4.90), with a mean of 4.04 (SD = 0.77). The response of peripheral viral load to DAPTA using a student's *t* test on change in viral load (regardless of serum or plasma specimen type) from baseline to 6 months was a mean change of −0.20 logs (SE = 0.11), as opposed to a mean change in peripheral load of +0.12 logs (SE = 0.11) in the placebo group. This difference was statistically significant ($t = -2.04$, $P = .047$), and the effect was equivalent to a relative difference between groups of 0.32 logs. As was done with the CSF viral load outcome measure, we conducted the Kolmogorov-Smirnov goodness-of-fit test on the peripheral viral load outcome ($n = 57$) in order to justify the use of the GLM procedure. This test yielded $D = 0.066$ ($P > .10$), indicating that the outcome measure was normally distributed. Thus, we employed a GLM procedure and controlled for the CD4 cell count (see Table 3). The overall model was statistically significant ($F_{(2,54)} = 3.49$, $P = .038$). The mean change in the DAPTA group in this model was −0.24 as opposed to a least square (LS) mean change in peripheral load of 0.16 in the placebo group ($t = -2.42$, $P = .019$), yielding a relative difference between groups on change in peripheral viral load of 0.40 logs. Adding control variables for age and years of education, the overall model showed a nonsignificant trend toward significance ($F_{(4,52)} = 2.18$, $P = .084$) in which the DAPTA indicator variable remained significant ($t = -2.41$, $P = .019$) with a similar LS mean difference between the two treatment groups (see Table 3). When we conducted the GLM testing, using the DAPTA indicator variable controlling for concomitant antiretroviral medication use as well as baseline CDC clinical disease stage, CD4 cell count, and specimen type (serum versus plasma), a nonsignificant overall model was obtained ($F_{(5,51)} = 1.51$, $P = .21$).

*Changes in serum versus plasma viral load*

To further ensure no effect of collapsing specimen source into a single peripheral viral load outcome measure, we conducted analyses on the subsamples with serum and with plasma results. In the serum subsample, the simple comparison of change was not statistically significant, with a change of −0.04 ($SE = .13$) in the DAPTA group and a change of 0.12 ($SE = .13$) in the placebo group ($t = -0.85$, $P = .41$). Adjusting for CD4 cell count yielded a nonsignificant overall model ($F_{(2,21)} = 0.43$, $p = .66$). Statistical power was not sufficient to conduct the more complex models used for the analyses above.

In plasma, there was a nonsignificant trend toward a difference on a simple comparison of change, −0.32 (SE = .17) in the DAPTA group and 0.12 (SE = .17) in the placebo group ($t = -1.84$, $P = .08$). This represents a relative difference of 0.44 logs. Adding controls for CD4 cell count, age, and educational level resulted in a nonsignificant overall model ($F_{(4,28)} = 1.54$, $P = .22$), likely due to the loss of statistical power. Yet, the DAPTA treatment indicator was the only significant predictor. The DAPTA group change was −0.37 (SE = .17) and change in the placebo group was 0.17 (SE = .17) ($\beta = -2.18$, $P = .04$), yielding a relative plasma viral load difference of 0.54 logs. As was true for serum viral load, statistical power was not sufficient to conduct the more complex models.

## Discussion

These results indicate that DAPTA given at a dose of 2 mg three times a day intranasally for 6 months was associated with no change in central viral load (CSF), although a decrement in peripheral viral load (serum and plasma samples) was observed in this limited subsample of the original randomized controlled clinical trial of HIV-1–infected patients with cognitive-motor impairment. The treatment effect estimate for peripheral viral load change increased from the simple comparison (0.32 logs) to the analysis controlling CD4 cell count (0.40 logs). Plasma viral load showed a greater DAPTA treatment effect estimate (0.44 logs) in the simple comparison and in the controlled analysis (0.54 logs) than serum viral load, although the latter was limited in statistical power. Thus, although collapsing plasma with serum viral load as "peripheral" was justified, treatment-associated changes were more closely related to changes in plasma viral load.

Within-assay variation is 0.15 to 0.2 logs and natural biological variation *in vivo* is 0.3 logs, yielding 0.45 to 0.50 logs in expected variation of HIV-1 load. Although an effect size just greater than this magnitude was observed in one analysis of limited power and such smaller effect sizes were typically observed with nucleoside reverse transcriptase inhibitor monotherapy, the other analyses conducted here on peripheral or plasma viral load generated smaller relative differences (0.32 to 0.44 logs). Hence, any potential effect attributable to DAPTA represents only the minimum change necessary to establish efficacy (Saag *et al*, 1996) and requires confirmation by future study.

Interestingly, DAPTA treatment had no effect on CSF load, though cognitive-motor impairment has been a primary treatment target for its use. One possibility for this result is that the CSF values were affected by differences in HIV-1 RNA stability over the time prior to freezing samples, the time the samples remained frozen at −70°C, and/or changes in temperature of stored samples (resulting in multiple freeze-thaw cycles). However, Singer et al. (2002) have shown that CSF HIV-1 load values using the Ultrasensitive AMPLICOR HIV-1 MONITOR test, version 1.5, remain within the range of intra-assay variability under each of these conditions. Further, the CSF copy number levels tested there were in the same range as those found in this trial. Another possibility

for the lack of change in CSF viral load in this study is that the Ultrasensitive AMPLICOR HIV-1 MONITOR test is known to show increased variance at the lower end of its range (Erali and Hillyard, 1999). This introduces the possibility that a lack of a viral load effect for DAPTA in the CSF was the result of an artifact of the decreased levels of CSF load with respect to plasma viral load at baseline. However, for plasma samples, it has been demonstrated that the version 1.5 kit used has greater sensitivity than the version 1.0 kit, with HIV-1 RNA levels near the 50 copy/ml lower limit of detection (Jackson *et al*, 2004). Moreover, Brambilla *et al* (2004) have shown the version 1.5 kit to be superior to the version 1.0 kit at a level as low as 15 HIV-1 RNA copies/ml plasma. Furthermore, our validation study suggested that version 1.5 performed similarly in CSF and plasma. In addition, the mean levels of viral load in CSF observed (although lower than plasma) were still relatively high. Hence, both the methods used and the ranges of CSF load obtained render an artifactual interpretation highly unlikely. One possibility for future investigation of a relationship of DAPTA treatment to a change in CSF viral load is to examine subgroups of treatment responders on neuropsychological performance who had more severe impairment and those with a CD4 cell count of 200-500 cells/$mm^3$ at baseline.

Alternatively, the specific reduction of central viral load may not be necessary to prevent or treat the HIV-1-associated cognitive-motor disorders. For example, if trafficking of activated, CD16+ or CD69+ monocytes from the bone marrow to the brain can be prevented in late stage disease, peripheral viral load suppression could effectively treat the brain (Gartner, 2000). Hence, the lack of an effect on CSF load herein is not necessarily counter to the use of DAPTA in the treatment of cognitive-motor disorder nor to the control of the viral reservoir in brain. When DAPTA was evaluated in the limited subsample on CSF monocyte counts, the DAPTA treatment indicator variable was not statistically significant, suggesting that DAPTA is ineffective in clearing HIV-1 from CSF monocytes, unlike the *in vitro* results. Moreover, the literature demonstrates that CSF viral load response to antiretroviral therapy is based on central nervous system (CNS) penetration (Antinori *et al*, 2002; Sacktor *et al*, 2001) and on antiretroviral medication experience (Antinori *et al*, 2002). The apparent central-peripheral treatment response divergence suggests that compartmentalization of CSF virus over time may be associated with resistance, highlighting the potential import of CSF resistance testing in patients lacking CART response on CSF viral load. It may be possible to avoid sampling CSF at all, as Brew *et al* (2004) have suggested that peripheral monocyte count may be a marker for CSF resistance.

Another caveat about DAPTA response reported here is that the present report was based upon a sample accrued for a randomized controlled trial not aimed at viral load endpoints. Of relevance, the mechanism of CCR5 antagonism by DAPTA in the periphery warrants further investigation. The small samples for the peripheral viral load analyses, particularly those on plasma, indicate yet another caveat in interpreting these findings. Future clinical trials with DAPTA should continue to aim at possible antiretroviral activity and the associated mechanism, to focus on plasma, and should have a sample size determined to be of sufficient statistical power *a priori*.

## Methods

*Design of the NIMH-sponsored, multisite, randomized, double-blind, placebo-controlled trial of HIV-1–associated cognitive-motor impairment*

*Overview:* The design and methods of the randomized, double-blind, placebo-controlled trial of DAPTA for HIV-1–associated cognitive-motor impairment were reported previously (Heseltine *et al*, 1998). Briefly, this was a multisite clinical trial (University of Southern California, Los Angeles [USC]; the University of Miami, Miami, FL [UM]; and the University of California at San Diego [UCSD]) designed to rigorously test whether HIV-1 seropositive participants ($N = 214$) with baseline evidence of cognitive-motor impairment would show improved neuropsychological (NP) performance in response to treatment with 2 mg of DAPTA intranasally three times a day for 6 months, compared to a placebo control group. Participants were required to have stable antiretroviral therapy (if prescribed) for at least 12 weeks prior to entry. Primary efficacy analyses were reported on $n = 143$ (66%) of those randomized (see Table 4 and the original report [Heseltine *et al*, 1998] for further details).

*Screening:* Prior to the baseline assessment, a screening evaluation was performed having three components—neuropsychological, psychiatric, and general medical. The neuropsychological component consisted of nine tests selected from the domains of language, abstract thinking, speed of information processing, learning and retention, and motor performance (Table 5). Cognitive dysfunction was defined by scores less than the corresponding population-based norms by 1.5 SDs or more on at least two tests or by 2.5 SDs or more on at least one test. The degree of impairment was defined as "severe" if scores on at least two of the neuropsychological screening tests were 1.5 SDs or more below the norm, with one of those tests being at least 2.5 SDs below the norm. Otherwise, the deficit was defined as "mild to moderate." Cognitive dysfunction must have been judged as likely to be due to HIV-1 infection.

Psychiatric screening evaluation was required to be negative for active alcohol or substance dependence (or abuse within the previous 3 months), history of psychotic disorder or bipolar disorder, a history of mental retardation or learning disability, treatment with psychoactive agents within 4 weeks, previous

**Table 4** Neuropsychological (NP) results of the NIMH-sponsored, randomized, double-blind, placebo-controlled clinical trial of intranasal DAPTA versus placebo

| *NP test performance outcomes* | *Model* | *DAPTA*[a] *Mean NP Change*[b] | *(SE)* | *Placebo*[a] *Mean NP Change*[b] | *(SE)* | P *value* |
|---|---|---|---|---|---|---|
| Global NP battery | Site-adjusted[c] | 0.24 | (0.05) | 0.16 | (0.03) | .18 |
| | CD4 and site-adjusted[d] | 0.26 | (0.04) | 0.15 | (0.04) | .072 |
| Cognitive domains | | | | | | |
| Verbal fluency | Site-adjusted | 0.14 | (0.09) | 0.15 | (0.08) | .90 |
| | CD4 and site-adjusted | 0.14 | (0.10) | 0.14 | (0.09) | .98 |
| Visuospatial ability | Site-adjusted | 0.17 | (0.06) | 0.25 | (0.06) | .34 |
| | CD4 and site-adjusted | 0.17 | (0.07) | 0.24 | (0.06) | .44 |
| Abstraction and flexibility of thinking | Site-adjusted | 0.34 | (0.07) | 0.23 | (0.04) | .18 |
| | CD4 and site-adjusted | 0.35 | (0.06) | 0.23 | (0.05) | .11 |
| Information processing speed | Site-adjusted | 0.23 | (0.07) | 0.14 | (0.05) | .27 |
| | CD4 and site-adjusted | 0.24 | (0.06) | 0.12 | (0.06) | .15 |
| Working memory | Site-adjusted | 0.23 | (0.09) | 0.08 | (0.07) | .15 |
| | CD4 and site-adjusted | 0.27 | (0.08) | 0.08 | (0.08) | .09 |
| Learning and retention | Site-adjusted | 0.19 | (0.07) | 0.11 | (0.06) | .44 |
| | CD4 and site-adjusted | 0.19 | (0.07) | 0.10 | (0.07) | .34 |
| Motor performance | Site-adjusted | 0.19 | (0.06) | 0.18 | (0.06) | .89 |
| | CD4 and site-adjusted | 0.22 | (0.06) | 0.18 | (0.06) | .59 |

*Outcome analysis on categorical NP change as a function of treatment group and baseline NP impairment status*[e]

| | | *Categorical rating of NP change* | | | |
|---|---|---|---|---|---|
| | *Treatment assignment* | *Improved* | *No significant change* | *Declined* | P *value*[f] |
| Full Sample | DAPTA | 19.7 | 71.2 | 9.1 | .02 |
| | Placebo | 10.4 | 68.8 | 20.8 | |
| NP Impaired | DAPTA | 32.0 | 56.0 | 12.0 | .02 |
| | Placebo | 3.5 | 75.9 | 20.7 | |
| NP Non-Impaired | DAPTA | 14.6 | 73.2 | 12.2 | .84 |
| | Placebo | 16.7 | 66.7 | 16.7 | |

[a]Sample size: $n = 143$; DAPTA $n = 66$, placebo $n = 77$.
[b]Z scores for NP change from baseline to post treatment (6 months) are used to define the continuous or categorical NP performance measures throughout.
[c]"Unadjusted" analysis: ANOVA by treatment assignment and study site.
[d]Adjusted analysis: ANCOVA by treatment assignment, study site, and baseline CD4 cell count.
[e] Presence of baseline NP impairment was defined by external NP norms, using the global NP deficit score at baseline (cutoff = 0.50). Outcome was defined by a global NP deficit score at 6 months, corrected for the baseline score [cf. Heseltine *et al* (1998)].
[f]The $p$ values below were tested using the Mantel-Haenszel chi-square test based upon these three 2 (treatment) × 3 (outcome) contingency tables.

DAPTA use, and inability to participate in neuropsychological testing or to comply with the instructions of medication administration. Medical screening evaluation was required to document a CD4 cell count $\leq$500 cells/mm$^3$ (UM and UCSD sites only); total granulocyte count $\geq$750 cells/mm$^{3;}$ hemoglobin level >8.0 g/L; platelet count $\geq$75,000; creatinine level $\leq$1.5 mg/dl; aspartate aminotransferase <5 times the upper limit of the normal range; bilirubin level <3.0 mg/dl; a prothrombin time >70% of the control; a negative urine pregnancy test (for women); an electrocardiogram and urinalysis that were either normal or consistent with a medically stable condition; need for less than two blood transfusions/month; absence of active acquired immunodeficiency syndrome (AIDS)–defining opportunistic infections, malignant neoplasms, or any underlying serious medical problems; an estimated survival time of at least 6 months; and agreement to a barrier method (for men) or a reliable method (for women) of contraception. Permitted antiretroviral regimens were based upon the standard recommended doses for nucleoside reverse transcriptase inhibitor monotherapy or dual combination therapy used at that time.

*Assessments*

Subjects were administered an extensive NP battery of 3.5 to 4 h in length at baseline and at 6 months in the controlled phase of the trial. The battery consisted of 23 tests in seven domains—verbal fluency; visuospatial ability; abstraction and flexibility of thinking; speed of information processing; working memory; learning and retention; and motor performance. In addition, a thorough neuropsychiatric assessment and a medical assessment (including a complete neurological examination) were done at these time points.

*Lumbar puncture*

A total of approximately 20 cc of CSF were obtained. Routine clinical studies were done, with the

**Table 5** Neuropsychological tests administered at screening by cognitive domain

| *Test* | *Measure* |
|---|---|
| *Language domain* | |
| AMNART (Grober *et al*, 1991) | IQ |
| *Abstraction and flexibility of thinking domain* | |
| Trail-Making Test—B (TMTB) (Reitan and Wolfson, 1985) | Time to complete<br>Time for C-W card |
| Stroop Test (Stroop, 1935) | |
| *Speed of information processing domain* | |
| Paced Auditory Serial Addition (PASAT) (Gronwall, 1977) | Number correct, series 1 |
| Visual Scanning and Discriminating Speed (VSDST) (Wilkie *et al*, 1990) | Time to complete (seconds) |
| Sternberg Memory Scanning Test (Sternberg,1996; Wilkie,1988) | Mean reaction time for correct trials |
| *Learning and retention domain* | |
| Benton Visual Retention Test (Benton, 1974) | Total correct |
| USC–Repeatable Episodic Memory (REMT) (Parker *et al*, 1995) | Total correct, trials 1–3 |
| *Motor performance domain* | |
| Grooved Pegboard (Klove, 1963) | Time (seconds), nondominant hand |

remaining CSF divided into 1-cc aliquots and frozen immediately at −70°C. (See Heseltine et al [1998] for details.)

*Storage of CSF and peripheral blood samples*
CSF and blood had been obtained from participants at baseline and 6 months. Peripheral samples were of plasma at USC and serum at UM and UCSD, due to change in study protocol. After the trial, samples were sent to a central repository for continued storage at −70°C.

CSF samples from 193 of the original 215 subjects (90% of those randomized) were available. For 92, a 6-month sample was also available. Plasma samples from 72 participants were available, with 33 having a 6-month sample. Serum samples from 78 participants were available, with 24 having a 6-month sample. Samples were re-coded to mask subject treatment assignment, site, and time of collection and were sent blindly to the University of Miami Miller School of Medicine (K.G.; D.A.) for assays of viral load, together with blinded repeat samples and HIV-1 seronegative control samples.

*Outcome measures*
*Primary outcomes: CSF and peripheral blood viral load assays:* CSF and peripheral viral loads were assayed under NIMH professional contract to Dr. Karl Goodkin. Plasma HIV-1 RNA copy number was measured using the UltraSensitive Roche HIV-1 MONITOR Test, version 1.5 (Roche Diagnostics, Branchburg, NJ) (range of 50 to 75,000 HIV-1 RNA copies/ml). An advantage of version 1.5 is its greater sensitivity to the non-B clades now becoming more common in the USA. When the upper limit was reached, the Standard AMPLICOR HIV-1 MONITOR test, version 1.5 (Branchburg, NJ), was used (range: 400 to 750,000 HIV-1 RNA copies/ml). The latter assay deletes the additional step required in the ultrasensitive assay entailing centrifugation at 23,600 × *g* for 2 h to concentrate the virus. Raw copy numbers were $\log_{10}$ transformed, as is standard in the literature and recommended for analytic purposes (Hirschhorn *et al*, 2005). Changes in central and peripheral viral load, from baseline to 6 months, were the outcome measures herein.

These viral load assays were performed under licensure approvals obtained from the manufacturer. Both assays employ a competitive reverse transcriptase (RT) PCR methodology. Samples were processed using a four-step procedure required by the training method provided by Roche Diagnostics: sample processing (HIV-1 concentration, manual RNA extraction, amplification, and combination of RNA and master mix solution), master mix preparation (RT-PCR reagent preparation), amplification (thermal cycling and reaction termination), and detection (plate hybridization, plate incubation, plate wash, and detection). The assay was set up as unidirectional, and the technician was required to change gloves and gown before entering each area. Internal standards were used for serum and CSF.

Forty-two samples exceeded 75,000 HIV-1 RNA copies/ml and required the Standard AMPLICOR HIV-1 MONITOR test, version 1.5 (Branchburg, NJ). For five, specimen volume did not allow this additional assay; these samples were quantified as 4.9 logs for the analyses reported herein. A total of 8.1% of the repository samples available for analysis initially failed the laboratory quality control; all were repeated successfully, and those from the baseline and six-month time points were incorporated into the analyses reported.

*Secondary outcome:* Because (1) data on brain-derived derived microglial cells indicate that CCR5 permits entry of HIV-1 into brain (He *et al*, 1997), (2) CCR5 is the major chemokine for monocyte-derived macrophages (MDMs); (3) monocytes carry HIV-1 from the periphery into brain; and (4) DAPTA inhibits infection of MDMs, we performed a secondary outcome analysis on the effect of DAPTA in reducing CSF monocyte count.

*Statistical analytic plan*
A Student's *t* test was used as a crude, preliminary test for treatment group differences. The generalized linear model (GLM) form of multiple regression analysis was used to conduct more refined analyses in which control variables were included. GLM is

widely cited in the methodological literature and is considered one of the major statistical developments in recent years. This analysis involves integration of aspects of multiple regression analysis and analysis of variance useful for analyzing linear and nonlinear effects of a mixed group of continuous and categorical predictor variables on a discrete or continuous dependent variable (McCullagh and Nelder, 1989). Use of antiretroviral medications at baseline was employed as a control variable, as it clearly relates to potential differences in both central and peripheral viral load between treatment groups. Baseline CD4 cell count was controlled, as it is well established to be related to plasma viral load (Saag *et al*, 1996). Age was controlled as well, as older HIV-1–seropositive individuals have been shown to have lower levels of plasma viral load than younger HIV-1–seropositive individuals in samples of those infected predominantly longer term (Goodkin *et al*, 2004), whereas older age has been associated with higher levels of plasma viral load in samples of older persons recently testing HIV-1 seropositive and thought to be recently infected as well (Operskalski *et al*, 1997). Educational level was controlled, as it relates to antiretroviral adherence (Escobar *et al*, 2003). In addition, CDC clinical disease stage (CDC, 1992) was controlled, as advanced disease stage is related to an increased likelihood of higher central and peripheral viral load due to prior regimen failures. As activated monocytes traffic from the bone marrow into the CNS (Brew *et al*, 2004), we added a predictor for the CSF monocyte count to the CSF viral load analysis. The expectation was for a positive relationship of peripheral to CSF viral load mediated at least partially by the egress of HIV-1–infected monocytes from the periphery to the CSF—as documented by Gisslen *et al* (1999). By conducting our analyses with and without this predictor, we were able to compare an analysis more specifically restricted to CNS-mediated changes in CSF viral load to an analysis directed to any treatment effect on CSF viral load (whether centrally or peripherally mediated). Due to the presence of missing CSF monocyte data, we used imputation techniques as well as a categorical indicator for missing data to allow analysis of the entire sample available for these analyses. Pearson's product-moment correlation was used to measure correlations for normally distributed variables, and a Spearman rank-order correlation was used for variables with significant deviations from a normal distribution. Data analyses were carried out by Constella Health Sciences (statistician: Patrick Crockett, PhD) under contract to the NIMH.

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