Raltegravir Treatment Intensification Does Not Alter Cerebrospinal Fluid HIV-1 Infection or Immunoactivation in Subjects onSuppressive Therapy

Viktor Dahl,1 Evelyn Lee,2 Julia Peterson,2 Serena S. Spudich,2,a Idris Leppla,2 Elizabeth Sinclair,2 Dietmar Fuchs,4 Sarah Palmer,1 and Richard W. Price2

1Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet and Swedish Institute for Infectious Disease Control, Stockholm, Sweden; 2Department of Neurology and 3Department of Medicine, University of California, San Francisco; and 4Division of Biological Chemistry, Biocentre, Innsbruck Medical University, Austria

Background. Despite suppression of plasma human immunodeficiency virus type 1 (HIV-1) RNA by antiretroviral therapy to levels below clinical assay detection, infection and immune activation may persist within the central nervous system and possibly lead to continued brain injury. We hypothesized that intensifying therapy would decrease cerebrospinal fluid (CSF) infection and immune activation.

Methods. This was a 12-week, randomized, open-label pilot study comparing addition of the integrase inhibitor raltegravir to no treatment augmentation, with an option for rollover to raltegravir. CSF and plasma were analyzed for HIV-1 RNA using a single-copy assay. CSF and blood immune activation was assessed by neopterin concentrations and CD4+ and CD8+ T-cell surface antigen expression.

Results. Primary analysis compared 14 intensified (including rollovers) to 9 nonintensified subject experiences. Median HIV-1 RNA levels in all samples were lower in CSF (<.3 copies/mL) than in plasma (<.9 copies/mL; P < .0001), and raltegravir did not reduce HIV-1 RNA, CSF neopterin, or CD4+ and CD8+ T-cell activation.

Conclusions. Raltegravir intensification did not reduce intrathecal immunoactivation or alter CSF HIV-1 RNA levels in subjects with baseline viral suppression. With and without raltegravir intensification, HIV RNA levels in CSF were very low in the enrolled subjects.

Clinical Trials Registration. NCT00672932.
HIV-1 CNS infection generally responds well to combination antiretroviral therapy (ART), so that when HIV-1 RNA levels in plasma become undetectable by standard assays (<50 copies/mL) the same is true of CSF [17, 18], although notable exceptions have been observed [10, 19]. Whereas ART largely prevents HAD [17, 18], less severe neurological impairment remains common in treated populations, and CNS injury may possibly continue despite ART [14, 22, 23]. Although the pathogenesis of this type of chronic injury is even less well understood than that of HAD, continued immune activation, despite suppressive therapy that reduces both plasma and CSF to levels below detection by standard assays, may be important [24].

One explanation tying these observations together is that CNS is a sanctuary site where HIV-1 replication continues at low levels that, despite being undetected by conventional assays, are sufficient to drive CNS immune activation and continued brain injury. To test this theory and assess an additional avenue of therapy, we undertook a randomized, open-label pilot clinical trial of treatment intensification adding the integrase inhibitor raltegravir to suppressive regimens [25, 26]. At the time the study began, raltegravir was a new agent to which the study participants would not have been previously exposed. It is a potent antiretroviral with a novel mode of action and generally favorable entry into the CNS [27–29]. The primary outcome measure was reduction in CSF neopterin, a pteridine metabolite produced largely by activated macrophages [30]. Its CSF concentration reflects CNS macrophage activation, increases with disease severity, and is especially elevated in HAD [11], and generally responds well to ART, although not always returning to normal levels [24, 31]. After the study began, we added measurement of HIV-1 RNA levels in CSF by a single-copy assay (SCA). Because of its sensitivity and precision, SCA has become the standard for studying the effect of treatment intensification on persistent plasma HIV-1 [32–34], but it had not been applied to CSF samples. We additionally measured cell surface markers of T-cell activation in CSF and blood [35].

METHODS

Study Design

This was a 1:1 randomized, open-label pilot study in which treated, virally suppressed subjects either added raltegravir 400 mg twice daily to their current regimens or received no additional treatment for 12 weeks. Those randomized to no intensification had the option to roll over to receive raltegravir for 12 weeks after the initial period of no added therapy. The study was approved by the University of California, San Francisco Committee on Human Research and was conducted according to the principles expressed in the Declaration of Helsinki. Written informed consent was obtained from all subjects.

Entry criteria included HIV-1 infection treated with ART for >2 years, at least 3 antiretroviral drugs (not including ritonavir to boost drug concentrations), plasma suppression to <50 copies/mL for >1 year, and plasma and CSF HIV-1 RNA <50 copies/mL at screening. Additional criteria included the following: >18 years of age; predicted medication adherence; no contraindications to lumbar puncture (LP); no active opportunistic infection or neurological disease confounding evaluations; no previous exposure or contraindications to raltegravir; and not taking immunomodulating drugs.

After providing consent, subjects underwent a screening evaluation that included LP and blood sampling, and standardized neurological assessments as previously described [17, 36, 37]. Those meeting entry criteria were randomized to either starting open-label raltegravir 400 mg twice daily for 12 weeks or no additional drug. At baseline and 4 and 12 weeks, subjects underwent full evaluation, including LP. A visit at 8 weeks included only blood sampling and brief clinical assessment for toxicity or clinical change. For subjects who rolled over, the 12-week visit was used as the baseline for subsequent raltegravir effect, and they underwent a similar schedule of evaluations at the subsequent 4, 8, and 12 weeks. For the main analysis, the subjects randomized to receive no raltegravir were compared with the combined group of those initially randomized to raltegravir and those rolling over to raltegravir. Treatment adherence was assessed by direct questioning and pill count. Theoretical CNS drug activity in the absence of raltegravir was examined using the CNS Penetration Effectiveness (CPE) score as recently updated [23].

CSF and Plasma HIV-1 RNA

For study entry, CSF and plasma HIV-1 RNA was measured using the Abbott RealTime HIV-1 Assay with a lower quantitative limit of 40 copies/mL. Although not initially planned, after the study was under way we added assessment of batch CSF (and plasma) HIV-1 RNA levels using a sensitive SCA method [38]. In brief, up to 8 mL of CSF or plasma, with a known amount of RCAS (an avian retrovirus) added as an internal standard, was centrifuged at 100,000g, and the pellet was extracted and subjected to complementary DNA synthesis followed by real-time polymerase chain reaction amplification of a 79-base pair region of HIV-1 Gag or a portion of the RCAS genome. HIV-1 RNA levels were determined using a standard curve constructed with HIV-1 of known RNA copy number. To ensure that the extraction process was successful, the level of RCAS was measured using a separate standard curve constructed with RCAS of known RNA copy number. HIV-1 RNA results by SCA each represent the median of triplicate determinations.

CSF Soluble Immunological and Other Outcome Measurements

Neopterin was measured in cell-free CSF and plasma by enzyme-linked immunoassay according to the manufacturer’s instructions.
(BRAHMS Aktiengesellschaft). CSF white blood cell (WBC) counts and differential, blood CD4+ and CD8+ T-cell counts, CSF and blood albumin used to compute the CSF-blood albumin ratio [39], CSF total protein, and blood metabolic profile were all performed in the San Francisco General Hospital Clinical Laboratories using standard methods.

**RESULTS**

**Subjects**

Of 37 subjects screened, 18 met criteria and were randomized. One subject in the raltegravir group was censored when a pharmacological study showed no drug in either plasma or CSF [27]. Six subjects randomized to no drug later rolled over to receive raltegravir. Primary analysis treated the rollover subjects as intensified to 9 nonintensified subjects. The baseline characteristics of the subjects are shown in Table 1, grouped as all subjects at entry, those not experiencing treatment intensification were 2-sided with values .05 considered significant. Descriptive and comparative statistical analyses were performed using GraphPad Prism 5 software (GraphPad).

**Statistics**

Changes from baseline to follow-up test intervals at 12 weeks for each of the outcome variables were compared independently by unpaired parametric or nonparametric t tests. Proportions were compared using Fisher exact test. All P values were 2-sided with values <.05 considered significant. Descriptive and comparative statistical analyses were performed using GraphPad Prism 5 software (GraphPad).

As defined by entry, all plasma and CSF HIV-1 RNA levels were <50 copies/mL. Blood CD4+ T-cell counts were relatively preserved (mean, 513 cells/µL). CSF WBCs and neopterin levels were also low, and CSF-blood albumin ratios were normal, indicating preservation of the blood-brain barrier [39]. CSF CD4+ and CD8+ T-cell activation measured by the percentage of cells coexpressing CD38 and HLA-DR and expressing CCR5 was higher in CSF than in blood and comparable to previous observations of treated suppressed patients [35, 43].

**Effects of Intensification on CSF HIV-1 Assessed by SCA**

Because SCA was originally developed for plasma samples, we conducted brief preliminary experiments to ensure that this method could also measure low levels of HIV-1 RNA in the CSF. First, we spiked 10 mL of CSF from an uninfected individual with 5 µL of a plasma sample from an HIV-1–infected individual with a known level of HIV-1 RNA. In the spiked CSF sample, we measured a median of 432 copies per well compared with 334 copies per well (SD 84) usually found in the positive control. To ensure efficient quantification at even lower levels, we spiked 2 uninfected CSF samples with 1 µL of the positive control and measured a median of 44 copies per well compared with 27 copies when the control was added to water (P = .10).

Because use of the SCA method with its optimization using a relatively large volume of CSF or plasma was not part of the original study plan, some of the samples were insufficient to detect the planned sensitivity of .3 copies/mL. Some of the assays also failed for technical reasons. Table 2 shows the results of the SCA assessment for all the intervals successfully tested. The differences in the limits of detection relate to the amount of fluid available for each assay. Thus, if there were <.3 copies detected, this implies that 7 mL of fluid was available. To evaluate this sample volume effect on the different samples, we examined the distribution of the available sample volumes, whether different between CSF and plasma or among the different treatment groups. The median volume for CSF was 6.5 mL and for plasma was 6 mL (P = .13, Mann–Whitney U test). The median for CSF samples without and with treatment intensification was 6.5 mL (P = .37); likewise, the medians for plasma samples obtained without and with treatment intensification were 6 mL (P = .73). Thus, although there were some differences in the available sample volumes, this did not appear to influence the overall results. As shown in Table 2, the amount of HIV-1 RNA in CSF was low in all of the CSF samples. At baseline, only 1 of the 16 CSF samples assessed was positive (with .5 copies/mL). This compared with 13 of 17 plasma samples with similar levels of detection. This low detection rate in CSF was noted in follow-up at weeks 4 (2 of 9) and 12 (0 of 6) in the nonintensified group and in weeks 4 (3 of 13) and 12 (2 of 11) in the raltegravir-treated groups, without difference in the frequency in the groups (P = .69, by Fisher exact test applied to the 4-week and 12-week results of the 2 groups).
In addition to the lack of an effect on the detection rate in CSF, substituting a value .1 copy below the limit of detection in the individual assay for quantitative approximation, there was no difference in the median values of CSF HIV-1 RNA concentrations at 4 and 12 weeks between the unintensified and the raltegravir-treated groups (\(P = .97\), Mann–Whitney U test). In both groups, this median was below the .3 copies detection limit.

Comparison of CSF to plasma underscored the low levels of HIV RNA in CSF. Thus, taking into account all samples, the frequency of viral detection in CSF was 8 of 56 (14%) compared with 32 of 50 (64%) in plasma (\(P < .001\), Fisher exact test). Using the rules for estimating the concentrations in CSF and plasma described above, the median for all CSF samples was .2 copies (interquartile range [IQR], 0.2–0.4), whereas for all plasma samples it was .9 copies (IQR, 0.3–2.2; \(P < .0001\), Mann–Whitney U test).

Effects of Intensification on Nonvirological Outcomes

Figure 1 shows the changes from baseline in CSF neopterin and some of the secondary outcome measures of the 2 comparison groups, whereas Table 2 compares the changes at 12 weeks. The CSF neopterin (Figure 1A) varied little over the course of treatment without significant difference in the changes between the groups. Likewise, changes in the blood neopterin (Figure 1B), CSF WBC counts (Figure 1C), blood CD4\(^+\) T-cell (Figure 1D) and CD8\(^+\) T-cell counts (data not shown), CSF-blood albumin ratios (Figure 1E), and QNPZ-4 scores did not differ between the comparison group (t test).

Differences between the 2 groups among the T-cell activation marker changes (Figure 2, Table 3) were noted in the blood CD8\(^+\) and CD 4\(^+\) CCR5 expression (Figure 2D and H) and in CSF CD4\(^+\) T-cell coexpression of CD38 and HLA-DR (2E). In each of these cases, the activation was higher in the raltegravir intensification arm. Changes in the other cell activation measurements between groups were not significant.

DISCUSSION

This study explored whether treatment intensification with an additional antiretroviral drug having a different mechanism of action from the drugs already being taken—in this case the integrase inhibitor raltegravir—would reduce CSF biomarkers
Our underlying mechanistic hypotheses centered on the capacity of raltegravir to further inhibit low levels of viral replication in the CNS despite clinically measured suppressive therapy and thereby reduce the residual intrathecal immune activation. Our main outcomes were CSF neopterin and CSF HIV-1 RNA measured by SCA, but we also measured CSF T-cell activation, WBC count, CSF-blood albumin ratio, and the QNPZ-4 index of neurological performance. None of these measures showed improvement with raltegravir augmentation in this well-treated group of subjects. None of these measures showed improvement with raltegravir augmentation and therefore of limited relevance to patients with low baseline CSF viral burden and immunoactivation. This was similar to the absence of systemic effects on the HIV-1 viral load in plasma and blood immune activation markers in this study and in larger studies [25, 33, 44–46], which also did not detect an effect in plasma and blood immune activation markers in this study and was similar to the absence of systemic effects on the HIV-1 viral load with low baseline CSF viral burden and immunoactivation. This explanation actually has 2 parts related to (1) residual ongoing viral replication in the CNS and (2) the mechanism of continued intrathecal immunoactivation. With respect to continued infection in treated patients, pathological studies do not generally detect active infection at autopsy in treated patients [47]. However, this is the first study to use a very sensitive SCA method to examine residual CSF HIV-1 RNA in very well-treated subjects, and it shows that indeed even at baseline there was little CSF virus despite detectible residual virus in many of the plasma samples, similar to that in other studies [25, 33, 34, 45, 46]. Thus, overall, HIV-1 RNA was detected in only 8 of 56 CSF samples (14%) compared with 32 of 50 (64%) plasma samples. Using the rough estimation method described earlier of subtracting .1 copies from the limits of detection, the median CSF copy number was .2 copies/mL while that of the plasma was near 9 copies/mL, a CSF-plasma relationship not much different from the usual 1:10 ratio found in untreated patients, although higher than in patients failing therapy in which the ratio is closer to 1:100 [6, 17, 35]. Overall, the very low levels of CSF HIV-1 RNA of immune activation and CSF HIV-1 RNA. Our underlying mechanistic hypotheses centered on the capacity of raltegravir to further inhibit low levels of viral replication in the CNS despite clinically measured suppressive therapy and thereby reduce the residual intrathecal immune activation. Our main outcomes were CSF neopterin and CSF HIV-1 RNA measured by SCA, but we also measured CSF T-cell activation, WBC count, CSF-blood albumin ratio, and the QNPZ-4 index of neurological performance. None of these measures showed improvement with raltegravir augmentation in this well-treated group of subjects with low baseline CSF viral burden and immunoactivation. This was similar to the absence of systemic effects on the HIV-1 viral load in plasma and blood immune activation markers in this study and in larger studies [25, 33, 44–46], which also did not detect an effect of intensification, with the exception of one that found an increase in 2–long terminal repeat circles, suggesting possible inhibition of low-level replication in a subset of patients [44]. The only measured changes in T-cell activation showed higher rather than lower levels in the intensified group, and in each case the level of difference was small and might be explained by the multiple comparisons.

There are at least 3 possible explanations for the lack of observed effect of intensification in this small study. First, raltegravir might not appreciably inhibit CNS infection. Although there is little direct study documenting the CNS efficacy of raltegravir in isolation, pharmacokinetic studies, including one involving some of the subjects included in this study [27], have shown that the drug commonly achieves CSF levels in the low therapeutic range, albeit well below those in plasma [28, 29]. In addition, depending on interpretation of therapeutically effective concentrations, drug exposure was likely sufficient to inhibit CNS replication, although the period of treatment may possibly have been too short to detect an effect.

Second, the underlying hypothesis might not be sound: there may be little or no continued HIV-1 replication within the CNS, and local viral replication may not cause the persistent immunoactivation in the CSF. This explanation actually has 2 parts related to (1) residual ongoing viral replication in the CNS and (2) the mechanism of continued intrathecal immunoactivation. With respect to continued infection in treated patients, pathological studies do not generally detect active infection at autopsy in treated patients [47]. However, the first study to use a very sensitive SCA method to examine residual CSF HIV-1 RNA in very well-treated subjects, and it shows that indeed even at baseline there was little CSF virus despite detectible residual virus in many of the plasma samples, similar to that in other studies [25, 33, 34, 45, 46]. Thus, overall, HIV-1 RNA was detected in only 8 of 56 CSF samples (14%) compared with 32 of 50 (64%) plasma samples. Using the rough estimation method described earlier of subtracting .1 copies from the limits of detection for each sample below these limits, the median CSF copy number was .2 copies/mL while that of the plasma was near 9 copies/mL, a CSF-plasma relationship not much different from the usual 1:10 ratio found in untreated patients, although higher than in patients failing therapy in which the ratio is closer to 1:100 [6, 17, 35]. Overall, the very low levels of CSF HIV-1

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**Table 2. Single-Copy Assay HIV RNA Results**

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Abbreviations: CSF, cerebrospinal fluid; HIV, human immunodeficiency virus; RAL, raltegravir.

* Samples with detectable HIV-1 RNA are emphasized by bold font.
RNA and the lack of further reduction by intensification suggest that there was indeed little active CNS viral replication in these subjects, with the general caveat that even our SCA applied to CSF may be insensitive to low-level infection within the brain parenchyma.

In untreated infection, virus detected in CSF may originate either from the bloodstream (so-called transitory or non-compartmentalized infection) or from local sources in the CNS (autonomous or compartmentalized infection) [9, 48]. To further elucidate the origins of the very low-level infection in treated subjects, it will be crucial to similarly explore the origins of CSF HIV found in these patients. While technically formidable, this will be essential to understanding the meaning of detecting CSF HIV in treated patients, particularly patients with higher amounts of virus than noted here. Is it spillover from the residual plasma virus or from long-lived memory CD4+ T cells, or is it released from local resident cells, macrophage-microglia, or even astrocytes? This issue may take on greater importance in relation to therapeutic viral eradication.

Stimulation of CSF neopterin by local infection is supported by the association of this pteridine with CNS infection and disease [11, 30] and with previous observations that an increase

![Graphs](https://academic.oup.com/jid/article-abstract/204/12/1936/1023329)
Figure 2. Cerebrospinal fluid (CSF) and blood T-cell activation changes with raltegravir intensification. Symbols are the same as in Figure 1. Panels indicate changes in CSF and blood CD8$^+$ T-cell activation as assessed by coexpression of CD38 and human leukocyte antigen (HLA)-DR on CD3$^+$ and CD8$^+$ lymphocytes (A and B); CSF and blood CD8$^+$ expression of CCR5 (C and D); CSF and blood CD4$^+$ T-cell activation (E and F); and CSF and blood CD4$^+$ CCR5 expression (G and H). *$P<.05$ as described in text.
in these low levels in CSF correlates with viral escape [10]. Thus, it is likely that local infection can "drive" CSF neopterin concentrations. However, the low residual levels seen in the broader experience of treated patients [11, 18] may also reflect other processes, including a continued immunological abnormality in the absence of local infection [49].

Third, the underlying hypothesis might not have actually been addressed because of the particular makeup of the subject group with minimal CNS infection and immunoactivation that left little room to discern a therapeutic effect. In addition to the very low levels of virus in CSF, CSF neopterin concentrations were also quite low, similar to our previous control experience (mean 5.3 nmol/L, SD 2.2), although higher than a larger series of HIV-uninfected individuals without neurological disease (4.2 nmol/L, SD 0.8) [11]. Whether the unanticipated low levels observed in our subjects related to their duration of treatment, their drug combinations (some of which included more than 3 drugs), treatment adherence, self-selection for a CNS intensification study, or other factors, they may have comprised an atypical or extreme group. This issue needs to be examined by further application of SCA to a larger cohort.

Nonetheless, our findings are consonant with a previous study of treatment intensification using either enfurvitide, maraviroc, or lopinavir/ritonavir over a shorter period of time (8 weeks) [50]. Although the proportion of CSF samples in which HIV-1 could be detected and the levels of HIV-1 detected were higher in that study using a different method for HIV-1 RNA detection, still no change in the CSF neopterin was detected.

In summary, despite the favorable characteristics of raltegravir for CNS treatment, we found no evidence that intensification reduced either intrathecal immunooactivation or CSF HIV-1 RNA in our subjects. This is not only consistent with the previous CSF report but also with systemic effects of treatment intensification [25, 33, 44–46]. A remarkable facet of our findings was the low amount of CSF viral RNA and neopterin detected and the levels of HIV-1 detected were higher in the subject group. This suggests that treatment can indeed be effective in reducing the CNS viral burden and intrathecal immune activation. It remains to be seen whether this is common or whether our subjects were indeed an unusual group.

### Notes

**Financial support.** This work was supported by the National Institutes of Health (NIH) (R21MH083520), Merck & Co (IISP ID: 33054), Swedish Doctors against AIDS Foundation, Foundation for AIDS Research, the Wallenberg Foundation, and the National Center for Research Resources via the University of California, San Francisco Clinical and Translational Sciences Institute (UL1 RR024131). The contents are solely the responsibility of the authors and do not represent the official views of the NIH or other funding agencies.
Potential conflicts of interest. R. W. P. has received funding from Merck to support this investigator-initiated research study and an honorarium from Abbott for a conference presentation. All other authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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