Central Nervous System Safety During Brief Analytic Treatment Interruption of Antiretroviral Therapy Within 4 Human Immunodeficiency Virus Remission Trials: An Observational Study in Acutely Treated People Living With Human Immunodeficiency Virus

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Background. The central nervous system (CNS) is a likely reservoir of human immunodeficiency virus (HIV), vulnerable to viral rebound, inflammation, and clinical changes upon stopping antiretroviral therapy (ART). It is critical to evaluate the CNS safety of studies using analytic treatment interruption (ATI) to assess HIV remission.

Methods. Thirty participants who started ART during acute HIV infection underwent CNS assessments across 4 ATI remission trials. ART resumption occurred with plasma viral load >1000 copies/mL. CNS measures included paired pre- vs post-ATI measures of mood, cognitive performance, and neurologic examination, with elective cerebrospinal fluid (CSF) sampling, brain diffusion tensor imaging (DTI) and magnetic resonance spectroscopy (MRS).

Results. Median participant age was 30 years old and 29/30 were male. Participants' median time on ART before ATI was 3 years, and ATI lasted a median of 35 days. Post-ATI, there were no differences in median mood scores or neurologic findings and cognitive performance improved modestly. During ATI, a low level of CSF HIV-1 RNA was detectable in 6 of 20 participants with plasma viremia, with no group changes in CSF immune activation markers or brain DTI measures. Mild worsening was identified in post-ATI basal ganglia total choline MRS, suggesting an alteration in neuronal membranes.

Conclusion. No adverse CNS effects were observed with brief, closely monitored ATI in participants with acutely treated HIV, except an MRS alteration in basal ganglia choline. Further studies are needed to assess CNS ATI safety in HIV remission trials, particularly for studies using higher thresholds to restart ART and longer ATI durations.

Keywords. central nervous system; HIV; analytic treatment interruption; acute HIV infection; HIV cure.

Analytic treatment interruption (ATI) is a component of human immunodeficiency virus (HIV) remission studies that evaluates the capacity to sustain viral suppression of antiretroviral therapy (ART). With advancing efforts toward HIV remission and cure, we must determine the clinical safety of ATI as a scientific approach considering the potential risks [1]. Discontinuing

ART was once thought to reduce associated toxicities, but the risks of this approach ultimately outweighed the benefits. Poor outcomes after prolonged and CD4⁺-guided ART interruption included increased morbidity, drug resistance, recurrent acute retroviral syndrome, and HIV transmission [2–6]. Recent, safer HIV remission trials use intensive monitoring and low, predefined plasma HIV RNA parameters for restarting ART, with the time to rebound as a study outcome [7–11].

Emerging work suggests that short periods of ATI may have minimal influence on systemic HIV reservoirs [12–14]. However, the impact of brief ATI on the central nervous system (CNS) remains unknown. Concern persists because the CNS is a possible site of viral resurgence, vulnerable to adverse laboratory and clinical effects with ART withdrawal. Studies during

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prolonged ATI with neurologic monitoring have demonstrated viral rebound in the cerebrospinal fluid (CSF), emergence of neuroinflammation, elevations in neuronal injury markers, and rare clinical meningitis after ART cessation [5, 15–20]. These CNS effects occurred with ART interruption lasting up to 5 months, often in individuals with low CD4⁺ nadirs and, in some cases, virologic failure. It is unknown whether participants with healthier immune status experience CNS risks when undergoing limited ATI with low-level plasma viral rebound. This is an urgent clinical issue for individuals on stable, suppressive ART deciding whether to participate in HIV remission studies.

To evaluate the CNS safety of closely monitored ATI in individuals with HIV with relatively preserved immunity, we prospectively examined neurologic and behavioral measures in 4 state-of-the-art HIV remission trials conducted at a single center in a cohort of participants who originally started ART during acute HIV infection [8, 11]. Where available, we analyzed paired CNS assessments of mood, cognitive functioning, neurologic findings, CSF, and neuroimaging. An additional objective was to identify whether rebounding virus found in the CSF during ATI derived from the CNS or from systemic HIV reservoirs.

METHODS

Participants and ATI Studies

Participants in Bangkok, Thailand, were initially enrolled in the parent RV254/SEARCH 010 study of individuals diagnosed during acute HIV infection (Fiebig stage I–V), rapidly started on ART, and then followed longitudinally (NCT00796146/NCT00796263) [21, 22]. Most participants in this study (83%, 25/30) started on efavirenz/tenofovir/lamivudine. Participants with stable plasma viral suppression who met specific study criteria were offered entry into 1 of 4 trials involving ATI that assessed the efficacy of interventions to achieve HIV remission without ART or delay time to viral rebound (very early ART RV411/NCT02614950; vorinostat RV409/NCT02475915; VRC01 antibody RV397/NCT02664415; Ad26.Mos.HIV and MVA-Mosaic vaccine RV405/NCT02919306; Figure 1; Table 1) [8, 11]. Participants in RV411 and RV397 were switched from efavirenz to ritonavir-boosted darunavir 4 weeks before ATI. Participants in RV409 and RV405 had efavirenz switched to a boosted protease inhibitor 2 weeks before ATI. All studies had viral load monitoring every 3–7 days. ART was resumed with confirmed plasma HIV-1 RNA >1000 copies/mL with additional trial conditions (Table 1). Included participants had paired CNS data collected at baseline before ATI (pre-ATI) while on stable ART with plasma viral load below the level of detection, and then a comparison measure either during ATI (off ART) or after ATI (post-ATI) when ART was restarted (Figure 1). During ATI, measures included assessments

before and during viral rebound, except for lumbar punctures (LPs), which were all performed during plasma viral rebound. Coordination challenges across the 4 ATI studies limited standardized timing of all CNS measures. We received study approval from institutional review boards at the University of California San Francisco, Yale University, Chulalongkorn University, the Walter Reed Army Institute of Research, and all other participating institutions. All participants provided written, informed consent for research.

Neurologic and Behavioral Measures

The parent RV254 study included neurologic and behavioral measures integrated as possible into the 4 ATI trials. Mood measures included validated Thai versions of the Patient Health Questionnaire-9 (PHQ-9), the Hospital Anxiety and Depression Scale (HADS), and a Distress Thermometer [23]. Not every ATI study could incorporate each CNS measure (Supplemental Table 1). Neuropsychological testing included 4 assessments sensitive to CNS effects of HIV: Color Trails 1 and Trail-making A (psychomotor speed); Color Trails 2 (executive functioning/set-shifting); and Grooved Pegboard, nondominant hand (fine motor function) [24]. We computed a composite neurocognitive performance (NPZ-global) from the mean *z* score of each test. Several ATI studies incorporated the National Institutes of Health (NIH) ToolBox Flanker subtest (reaction time during response inhibition) before, during, and after ATI [25]. Participants underwent a standardized neurologic assessment based on the AIDS Clinical Trials Group Macro Neurological Examination [24]. Participants underwent optional LP pre-ATI and during ATI when plasma HIV RNA was >20 copies/mL. CSF HIV RNA analysis occurred at 2 laboratories: CSF from RV409 at the HIV Netherlands Australia Thailand Research Collaboration laboratory in Bangkok (lower level of quantification [LLQ] of 20 copies/mL); the other 3 studies at the Military HIV Research Program laboratory in Silver Spring, Maryland (LLQ of 80 copies/mL; Roche COBAS AmpliPrep/COBAS TaqHIV-1 Test v2.0 diluted 4-fold because of low CSF volumes). CSF immune activation markers measured at Military HIV Research Program include neopterin (EIA), a marker of macrophage activation; chemokines CCL2/MCP-1 and CXCL10/IP-10 (Luminex platform), which regulate trafficking of immunologic cells; CD163 (Luminex), a marker of monocyte/macrophage lineage cells; and s100B (Luminex), a marker of glial activation. Brain magnetic resonance imaging (MRI) scans were optional pre-ATI and during ATI, including diffusion tensor imaging assessing white matter tract integrity performed on a Philips Ingenia 3T MRI scanner with a 15-channel volume head coil for signal excitation and reception following previous procedures [26]. Brain proton magnetic resonance spectroscopy (MRS) was acquired pre-ATI and either during ATI (RV411) or post-ATI (RV409) measuring CNS inflammation and glial function using vendor-specific single

Figure 1. Timeline of human immunodeficiency virus (HIV) infection, antiretroviral therapy (ART), and analytic treatment interruption (ATI) by study. Study intervention is listed in blue, with RV409, RV397, and RV405 also including a placebo arm detailed in Table 1. Information at the bottom of the figure indicates median timing between events across all ATI studies, as well as ART resumption criteria. bNAb, broadly neutralizing antibody.

voxel. ^1H -MRS PRESS sequence with the following acquisition parameters: echo time/repetition time = 35/1500 ms; 2048 data points; 128 total averages; $20 \times 20 \times 20 \text{ mm}^3$ voxel at the left basal ganglia. Limited samples prevented other voxel analysis. Water-unsuppressed spectra were acquired with similar parameters and 16 averages. LCModel (version 6.2) was used to quantify brain metabolites using GAMMA simulated reference basis sets [27, 28]. Fittings were performed between 4.0 and 0.5 ppm. Quantified metabolites total n-acetyl aspartate, total choline, myo-inositol, and glutamate + glutamine were included only if the signal-to-noise ratio was >4 and their percent standard deviations were $<20\%$. Metabolites were expressed as a ratio to stable total creatine levels.

Statistical Analyses

Numerical results were presented as medians, interquartile ranges (IQRs) and total ranges. Paired data was analyzed using 2-tailed Wilcoxon matched signed-rank test, and analysis of

variance for multiple time points. Approximate 95% confidence intervals for median values were included to account for variability of the data and small sample size, with actual median confidence level stated. Chi-squared analyses were used to compare proportions.

RESULTS

Thirty participants (29 males) with paired CNS data were included. Pre-ATI, all were stable on ART for a median of 3 years, had a plasma HIV-1 HIV RNA <20 copies/mL, with a median CD4^+ cell count of 695 cells/ μL (Table 2). Median ATI duration was 35 days until ART was restarted, and none experienced acute retroviral syndrome (Table 2; Supplemental Table 1).

Neurologic, Cognitive, and Mood Measures Pre- and Post-ATI

There were no differences in the occurrence of neurologic symptoms ($n = 28$, Table 3). The median NPZ-global score

improved post-ATI, with increases in Color Trails 1 and 2 (n = 18; [Table 3](#); [Supplemental Figure 1A–C](#)) but not in other assessments. Flanker metrics did not change in computed score or average response time (n = 16). Comparing pre- vs post-ATI mood assessments (n = 14), there was no group change in PHQ-9 depression score, HADS depression or anxiety scores, or Distress Thermometer rating ([Table 3](#), [Supplemental Figure 1D–E](#)).

Detection of CSF HIV RNA During ATI

CSF HIV RNA assays were performed for all 20 participants who underwent LP during plasma viral rebound; of these, 6 (30%) revealed detectable HIV RNA. Two were in the active drug arm of RV409 (vorinostat, hydroxychloroquine, maraviroc before ATI): 1 with CSF HIV RNA of 25 copies/mL at 29 days into ATI (plasma HIV RNA 329 copies/mL) and the other CSF HIV RNA 42 copies/mL 32 days into ATI (plasma HIV RNA 35 796 copies/mL; LLQ = 20 copies/mL). Additionally, 4 participants in RV405 had detectable CSF HIV RNA at the time of restarting ART, although 3 were unquantifiable (LLQ = 80 copies/mL). One participant who received placebo had CSF HIV RNA <80 copies/mL 31 days into ATI (plasma HIV RNA 111 812 copies/mL). Three received the Ad26.Mos.HIV and MVA-Mosaic heterologous vaccine regimen before ATI. Two of

these had CSF HIV RNA <80 copies/mL 36 and 44 days into ATI (plasma HIV RNA 6585 and 83 014 copies/mL, respectively) and 1 had a quantifiable CSF HIV RNA of 424 copies/mL at 50 days into ATI (plasma HIV RNA 59 890 copies/mL). Because of low CSF HIV RNA levels, viral sequencing could not be performed to identify origin of the CSF virus during ATI. Participants with detectable CSF HIV RNA during ATI had no change in neurological findings (n = 6) or neuropsychological test scores (n = 5). Other CNS metrics for this subgroup were not analyzed because of limited data (n < 3).

Assessments of CNS Inflammatory and Neuroimaging Changes With ATI

There were no alterations in CSF white blood cell count or protein levels pre- and during ATI (n = 12). There were also no differences in soluble CSF immune activation markers pre-ATI vs during ATI for neopterin, CCL2/MCP-1, CXCL10/IP-10, CD163, and s100B (n = 8; [Table 3](#), [Supplemental Figure 1F–H](#)). None of the participants with detectable CSF HIV RNA were in the subset (n = 8) of those with measured CSF immune activation markers in this study. There were no differences in brain diffusion tensor imaging measures pre- vs during ATI in fractional anisotropy or mean, radial, or axial diffusivity (n = 12, [Figure 2A](#)). MRS data from the single basal ganglia voxel identified a mild worsening of total choline from pre-ATI to either

Table 1. Characteristics of HIV Remission Studies Incorporating ATI With Paired CNS Measures

Study	Intervention	n	Inclusion Criteria	ART Resumption Criteria
RV411	Observational ATI in Fiebig I	6	Started ART during Fiebig I acute HIV	Plasma HIV-1 RNA >1000 cps/mL
NCT02614950	No study drug intervention		On ART for 96+ weeks Plasma HIV-1 RNA <50 cps/mL for 48+ weeks CD4 ⁺ T cells ≥400 cells/μL Integrated HIV DNA <10 cps/10 ⁶ in PBMCs	Increase in >0.5 log plasma HIV-1 RNA 1× plasma HIV-1 RNA >10 000 cps/mL CD4 ⁺ T cell <350 cells/mm ³ CD4 ⁺ T cell count decline >50% from baseline
RV409	2:1 randomized, placebo-controlled, open-label	8	Started ART during Fiebig I–V acute HIV	Plasma HIV-1 RNA >1000 cps/mL ×2 samples
NCT02475915	Vorinostat, hydroxychloroquine, maraviroc for 10 weeks before ATI	(7 study drugs; 1 placebo)	On ART for 48+ weeks Plasma HIV-1 RNA <50 cps/mL for 28+ weeks CD4 ⁺ T cells ≥450 cells/μL, 2× in last 6 months	
RV397	3:1 randomized, placebo-controlled, double blind	10	Started ART during Fiebig I–III acute HIV	Plasma HIV-1 RNA >1000 cps/mL ×3 days
NCT02664415	VRC01 broadly neutralizing HIV antibody at start of ATI and every 3 weeks during ATI	(8 study drugs; 2 placebo)	On ART for 24+ months Plasma HIV-1 RNA <50 cps/mL for 3× measures CD4 ⁺ T cells of ≥400 cells/μL Integrated HIV DNA <10 cps/10 ⁶ in PBMCs	Clinical evidence of disease progression
RV405	2:1 randomized, placebo controlled	6	Started ART during Fiebig I–IV acute HIV	Plasma HIV-1 RNA >1000 cps/mL ×2, 1+ week apart
NCT02919306	Ad26.Mos.HIV and MVA-mosaic vaccine at 0, 12, 24, and 48 weeks before ATI	(5 study drugs; 1 placebo)	On stable ART for at least 4 weeks Plasma HIV-1 RNA <50 cps/mL for 48+ weeks CD4 ⁺ T cells of ≥400 cells/μL	CD4 ⁺ T cell <350 cells per mm ³ ×2, 1 week apart Clinical evidence of disease progression Diagnosis of acute retroviral syndrome

Abbreviations: ART, antiretroviral therapy; ATI, analytic treatment interruption; CNS, central nervous system; cps/mL, copies per milliliter; HIV, human immunodeficiency virus; PBMCs, peripheral blood mononuclear cells.

Table 2. Demographics of n = 30 Participants in ATI Trials With CNS Monitoring

Age at ATI (years)	30 (25, 35; 21–52)
Sex	29 male, 1 female
%Fiebig I at ART initiation (n)	26.7 (8)
%Fiebig II at ART initiation (n)	23.3 (7)
%Fiebig III at ART initiation (n)	36.7 (11)
%Fiebig IV at ART initiation (n)	13.3 (4)
Log10 plasma HIV RNA at ART initiation	5.5 (4.6, 6.4; 3.3–7.5)
Est. infection duration at ART start (days)	20 (15, 25; 9–32)
ART duration before ATI (years)	3.0 (2.5, 4.7; 1.8–5.9)
Plasma HIV RNA before ATI	All <20 cps/mL
CD4 ⁺ T cells/μL before ATI	695 (555, 812; 402–1211)
Highest plasma HIV RNA during ATI	5913 (3241, 25 296; 1154–111 812)
Days of ATI until ART resumption	35 (22,38; 14–69)

Unless otherwise described, data are presented as median (IQR; range).

Abbreviations: ART, antiretroviral therapy; ATI, analytic treatment interruption; CNS, central nervous system.

during ATI (n = 5) or post-ATI (n = 3) ($P = .047$; median 0.217 [IQR: 0.202, 0.228] vs 0.237 [IQR: 0.224, 0.242]; [Figure 2B](#)), with no differences in other metabolites.

DISCUSSION

In participants with very early ART intervention sustained for a median of 3 years, we identified that a brief, closely monitored ART interruption resulted in no adverse clinical CNS complications. Neurocognitive performance improved modestly after ATI on tests of psychomotor speed and executive functioning/set-shifting. In 30% of sampled participants, we observed low levels of CSF HIV RNA during plasma viral rebound in ATI. We found a modest alteration in 1 MRS measure of cellular turnover during/post-ATI without a change in neurologic findings, highlighting the need for additional ATI studies examining brain MRS studies.

In previous literature, treatment interruption has been associated with detectable HIV in CSF and elevations in CSF lymphocytic cell count preceded by increases in chemokine CCL2/MCP-1 [15, 16]. One study suggests that axonal injury can occur in asymptomatic individuals after stopping ART, measured via elevations in CSF neurofilament light chain protein [18]. Similar to our findings, 1 long ATI study found participants' neuropsychological test performance actually improved over 2 years off treatment [19]. Likewise, a study of five patients who underwent 12 weeks of ATI did not find any decline on standardized neurologic assessments [20]. More fulminant neurologic sequelae during ART interruption, including meningitis or meningoencephalitis, have been reported but are uncommon [5].

In terms of the improved neurocognitive performance, participants were exposed to this testing battery multiple times in the parent study, minimizing practice effects. Although viral suppression is broadly thought to improve cognitive functioning, cessation of efavirenz may have contributed to this

Table 3. Paired Neurologic, Neuropsychological, Mood, and CSF Immune Activation Marker Data Across ATI

CNS Measure	Sample	Pre-ATI	During ATI	Post-ATI	P Value
% with any neurologic findings	n = 28	25%	–	18%	$P = .515$
Median no. neurologic findings (IQR, range)	n = 28	0 (0–0.75; 0–7)	–	0 (0–0.75; 0–5)	$P = .305$
Median composite NPZ-global (96.9% CI)	n = 18	0.95 (.33–1.30)	–	1.13 (.61–1.52)	$*P < .001$
Median Color Trails 1 z score (96.9% CI)	n = 18	1.47 (.64–1.73)	–	1.54 (1.16–2.01)	$*P = .040$
Median Color Trails 2 z score (96.9% CI)	n = 18	1.06 (.57–1.25)	–	1.20 (.90–1.62)	$*P = .007$
Median Grooved Pegboard z score (96.9% CI)	n = 18	0.71 (.41–1.10)	–	0.86 (.23–1.23)	$P = .064$
Median Trails A z score (96.9% CI)	n = 18	1.45 (.73–1.80)	–	1.36 (1.01–1.75)	$P = .890$
Flanker computed score (97.9% CI)	n = 16	8.87 (8.27–9.30)	8.96 (8.44–9.56)	9.14 (8.36–9.59)	$P = .143$
Flanker average response time (97.9% CI)	n = 10	0.76 (.56–.94)	0.75 (.60–.88)	0.78 (.55–.88)	$P = .657$
Median PHQ-9 depression score (98.7% CI)	n = 14	3.5 (0–8)	–	5.0 (2–7)	$P = .510$
Median HADS depression score (98.7% CI)	n = 14	1.0 (0–3)	–	1.5 (0–6)	$P > .999$
Median HADS anxiety score (98.7% CI)	n = 14	3.0 (2–7)	–	3.0 (2–9)	$P = .677$
Median distress thermometer rating (98.7% CI)	n = 14	1.5 (.5–4.0)	–	2.5 (1.5–6.3)	$P = .252$
CSF neopterin pg/mL (99.2% CI)	n = 8	527 (332–1284)	504 (221–909)	–	$P > .999$
CSF CCL2/MCP-1 pg/mL (99.2% CI)	n = 8	690 (448–1024)	644 (431–1281)	–	$P = .461$
CSF CXCL10/IP-10 pg/mL (99.2% CI)	n = 8	428 (170–2220)	361 (216–709)	–	$P = .742$
CSF CD163 pg/mL (99.2% CI)	n = 8	5991 (5286–9851)	6238 (5264–6939)	–	$P = .945$
CSF s100B pg/mL (99.2% CI)	n = 8	1137 (887–1998)	1214 (896–2357)	–	$P = .078$

Unless otherwise described, data are presented as median values with approximate 95% confidence interval (CI) of the median.

Abbreviations: ATI, analytic treatment interruption; CNS, central nervous system; CSF, cerebrospinal fluid; HADS, Hospital Anxiety and Depression Scale; IQR, interquartile range; NPZ-global, composite neuropsychological test z score; PHQ-9, Patient Health Questionnaire.

improvement and may have masked adverse CNS effects. Self-reported mood assessments showed no group-level increase post-ATI, with few participants scoring above clinically relevant thresholds. Some individuals exhibited increased mood symptoms after viral resurgence, consistent with work describing disappointment with rapid viral rebound in the RV411 HIV remission trial [29].

Participants with paired MRS data (n = 8) revealed evidence of mild cell membrane damage in the basal ganglia

Figure 2. Neuroimaging findings in analytic treatment interruption (ATI). *A*, Brain diffusion tensor image magnetic resonance imaging measures in participants ($n = 12$) pre-ATI on treatment (initial acquisition) compared with a time point during or after treatment interruption (follow-up). Top to bottom images are representative coronal, axial, and sagittal slices revealing no significant voxels (no red) found between the 2 time points. Mean fractional anisotropy (FA) skeleton (cyan) is shown superimposed on the mean FA template for entire ATI group to demonstrate the coverage of the tested white matter tracts. *B*, Magnetic resonance spectroscopy (MRS) basal ganglia for total choline ($n = 8$). Red dots indicate paired MRS data with end point collected post-ATI ($n = 3$, mean 66 days after resuming antiretroviral therapy [ART]). Larger red dots indicate the only participant with MRS data where cerebral spinal fluid human immunodeficiency virus RNA was detected during ATI; this MRS was performed 49 days after resuming ART.

with ATI. There was no extended MRS follow-up and we do not know whether this resolved with additional time on ART. However, we measured no increase in 5 CSF markers of neuroinflammation during ATI or any increase in CNS clinical sequelae. A similar stability in CSF inflammatory markers across ATI was observed in $n = 7$ participants in RV409, assessed using different assays in a separate study [30]. Post-ATI CSF inflammatory markers were not measured in this work; however, rhesus macaques inoculated with SHIV-1157ipd3N4 who underwent treatment interruption had no elevations in CSF IL15, neopterin, CCL-2/MCP-1, or CXCL-10/IP-10 at 12 weeks after plasma viral rebound [31]. Further, higher numbers of CD3⁺ cells were seen in the brains of 3 of 5 macaques and greater CD68⁺/CD163⁺ was seen in 1 macaque before sacrifice, compared with 4 infected macaques that did not undergo ATI [31]. This contrasts with a brain fluorodeoxyglucose-positron emission tomography analysis in simian immunodeficiency virus (SIV)-infected macaques (SIVmac251, $n = 4$; SIVE660, $n = 3$) who underwent ATI: within 1 month, brain glucose metabolism and CSF inflammatory markers IL2 and IL15 increased, suggesting neuroinflammation in the context of viral rebound [32]. These findings, along with older studies of sustained ATI, imply that HIV remission studies with more liberal parameters for ART resumption or longer periods off treatment may have adverse CNS effects. This also highlights the value of planning parallel

animal model studies with tissue-based CNS measures with HIV remission trials [1].

The absence of clinically relevant CNS alterations in these studies is reassuring, but the relative CNS safety of ATI is unknown for other contexts. These study participants uniquely had early initiation of ART during Fiebig I-V, likely associated with lower viral reservoir size and potentially distinct virologic and immunologic characteristics limiting CNS sequelae with ATI. To more fully characterize the CNS safety of ATI, longitudinal CNS evaluations should include participants who started ART during the chronic phase of HIV infection, and should assess remission trials with longer durations off ART and with higher plasma HIV RNA thresholds for restarting ART. Potential effects on inflammation, viral replication, and tissue injury in target organs such as the CNS remain a significant concern to stakeholders [1]. We provide proof of concept for implementing prospective CNS assessments in future HIV remission trials involving ATI.

A potential study contribution was to examine sources of CNS viral rebound during ATI. The rebounding CSF virus detected may derive from the CNS rather than peripheral systemic reservoirs, as unique CNS viral populations have been identified under this circumstance [17]. We also questioned whether any participants would display higher CSF than plasma HIV RNA levels during ATI, suggesting CNS viral reservoirs may seed plasma viral rebound; this was not observed. Fewer than

one-third of sampled participants had relatively low levels CSF HIV RNA during plasma viral rebound, consistent with earlier observations that CSF tends to rebound later than in plasma (in 1 study, a mean of 8 days later) and at lower levels [15, 18]. With low CSF HIV RNA levels, standard sequencing techniques could not be used to compare with plasma HIV RNA for viral reservoir origins or viral compartmentalization. The conservative criterion to restart ART in these HIV remission studies may have restricted the host immune system from independently controlling the virus, thus limiting the ability to assess the full picture of CSF viral rebound and CNS HIV reservoirs. Future HIV remission studies using ATI will provide an unmatched opportunity to assess these viral rebound dynamics and reservoirs in the CNS compartment.

There are a number of limitations to the present work. The sample size was small and further limited by the lack of inclusion of all CNS measures across the 4 ATI studies. The CNS measures were not collected at uniform time intervals in all studies (Supplemental Table 1), potentially introducing bias through different immunobiological states among participants, particularly in the post-ATI time point where individuals had either a declining or undetectable plasma HIV RNA. These differences introduced variability and potentially obscured transient, adverse CNS findings during ATI. Another consideration is that 3 of 4 ATI studies exposed some participants to a drug intervention that potentially influenced the CNS measures, perhaps through viral escape or neuroinflammation. The VRC01 broadly neutralizing antibody intervention in RV397 may have caused CNS immune or virologic effects; however, this drug is not likely to penetrate into the CNS, and in the parent study, VRC01 did not result in HIV remission at 24 weeks (the primary outcome) or lead to significant systemic immunological benefits [11, 33]. There were no differences in CNS measures when analyzing data only from participants who received no study drug intervention ($n = 10$). Despite the clinical heterogeneity in the ATI trial conditions, the reported confidence intervals include this variability and rule out large effect sizes. This work included only 1 female, which obscured detection of sex differences. Almost all participants were young in age, with high CD4⁺ cell counts who started treatment very early in the course of HIV infection and likely a limited HIV reservoir size; these issues restrict the generalizability to other populations. Though the brief neuropsychological test battery has been sensitive to detecting impairment during acute infection, it may overlook subtle deterioration in untested cognitive domains [34]. Collectively, these issues limit our ability to definitively state there are no adverse CNS outcomes with brief, closely monitored ATI, and highlight the need for future work in the field. Despite these limitations, we hope these findings encourage future ATI trials to incorporate coordinated neurologic measures to further assess safety and efficacy of CNS viral eradication strategies [35].

CONCLUSIONS

CNS assessments spanning brief, monitored ATI in HIV remission studies revealed no adverse clinical outcomes and only low levels of CSF HIV RNA rebound in a minority of participants originally diagnosed and treated during acute HIV infection. A subset of participants with MRS data revealed a mild worsening in a measure of cell turnover in the basal ganglia, suggesting injury. CNS assessments are feasible to integrate in HIV remission studies involving ATI and are critical to incorporate moving forward, particularly in studies using higher plasma HIV RNA thresholds for ART resumption, longer durations of ATI, and study populations in which ART was not initiated very early.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author contributions. J. H.: literature search, study design, data collection, data analysis, data interpretation, and writing; C. M.: literature search, data collection, and writing; D. J. C. and E. K.: study design, data collection, and writing; M. d. S. and T. A. C.: study design and writing; P. C.: data collection, data analysis, and writing; C. S.: study design, data collection, and writing; J. I.: data collection, data analysis, and writing; K. B. and S. T.: data collection and writing; S. P.: study design, data collection, and writing; N. C.: data collection, data analysis, and writing; V. V., M. S., F. T.: study design and writing; S. J. K., B. M. S., L. L. J.: data analysis, data interpretation, and writing; N. D.: data collection, data analysis, data interpretation, and writing; N. S.: study design, data analysis, data interpretation, and writing; V. S.: data analysis, data interpretation, and writing; N. L. M. and M. L. R.: study design, data interpretation, and writing; S. V.: study design and data interpretation; J. A., P. P., N. P., R. P.: study design, data interpretation, and writing; S. S.: literature search, study design, data analysis, data interpretation, and writing.

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