BRIEF REPORT

The Virologic and Immunologic Effects of Cyclosporine as an Adjunct to Antiretroviral Therapy in Patients Treated during Acute and Early HIV-1 Infection

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Acute human immunodeficiency virus type 1 (HIV-1) infection is characterized by high levels of immune activation. Immunomodulation with cyclosporine combined with antiretroviral therapy (ART) in the setting of acute and early HIV-1 infection has been reported to result in enhanced immune reconstitution. Fifty-four individuals with acute and early infection were randomized to receive ART with 4 weeks of cyclosporine versus ART alone. In 48 subjects who completed the study, there were no significant differences between treatment arms in levels of proviral DNA or CD4$^+$ T cell counts. Adjunctive therapy with cyclosporine in this setting does not provide apparent virologic or immunologic benefit.

Acute human immunodeficiency virus type 1 (HIV-1) infection is characterized by high levels of viremia resulting from rapid rounds of viral replication. Destruction of CD4$^+$ T cells in massive numbers, particularly in the gastrointestinal tract, is associated with significant immune activation with high levels of circulating inflammatory cytokines [1–3]. The initiation of antiretroviral therapy (ART) during acute infection results in prompt and highly effective suppression of viral replication, increases in levels of circulating CD4$^+$ T cells and partial reconstitution of the mucosal pathology that characterizes acute infection [4–6]. Moreover, treatment during acute infection also ameliorates symptoms, shortens the duration of the acute illness, and may provide some long-term virologic and immunologic benefit [7–9].

Cyclosporine suppresses the cellular immune response by inhibiting interleukin (IL)–2 transcription after T cell receptor–mediated signal transduction, thus inhibiting IL-2–induced activation and proliferation [10, 11]. Because highly activated CD4$^+$ T cells are the preferred target for HIV-1 replication, inhibiting activation during primary infection may reduce the numbers of susceptible viral targets. This would in turn preserve immune function, particularly HIV-1–specific immune responses, because it has been shown that HIV-1–specific CD4$^+$ T cells are preferentially infected during HIV-1 infection [12]. Rizzardi et al [13] proposed the use of low-dose cyclosporine during acute HIV infection and reported that 8 weeks of low-dose cyclosporine as an adjunct to ART in 9 newly infected individuals resulted in dramatically improved and sustained levels of CD4$^+$ T cells and HIV-1–specific responses, when compared with historical controls.

On the basis of these results, we performed a randomized, open-label study of cyclosporine for 4 weeks in combination with ART in subjects identified and treated during acute and early HIV-1 infection. We hypothesized that we would confirm higher CD4$^+$ T cell levels, a sustained reduction in levels of immune activation, and a reduction in the number of cells harboring proviral DNA in the cyclosporine-treated patients.

Methods. Patients enrolled in the trial were ⩾18 years old

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and had acute and early infection, based on laboratory criteria within 28 days of screening. The presence of plasma viremia >50,000 copies/mL in the presence of an absent or indeterminate antibody response was considered acute infection. Patients with a positive serologic response were classified as having early infection and separated into 2 groups, those with ⩽3 bands and those with 4–5 bands on Western blot analysis. All study sites received approval from local institutional review boards, and all participants gave written informed consent.

Patients were assigned to 1 of 2 arms, according to a 2:1 randomization scheme in this open-label study. In both arm A and arm B, subjects received ART, including 1 tablet of fixed-dose combination of zidovudine (300 mg)–lamivudine (150 mg)–abacavir (300 mg) (Trizivir) together with 3 soft-gel capsules of fixed-dose combination lopinavir (133.3 mg)–ritonavir (33.3 mg) (Kaletra), taken twice daily with food. Subjects assigned to arm A also received a 4-week course of liquid cyclosporine (0.3 mg/kg based on ideal body weight), and those in arm B received the ART alone. Cyclosporine levels were monitored at day 3 and weeks 1, 2, and 3, with target trough concentrations of 250–450 ng/mL.

Genotypic testing on baseline viruses for resistance was performed in pretreatment plasma samples from all patients. Those with resistance to lopinavir-ritonavir or to 2 additional components of the treatment regimen were removed from the study. Study visits were conducted at day 3, at weeks 1–3, and 4, and every 4 weeks through week 48. All participants were required to continue taking lopinavir-ritonavir throughout the first 4 weeks of study, after which substitutions were allowed on the basis of patient or investigator preference if treatments with ⩾3 fully active agents were maintained, on the basis of baseline resistance testing, and if treatment regimens conformed to preferred treatment guidelines.

HIV-1 RNA levels were measured using the Roche COBAS Amplicor assay, with a lower limit of detection of 50 copies/mL plasma. Proviral DNA levels were determined at a single site using a modification of published real-time polymerase chain reaction methods [14]. Routine ($CD4^+$ and $CD8^+$ T cell quantitation) and advanced T cell subsets were measured locally. Advanced flow studies were performed by flow cytometry incorporating the following markers: CD45RA, CD38, HLA-DR, and Ki-67.

The study was designed to enroll 50 subjects, with an anticipated dropout rate of 10%, yielding 45 evaluable patients. The level of proviral DNA at week 48 was the primary end point. This resulted in 84% power to detect a difference of 0.5 $\log_{10}$ between treatment arms. Fisher's exact test was used for comparisons of entry criteria and sex, race, and ethnicity. The Wilcoxon rank test was used to compare age, $CD4^+$ T cell and subset levels, and proviral DNA levels at weeks 12, 24, and 48. The Gehan-Wilcoxon rank test was used for comparisons between arms for HIV-1 RNA levels and time to virologic suppression, with adjustment for censoring below the limit of detection of 50 copies/mL. The interquartile ranges (IQRs) were reported for the various parameters when appropriate. The geometric mean and 95% confidence intervals (CIs) of proviral DNA levels were computed for each arm at weeks 12, 24, and 48, as were the means and 95% CIs for $CD4^+$ T cell values.

Table 1. Baseline Characteristics in 41 Subjects Completing the Trial

Characteristics	Arm A (n = 28)	Arm B (n = 13)
Age, median years (IQR)	36 (30–40)	35 (30–43)
Sex		
Male	27 (96)	13 (100)
Female	1 (4)	0 (0)
Race/ethnicity		
White	20 (71)	10 (77)
Hispanic	8 (29)	1 (8)
African American	0 (0)	1 (8)
Other	0 (0)	1 (8)
Baseline $CD4^+$T cell count, median cells/mm^3 (IQR)	407 (340–587)	490 (399–623)
Baseline $\log_{10}$ HIV RNA, median copies/mL (IQR)	5.0 (4.9–5.8)	4.9 (4.5–5.8)
Serologic entry criteria		
Negative EIA result	7 (25)	3 (23)
Positive EIA result		
0–3 bands on Western blot analysis	16 (57)	7 (54)
4–5 bands on Western blot analysis	5 (18)	3 (23)

NOTE. Data are no. (%) of patients, unless otherwise indicated. Patients in arm A received antiretroviral therapy (ART) plus cyclosporine, and patients in arm B received ART only. EIA, enzyme immunoassay for human immunodeficiency virus (HIV); IQR, interquartile range.

The square root transformation was used to improve the symmetry of the $CD4^+$ T cell distribution. A similar analysis was done for additional T cell subsets.

Results. Fifty-four individuals were randomized to treatment (36 into arm A and 18 into arm B). Forty-one of 54 completed 44–48 weeks of therapy and were included in the analysis; they are described in Table 1. Thirteen subjects failed to complete the study, 8 subjects (22%) in arm A and 5 (28%) in arm B ($P > .99$). Reasons for patient discontinuation included nonadherence with study medications or procedures ($n = 4$), unavailability for follow-up ($n = 3$), protocol-defined virologic failure due to nonadherence ($n = 2$), adverse events ($n = 1$), baseline resistance to lopinavir-ritonavir ($n = 1$), suicide ($n = 1$), and investigator preference ($n = 1$).

Of the 41 patients completing the trial, 15 remained on their originally prescribed ARV regimen. Of the 26 patients changing therapy, all remained on a potent ART regimen, consisting minimally of agents anchored by either efavirenz or a ritonavir-enhanced protease inhibitor. There were no significant differences in changes between arms. Of 28 subjects in arm A who completed the trial, 27 competed cyclosporine treatment. One subject stopped taking cyclosporine before the end of week 1 because of nausea. Therapeutic levels were documented in 20 (74%) of 27 subjects by week 2 and 23 (85%) of 27 by week 3. Four subjects did not achieve target concentrations, with 3 above target and 1 below. The mean cyclosporine levels in these 27 subjects in arm A were 321, 431, 363, and 362 ng/mL at day 3 and weeks 1–3, respectively.

Levels of plasma HIV-1 RNA were measured longitudinally in all patients (Figure 1*A*). A rapid and sustained reduction in plasma viremia was seen in all patients analyzed. The median time to HIV-1 RNA levels below detection (50 copies/mL) was 15 weeks in arm A (IQR, 12–20 weeks) and 16 weeks in arm B (IQR, 12–20 weeks) ($P = .58$). Interestingly, at week 4 there was a significant higher viral load in the patients receiving cyclosporine (3.37 vs 2.75 log copies/mL; $P = .041$). This difference was not sustained, and its importance is unclear.

Proviral DNA levels were measured in $CD4^+$ T cells in patients at weeks 12, 24 and 48 (Figure 1*B*). Levels could be quantified in 36 of 41 subjects. Quantification failed in 2 subjects, and not enough cells were available for analysis in 3 subjects. Among the 36 subjects in whom proviral DNA levels were analyzed, 24 were in arm A and 12 in arm B, consistent with the 2:1 randomization scheme. There were no significant differences between levels of proviral DNA at weeks 12, 24, and 48 between treatment arms, either when expressed as median $\log_{10}$ copies/10^6 $CD4^+$ T cells (1.88 vs 1.92 [$P = .84$], 2.12 vs 1.96 [$P = .34$], and 2.22 vs 2.13 [$P = .70$], respectively) or when expressed as mean copies/10^6 $CD4^+$ T cells (130 vs 115 [$P = .70$], 105 vs 92 [$P = .34$], and 95 vs 80 [$P = .84$]). The mean differences between arms A and B in the change from baseline

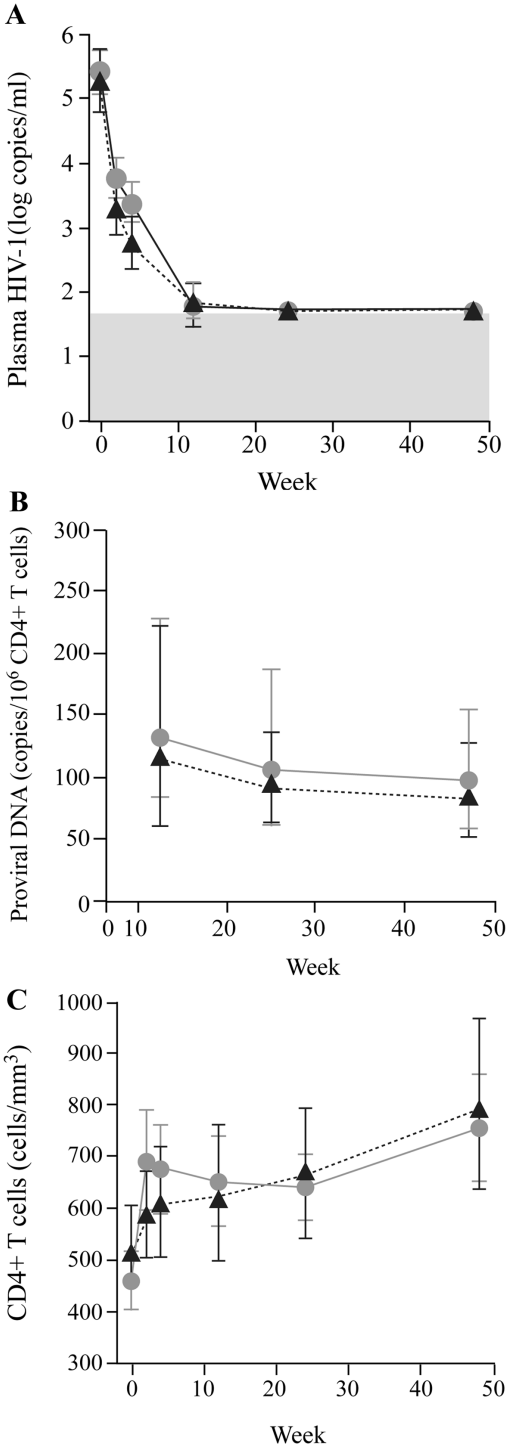

Figure 1. *A,* Longitudinal mean $\log_{10}$ human immunodeficiency virus type 1 (HIV-1) RNA levels in patients completing the study. *B,* Mean proviral DNA levels at weeks 12, 24, and 48. *C,* Longitudinal mean absolute $CD4^+$T cell counts. In all panels, solid gray lines and gray circles represent mean values for patients in arm A; dashed black lines and black triangles represent mean values for patients in arm B; error bars, 95% confidence intervals.

were 0.05 (95% CI, −0.33 to 0.44), 0.08 (95% CI, −0.24 to 0.36), and 0.07 (95% CI, −0.22 to 0.36) $\log_{10}$ copies/10^6 $CD4^+$ T cells, respectively, at weeks 12, 24, and 48. There were no differences in proviral DNA levels between arms A and B at any time point, and no significant difference between arms in the decay of proviral DNA from weeks 12 to 48 (−0.14 and −0.04 $\log_{10}$ copies/10^6 $CD4^+$ T cells for arms A and B, respectively; $P = .99$).

Mean absolute $CD4^+$ T cells counts did not differ between arms A and B at baseline or at weeks 2, 4, 12, 24, or 48 ($P = .24$, 0.28, .43, .78, .75, and .73, respectively) (Figure 1*C*). The increases in $CD4^+$ T cell counts at week 48 were 301 and 295 cells/mm^3 in arms A and B, respectively ($P = .95$). The levels of activation in the memory ($CD45RA^-$) $CD4^+$ T cell population were determined with both CD38 and HLA-DR staining. No differences were noted between arms. One measurable effect of cyclosporine was a marked and statistically significant reduction in absolute numbers of cells positive for Ki-67 staining in the memory $CD4^+$ T cell population at weeks 2 and 4 (11 vs 24 [$P = .04$] and 9 vs 25 cells/mm^3 [$P = .01$]), consistent with the ability of cyclosporine to inhibit IL-2–induced T cell proliferation. A more striking difference was seen between treatment arms in Ki-67 staining in the $CD8^+CD45RO^+$ population at weeks 2 and 4 (15 vs 66 and 12 vs 49 cells/mm^3; $P < .001$ for both).

Discussion. This clinical trial was designed to test the effectiveness of low-dose cyclosporine as an adjunct to ART during acute and early HIV-1 infection. Based on the report by Rizzardi et al [13], we expected to see dramatic immunologic benefit and hypothesized that reductions in activation and proliferation would reduce the numbers of cells harboring proviral DNA. However, we measured no effect of cyclosporine on the quantitative immunologic response, sustained levels of immune activation, or the virologic response. Although we generated proviral DNA results in fewer than the projected 45 subjects, the between-arm difference in proviral DNA levels at week 48 was small, and the 95% CI excluded differences ⩾0.4 log at weeks 12, 24, and 48, showing that the study had adequate power to detect clinically relevant treatment differences.

We chose a 4-week as opposed to an 8-week treatment period, but Rizzardi et al [13] reported significant differences in $CD4^+$ T cell levels as early as 1 to 2 weeks into treatment, which were sustained at week 48. Such differences were not seen in our study. Furthermore, findings in a recent study by Miro et al [15], in which newly infected patients received 8 weeks of low-dose cyclosporine with ART, support our findings of no significant difference in $CD4^+$ T cell levels early in the course of therapy. The most likely explanation for the contradictory results seems to be that Rizzardi and colleagues treated only 9 patients with cyclosporine and ART and compared these results with those in historical controls, patients treated with ART alone. Our current study had a more robust sample size and was randomized, resulting in a matched control group.

In summary, this randomized, clinical trial failed to document any virologic, immunologic, or clinical benefit of immunosuppression with cyclosporine as an adjunct to ART in patients identified and treated during acute and early HIV-1 infection. Target measured concentrations of cyclosporine and evidence of reduced proliferation of T cells during treatment rule out insufficient drug levels as an explanation for the lack of benefit. We therefore conclude that this particular modality is not indicated in the management of acute and early HIV-1 infection.

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AIDS Clinical Trials Group Study 5216 study sites and team. MetroHealth: Mary Wild; Beth Israel Medical Center: Manuel Revuelta and Stanley Yancovitz; New York University/New York City Health and Hospitals Corporation at Bellevue: Karen Cavanagh and Margie Vasquez.

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