A Proof-of-Concept Study of Short-Cycle Intermittent Antiretroviral Therapy with a Once-Daily Regimen of Didanosine, Lamivudine, and Efavirenz for the Treatment of Chronic HIV Infection

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Background. We previously demonstrated that short-cycle structured intermittent therapy (SIT; 7 days without therapy followed by 7 days with antiretroviral therapy [ART]) with a ritonavir-boosted, indinavir-based, twice-daily regimen maintained suppression of plasma HIV viremia while reducing serum levels of lipids. Adherence to such a regimen may be problematic for certain patients.

Methods. Eight patients with a history of receiving combination ART that maintained suppression of plasma HIV RNA to <50 copies/mL received a once-daily SIT regimen of didanosine, lamivudine, and efavirenz.

Results. For 7 patients, suppression of plasma HIV RNA to <50 copies/mL was maintained for 60–84 weeks. Four patients with adequate samples had no evidence for an increase in plasma viremia for up to 72 weeks, by use of an assay with a limit of detection of <1 copy/mL. The lack of rebound viremia may be the result of the persistence of efavirenz in plasma on day 7 of the no-therapy period, as was detected in 7 of 7 patients. There was no significant change in CD4+ T cell counts or serum hepatic transaminase or lipid levels.

Conclusion. A once-daily short-cycle SIT regimen maintained suppression of plasma HIV RNA while preserving CD4+ T cell counts. Such a regimen may have importance in resource-limited settings where the monetary cost of continuous ART is prohibitive.

Combination antiretroviral therapy (ART) has significantly reduced HIV-associated morbidity and mortality among patients who have access to such therapy [1]. Unfortunately, long-term continuous ART may not be sustainable for many individuals and nations, because of associated drug toxicities [2–15], difficulty with adherence [16, 17], and a prohibitive monetary cost [18]. Therefore, although ART provides significant clinical benefit, it is important to explore alternative strategies to reduce cost and toxicity and, possibly, to promote adherence.

We previously demonstrated that short-cycle structured intermittent therapy (SIT) involving a potent dual protease inhibitor (PI)–based regimen of 7 days without ART, followed by 7 days with ART, maintained suppression of plasma levels of HIV RNA, preserved CD4+ T cell counts, and reduced serum levels of lipids in a pilot study of 10 individuals [19]. It was clear from the pilot study that strict adherence to the regimen of 7 days without ART, followed by 7 days with ART, was essential to success in maintaining suppression of plasma HIV RNA; patients who periodically extended, by several days, the period when they were not receiving ART experienced transient increases in plasma viremia [19]. These results were consistent with reports of the occurrence of rebound levels of plasma HIV RNA after ART was interrupted for >7 days [20–22]. Therefore, the clinical
Table 1. Clinical characteristics of the patients.

<table>
<thead>
<tr>
<th>Patient</th>
<th>ART regimen received before the study</th>
<th>CD4+ T cell count, cells/mm³</th>
<th>Plasma HIV RNA level before receipt of ART,⁴⁰⁰ copies/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>601</td>
<td>ddT, 3TC, EFV</td>
<td>605</td>
<td>859</td>
</tr>
<tr>
<td>602</td>
<td>ddT, 3TC, NFV</td>
<td>373</td>
<td>650</td>
</tr>
<tr>
<td>603</td>
<td>AZT, 3TC, EFV</td>
<td>466</td>
<td>538</td>
</tr>
<tr>
<td>604</td>
<td>ddl, ddT, EFV</td>
<td>350</td>
<td>834</td>
</tr>
<tr>
<td>605</td>
<td>ddl, 3TC, EFV</td>
<td>337</td>
<td>495</td>
</tr>
<tr>
<td>606</td>
<td>ddT, 3TC, NVP</td>
<td>543</td>
<td>1324</td>
</tr>
<tr>
<td>607</td>
<td>AZT, 3TC, IDV</td>
<td>508</td>
<td>723</td>
</tr>
<tr>
<td>608</td>
<td>AZT, 3TC, ABC, EFV</td>
<td>72</td>
<td>326</td>
</tr>
</tbody>
</table>

NOTE. ABC, abacavir; ART, combination antiretroviral therapy; AZT, zidovudine; ddI, didanosine; ddT, stavudine; EFV, efavirenz; NA, not available; NFV, nelfinavir; NVP, nevirapine; IDV, indinavir; 3TC, lamivudine.

⁴ May have been obtained while patients were receiving either monotherapy or dual therapy.

³ Median, 420 CD4+ cells/mm³.

³ Median, 687 CD4+ cells/mm³.

⁴ Median, 24,802 HIV RNA copies/mL.

The applicability of short-cycle SIT may be limited because of the difficulties of strict adherence to the prescribed regimen.

It has been suggested that ART regimens that require once-daily dosing may promote adherence [23]. In this regard, it is possible that a once-daily regimen of short-cycle SIT may result in greater adherence. In addition, a once-daily regimen of didanosine, lamivudine, and efavirenz has been proven to have efficacy in small clinical studies [24, 25]. Therefore, we evaluated the effects of a short-cycle SIT regimen of 7 days without ART, followed by 7 days with once-daily administration of didanosine, lamivudine, and efavirenz, on plasma levels of HIV RNA, certain immunologic parameters, and drug toxicities. Because there have been reports of drug resistance to nonnucleoside reverse-transcriptase inhibitors (NNRTIs) during treatment interruptions in which relatively high levels of rebound plasma HIV RNA were observed during the period of interruption [26, 27], we evaluated the pharmacokinetics of efavirenz during short-cycle SIT and the emergence of mutations associated with drug resistance.

PATIENTS AND METHODS

Patient characteristics, antiretroviral-drug regimen, and clinical assays. Enrolled in the study were 8 HIV-infected patients who had a CD4+ T cell count >300 cells/mm³, who were receiving an ART regimen that included ≥3 drugs, and who had plasma HIV RNA levels of <500 copies/mL for >6 months and of <50 copies/mL during screening. Patients who had a clinical history consistent with drug resistance were excluded from the study; however, individuals who had received antiretroviral drugs before receiving optimal combination ART were eligible for the study. Patients with a history of an opportunistic infection other than oral candidiasis or mucocutaneous Kaposi sarcoma were excluded from the study. Six of 8 patients were receiving an NNRTI-based regimen before enrollment. Thirty days before enrollment, all patients were receiving an efavirenz-based regimen. A summary of relevant patient characteristics is provided in table 1. At the time of enrollment, all patients began following a regimen of 7 days without ART, followed by once-daily administration of didanosine (200 mg), lamivudine (300 mg), and efavirenz (600 mg) for 7 days; all antiretroviral drugs were discontinued at the same time during the periods of treatment interruption.

Patients underwent laboratory evaluations every 4 weeks during the first 48 weeks of the study and at least every 12 weeks after 48 weeks. All evaluations were performed after the period during which patients were not receiving ART. Laboratory evaluations included assays of the plasma HIV RNA levels, as determined by branched DNA (bDNA) assay (Bayer; limit of detection, 50 copies/mL), and examination of lymphocyte subsets, including analysis of lymphocyte activation, by use of standard flow cytometric analysis. Additional assays of plasma HIV RNA levels, which had a limit of detection of <1 copy/mL, were performed when there was sufficient plasma to perform the assay at baseline, at week 52, and at week 72. For certain patients, plasma samples were pelleted by ultracentrifugation and extracted, and HIV-1 RNA was evaluated using a recently described real-time polymerase chain reaction (PCR) assay with a limit of quantitation of 1 copy of HIV-1 RNA/mL, a false-positive rate of 0, and a false-negative rate of <0.1, as reported elsewhere [28].

The study protocol was approved by the internal review board of the National Institute of Allergy and Infectious Diseases (Bethesda, MD). Patients were required to provide written, informed consent before enrollment.

Parameters of toxicity. Measurement of levels of fasting to-
Figure 1. Effects of once-daily short-cycle intermittent therapy on plasma levels of HIV RNA. Eight patients who were receiving cycles of no antiretroviral therapy (ART) for 7 days, followed by ART for 7 days, had plasma levels of HIV RNA measured at the end of periods when ART was not received (i.e., “study period terminated”). The results are shown as the no. of HIV RNA copies per milliliter of plasma, as determined by branched DNA (bDNA) assays. All values of >50 HIV RNA copies/mL of plasma are indicated at the designated time point. Patient 602 received a diagnosis of acute hepatitis, and ART was temporarily discontinued for 12 weeks. Patients 601 and 606 voluntarily withdrew from the study at weeks 64 and 24, respectively.

Plasma concentration of efavirenz. The plasma concentration of efavirenz was determined using a validated liquid chromatographic/ultraviolet spectrometry method. The inter- and intraday coefficient of variation was <15%.

Resistance to ART drugs. CD8+ T cell–depleted peripheral blood mononuclear cell (PBMC) populations from each patient were stimulated in culture with anti-CD3 for 21 days. If replication-competent virus was produced, as determined by ELISA, then these samples were analyzed for point mutations by use of TrueGene’s HIV-1 genotyping kit (Visible Genetics), as per the manufacturer’s instructions. The TrueGene HIV-1 genotyping kit was also used to evaluate plasma samples obtained at time points when HIV RNA levels were >1000 copies/mL.

To evaluate resistance in a quantitative and more sensitive way, oligonucleotide ligation assay (OLA) was performed, as described elsewhere [29], on CD8+ T cell–depleted PBMCs. In brief, PCR amplicons were subjected to a mixture of 3 differentially labeled oligonucleotides: a wild-type (wt) oligonucleotide labeled at the 5′ end with fluorescein, a drug-resistant mutant oligonucleotide labeled at the 5′ end with digoxigenin, and a common oligonucleotide that binds the PCR amplicons adjacent to the mutant or wt oligonucleotide. The common oligonucleotide is phosphorylated at its 5′ end and has a biotin tag on its 3′ end. All oligonucleotides were highly purified and salt free and were obtained from MWG-Biotech. Ten cycles of ligation were performed using a thermostable ligase (Ampligase DNA ligase; Epicentre Technologies). The ligation products were bound to streptavidin-coated plates, denatured with NaOH, and exposed to a mixture of anti-fluorescein–alkaline phosphatase (AP) and anti-digoxigenin-peroxidase (POD) antibodies (Roche Diagnostics). AP was detected using an ELISA-based AP substrate-amplifier system (Invitrogen). POD was detected using 3,3′,5,5′-tetramethylbenzidine (Sigma-Aldrich), followed by 0.3 mol/L H2SO4. Spectrophotometric absorbances were read at 490 nm, for AP, and at 450 nm, for POD.

Statistics. Because this was a proof-of-concept study, and because we had noted efficacy among 8 patients who continued following the study regimen in our previous trial [19], we evaluated the use of once-daily short-cycle SIT for 8 patients. The Wilcoxon signed rank test was used to test the paired differences in values obtained from baseline to week 48 for significance. Adjustment of P values for multiple testing was done using the Bonferroni method.

RESULTS

Effect of a once-daily short-cycle SIT regimen on plasma levels of HIV RNA. Seven patients received no therapy for 7 days,
followed by a once-daily regimen of ART for 7 days, for 60–84 weeks (30–42 cycles). One patient (patient 606) withdrew from the study, at week 24, for personal reasons; this patient had maintained a plasma HIV RNA level of <50 copies/mL at each measurement (figure 1). All patients who remained in the study had a plasma HIV RNA level of <50 copies/mL at each measurement, while they remained on the study schedule; there were no “blips” of plasma viremia (i.e., occasional positive measurements of plasma in virus) >50 copies/mL. Of note, all measurements of plasma levels of HIV RNA were performed after the period when patients were not receiving ART. Patient 602 had therapy interrupted for 12 weeks as a result of significantly elevated hepatic transaminase levels caused by the presence of acute hepatitis secondary to primary syphilis. This patient experienced a rebound in the plasma HIV RNA level to a peak of 25,320 copies/mL at week 28, before the resumption of ART with the SIT regimen at week 32; subsequently, the patient maintained a plasma HIV RNA level of <50 copies/mL from week 44 to week 72, while receiving short-cycle SIT. Patient 601 elected to permanently discontinue receiving ART at week 64; at week 68, the patient had the plasma HIV RNA level rebound to 3762 copies/mL.

To further evaluate the effects of short-cycle SIT on plasma levels of HIV RNA, we used an assay that had a limit of detection of <1 copy/mL. As shown in table 2, there was no evidence for a substantial change in plasma viremia during weeks 48–72 of SIT, for 5 patients who had adequate material with which to perform the assay. At week 68, patient 601 had a plasma HIV RNA level of 2446 copies/mL, according to this assay, compared with a plasma HIV RNA level of 3762 copies/mL, according to the bDNA method. Therefore, the low levels detected by this ultrasensitive assay were not a result of an inability to amplify HIV RNA.

**Plasma levels of efavirenz and evaluation of resistance to ART.** The emergence of genotypic mutations associated with resistance to NNRTIs and/or lamivudine has been reported among patients who received long-cycle SIT and experienced a rebound of HIV plasma viremia [27, 30]. Selective pressure for the emergence of resistance to ART in patients receiving a SIT regimen containing NNRTIs could occur during the period when patients are not receiving ART, either as a result of levels of these drugs being suboptimal because of the prolonged half-life of NNRTIs in serum and/or plasma [31, 32] or as a result of the prolonged intracellular half-life of lamivudine [33], coupled with the low genetic barrier to resistance of these agents [34]. Therefore, for 7 patients, we determined the plasma levels of efavirenz on day 7 of the period when patients were not receiving ART (figure 2). The plasma levels ranged from 70 to 720 ng/mL (therapeutic level, ~1000 ng/mL). Alternatively, selective pressure could occur during the first few days after resumption of ART, in the presence of suboptimal concentrations of antiretroviral drugs possibly associated with increased HIV replication. Therefore, for 6 patients, we evaluated the plasma concentration of efavirenz during the first 3 days of resumption of ART. All patients had therapeutic levels of efavirenz within 36 h of resumption of ART, and 4 of 6 patients had therapeutic levels within 12 h of resumption of ART (data not shown).

For the 2 patients who had breakthrough plasma viremia due to prolonged interruptions of ART, we performed standard antiretroviral drug–resistance assays at week 68 (for patient 601) and week 28 (for patient 602); neither patient had evidence of resistance at these time points (data not shown). To further evaluate the risk of emergence of resistance to ART in association with use of a once-daily regimen of 7 days without ART, followed by 7 days with ART, we attempted to induce replication-competent HIV from 10⁷ CD8⁺ T cell–depleted PBMCs obtained from 7 patients at baseline, at week 24, and at week 48 or 52. For 5 of 7 patients, HIV RNA was not detected in the supernatants at any time point. There was no evidence of resistance to antiretroviral drugs, as determined by standard assays, for the 2 patients with sufficient levels of HIV RNA to complete the analysis (data not shown).

For 3 of the 7 patients, provirus HIV RNA could not be detected by quantitative PCR, despite the fact that ≥3 independent attempts were made per time point, with up to 1 μg of DNA used per attempt. To evaluate the presence of point mutations by use of a more sensitive and quantitative genotypic assay, the 4 patients (patients 601, 603, 604, and 608) who had detectable provirus copy numbers, according to quantitative PCR, were subjected to OLA at 100 copies/time point (except for patient 603, who had only 70 copies/time point). Patients 601, 604, and 608 failed to show point mutations for K103N or M184V (mutations associated with resistance to efavirenz and lamivudine, respectively) at any time point (figure 3A, 3C, and 3D). Although there was no evidence for these mutations either at baseline or at week 24, for patient 603, there was evidence, at week 48, for an M184V mutation in a single amplicon of the 3 amplicons from that time point (figure 3B). The presence of this mutation in this amplicon, as well as its absence in other amplicons, was confirmed by sequence analysis (data not shown). However, at week 80, there was no evidence for the M184V mutation in 3 amplicons, as determined by OLA (figure 3B) or sequence analysis (data not shown).

**Effect of a once-daily short-cycle SIT regimen on certain immunologic parameters.** The median absolute CD4⁺ T cell count of 748 cells/mm³ at baseline was not significantly different from the median absolute CD4⁺ T cell count of 740 cells/mm³ at week 48, for the 6 patients who continued following the study regimen for 48 weeks (P > .5). In addition, the median CD4⁺ T cell percentage at baseline (39%) was not significantly different from the median CD4⁺ T cell percentage at week 48 (38%) (P > .5). No patient had a significant decrease in the
### Table 2. Hypersensitive plasma HIV RNA assay.

<table>
<thead>
<tr>
<th>Patient, study time point</th>
<th>Plasma level of HIV RNA, copies/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>601</td>
<td></td>
</tr>
<tr>
<td>Week 0</td>
<td>2</td>
</tr>
<tr>
<td>Week 52</td>
<td>1</td>
</tr>
<tr>
<td>Week 68</td>
<td>2446</td>
</tr>
<tr>
<td>603</td>
<td></td>
</tr>
<tr>
<td>Week 0</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Week 52</td>
<td>NA</td>
</tr>
<tr>
<td>Week 72</td>
<td>3</td>
</tr>
<tr>
<td>604</td>
<td></td>
</tr>
<tr>
<td>Week 0</td>
<td>1</td>
</tr>
<tr>
<td>Week 48</td>
<td>1</td>
</tr>
<tr>
<td>606</td>
<td></td>
</tr>
<tr>
<td>Week 0</td>
<td>4</td>
</tr>
<tr>
<td>Week 52</td>
<td>1</td>
</tr>
<tr>
<td>609</td>
<td></td>
</tr>
<tr>
<td>Week 0</td>
<td>2</td>
</tr>
<tr>
<td>Week 52</td>
<td>1</td>
</tr>
</tbody>
</table>

**NOTE.** NA, not available.

absolute CD4+ T cell count (determined as a decrease of >30%) or percentage (determined as a decrease of >3%) from week 0 to week 48 (data not shown). There was no difference in the median absolute CD8+ T cell count and the CD8+ T cell percentage at baseline (688 cells/mm² and 40%, respectively), compared with the corresponding values at week 48 (809 cells/mm² and 40%, respectively) (P > .5 for both; data not shown). In addition, similar to the findings of our previous report of the use of short-cycle SIT with a dual-PI regimen [19], and in contrast to the findings of our previous report of long-cycle SIT [26], there was no significant change in the percentage of CD8+ T cells that expressed the activation markers DR or CD38 at baseline or at week 48 (P > .5 for both) (data not shown).

**Effect of once-daily short-cycle SIT on serum lipid and liver transaminase levels.** The median serum triglyceride level at baseline (197 mg/dL; range, 91–279 mg/dL) was not significantly different from the median level at week 48 (171 mg/dL; range, 60–270 mg/dL) (P > .5). At baseline, 3 of 6 patients had serum triglyceride levels >200 mg/dL, compared with 2–6 patients at week 48. The median serum level of total cholesterol at baseline (191 mg/dL; range, 165–265 mg/dL) was not significantly different from the median level at week 48 (192 mg/dL; range, 163–219 mg/dL) (P > .5). At baseline and at week 48, total cholesterol levels >200 mg/dL were found for 2 of 6 patients. The median serum level of LDL cholesterol at baseline (119 mg/dL; range, 94–204 mg/dL) was not significantly different from the median level at week 48 (126 mg/dL; range, 77–157 mg/dL) (P > .5). At baseline, 2 of 6 patients had serum levels of LDL cholesterol >150 mg/dL, compared with 1 of 6 patients at week 48.

There was no significant difference in the median hepatic transaminase ALT or AST levels at baseline (29 IU/L [range, 18–46 IU/L] and 27 IU/L [range, 21–41 IU/L, respectively) and the median levels at week 48 (23 IU/L [range, 16–43 IU/L] [P > .5] and 28 IU/L [range, 19–31 IU/L] [P > .5], respectively). At baseline, 2 of 6 patients had elevated levels of ALT (>41 IU/L), compared with 1 of 6 patients at week 48. At baseline, 1 of 6 patients had elevated levels of AST (>34 IU/L), compared with 0 of 6 patients at week 48.

**DISCUSSION**

The present proof-of-concept study demonstrated that, for 7 patients, short-cycle SIT administered as 7 days without ART, followed by 7 days with an ART regimen of once-daily didanosine, lamivudine, and efavirenz, maintained suppression of plasma HIV RNA for 60–84 weeks. In addition, 1 patient who withdrew from the study for personal reasons maintained suppression of plasma HIV RNA for 24 weeks. These data for a once-daily regimen are similar to data from our previous report of a pilot study of the use of a twice-daily regimen of stavudine, lamivudine, and ritonavir-boosted indinavir for 10 patients [19]. We further demonstrated that there was no substantial change in the plasma HIV RNA level during 48–72 weeks of short-cycle SIT (24–36 cycles), using an assay with a limit of detection of <1 copy/mL. In addition, similar to the results of the pilot study, in which a dual-PI regimen was used [19], there was preservation of CD4+ T cells and no evidence of generalized activation of CD8+ T cells in the present study.

Of note, in contrast to the short-cycle SIT study with a dual-PI regimen, in which there were infrequent blips of plasma HIV RNA levels of >50 copies/mL, there were no such detectable blips in any patients treated with a once-daily regimen of short-
Once-Daily Intermittent ART for HIV

Figure 3. Effects of once-daily short-cycle intermittent therapy on the development of drug resistance. An oligonucleotide ligation assay was performed at weeks 0, 24, and 48 (or at week 52, where noted) on amplified provirus obtained from CD8+ T cell–depleted peripheral blood mononuclear cells from patients 601 (A), 603 (B), 604 (C), and 608 (D). The results are shown as the mean of 3 independent optical density readings obtained, at 490 nm, for each time point. Patient 603 showed an M184V mutation in 1 of 3 independent amplicons at week 48; thus, there is a relatively large error bar at that time point. The presence of M184V was not detected at the other time points that were evaluated.

cycle SIT. This discrepancy might be the result of the relatively small sample sizes in the 2 proof-of-concept studies. However, it is unlikely that the underlying cause of the discrepancy is a difference in the replication capacity of HIV in these individuals. The median HIV RNA levels before initiation of combination ART were similar in the 2 patient groups: 24,802 copies/mL in the present study and 55,200 copies/mL in the previous study [19]. In addition, for patients 601 and 602, an interruption of therapy for >7 days resulted in significant increases in plasma viremia, confirming the replication capability of the virus. The most likely explanation for the lack of blips in plasma viremia is the relatively long half-life of the antiretroviral drugs used in the once-daily regimen in the present study. It has been reported that both didanosine and lamivudine can persist in the intracellular compartment for 3–4 days [33, 35]. In addition, in the present study, we demonstrated that efavirenz could be detected in the plasma of 7 of 7 patients at the end of the period when ART was not received and that efavirenz could even be detected at relatively high concentra-

tions in certain patients. Therefore, with the regimen used in the present study, ART could remain active during the period when treatment was not received and could contribute to the lack of blips in plasma HIV RNA and the maintenance of very low levels of HIV RNA, by use of an assay with a limit of detection of <1 copy/mL.

Although the potential persistence of didanosine and lamivudine in the intracellular compartment, as well as the persistence of efavirenz in plasma (figure 2), might contribute to the lack of blips in plasma HIV RNA during once-daily short-cycle SIT, there is a potential danger associated with this persistence of drug levels. In this regard, the persistence of suboptimal concentrations of certain antiretroviral drugs after repeated interruptions of therapy may increase the risk of emergence of genetic mutations associated with resistance to antiretroviral drugs in situations where a degree of viral replication does occur during periods of drug interruption.

We and others have reported that the emergence of mutations associated with resistance to lamivudine and NNRTIs oc-
curred in patients who were receiving these antiretroviral drugs and who underwent cyclic interruptions of therapy of >3 weeks [26, 27, 30]. Therefore, we performed extensive analyses of the emergence of resistance in the present study. Because all of the patients entered the study with plasma HIV RNA levels <50 copies/mL, and because the plasma HIV RNA levels remained at <50 copies/mL while the patients were receiving SIT, we induced the expression of HIV RNA and evaluated provirus DNA from PBMCs by use of the highly sensitive, quantitative technique OLA. There was no evidence for the emergence of resistance to antiretroviral drugs during weeks 48–80 (24–40 cycles) of SIT. Although patient 603 had evidence for an M184V mutation in a single amplicon at week 52, by OLA, that mutation was not identified at week 80, and the patient maintained a plasma HIV RNA level of <50 copies/mL during the 28 weeks of observation after the mutation was identified. The identification of the mutation at week 52 was likely a result of a high sensitivity of the assay and of the use of provirus DNA rather than HIV RNA; there is general consensus that nearly all mutations that have occurred during the course of HIV infection are archived in the PBMCs of the patient [36]. The emergence of clinically significant resistance and repeated identification, by assays, of resistance would indicate that selective pressure has effectively enhanced those mutations; in this particular patient, we found no evidence for such selective pressure during short-cycle SIT.

The persistence of antiretroviral-drug levels during the period in the pilot study when patients were not given drugs, as well as the reported potency of efavirenz-based and ritonavir-boosted indinavir-based regimens, has likely contributed to the discrepancy between our results and those of reports of a higher percentage of patients who had rebounds of plasma HIV RNA of >50 copies/mL within days of discontinuation of ART and who had limited success with an approach involving 7 days with ART, followed by 7 days without ART [37, 38]. In those studies, there was a relatively high percentage of patients who were treated with less potent regimens that consisted of nelfinavir [37] or ritonavir-boosted saquinavir-based [38] ART. In this regard, it may be important to use the most potent ART regimen possible during studies of short-cycle SIT. In addition, in 1 study, the patients were highly experienced with several suboptimal regimens before receiving combination ART [38]. Therefore, prior experience with antiretroviral drugs and the potency of such drugs may be important factors in the success of short-cycle SIT.

Although there was not a significant decrease in CD4+ T cells, the lack of an increase in CD4+ T cells over 48 weeks may represent an important alteration, compared with continuous therapy. Randomized, controlled trials are necessary to evaluate this possibility.

In contrast to our previous report of a significant decrease in serum levels of lipids with the use of a dual-PI–based short-cycle SIT regimen [19], there was no significant difference in the median serum levels of triglycerides, total cholesterol, and LDL cholesterol in the present study that used a once-daily, efavirenz-based, short-cycle SIT strategy. The most likely explanation for this discrepancy is the relatively low serum level of lipids noted at baseline in the patients studied. This may be the result of the fact that 6 of 8 patients in the present study were receiving an NNRTI-based regimen before enrollment in the study; in certain studies, NNRTI-based ART regimens have been reported to be associated with a lower incidence of elevated total and LDL cholesterol levels than are PI-based ART regimens [39–42]. In this regard, the median levels of total cholesterol, LDL cholesterol, ALT, and AST at baseline were close to, or within, the normal range for these parameters. In addition, the absence of a decrease in serum triglyceride levels with 50% less drug may support the concept that abnormalities of these levels are idiopathic in patients treated with efavirenz. It was also possible that the persistence of intracellular and plasma concentrations of the drugs used in this study could have prevented a decrease in these parameters of toxicity as a result of treatment interruptions. Finally, although patients may experience systemic side effects during the first days to weeks of receiving treatment with efavirenz, recrudescence of such symptoms was not observed in this limited number of patients during cycles of therapy and no therapy.

In conclusion, we demonstrated, in a proof-of-concept study, that short-cycle SIT with a once-daily regimen of didanosine, lamivudine, and efavirenz maintained suppression of plasma levels of HIV RNA for 60–84 weeks (30–42 cycles) while preserving CD4+ T cells counts. In addition, there was no evidence for the emergence of resistance to antiretroviral drugs. However, the need for strict adherence to the regimen is necessary, and the feasibility of such an approach will require studies of larger numbers of patients for extended periods. A once-daily regimen may allow for enhanced adherence in general, compared with the twice-daily regimen that we used in a previous study [19]. In addition, although the long half-life of the drugs that were used posed hazards in long-cycle interruption strategies, it may be ideal in short-cycle approaches if patients can remain adherent. If the safety and efficacy of short-cycle SIT ultimately are demonstrated in clinical settings, it might prove to be an important strategy to expand therapy in resource-limited settings. In this regard, randomized, controlled clinical trials are being conducted in various sites in the United States and other countries to evaluate the clinical usefulness of short-cycle SIT.

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References


