Antiviral Activity of Lamivudine in Salvage Therapy for Multidrug-Resistant HIV-1 Infection

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Background. Maximum suppression of virus replication is often not achievable for persons infected with multidrug-resistant human immunodeficiency virus type 1 (HIV-1). Available data suggest that lamivudine contributes to partial viral suppression, despite the presence of M184V mutations and high-level phenotypic lamivudine resistance.

Methods. Selective lamivudine withdrawal was studied in 6 subjects who had incomplete viral suppression during antiretroviral treatment for multidrug-resistant HIV-1 infection.

Results. Plasma levels of HIV-1 RNA increased to 0.5 log_{10} copies/mL above baseline 6 weeks after the withdrawal of lamivudine treatment (P = .04), even though reversion of lamivudine resistance was not yet detected. Early increases in plasma levels of HIV-1 RNA after lamivudine withdrawal were associated with the presence of the T215Y/F mutation and broad phenotypic resistance to nucleoside reverse-transcriptase inhibitors at baseline. Genotypic and phenotypic reversion of lamivudine resistance was detected in 4 subjects 8–14 weeks after withdrawal of lamivudine therapy. The duration of lamivudine withdrawal ranged from 8 to 22 weeks; all subjects resumed lamivudine treatment. Plasma levels of HIV-1 RNA were 0.6 log_{10} copies/mL above baseline (P = .03) when lamivudine therapy was resumed. After the resumption of lamivudine treatment, plasma HIV RNA levels decreased to baseline levels in 3 subjects but remained elevated in 3 subjects who had evolution of increased antiretroviral drug resistance during the period of lamivudine withdrawal. Safety concerns raised by this latter finding led to permanent closure of the study.

Conclusions. In select cases of multidrug-resistant HIV-1 infection, lamivudine contributes to suppression of HIV-1 replication, despite the presence of M184V mutations and lamivudine resistance.

Treatment options for persons infected with multidrug-resistant HIV-1 are limited, and, in many cases, suppression of plasma levels of HIV-1 RNA to less than detectable levels is not possible. However, the accumulation of drug-resistance mutations in reverse transcriptase (RT) and protease (PR) is sometimes associated with plasma HIV-1 RNA levels that are less than pretreatment levels. In addition, antiretroviral drugs may have partial activity, even in the presence of substantial resistance. Although complete viral suppression may not be achievable in such patients, maintenance of even partial viral suppression provides a clinical benefit [1].

High-level lamivudine resistance is conferred by mutations at RT codon 184 that produce a M184V or M184I substitution in the conserved YMDD motif of the RT polymerase domain [2]. Despite the rapid appearance of the 184V mutation during lamivudine therapy, plasma HIV-1 RNA levels remain partially suppressed when lamivudine therapy is continued [3–5]. Withdrawal of lamivudine monotherapy or discontinuation of treatment with multidrug regimens that include lamivudine in persons infected with HIV-1 with the 184V mutation is associated with increased plasma levels of HIV-1 RNA and reversion to wild-type 184M virus [6–8]. Explanations for the apparent paradox between the development of high-level lamivudine resistance and continued partial suppression of HIV-1 rep-
lication are that the 184V mutation causes decreased viral replication fitness and increased susceptibility to other nucleoside analogues. Alternatively, lamivudine could have residual antiviral activity against viruses that contain the M184V/I mutation, despite high-level phenotypic resistance [9]. To assess the potential antiviral activity of lamivudine against lamivudine-resistant virus, we conducted a pilot study of withdrawal of lamivudine therapy (hereafter, “lamivudine withdrawal”) among patients infected with lamivudine-resistant virus.

PATIENTS AND METHODS

The results of 2 prospective studies of open-label withdrawal of the lamivudine component of the antiretroviral regimens conducted at the University of Colorado General Clinical Research Center and Stanford University School of Medicine were combined. Both studies followed the US Department of Health and Human Services Guidelines for human experimentation and were approved by the respective institutional review boards.

The entry criteria for the University of Colorado study were as follows: (1) receipt of an antiretroviral regimen of ≥3 drugs that included zidovudine (or stavudine), lamivudine, and at least 1 protease inhibitor (PI) and/or nonnucleoside reverse-transcriptase inhibitor (NNRTI); (2) plasma HIV-1 RNA level of ≥1000 copies/mL within 60 days before study entry; (3) screening RT/PR genotype showed the M184V mutation, at least 3 thymidine analogue resistance mutations (i.e., M41L, D67N, K70R, L210W, T215Y/F, or K219Q), and evidence of resistance to other drugs that were part of the current regimen; (4) cumulative duration of antiretroviral treatment of ≥24 months, with failure of at least 2 previous antiretroviral regimens; (5) no satisfactory alternative regimens available, or the subject did not wish to initiate a new regimen; (6) willingness to continue the current regimen without lamivudine; (7) ability and willingness to give written, signed, informed consent; and (8) age ≥18 years. Potential subjects were excluded on the basis of the following criteria: (1) current abacavir, didanosine, or zalcitabine use, because these drugs also may select for the 184V genotype; (2) treatment with an immune modulating agent within 30 days before study entry; (3) treatment for an opportunistic infection within 14 days before study entry or presence of a malignancy requiring systemic chemotherapy; (4) pregnancy or breastfeeding; (5) active drug or alcohol abuse that would interfere with the study requirements.

Entry criteria for the Stanford University study were as follows: (1) CD4 lymphocyte count of ≥200 cells/mm³; (2) plasma HIV-1 RNA level of >500 copies/mL; (3) receipt of stable antiretroviral therapy including lamivudine for ≥3 months; (4) stable plasma HIV-1 RNA levels, defined as 2 consecutive values within 0.5 log₁₀ copies/mL within 3 months; (5) presence of the M184V mutation; and (6) age ≥18 years. Subjects were excluded on the basis of the following criteria: (1) receipt of a regimen that included abacavir or didanosine, (2) active opportunistic infections, (3) chronic hepatitis B virus infection, or (4) an anticipated change in antiretroviral therapy within the subsequent 3 months.

Evaluations. At the time of study entry (day 0), subjects were asked to discontinue taking lamivudine but to continue taking all other antiretrovirals in their pre-entry regimen. After withdrawal of lamivudine therapy, CD4⁺ lymphocyte counts and plasma HIV-1 RNA levels were determined, and genotypic resistance tests were performed at weeks 1, 2, and 3 (University of Colorado study only) and 4, 6, 8 and then every 2 weeks (Stanford University study) or 4 weeks (University of Colorado study). At each visit, current antiviral therapy was documented to verify that patients continued the nonlamivudine component of their regimens, and subjects were counseled to continue adherence with this therapy. Resumption of therapy with lamivudine was required if any of the following events occurred: (1) subject had a reduction in CD4⁺ cell counts of 200 cells/mm³ or to ≤50% of baseline, (2) subject sustained plasma levels of HIV-1 RNA of >1.0 log₁₀ copies/mL above baseline (University of Colorado study only), or (3) subject missed ≥2 study visits (Stanford University study only). Subjects who resumed lamivudine therapy were asked to continue study follow-up.

Antiretroviral drug susceptibility assays. The nucleotide sequence of RT and PR was determined by consensus sequence analysis with the TruGene HIV-1 Sequencing Kit and OpenGene DNA Sequence Analysis System (Bayer Nucleic Acid Diagnostics) or Applied Biosystems Big Dye v3.1 (Applied Biosystems). Clonal analysis of HIV-1 RT was conducted by RT-PCR amplification, as described elsewhere [10]. All RT-PCRs included samples that contained no RNA and a sample that contained 4000 median tissue culture infective doses of HIV-1 reference strain NL4-3. Phylogenetic trees were constructed to rule out the occurrence of PCR contamination [11].

Phenotypic susceptibility to antiretrovirals (i.e., 50% inhibitory concentration [IC₅₀]) and the contribution of RT/PR to replication capacity (RC) were measured by the PhenoSense HIV Drug Resistance Assay (ViroLogic). Specimens collected at baseline and during interruption of lamivudine treatment from individual subjects were assayed concurrently to eliminate potential interassay variation.

Measurement of antiretroviral drug concentrations. Lamivudine, stavudine and zidovudine concentrations in plasma were determined by reverse-phase high-performance liquid chromatography (HPLC) [12, 13]. A simultaneous reversed-phase HPLC assay for the determination of indinavir, amprenavir, nelfinavir, AG 1402 (active metabolite of nelfinavir), saquinavir, ritonavir, efavirenz, and lopinavir was performed as described elsewhere [14].

Statistical methods and data analysis. Analyses used log₁₀-transformed plasma HIV-1 RNA data. Comparisons used a
Wilcoxon signed-rank test for paired continuous variables, a Mann-Whitney test for groups of continuous variables, and Fisher’s exact test for categorical variables. Statistical comparisons used Statview (Abacus Concepts) and assumed a 2-sided significance level of .05. Ninety-five percent CIs based on the binomial distribution were computed with Splus, version 6.2 (Insightful).

Safety monitoring. A planned safety review was conducted by an independent monitor after enrollment of the fourth subject at the University of Colorado. The monitor noted that plasma levels of HIV-1 RNA did not return to baseline levels after resumption of lamivudine therapy in 3 subjects and that further evolution of antiretroviral drug resistance occurred during the period of lamivudine withdrawal. The monitor recommended permanent closure of the study.

RESULTS

Subject characteristics. Six men (median age, 48 years; range, 43–64 years) participated in the studies (table 1). All subjects had virus with nucleoside reverse-transcriptase inhibitor resistance, including the M184V mutation, as determined by consensus sequence analysis, and high-level phenotypic lamivudine resistance. Resistance-associated mutations in PR (D30N in subjects 1 and 2; M46I, I84V, and L90M in subject 3; K20R, I54V, A71T, and V82A in subject 4; M46I and I84V in subject 5; and M46I and V82A in subject 6) and decreased phenotypic susceptibility (30- to 231-fold increased IC50) to the PI component of the antiretroviral regimen were also present. At the time of entry into the study, all subjects had plasma HIV-1 RNA levels of >10,000 copies/mL (median, 20,000 copies/mL), and plasma HIV-1 RNA levels were within ±0.5 log10 copies of baseline levels 8–12 weeks before lamivudine withdrawal for 5 of 6 subjects (figure 1).

Effects of lamivudine withdrawal. All subjects discontinued treatment with lamivudine but continued treatment with the other components of their antiretroviral regimens. All subjects had quantifiable plasma lamivudine concentrations at the time of entry into the study (range, 374–2026 ng/mL) and undetectable plasma lamivudine concentrations at week 6 (<20 ng/mL). The expected thymidine analogues, PR inhibitors and NNRTI listed for each subject in table 1 were detected at both week 0 and week 6.

The duration of lamivudine withdrawal ranged from 8 weeks (for subjects 2 and 5) to 22 weeks (for subject 6). During lamivudine withdrawal, subject 1 had acute antibiotic-associated colitis at week 6 that responded to treatment for Clostridium difficile infection. Viral load measurements were not performed during the period of acute illness, and plasma HIV-1 RNA levels determined 2 weeks before onset and 4 days after the resolution of symptoms were similar (4.7 vs. 4.9 log10 copies/mL, respectively). No other serious adverse events occurred.

The 184V→M reversion was detected at least once by consensus sequence analysis of plasma virus in 4 subjects during the period of lamivudine interruption (subjects 1, 3, 4, and 6; figure 1). The median time to first detection of 184V→M was 12 weeks after lamivudine withdrawal (range, 8–14 weeks).

### Table 1. Characteristics of and laboratory values at baseline for 6 subjects who had incomplete viral suppression during antiretroviral treatment for multidrug-resistant HIV-1 infection.

<table>
<thead>
<tr>
<th>Subject</th>
<th>CD4 cell count, cells/mm³</th>
<th>Plasma HIV-1 RNA level, copies/mL</th>
<th>Reverse transcriptase inhibitors</th>
<th>Replication capacity, %</th>
<th>Reverse transcriptase/protease phenotype IC₅₀ fold change</th>
<th>ZDV</th>
<th>D4T</th>
<th>3TC</th>
<th>ABC</th>
<th>ddI</th>
<th>TDF</th>
<th>EFV</th>
<th>NFV</th>
<th>LPV</th>
<th>IDV</th>
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<tr>
<td>1</td>
<td>360³</td>
<td>18,197²</td>
<td>D4T, 3TC, NFV</td>
<td>41L, 67N, 70R, 118I, 184V, 215F</td>
<td>53</td>
<td></td>
<td>186</td>
<td>4.5</td>
<td>-300</td>
<td>10</td>
<td>2.3</td>
<td>2.0</td>
<td>0.2</td>
<td>231</td>
<td>5.5</td>
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<tr>
<td>2</td>
<td>577³</td>
<td>35,481²</td>
<td>ZDV, 3TC, NFV</td>
<td>70R, 118I, 184V, 215F, 219F, 219E</td>
<td>117</td>
<td></td>
<td>4.7</td>
<td>1.4</td>
<td>-300</td>
<td>4.2</td>
<td>1.7</td>
<td>1.0</td>
<td>0.3</td>
<td>153</td>
<td>1.4</td>
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<tr>
<td>3</td>
<td>163³</td>
<td>25,547²</td>
<td>D4T, 3TC, LPV, RTV(LD)</td>
<td>41L, 184V, 210W, 215Y</td>
<td>29</td>
<td></td>
<td>9.5</td>
<td>1.9</td>
<td>-300</td>
<td>5.4</td>
<td>1.4</td>
<td>1.2</td>
<td>0.3</td>
<td>8.6</td>
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<td>67N, 70R, 103N, 184V, 219E</td>
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<td></td>
<td>2.2</td>
<td>1.1</td>
<td>-300</td>
<td>2.4</td>
<td>1.2</td>
<td>0.8</td>
<td>0.97</td>
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<tr>
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<td>D4T, 3TC, IDV</td>
<td>41L, 69D, 118I, 184V, 210W, 215Y</td>
<td>53</td>
<td></td>
<td>27</td>
<td>3.2</td>
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<td>0.4</td>
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<td>ZDV, 3TC, EFV, IDV, RTV(LD)</td>
<td>67N, 70R, 103N, 184V, 210W, 215Y</td>
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<td></td>
<td>5.2</td>
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<td>-300</td>
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<td>1.3</td>
<td>0.9</td>
<td>0.99</td>
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</table>

**NOTE.** ABC, abacavir; ddI, dideoxynosine; D4T, stavudine; EFV, efavirenz; IDV, indinavir; LPV, lopinavir; NFV, nelfinavir; RTV(LD), low-dose ritonavir; TDF, tenofovir; ZDV, zidovudine; 3TC, lamivudine. Subjects 1, 2, 3, and 4 were studied at the University of Colorado Health Sciences Center. Subjects 5 and 6 were studied at Stanford Medical School.

* Mutations in HIV-1 reverse transcriptase associated with antiretroviral drug resistance, according to International AIDS Society–USA criteria.

* HIV-1 protease and reverse transcriptase replication capacity relative to the HIV-1 NL4-3 control, measured by the PhenoSense assay.

* Fold-change in the concentration of the drug that inhibits virus replication by 50% in the PhenoSense assay. Values in boldface indicate reduced phenotypic susceptibility, defined as a fold-change that exceeded the defined cutoff for full susceptibility.

* Mean of values determined at screening (i.e., week –1) and study entry (i.e., week 0).
Figure 1. Effects of lamivudine (3TC) withdrawal and resumption of therapy on CD4+ lymphocyte count, plasma HIV-1 RNA levels, and reverse-transcriptase codon 184 genotype. CD4+ lymphocyte counts and plasma HIV-1 RNA levels at baseline are provided in table 1. Horizontal dashed lines, baseline values; vertical dashed lines, time of lamivudine withdrawal at week 0; arrows, the time of lamivudine treatment resumption; open circles, gray circles, and black circles, time points at which the plasma virus was had 184V, 184M/V, and 184M mutations, respectively, as determined by consensus sequence analysis. Panels A and B, patient 1; C and D, patient 2; E and F, patient 3; G and H, patient 4; I and J, patient 5; K and L, patient 6.
184V→M reversion was first detected as a 184V/M mixture in subjects 2, 3, and 4, followed by detection of only 184M at the next time point (figure 1A, 1F, 1H, and 1L). Only the 184V/M mixed mutation was detected in subject 1, who resumed treatment with lamivudine after the week 12 visit (figure 1B). In these 4 subjects, there was a trend toward increased plasma HIV-1 RNA levels at the time the 184M mutation first appeared (median, +0.3 log_{10} copies/mL; \( P = .07 \), by the paired Wilcoxon signed rank test).

The 184V→M reversion was associated with 33-fold increased lamivudine susceptibility and 2.2-fold increased abacavir susceptibility. On the other hand, the 184V→M reversion was associated with 10-fold decreased zidovudine and 2-fold decreased tenofovir susceptibility. The IC_{50} for lamivudine, abacavir, zidovudine, and tenofovir remained unchanged during lamivudine withdrawal in the 2 subjects who did not have the 184V→M reversion. Susceptibility to didanosine, stavudine, efavirenz, and protease inhibitors was not affected by 184V→M. Median susceptibility to lamivudine, abacavir, zidovudine, and tenofovir returned to baseline levels in association with the reappearance of the 184V mutation after resumption of lamivudine. Median RT/PR replication capacity was 41% at entry, 60% at the time of the 184V→M reversion, and 38% at the time of the reappearance of the 184V mutation after treatment with lamivudine was resumed (\( P = .07 \) and \( P = .9 \) for paired Wilcoxon signed rank comparisons of the latter values with values at entry, respectively).

During lamivudine withdrawal, CD4+ lymphocyte counts remained near baseline values (figure 1A, 1C, 1E, 1G, and 1K), except in subject 5, who had a sustained 30% decreased lymphocyte count (figure 1J). The 184V→M reversion was not associated with changes in CD4+ lymphocyte counts.

Median plasma HIV-1 RNA levels increased 0.5 log_{10} copies/mL above baseline levels at 6 weeks after lamivudine withdrawal (\( P = .04 \), by paired Wilcoxon signed-rank test), even though the 184V→M reversion was not detected in any subject at or before week 6 by consensus sequence analysis (figure 1). Two patterns of plasma virus load trajectories were observed in individual subjects through week 6 of lamivudine interruption, before the first detection of 184V→M reversion. Plasma HIV-1 RNA levels were at or below baseline values in subjects 4 and 6 through week 6 (figure 1H and 1L). Subject 4 subsequently had increased plasma virus load when the 184V→M reversion occurred (+0.3 log_{10} copies/mL at weeks 12 and 14). Although the 184V→M reversion occurred at week 12 in subject 6, plasma HIV-1 RNA levels remained near baseline values.

Subjects 1, 2, 3, and 5 had early increased levels of plasma HIV-1 RNA, beginning within several weeks after lamivudine withdrawal (figure 1B, 1D, 1F, and 1I) even though 184M was not detected in the plasma virus by consensus sequence analysis. The 184M mutation was not detected by sequence analysis of 12–14 molecular clones (95% CI for frequency of the 184M mutation, 0–0.3 for 12 clones and 0–0.2 for 14 clones) derived from the plasma of each of these 4 subjects at the last time during lamivudine withdrawal that the 184M mutation was not detected by consensus sequence analysis of the plasma virus.

Early increases in plasma HIV-1 RNA levels after lamivudine interruption were associated with T215Y/F and M41L mutations at study entry (subjects 1, 2, 3, and 5; table 1) and a greater frequency of reduced phenotypic susceptibility to drugs in the nucleoside reverse-transcriptase inhibitor class, excluding lamivudine (75% vs. 10%; \( P = .001 \), by Fisher’s exact test). No differences in plasma antiretroviral drug concentrations were observed in subjects with early or delayed increases of plasma HIV-1 RNA levels after lamivudine withdrawal.

**Lamivudine resumption.** All subjects resumed lamivudine therapy, either at their own request or at the recommendation of their primary care provider. No subject met the CD4+ cell count or plasma HIV-1 RNA level criteria for mandated resumption. At the time that treatment with lamivudine was resumed, median plasma HIV-1 RNA levels were 0.6 log_{10} copies/mL above baseline levels (range, 0.1 to 0.6 log_{10} copies/mL above baseline levels; \( P = .03 \), by paired Wilcoxon signed-rank test). Eight weeks after treatment with lamivudine was resumed, plasma HIV-1 RNA levels were not significantly different from levels at the time of study entry (\( P = .2 \)), and only the 184V mutation was detected in plasma by consensus sequence analysis.

The response of plasma HIV-1 RNA to lamivudine resumption in individual subjects was dichotomous and was related to the evolution of increased antiretroviral drug resistance during lamivudine interruption. Plasma HIV-1 RNA levels remained at least 0.3 log_{10} copies/mL above baseline levels at all visits after lamivudine resumption for subjects 1, 2, and 3 (figure 1B, 1D, and 1F), who had increased phenotypic antiretroviral drug resistance during lamivudine withdrawal. Virus from subject 1 acquired a K219Q mutation in RT during lamivudine withdrawal and had persistently increased stavudine resistance (stavudine IC_{50} increased 2-fold from baseline levels) after treatment with lamivudine was resumed, despite the reappearance of the M184V mutation. Virus from subject 3 acquired the L10F and G73S mutations in protease and a V118I/V mixed mutation in RT during lamivudine withdrawal; resistance to stavudine and lopinavir increased 2-fold above baseline levels, even after treatment with lamivudine was resumed and the M184V mutation returned. Virus from subject 2 did not have the 184V→M reversion or the new appearance of other mutations in RT associated with drug resistance but had increased zidovudine resistance during lamivudine withdrawal (zidovudine IC_{50} increased 2-fold from baseline levels).
DISCUSSION

Lamivudine contributed a suppression of plasma HIV-1 RNA levels of $\sim 0.5 \text{log}_{10}$ despite high-level phenotypic resistance to lamivudine and thymidine analogues. This finding is consistent with the findings of previous studies in which lamivudine monotherapy, despite the presence of the M184V mutation, provided a 0.5- and 0.6-$\text{log}_{10}$ suppression of plasma HIV-1 RNA levels in either antiretroviral-naïve or treatment-experienced persons, respectively [3, 15]. Given that a 0.5-$\text{log}_{10}$ decrease of plasma HIV-1 RNA levels during partially suppressive antiretroviral therapy is associated with an $\sim 50\%$ reduction of risk of clinical disease progression [16–18], lamivudine could provide benefit in persons with the M184V mutation and limited therapeutic alternatives.

Even though the present study did not include a comparable group of control subjects who continued to receive lamivudine therapy during the follow-up period, several findings suggest that the observed increased plasma virus load was a direct effect of lamivudine withdrawal. First, the observed virus load increases exceeded the expected interassay variation for plasma HIV-1 RNA quantification [19, 20]. Second, plasma HIV-1 RNA levels were stable before lamivudine withdrawal, and increased plasma HIV-1 RNA levels were temporally related to lamivudine withdrawal and/or 184V$\rightarrow$M reversion. Third, the presence of all prescribed antiretroviral drugs in plasma at study entry and week 6 and the absence of lamivudine at week 6 provides objective evidence of adherence to study procedures. Finally, the reversibility of virus load increases after resumption of lamivudine therapy in the subjects who did not develop increased antiretroviral drug resistance provides additional evidence for a direct effect of lamivudine on plasma levels of HIV-1 RNA.

Our finding that plasma HIV-1 RNA levels increased prior to the 184V$\rightarrow$M reversion in 4 subjects suggests that lamivudine contributed to partial viral suppression in salvage therapy, despite the presence of the 184V mutation. This finding is consistent with the results of other studies in which early increases in plasma virus load occurred after withdrawal of other nucleoside reverse-transcriptase inhibitors, but not after withdrawal of protease inhibitors or NNRTIs [21, 22]. Collectively, these observations suggest direct residual antiviral activity of nucleoside analogues against resistant virus in some patients. Because lamivudine acts synergistically with thymidine analogues against zidovudine-resistant HIV-1 [23] and partially inhibits lamivudine-resistant RT [9], there are established mechanisms for direct effects of lamivudine on lamivudine- and zidovudine-resistant HIV-1. Loss of either lamivudine or thymidine analogue synergy and/or residual RT inhibition by lamivudine withdrawal would be expected to result in prompt increases in HIV-1 replication, as occurred in our study. The trends toward increased plasma HIV-1 RNA levels and replication capacity at the time of 184V$\rightarrow$M reversion in some subjects suggest that continued lamivudine therapy also provided partial viral suppression by maintenance of the 184V mutation. The potential virologic benefits of maintaining the 184V mutation include increased thymidine analogue and tenofovir susceptibility and decreased RT replication fitness.

Our findings differ with the results of the COLATE trial [24], which found that inclusion of lamivudine in a new treatment regimen after failure of a lamivudine-containing regimen does not provide additional virologic benefit. It is important to point out that all subjects in our study had incomplete suppression of virus replication while they were receiving a lamivudine-containing regimen. In contrast, subjects in COLATE received a new antiretroviral regimen of $\geq 3$ drugs with or without lamivudine, and the majority of subjects had suppression of plasma HIV-1 RNA levels to $\leq 400$ copies/mL while they were receiving the new regimen. It is possible that the effects of adding 3 new drugs to the antiretroviral regimens in the COLATE treatment arms overshadowed any benefit of continued lamivudine treatment.

Generalization of the results of the present study is limited for several reasons. First, the present study was designed to be a pilot study of relatively homogenous subjects. All subjects had multiple thymidine analogue resistance mutations at the time of study entry and received a thymidine analogue throughout the course of lamivudine withdrawal, and no subjects received abacavir or didanosine during the study. Because lamivudine monotherapy provides suppression of plasma HIV-1 RNA levels of $\sim 0.5 \text{log}_{10}$ in patients with a M184V mutation without thymidine analogue mutations [6], it is unlikely that the antiviral effects of lamivudine in salvage therapy require concomitant administration of a thymidine analogue or the presence of thymidine analogue resistance mutations. The increase in plasma HIV RNA levels after lamivudine withdrawal noted in the present study suggests that lamivudine contributes to partial viral suppression during salvage antiretroviral therapy in a select group of patients who have resistance to both lamivudine and other nucleoside reverse-transcriptase inhibitors.

Acknowledgments

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References


