The short-term effects of stavudine (d4T) plus lamivudine (3TC) were evaluated among 48 human immunodeficiency virus–infected patients for whom zidovudine therapy had failed or who could not tolerate zidovudine. Patients were followed for 8 weeks after initiation of open-label d4T plus 3TC. Four patients discontinued therapy, because of neutropenia (1), hepatitis (1), or neuropathy (2). Reduction in virus load was \( -0.86 \, (0.3 \, to \, -3.4) \, \log_{10} \, \text{copies/mL} \) and CD4 cell increase was \( 30 \, (100 \, to \, 290) \, \text{cells/mm}^3 \). Virologic response was associated with a higher CD4 cell count, no prior exposure to d4T and 3TC, and no previous AIDS-defining illness. Virus load reduction for patients naive to 3TC and d4T was \( -1.47 \, (-0.14 \, to \, -3.37) \, \log_{10} \, \text{copies/mL} \). Short-term use of d4T plus 3TC is safe, well-tolerated, and associated with virologic and substantial immunologic benefits. Further evaluation of d4T and 3TC in combination is warranted.

A number of controlled trials have now demonstrated that combination antiretroviral therapy is generally more effective than monotherapy for the treatment of human immunodeficiency virus (HIV) infection [1–6]. Survival benefit and improvement of clinical outcomes have been correlated with short-term changes in surrogate markers of HIV disease progression [7–9]. In particular, recent evidence suggests that changes in plasma virus load within the first 2 months of therapy are predictive of long-term clinical outcome [10–12]. These developments have led to a substantial revision of our therapeutic approach based on the use of combination antiretroviral therapy regimens and ongoing monitoring of plasma virus load and CD4 cell counts [13].

The effect of specific therapeutic regimens on clinical outcomes and survival is currently being assessed in long-term clinical trials. Unfortunately, the realities of HIV management cannot wait for definitive answers and often rely on preliminary results to guide therapeutic decisions [13]. In the recent past, patients who could not tolerate or who could no longer benefit from zidovudine therapy have often received treatment with untested combinations. Despite the lack of controlled clinical data, stavudine (d4T) and lamivudine (3TC) have often been combined under these circumstances on the basis of their well-characterized antiviral effect, nonoverlapping toxicities, and favorable safety and pharmacokinetic profiles [14–24].

We report here the results of a pilot open-label study of the short-term tolerability and antiviral effect of d4T and 3TC combination therapy in the setting of advanced HIV infection among patients who were zidovudine-intolerant or no longer benefiting from zidovudine therapy.

### Methods

**Study design.** This was an open-label pilot study conducted within the frame of the 3TC (NUCA 3004) and the d4T (BMS A1455-903) open-label programs. Eligible patients had documented HIV infection and were either zidovudine-intolerant or clinically progressing despite zidovudine therapy. Clinical progression was defined by a lack of CD4 cell response to antiretroviral therapy, clinical disease progression, or both. Patients were \( \geq 18 \) years old and had the following: CD4 cell count \( < 400 \) cells/mm\(^3\), hemoglobin concentration \( \geq 80 \, \text{g/L} \), absolute neutrophil count \( \geq 0.6 \times 10^{9}/\text{L} \), platelet count \( \geq 25 \times 10^{9}/\text{L} \), liver function tests \( < 5 \) times the upper limit of normal, and serum creatinine level \( < 265 \, \mu\text{mol/L} \). Women of childbearing age had a negative pregnancy test before enrollment. Patients were excluded if d4T or 3TC were contraindicated according to contemporary product monographs. Use of antiretroviral agents other than d4T and 3TC was not permitted. Concomitant medication use was allowed unless it was specifically contraindicated for use with d4T and 3TC.

**Study medications.** Dosage of 3TC was as assigned by the 3TC open-label program (NUCA 3004) before November 1995: either 150 or 300 mg orally twice daily. After November 1995, 3TC was used at a standard of 150 mg orally twice daily. Dosage of d4T was as assigned by the d4T open-label program (BMS A1455-903): either 20 or 40 mg orally twice daily (15 or 30 mg
twice daily for persons whose body weight was <60 kg and >40 kg). After December 1995, standard weight-adjusted doses of d4T were used (40 mg orally twice daily for patients ≥60 kg and 30 mg orally twice daily for patients <60 kg).

Follow-up. At baseline, patients underwent a full clinical evaluation, including a physical examination, medical history, and detailed history of prior exposure to antiretroviral agents. Patients also had two plasma HIV RNA measurements, a CD4 cell count, complete blood count and differential, liver function tests, and serum creatinine and amylase determinations at baseline. Patients were seen at weeks 2, 4, and 8. At each visit, clinical status, compliance, and side effects were evaluated by use of a targeted questionnaire. Plasma HIV RNA, CD4 cell count, and laboratory safety tests were also repeated at each visit. Adverse reactions were categorized as grade 1–4 in accordance with the AIDS Clinical Trials Group definitions, and their relationship to the study medications was characterized as unlikely, possible, or probable by a single observer [25].

Laboratory methods. CD4 and CD8 lymphocyte counts were made at St. Paul’s Hospital’s clinical laboratory by use of standard flow cytometry [26]. HIV RNA plasma levels were measured by RNA polymerase chain reaction (AmpliCoc HIV Monitor Kit; Roche Diagnostic Systems, Mississauga, Canada) [27] at the Clinical Retrovirology Laboratory, British Columbia Centre for Excellence in HIV/AIDS. All samples with values ≤500 copies/mL were reanalyzed by use of the Ultra-Direct assay (Roche Molecular Systems, Alameda, CA) [28]. This allowed us to extend the lower sensitivity of the plasma HIV RNA determinations to 20 copies/mL.

Statistical methods. Baseline characteristics were summarized for the entire study group. Ultra-Direct plasma HIV RNA results below the limit of quantification of the assay (20 copies/mL) were reported as 20 copies/mL for analytical purposes. A virologic response was prospectively defined as ≥0.5 log10 reduction in plasma HIV RNA from baseline to week 4 [10]. Characteristics of patients with and without a virologic response were compared by use of two-sample tests of proportions for categorical variables and Wilcoxon rank sum tests for continuous variables. Logistic regression was used to determine the predictors of having a virologic response.

Results

Patients’ characteristics. A total of 71 consecutive patients were screened, and 58 were enrolled between October 1995 and April 1996. Five patients discontinued the study before week 4: 1 died (cryptococcal meningitis), 1 was found to have a testicular carcinoma and chemotherapy was begun, 1 developed Pneumocystis carinii pneumonia, and 2 stopped both study drugs because of a newly developed rash. A further 5 subjects failed to comply with the protocol: 2 did not start medications for personal reasons and 3 failed to return to their appointments. These 10 patients are not included in the analyses. The remaining 48 patients are henceforth referred to as the study group.

A full 94% of patients were homosexual men; median age was 39 years, and one-third of patients had a prior AIDS-defining illness. Initial median CD4 cell count was 135 (range, 0–380) cells/mm³. Median baseline plasma virus load was 4.7 (range, 3.0–6.7) log10 copies/mL. Median prior exposure to zidovudine was 12 (range, 1–108) months. At baseline, 18 patients were naive to both d4T and 3TC, 15 patients had received d4T, and 15 had received 3TC. Median prior exposure was 15 (range, 0.75–25) and 6 (range, 1–14) months for d4T- and 3TC-pretreated patients, respectively. Eleven patients took 20 mg of d4T orally twice daily, and 1 took 300 mg of 3TC orally twice daily. Hence, 37 and 47 patients took currently recommended doses of d4T and 3TC during the study.

Safety and tolerability. Simultaneous administration of 3TC and d4T was generally well-tolerated. During the study period, 11 patients manifested new or worsening neutropenia. However, only 1 patient had grade 3 neutropenia leading to 3TC discontinuation, and this occurred shortly after chemotherapy for Kaposi’s sarcoma was initiated. Mild elevations of liver enzymes (grade 1 or 2) occurred in 12 patients. In 4 of them, the liver enzyme elevations were considered to be related to the study medications. In all of these 12 cases, however, study medications were continued even beyond the study period without ill consequences. One further patient had a grade 3 elevation of liver enzymes at the completion of the study, leading to d4T discontinuation after week 8. Grade 1 peripheral neuropathy developed in 9 patients. An additional patient developed grade 3 peripheral neuropathy leading to discontinuation of the study medications.

Virologic and immunologic effects. As shown in figure 1, the median change in plasma HIV RNA at week 8 was −0.86 (range, +0.3 to −3.4) log10 copies/mL. The maximum median change in plasma HIV RNA was −1.5 (range, +0.2 to −2.4) log10 copies/mL at week 2. Nineteen patients (40%) had reached the plasma HIV RNA nadir by week 2. At week 8, plasma HIV RNA was below the level of quantification of the Amplicor Monitor HIV assay (400 copies/mL) in 7 (16%) of 45 patients and below the level of quantification of the Ultra-Direct assay (20 copies/mL) in 2 (4%) of 45 patients. Median log10 HIV RNA change at week 8 was −1.47, −0.88, and −0.42 copies/mL for patients naive to both study drugs, 3TC, and d4T, respectively.

The median increase in CD4 cell count at week 8 was 30 (range, −100 to +290) cells/mm³. Thirty-nine patients (81%) demonstrated an improvement in CD4 cell count during the study.

Predictors of virologic response. A full 34 patients (71%) demonstrated a virologic response (≥0.5 log10 decrease in plasma HIV RNA at week 4). Baseline characteristics of responders and nonresponders are compared in table 1. As shown in table 2, higher CD4 cell counts at baseline, no prior exposure to d4T and 3TC, or no previous AIDS-defining illness were associated with a greater likelihood of virologic response with d4T plus 3TC. Other variables, such as baseline plasma HIV RNA and the nature or length of prior exposure to other nucleosides, were not significantly associated with the likelihood of
such a response. Of note, similar results were obtained when a virologic response was defined as having a \( \geq 0.5 \) or \( \geq 1 \) log\(_{10}\) reduction in plasma HIV RNA at 4 or 8 weeks.

**Discussion**

In this pilot study, we have shown that d4T plus 3TC in combination are generally safe and well-tolerated and have substantial antiviral effect, even among heavily pretreated patients with advanced disease. Despite the fact that our study group was at increased risk for the development of bone marrow suppression and peripheral neuropathy, the number of patients with grade 3 or 4 toxicities was within rates reported in previous studies with d4T or 3TC monotherapy [16–19, 29]. The magnitude of the virologic and immunologic response seen in our study group is also noteworthy. We observed a median decrease in plasma HIV RNA of 0.86 log\(_{10}\) and a median increase in CD4 cell count of 30 cells/mm\(^3\) despite the fact that two-thirds of our study group had been previously exposed to one or both study drugs.

Synergistic activity, delay in drug resistance, and lack of overlapping toxicity are the main principles for combining antiretroviral agents. A synergistic interaction between d4T and 3TC has been suggested in vitro [30]. In vivo, use of the combination may delay the appearance of the M184V mutation, which confers resistance to 3TC [31]. Furthermore, d4T and 3TC have been shown to have nonoverlapping toxicities [16–19, 29]. In addition, d4T and 3TC are attractive compounds for combination therapy.

### Table 1. Comparison of baseline characteristics of responders and nonresponders by 2-sample tests of proportion and Wilcoxon rank sum tests.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Responders (n = 34)</th>
<th>Nonresponders (n = 14)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>32 (94)</td>
<td>13 (93)</td>
<td>.99</td>
</tr>
<tr>
<td>Homosexual or bisexual</td>
<td>29 (84)</td>
<td>13 (93)</td>
<td>.66</td>
</tr>
<tr>
<td>Prior AIDS-defining illness at baseline</td>
<td>8 (24)</td>
<td>8 (57)</td>
<td>.04</td>
</tr>
<tr>
<td>Median age (years)</td>
<td>40 (28–72)</td>
<td>38 (28–51)</td>
<td>.03</td>
</tr>
<tr>
<td>CD4 cell count/mm(^3)</td>
<td>195 (0–380)</td>
<td>65 (20–310)</td>
<td>.04</td>
</tr>
<tr>
<td>Log(_{10}) HIV RNA copies/mL</td>
<td>4.7 (3.0–6.8)</td>
<td>4.7 (4.1–5.6)</td>
<td>.49</td>
</tr>
<tr>
<td>Months prior zidovudine</td>
<td>12 (1–72)</td>
<td>15 (1–108)</td>
<td>.43</td>
</tr>
<tr>
<td>Months prior didanosine</td>
<td>16 (1–36)</td>
<td>23 (1–40)</td>
<td>.52</td>
</tr>
<tr>
<td>Months prior zalcitabine</td>
<td>11 (3–32)</td>
<td>6 (2–29)</td>
<td>.55</td>
</tr>
<tr>
<td>Months prior didanosine and zalcitabine*</td>
<td>19 (3–48)</td>
<td>26 (1–44)</td>
<td>.95</td>
</tr>
<tr>
<td>Months prior 3TC</td>
<td>2 (1–6)</td>
<td>8 (4–14)</td>
<td>.004</td>
</tr>
<tr>
<td>Months prior d4T</td>
<td>1 (1–24)</td>
<td>5 (1–25)</td>
<td>.84</td>
</tr>
<tr>
<td>Low dosage of d4T</td>
<td>7 (21)</td>
<td>4 (29)</td>
<td>.71</td>
</tr>
</tbody>
</table>

NOTE. Data are no. (%) or median (range) as appropriate.

* Cumulative.

### Table 2. Multivariate logistic regression models for predicting virologic response at week 4.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Odds ratio</th>
<th>P</th>
<th>Confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prior exposure to d4T</td>
<td>0.10</td>
<td>.06</td>
<td>(0.01–1.12)</td>
</tr>
<tr>
<td>Prior exposure to 3TC</td>
<td>0.04</td>
<td>.008</td>
<td>(0.00–0.43)</td>
</tr>
<tr>
<td>Prior AIDS-defining illness</td>
<td>0.17</td>
<td>.06</td>
<td>(0.03–1.06)</td>
</tr>
<tr>
<td>Baseline CD4 cell count (/100 cells)</td>
<td>2.39</td>
<td>.06</td>
<td>(0.96–5.95)</td>
</tr>
</tbody>
</table>
for use in combination because they can be given orally twice daily, require no dietary restrictions, and have no substantial drug interactions.

We limited our evaluation of d4T plus 3TC to an 8-week period, in the setting of a preliminary pilot study. We cannot speculate about the durability of the response to this combination nor how it would fare as part of a triple combination. However, clinical studies assessing the immunologic and virologic effects of double nucleoside combinations have consistently shown that maximal antiviral effect is generally achieved within 4 weeks of starting therapy [1, 32, 33]. Furthermore, subanalysis of the AIDS Clinical Trials Group protocol 175 study demonstrated that treatment-related reductions in plasma HIV RNA level by week 8 were predictive of long-term clinical outcome [11]. In this context, our data demonstrated a 0.86 log_{10} reduction in plasma HIV RNA at week 8, with a range of +0.3 to −3.4, with the majority of patients exhibiting a significant response. Despite this, long-term follow-up evaluation of d4T plus 3TC will be needed to characterize the longevity of this response.

Our study targeted a population with limited therapeutic options. As a result, we studied patients with varying degrees of prior exposure to the study drugs, a situation often faced in the clinical setting. Using a multivariate analysis, we identified predictors of a virologic response to the study drugs. A higher CD4 cell count at baseline, the absence of prior AIDS-defining illness, and the absence of prior exposure to 3TC and d4T were associated with a virologic response, a finding consistent with that observed with current therapeutic guidelines [13]. It is quite likely that nonresponders with prior exposure to 3TC may have harbored the M184V mutation conferring resistance to 3TC [34–37], therefore compromising the suggested synergistic or additive effect of d4T. Of particular interest, our data demonstrate that prior didanosine or zalcitabine use did not preclude the ability to achieve an antiviral response with d4T plus 3TC.

Our results are remarkably consistent with those of the Altis trial, recently presented in preliminary form by Katlama et al. [24]. In brief, this study also showed substantial antiviral effect of d4T and 3TC, which was greater among antiretroviral therapy–naive patients. Key differences between our patient population and that included in the Altis trial relate to stage of disease and extent of prior antiretroviral therapy use, our patients being generally more advanced and more heavily pretreated.

In summary, our results demonstrate that d4T in combination with 3TC is generally safe and well-tolerated and has substantial antiviral effect, even among heavily pretreated patients with advanced disease who were zidovudine-intolerant or clinically progressing while being treated with zidovudine. We further identified patients with higher CD4 cell counts at baseline, no previous AIDS-defining illness, and no prior exposure to d4T and 3TC as more likely to achieve a virologic response. This can be useful in clinical practice to optimize the use of this regimen, until results of ongoing long-term controlled trials become available.

Acknowledgments

We are indebted to J. Sninsky and S. Kwong (Roche Molecular Systems) for the Ultra-Direct assay.

References