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The relative potency and tolerability of multidrug regimens used to treat infants and children infected with human immunodeficiency virus type 1 (HIV-1) are largely unknown. In Pediatric AIDS Clinical Trials Group (PACTG) Protocol 377, 181 infants and children were assigned to receive stavudine (d4T) plus nevirapine (NVP) and ritonavir (RTV); d4T plus lamivudine (3TC) and nelfinavir (NFV); d4T plus NVP and NFV; or d4T plus 3TC, NVP, and NFV. Eleven additional children received d4T and NVP plus NFV given twice daily. All subjects had not previously received protease inhibitors or nonnucleoside reverse-transcriptase inhibitors and all had been immunologically stable while receiving reverse-transcriptase inhibitor therapy. After 48 weeks of therapy, 17 (41%) of 41 subjects receiving d4T-NVP-RTV, 13 (30%) of 44 receiving d4T-NVP-NFV, 21 (42%) of 50 receiving d4T-3TC and NFV (3 times daily), and 22 (52%) of 42 receiving d4T-3TC-NVP-NFV were still receiving their assigned therapy and had HIV-1 RNA suppression to ≤400 copies/mL. These regimens were similar in their drug activity, but the 4-drug regimen offered slightly more durable suppression of viremia.

Antiretroviral therapy with reverse-transcriptase inhibitors (RTIs) prolongs survival and lessens the morbidity of HIV-infected children [1, 2]. Age-dependent changes in the body composition and drug metabolism of pediatric patients, the lack of appropriate formulations of some medications, and other practical considerations have complicated the evaluation of other antiretroviral agents for pediatric use. Pediatric AIDS Clinical Trials Group (PACTG) Protocol 377 was undertaken to evaluate promising combinations of a nucleoside-analogue RTI with an inhibitor of the HIV type 1 (HIV-1) protease, a second nucleoside-analogue RTI, and/or a nonnucleoside-analogue RTI. The therapeutic objective was to pro-

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duce a prolonged decrease in the plasma HIV-1 RNA concentration, in addition to clinical and immunological improvement, by use of regimens with an acceptable safety profile. A substudy was designed to compare the pharmacokinetics, safety, and virological response associated with nelfinavir (NFV) regimens given twice daily or 3 times daily. The initial virological responses seen in the first 24 weeks of treatment have been recently described elsewhere [3]. We present information on the antiviral activity and relative safety of the 4 therapeutic regimens assessed after 48 weeks of follow-up.

SUBJECTS, MATERIALS, AND METHODS

Subjects and study design. The PACTG 377 study, a multicenter, randomized clinical trial, compared a change in treatment from currently used nucleoside-analogue therapy to 1 of 4 drug regimens that contained stavudine (d4T): nevirapine (NVP) plus ritonavir (RTV), lamivudine (3TC) plus NFV, NVP plus NFV, and 3TC with both NVP and NFV. Concurrently, 11 additional children participated in a fifth treatment arm of d4T plus 3TC plus NFV, in which the NFV was given twice daily, rather than 3 times a day, as in the main portion of the study. All subjects were infected with HIV, were 4 months to 17 years old, had a stable CD4 cell number or percentage, remained in Centers for Disease Control and Prevention immune category 1 or 2 during the 4 months before study entry [4], and were receiving the same antiretroviral therapy for the 16 weeks before study entry. None had previously received d4T, 3TC, protease inhibitors, or nonnucleoside RTIs. Exclusion criteria included the following: current grade 3 or 4 (severe or life-threatening) laboratory test abnormalities (as judged by protocol-specified, standard pediatric toxicity criteria), active opportunistic or serious bacterial infection, and current diagnosis of malignancy or pregnancy.

Subject randomization was designed to first complete the accrual of subjects into a series of concurrent pharmacology substudies involving 6–12 children who were receiving each of the treatment regimens. Children who weighed <30 kg and could swallow tablets were offered the opportunity to participate in the small study of d4T plus 3TC plus NFV given twice daily, up to a maximum of 12 assessable children. Subject to the above constraints, randomization was stratified by CD4 percentage (<25% vs. ⩾25%) and by age at study entry (<2 years vs. ⩾2 years).

Dosages of medication dispensed were as follows: 3TC, 4 mg/kg b.i.d. (maximum dosage, 150 mg per dose b.i.d.); d4T, 1 mg/kg b.i.d. (if body weight was <30 kg), 30 mg b.i.d. (if body weight was ⩾30 but <60 kg), or 40 mg b.i.d. (if body weight was ⩾60 kg); RTV, 400 mg/m^2 b.i.d.; and NVP, 120 mg/m^2 q.d. for 14 days, followed by 120 mg/m^2 b.i.d. thereafter. In the main treatment arms, NFV, 30 mg/kg t.i.d. was given to children who weighed <30 kg. Children who weighed ⩾30 kg received 27–33 mg of NFV per kg of body weight, as per a dosing chart. In the substudy of NFV administered twice daily, NFV tablets ~55 mg/kg b.i.d., were given to children. The maximum dosage of NFV was either 1250 mg per dose t.i.d. or 1500 mg per dose b.i.d. Drugs were available in both pediatric and adult formulations. Pharmacokinetic evaluations were not performed in real time and did not affect medication schedules during the period of follow-up described in this report.

The primary objective of the study was to evaluate the 4 main combination treatment regimens with respect to RNA response, safety, and tolerance. "Primary virological failure" at week 12/16 was defined as failure to achieve either undetectable plasma RNA or RNA suppression on at least 2 of the 3 RNA determinations performed at weeks 8, 12, and 16. "Undetectable plasma RNA" was defined as an RNA level of ⩽400 copies/mL, and "RNA suppression" was defined as an RNA level of ⩽400 copies/mL or an RNA level of <10,000 copies/mL plus at least a 2-log decrease in RNA from the baseline level. A "subsequent virological failure" was defined by an RNA level of >10,000 copies/mL that was also a 0.75-$\log_{10}$ increase above the RNA nadir, which was confirmed 1 month later. The "RNA nadir" was defined as the average of the $\log_{10}$ transformation of the 2 lowest RNA determinations at weeks 8, 12, and 16.

The duration of study treatment initially planned for each child was 48 weeks, but it was extended to 96 weeks for children who were still following their initial study treatment regimen. Study treatment was discontinued for children who experienced virological failure or disease progression, or who had a persistent drug-related toxicity of grade 3 or greater. Such children were offered the best available therapy, at their own discretion (or that of their guardian or doctor), but they remained in the study for 48 weeks, if treatment was prematurely discontinued before week 48, or for the full study period of 96 weeks, if treatment was prematurely discontinued after week 48. In some cases, the best available therapy was considered to be the same as the randomized study regimen.

A total of 193 HIV-infected children from 50 sites were enrolled in the study. The institutional review board at each institution approved the study, and informed consent was obtained from all patients and their parents or legal guardians. Research was conducted according to the human experimental guidelines of the United States Department of Health and Human Services and those of the authors' institutions. Subject enrollment began in December 1997. An independent study monitoring committee reviewed the present study 3 times. In September 1998, the study monitoring committee recommended that study accrual be stopped prematurely because the rate of accrual had slowed and of a lower than anticipated aggregate primary end point. At that time, the aggregate proportion of children with undetectable RNA at week 12/16 was 47%.

Table 1. Patient characteristics at baseline, by treatment group.

Characteristic	d4T-NVP-RTV (n = 41)	d4T-NVP-NFV (n = 44)	d4T-3TC-NVP-NFV (n = 44)	d4T-3TC plus NFV (t.i.d.) (n = 52)	d4T-3TC plus NFV (b.i.d.) (n = 11)	All (n = 192)
Age, median years	4.7	6.2	7.0	6.5	7.8	6.2
Female sex	49	55	57	54	73	55
Race/ethnicity						
Black, non-Hispanic	66	68	45	67	91	64
Hispanic	29	23	36	23	9	27
White, non-Hispanic	5	9	18	10	0	10
Previous antiretroviral therapy						
Didanosine	35	40	38	29	36	35
Zidovudine-didanosine	60	49	57	69	64	60
Other	5	12	5	2	0	5
CD4 cell count, cells/μL						
Median	733	602	714	723	710	696
<500	22	32	32	31	18	29
⩾1000	32	20	27	40	18	30
CD4 percentage						
Median	28	28	27	30	29	29
<15	5	5	2	2	9	4
15–25	22	36	39	33	27	32
⩾25	73	59	59	65	64	64
HIV RNA copy number, $\log_{10}$						
Median	4.38	4.32	4.38	4.50	4.38	4.39
<3	2	2	11	4	0	5
⩾3 but <4	24	32	23	21	18	24
⩾4 but <5	46	52	50	48	64	50
⩾5	27	14	16	27	18	21

NOTE. Data are percentages of patients, unless indicated otherwise. 3TC, lamivudine; d4T, stavudine; NFV, nelfinavir; NVP, nevirapine; RTV, ritonavir.

Study evaluations. Evaluations were performed at a preentry visit that occurred within 14 days before randomization at study entry, and follow-up visits were conducted every 4 weeks while the children remained in the study. During these visits, a medical history was obtained, physical examination and a complete blood count with a differential count and serum chemistry analysis (creatinine, total bilirubin, alanine aminotransferase, aspartate aminotransferase, total amylase, cholesterol, triglycerides, creatine phosphokinase, uric acid, and glucose). Lymphocyte surface markers were evaluated at preentry and entry visits, at study weeks; 4, 8, 12, 16, and 24; and every 12 weeks thereafter. These evaluations were performed by local laboratories participating in the National Institute of Allergy and Infectious Diseases (NIAID; Bethesda, MD) Flow Cytometry Quality Assurance Program [5]. Specimens were obtained for HIV-1 RNA evaluation at preentry and entry; at study weeks 4, 8, 12, 24, 36, 44, and 48; and every 12 weeks thereafter. In addition, a specimen was obtained at week 16 if the results for specimens obtained at weeks 8 and 12 were discordant with respect to undetectable HIV-1 RNA or RNA suppression, as defined in the "subjects and study design" section. HIV-1 RNA copy number was assessed using the Roche Amplicor Monitor Assay (Roche Diagnostics) [6]; assessment was done by the staff of a single laboratory at Johns Hopkins University (Baltimore, MD), whose proficiency in the performance of this assay had been certified by the NIAID Virology Quality Assurance program [7]. Specimens obtained from children during the period from the preentry visit through week 12 were assayed in a batched fashion for HIV-1 RNA copy number; subsequently obtained specimens were run at the specified individual time points. The lower limit of assay quantification for RNA was 400 copies/mL.

All adverse events were graded by use of protocol-specified, standard toxicity criteria for children. Only the toxicity events that occurred during therapy or <60 days after administration of the initial study medication was terminated were included in the analysis.

Statistical analysis. Fifty children were to be entered into

Table 2. Proportion of children experiencing virological failure, by treatment group.

Week	d4T-NVP-RTV (n = 41)	d4T-NVP-NFV (n = 44)	d4T-3TC-NVP-NFV (n = 42)	d4T-3TC-NFV (n = 50)
12/16[a]	44	50	36	42
24[b]	46	52	36	42
36[b]	54	61	45	48
48[b]	49	57	40	40

NOTE. All patients had an RNA level of >400 copies/mL at baseline. No significant pairwise differences were found among treatment groups. 3TC, lamivudine; d4T, stavudine; NFV, nelfinavir; NVP, nevirapine; RTV, ritonavir.

[a] Failure to achieve either undetectable plasma RNA (RNA level ≤400 copies/mL) or RNA suppression (RNA level ≤400 copies/mL or ≥2-log decrease in RNA from baseline level that is <10,000 copies/mL) on at least 2 of the 3 RNA level determinations at weeks 8, 12, and 16.

[b] RNA level >10,000 copies/mL, which was also a 0.75 $\log_{10}$ increase above the RNA nadir (the average of the $\log_{10}$ transformation of the 2 lowest determinations of RNA level at weeks 8, 12, and 16), which was confirmed a month later.

each of the 4 treatment arms in the present study. This would ensure that the estimate of the proportion of children reaching an undetectable level of RNA (≤400 copies/mL) would be within 15% of the true proportion with 95% confidence.

Some patients enrolled in the study experienced virological failure and subsequently had their randomized study treatment discontinued. These patients remained in the study but received nonprotocol alternative antiretroviral treatment. To avoid confounding of the study-related virological evaluations by nonstudy antiretroviral treatment, we classified a child as having an undetectable level of RNA at a specific study week only if the child was still receiving the initial randomized study treatment at that time. Children who had an HIV RNA value of ≤400 copies/mL at week 48 and also had study treatment discontinued at week 48 as a result of completion of protocol treatment were not considered to have virological failure. For the purpose of the analysis, at each time point, children whose RNA value was missing were considered to have virological failure.

Comparisons among treatment groups used Fisher's exact test for nominal categorical variables, the Wilcoxon exact test for ordinal categorical variables, and the Wilcoxon/Kruskal-Wallis test for continuous variables [8]. Kaplan-Meier curves are presented for the "time-to-event" variables, and comparisons in the time-to-event analyses were done with the log-rank statistic to test the differences among treatment groups [9]. The baseline RNA value was defined as the geometric mean of the RNA values obtained at the preentry visit and at study entry. Data for 4 patients with undetectable baseline RNA values could not contribute to the determination of the change from a de-

Table 3. Proportion of children receiving original randomized treatment who had an RNA level of ≤400 copies/mL, by treatment group.

Week	d4T-NVP-RTV (group 1; n = 41)	d4T-NVP-NFV (group 2; n = 44)	d4T-3TC-NVP-NFV (group 3; n = 42)	d4T-3TC-NFV (group 4; n = 50)
4	44	39	40	34
8	49	52	57	42
12[a]	44	48	69	44
24[b]	39	41	67	48
36[c]	37	34	60	52
48[d]	41	30	52	42

NOTE. All patients had an RNA level of >400 copies/mL at baseline. 3TC, lamivudine; d4T, stavudine; NFV, nelfinavir; NVP, nevirapine; RTV, ritonavir.

[a] Fisher's exact P values for pairwise comparisons were $P = .03$, for groups 1 and 3; $P = .02$, for groups 3 and 4; and $P = .052$, for groups 2 and 3.

[b] Fisher's exact P values for pairwise comparisons were $P = .02$, for groups 1 and 3, and $P = .02$, for groups 2 and 3.

[c] Fisher's exact P values for pairwise comparisons were $P = .049$, for groups 1 and 3, and $P = .03$, for groups 2 and 3.

[d] Fisher's exact P value for pairwise comparison was $P = .048$, for groups 2 and 3.

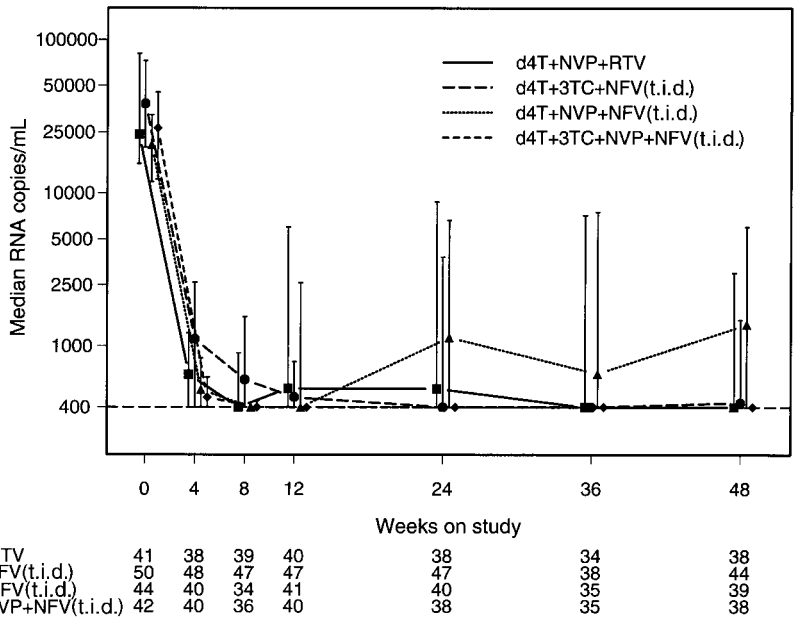

Figure 1. Comparison of the 4 combination treatment regimens, by median human immunodeficiency virus type 1 (HIV-1) RNA levels. Bars denote the 95% CIs. The number of assessable subjects at each visit is listed below the figure. Four patients had undetectable HIV-1 RNA levels at baseline and are excluded from this analysis. 3TC, lamivudine; d4T, stavudine; NFV, nelfinavir; NVP, nevirapine; RTV, ritonavir.

tectable to an undetectable RNA level; therefore, they were excluded from that analysis. All analyses were done on the basis of an intent-to-treat approach [10]. For the figures, a value of 400 RNA copies/mL was imputed for RNA values below the level of detection, and 95% CIs were given [11].

The primary objective of the study was to evaluate the 4 combination treatment regimens. Because no standard regimen existed at that time, all 6 pairwise treatment regimen comparisons were reported. All *P* values were 2-sided and were not adjusted for multiple comparisons. A conservative method of adjustment for multiple comparisons is the Bonferroni method [10]. With this method, the observed *P* value is multiplied by the number of comparisons, and if the result is still statistically significant, then it should be considered reliable.

RESULTS

Study population. A total of 193 children were entered into the study from December 1997 through September 1998. No information was available for 1 child who, as a result of a family crisis, never started receiving treatment. Data for the remaining 192 children were included in this analysis. Data on patient characteristics at baseline are listed in table 1. There were no significant differences among the main 4 treatment groups with respect to these characteristics, except for the comparison between the median age of children receiving d4T-NVP-RTV and that of the children receiving the 4-drug regimen (4.7 years vs. 7.8 years of age; $P = .03$). This difference probably resulted

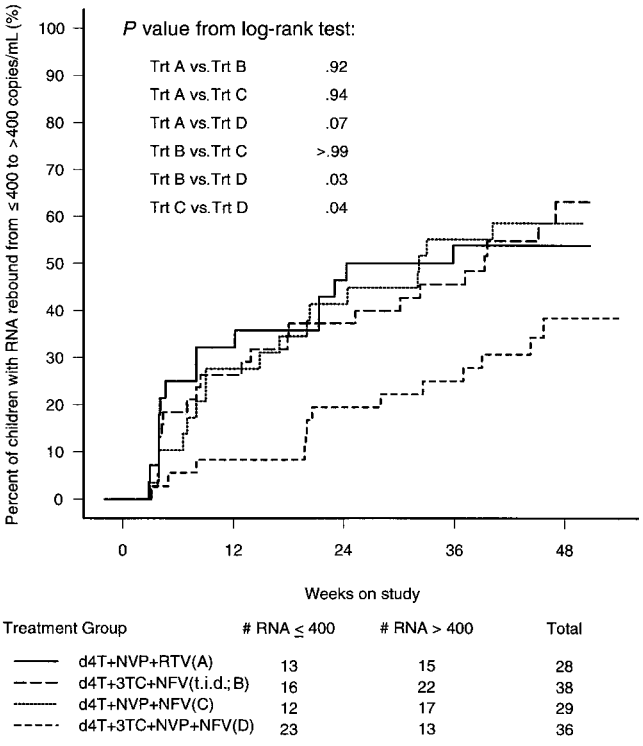

Figure 2. Kaplan-Meier analysis of time to rebound of human immunodeficiency virus type 1 (HIV-1) RNA level to ⩾400 copies/mL, comparing the 4 combination treatment regimens. Includes data from patients with undetectable viral RNA at baseline. 3TC, lamivudine; d4T, stavudine; NFV, nelfinavir; NVP, nevirapine; RTV, ritonavir; Trt, treatment.

Table 4. Proportion of children receiving original randomized treatment who had an HIV RNA level of ≤400 copies/mL, by baseline HIV RNA level.

Baseline HIV RNA level, log_{10}	Percentage of patients with RNA level of ≤400 copies/mL		
	At week 12[a]	At week 24[b]	At week 48[c]
2.6–3.0	80 (4/5)	60 (3/5)	60 (3/5)
3.0–4.0	67 (30/45)	64 (29/45)	62 (28/45)
4.0–5.0	48 (43/89)	42 (37/89)	33 (29/89)
5.0–6.0	34 (13/38)	45 (17/38)	34 (13/38)

NOTE. Data are percentage of patients (no. of patients with RNA ≤400 copies/total no. of patients in group). All patients had an RNA level of >400 copies/mL at baseline.

[a] $P = .001$, by Wilcoxon exact test for comparisons among baseline HIV RNA levels.

[b] $P = .046$, by Wilcoxon exact test for comparisons among baseline HIV RNA levels.

[c] $P = .004$, by Wilcoxon exact test for comparisons among baseline HIV RNA levels.

from the accrual scheme, which initially emphasized enrollment into 10 pharmacokinetic substudies. Approximately 95% of the children had previously received treatment with didanosine, and 60% had received zidovudine (ZDV). As of 13 October 1999, study follow-up ranged from 1 month to 20 months (median, 11.3 months; range for the 4 treatment groups, 11.1–12.0 months). There were significant differences among treatment groups with respect to the length of study follow-up ($P = .002$). This was mainly because of the large proportion of children receiving d4T-3TC and NFV (3 times daily) or d4T-NVP-NFV who left the study at week 48 because of completion of the protocol.

HIV RNA response to treatment. Overall, 88 (50%) of 177 children on the 4 main treatment groups achieved an undetectable HIV-1 RNA copy number, and 101 (57%) experienced HIV RNA suppression at week 12/16. There were no significant pairwise differences among the treatment groups with respect to these end points [3]. The proportion of children in the 4 main treatment groups who experienced virological failure was 44% (78 of 177 children) at week 24, 52% (92 of 177) at week 36, and 46% (82 of 177) at week 48 (table 2). A pairwise comparison of data for patients in the treatment arms failed to reveal any significant differences in failure rates.

The proportion of children in the 4 main treatment groups who had sustained suppression of the HIV RNA level at ≤400 copies/mL and who were still receiving their protocol-assigned treatment was 51% (90 of 177 children) at week 12, 49% (86 of 177) at week 24, 46% (81 of 177) at week 36, and 41% (73 of 177) at week 48 (table 3). At week 12, more patients receiving the 4-drug treatment regimen had an HIV-1 RNA ≤400 copies/mL (29 [69%] of 42 patients) than did those receiving d4T-NVP-RTV (18 [44%] of 41; $P = .03$) or d4T-3TC and NFV 3 times daily (22 [44%] of 50; $P = .02$) (table 3). The comparison between patients receiving the 4-drug regimen and those receiving d4T-NVP-NFV was of borderline significance at week 12 ($P = .052$). At weeks 24 and 36, the proportions were also higher for patients receiving the 4-drug treatment regimen (67% at week 24 and 60% at week 36), compared with those

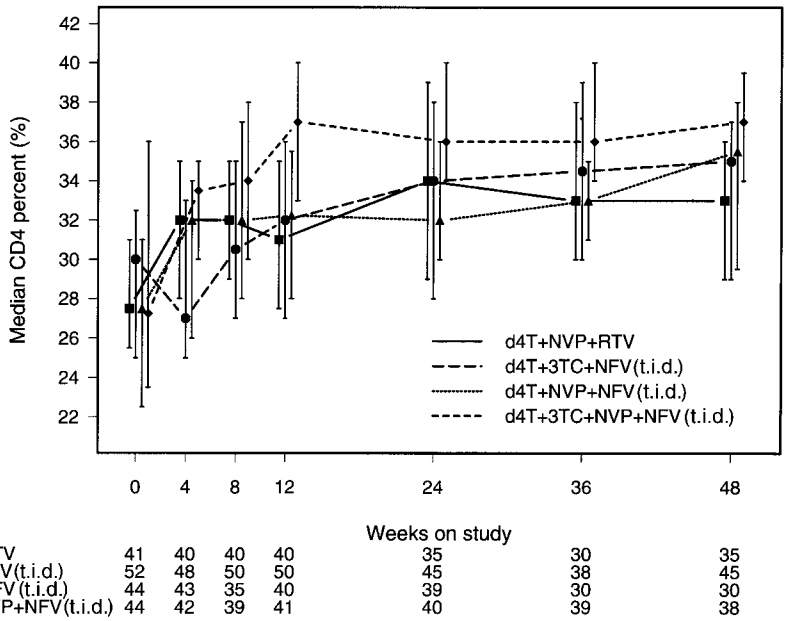

Figure 3. Median percentage of $CD4^+$ lymphocytes in peripheral blood, by treatment regimen. Bars denote the 95% CIs. 3TC, lamivudine; d4T, stavudine; NFV, nelfinavir; NVP, nevirapine; RTV, ritonavir.

Table 5. Common adverse effects by treatment group.

Adverse effect	Proportion of patients with moderate or worse adverse effects, by regimen			
	d4T-NVP-RTV (n = 41)	d4T-NVP-NFV (n = 44)	d4T-3TC-NVP-NFV (n = 44)	d4T-3TC-NFV (n = 52)
Rash/skin	27	41[a]	32	17[a]
Nausea/vomiting	29	32	18	15
Fever	24	30[b]	20	10[b]
Gastrointestinal	10	25	18	19
Hepatic	12	14	18	23
Respiratory	12	18	23	15
Neutropenia	17	9	14	23

NOTE. 3TC, lamivudine; d4T, stavudine; NFV, nelfinavir; NVP, nevirapine; RTV, ritonavir.

[a] $P = .01$, by Fisher's exact test.

[b] $P = .02$, by Fisher's exact test.

receiving d4T- NVP-RTV (39% at week 24, $P = .02$, and 37% at week 36, $P = .049$) or d4T-NVP-NFV (41% at week 24, $P = .02$, and 34% at week 36, $P = .03$). At week 48, the proportion of patients receiving initial treatment who had an RNA level of ≤400 copies/mL was significantly greater for patients receiving the 4-drug combination regimen (52% [22 of 42 patients]), than for those receiving d4T-NVP-NFV (30% [13 of 44]; $P = .048$). The median HIV RNA level over time, for each treatment group is shown in figure 1.

For some children, HIV-1 RNA became detectable again after initial suppression to ≤400 copies/mL. Figure 2 depicts Kaplan-Meier curves of the time to the first rebound of >400 RNA copies/mL for the 4 children with an RNA level of ≤400 copies/mL at baseline and the 123 children with suppression to this level at study follow-up. Suppression of HIV RNA was maintained for a significantly longer period with the 4-drug treatment regimen than with d4T-3TC and NFV (given 3 times daily [$P = .03$]) and d4T-NVP-NFV ($P = .04$).

A higher baseline virus load was associated with a higher rate of virological failure (table 4). Children with a lower HIV RNA copy number at baseline were more likely to receive the initial study treatment throughout the study. Thirty-one (62%) of 50 children with a baseline HIV RNA level of 2.6–4 $\log_{10}$ copies/mL had an HIV RNA count of ≤400 copies/mL at week 48, compared with 34% (13 of 38) of children who had a baseline HIV RNA level of 5–6 $\log_{10}$ copies/mL ($P = .004$).

Nearly all patients (96%) had ≥15% $CD4^+$ lymphocytes at baseline. When all 4 treatment groups were combined, no differences in the rate of virological failure were observed between those with evidence of moderate immunodeficiency (15%–25% $CD4^+$ lymphocytes), and those with higher CD4 percentage values.

$CD4^+$ T cell response to treatment. Differences in CD4 percentages of patients receiving the d4T-NVP-RTV treatment regimen and those receiving the 4-drug treatment regimen were noted at week 12 ($P = .03$) (figure 3), and differences in the $CD4^+$ T cell counts of patients in groups receiving d4T-NVP-RTV and d4T-3TC plus NFV (3 times daily) were noted at week 24 ($P = .01$). However, there were no significant pairwise differences among treatment groups with respect to the change in the CD4 cell count and CD4 percentages between baseline and week 48.

Adverse events. Overall, 149 (78%) of 192 children experienced moderate or worse (grade, ≥2) toxicity while receiving initial therapy, and 44 (23%) of 192 experienced severe (grade, ≥3) or worse toxicity. There were no significant differences between the treatment groups with respect to overall rates of toxicity. The most commonly observed adverse events were skin rash (53 [28%] of 192 children; for those with a toxicity grade ≥2, diffuse maculopapular rash, dry desquamation, or worse); nausea/vomiting (44 [23%] of 192 children; for a toxicity grade ≥2, more than 3 episodes of vomiting per day, duration >3 days, nausea: decreased oral intake); and temperature ≥38.5°C (40 [21%] of 192) (table 5). Skin rash of moderate or worse severity occurred more frequently among children assigned to a treatment regimen that contained NVP (33%) than among those following one of the other treatment regimens (16%; $P = .02$). Children assigned to the d4T-NVP-NFV treatment regimen had temperature ≥38.5°C (30%) more often than did children assigned to the d4T-3TC and NFV (3 times daily) treatment regimen.

Discontinuation of study drugs. Administration of initial randomized study treatments was permanently discontinued for children with (1) an HIV RNA copy number >10,000 copies/mL (32 [17%] of 192 children); (2) toxicity or medication intolerance (13 [7%] of 192); or (3) other reasons, including poor adherence to the study regimen and parental request for withdrawal of the patient from the study (29 [15%] of 192). Permanent cessation of the initial treatment as a result of virological failure was much less common for patients receiving

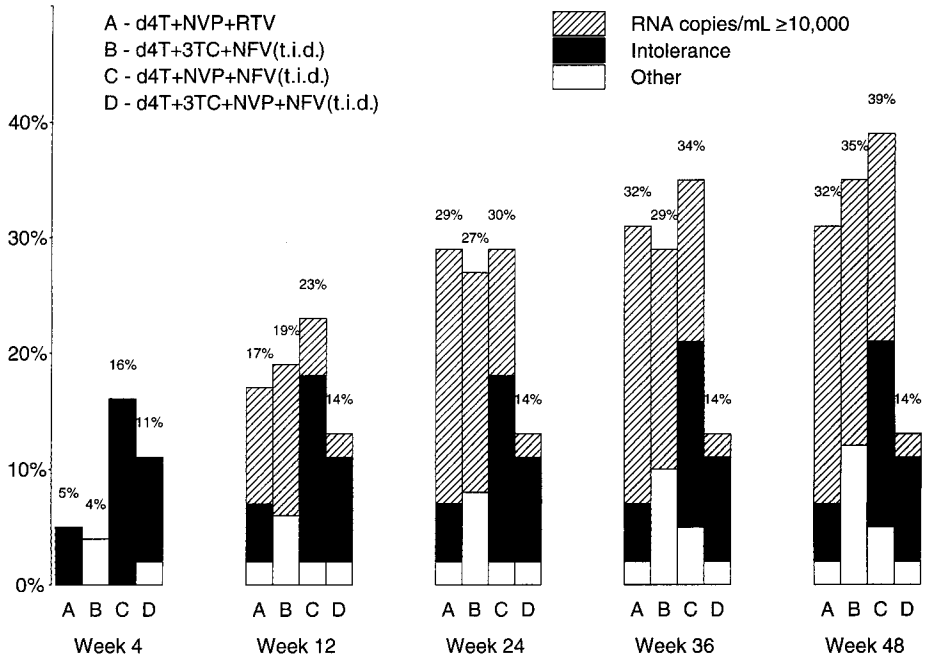

Figure 4. Distribution of reasons for permanent cessation of initial treatment, by treatment group. 3TC, lamivudine; d4T, stavudine; NFV, nelfinavir; NVP, nevirapine; RTV, ritonavir.

the 4-drug treatment regimen than for patients in the other treatment groups (figure 4); only 2 children who received the 4-drug combination regimen had their treatment prematurely terminated because of virological failure. In contrast, a lack of HIV-1 RNA response (RNA level, ⩾10,000 copies/mL) led to most treatment discontinuations for those receiving d4T-NVP-RTV or d4T-3TC-NFV. The only significant difference in the rates of study treatment discontinuation as a result of virological failure was found in a comparison of patients receiving the d4T-NVP-RTV and 4-drug combination regimens ($P = .02$).

Intolerance was the main reason for permanent discontinuation of treatment for both the d4T-NVP-NFV treatment regimen and the 4-drug treatment regimen. Thirteen (10%) of 129 patients who received a regimen that contained NVP discontinued treatment because grade 2 or 3 rash developed. In comparison, none of the 63 patients receiving d4T-3TC-NFV stopped receiving the study drug because of rash ($P = .006$). Moreover, 7 (16%) of 44 subjects receiving d4T-NVP and NFV (3 times daily) but none of the 52 subjects receiving d4T-3TC and NFV (3 times daily) had the study regimen discontinued because of grade 2 or 3 rash ($P = .003$).

d4T-3TC and NFV (twice daily). A comparison of the subjects receiving d4T-3TC and NFV (twice daily) with those receiving d4T-3TC and NFV (3 times daily), showed similar rates of virological response and virological failure. Four (36%) of 11 children receiving d4T-3TC and NFV (twice daily) experienced virological failure, which occurred at week 12. The proportion of patients receiving initial treatment who had ⩽400 RNA copies/mL was 55% at all weeks—except for week 24, when the proportion was 64%; this finding was comparable to that seen for patients receiving d4T-3TC and NFV (3 times daily). Adverse events reported by patients receiving d4T-3TC and NFV (twice daily) had similar incidence rates; they include rash/skin problems (9%); nausea/vomiting (18%); fever (27%); and gastrointestinal (27%); hepatic (18%); and respiratory (18%) problems. No patients had neutropenia.

DISCUSSION

Antiretroviral therapy for HIV-infected children has changed markedly in the past several years, with several studies having demonstrated the potency of regimens that contain nonnucleoside RTIs or inhibitors of the viral protease [2, 3, 12–17]. Undoubtedly, therapy will continue to evolve at a rapid pace. Nevertheless, protease and RTIs remain the mainstay of therapy for HIV-infected adults and children [18, 19].

This study was the second study in a planned series of trials to evaluate promising combinations of these agents to identify well-tolerated and potent regimens. Here, we focus on the results after week 24, because the results up to week 24 have been reported elsewhere [3]. The treatment regimens in this study were reasonably well tolerated for >48 weeks, but moderate to severe toxicities were frequently encountered. Skin rashes were more frequently seen in patients in study treatment arms that contained NVP. However, adverse reactions seldom led to the discontinuation of study drugs, and virological failure was the

most common cause of discontinuation of the treatment assigned at the time of randomization. There was no significant difference between the toxicity profile of the regimen that contained NFV given twice daily and that of the same regimen that contained NFV given 3 times daily.

Overall, 50% of the children in the 4 main treatment groups achieved an undetectable HIV-1 RNA level, and 57% met the definition of having virological suppression. These results may be compared with responses seen in the recently completed AIDS Clinical Trials Group 338 study, which enrolled a similar population of antiretroviral drug–experienced, clinically stable children [20]. In that study, 42% of children receiving a 3-drug combination of ZDV, 3TC, and RTV, but only 26% of patients receiving RTV plus d4T had plasma viral RNA levels of <400 copies/mL after 48 weeks of treatment. Thus, there was evidence of the superiority of the 3-drug regimen that consisted of 2 nucleoside RTIs and a protease inhibitor. In the current study, we found that the 4-drug regimen d4T-3TC-NVP-NFV was superior to the 3-drug treatment regimens, although statistical significance was achieved only for comparison with d4T-NVP-NFV. When adjusted for multiple comparisons, however, this comparison was no longer statistically significant.

These virological responses are also comparable to those seen in a recent study of NFV and the nonnucleoside RTI efavirenz given in combination with nucleoside RTIs [14]. In the efavirenz study, ~76% of patients had plasma HIV-1 RNA levels of <400 copies/mL after 48 weeks of therapy. As in our study, a lower plasma HIV-1 RNA level at baseline was correlated with the likelihood of maintaining suppression of the plasma viral RNA to levels below detection.

Although minor differences in T cell parameters were initially seen among the different treatment arms, no significant differences were observed after 48 weeks of follow-up. This is not surprising because patients with severe immunosuppression were excluded from this trial. Moreover, the lack of significant differences may also indicate the inherent similarity of the study regimens for the treatment of children who have moderate immunosuppression. Note that virological responses and T cell changes were similar for those given the regimens including NFV either 2 or 3 times daily. Twice-daily dosing may make antiretroviral therapeutic regimens more manageable for some patients.

It is, however, disappointing that sustained suppression of viremia was seen in only approximately half of all patients. A variety of factors may have attenuated the therapeutic response seen in individual subjects. It has been noted repeatedly that HIV infection in infants and in young children is an explosive process characterized by a sustained high level of viremia [21–24]. Immunological immaturity and rapid somatic growth of early childhood may contribute to the unchecked nature of HIV replication in these young children. Recent and ongoing studies of the kinetic behavior of virus and cellular populations in children are likely to help clarify this issue [25, 26]. Antiinfective therapy in children is often complicated by the need to adjust for differences in body composition, metabolism, and clearance of therapeutic agents. Analysis of a series of nested pharmacokinetic substudies performed in the course of this trial may lead to a refinement of the dosing regimens used in this study.

In addition, previous antiretroviral therapy and the selection of drug-resistant variants probably contributed to virological failure in some cases. Although patients were not enrolled in the trial if they had experience with any of the study agents, one recent report has indicated that mutations that confer resistance to ZDV may confer resistance to d4T [27]. Genotypic analysis is currently underway to examine the frequency of resistance-associated mutations in HIV-1 plasma RNA at baseline and at the time of treatment failure in each of the study arms. Finally, adherence is likely to play a role in the success of regimens used in this study. Analysis of data from the pharmacokinetic substudies and from responses to an adherence questionnaire collected during this study may allow for a comprehensive assessment of factors that contribute to the success or failure of treatment regimens for HIV-infected children.

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