

ORIGINAL ARTICLE

## A Trial of Three Antiretroviral Regimens in HIV-1–Infected Children

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### ABSTRACT

#### BACKGROUND

Depletion of CD4 T-cell counts or progression of human immunodeficiency virus (HIV) disease occurs rapidly in children, but few data address the efficacy of aggressive therapy for HIV-infected children.

#### METHODS

We evaluated the safety, tolerability, and activity of three regimens of antiretroviral therapy in a multicenter, open-label, phase 1–2 trial. Children infected with HIV type 1 (HIV-1) were stratified at entry according to age — three months or younger (early therapy) or older than three months (delayed therapy) — and assigned sequentially to one of three regimens. Children continued to receive treatment for up to 200 weeks if the plasma HIV-1 RNA level was less than 1000 copies per milliliter by 16 weeks.

#### RESULTS

Plasma HIV-1 RNA levels fell from a median of 5.3 log copies per milliliter (range, 3.3 to 6.4 log copies per milliliter) at baseline to less than 1000 copies per milliliter at 16 weeks in 32 of 52 infants (62 percent). Plasma HIV-1 RNA levels were below 400 copies per milliliter at 48 weeks in 26 infants (50 percent) and at 200 weeks in 23 infants (44 percent). An intention-to-treat analysis revealed that significantly more children who received stavudine, lamivudine, nevirapine, and nelfinavir had plasma HIV-1 RNA levels of less than 400 copies per milliliter at 48 weeks (83 percent) and 200 weeks (72 percent) than children who received reverse-transcriptase inhibitors alone ( $P=0.001$  and  $P=0.01$ , respectively). Fewer infants in the delayed-therapy group than in the early-therapy group (30 percent vs. 60 percent) had plasma HIV-1 RNA levels of less than 400 copies per milliliter at 200 weeks ( $P=0.03$ ). Treatment-associated adverse effects were infrequent.

#### CONCLUSIONS

In this phase 1–2 trial involving HIV-1–infected children, an age of three months or younger at the initiation of therapy and treatment with stavudine, lamivudine, nevirapine, and nelfinavir were associated with improved long-term viral suppression. Larger, randomized trials are required to define the optimal time to initiate therapy and the optimal regimen for these infants.

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**A**MONG PATIENTS INFECTED WITH HUMAN immunodeficiency virus type 1 (HIV-1), viral replication plays an important role in the depletion of CD4 T cells and disease progression.<sup>1,2</sup> Few studies have addressed the optimal time to initiate antiretroviral therapy in infants. Timing is important because the depletion of CD4 T cells and disease progression occur more rapidly in children than in adults and because there are no reliable clinical or laboratory indicators of the risk of progression during the first year of life.<sup>3-7</sup> However, limited data address the efficacy of aggressive early therapy for HIV-1-infected infants. We therefore evaluated the rate and durability of viral suppression during the four years after the initiation of combination antiretroviral therapy in infants, as well as factors influencing the suppression of viral replication.

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## METHODS

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### STUDY DESIGN AND SUBJECTS

The Pediatric AIDS Clinical Trials Group Protocol 356 (PACTG 356) was an open-label, multicenter, phase 1-2 trial. Infants were eligible to enroll if at least one blood sample was positive for HIV-1 on the basis of a polymerase-chain-reaction (PCR) assay or culture and if HIV-1 was isolated from peripheral-blood mononuclear cells in another blood sample before therapy. Enrollment occurred between May 1997 and November 1998 at 25 clinical sites in the United States and Puerto Rico. Enrollees were stratified according to age — three months or younger (early therapy) and older than three months (delayed therapy) — and were sequentially enrolled in one of three treatment regimens, for a total of six cohorts (Table 1). All the children had received less than 10 weeks of prior therapy with nucleoside reverse-transcriptase inhibitors, and none had ever received protease inhibitors or nonnucleoside reverse-transcriptase inhibitors.

Height and weight were measured, a physical examination was performed, and laboratory evaluations (a complete blood count with a differential count, liver-function tests, and measurement of the serum glucose level, plasma HIV-1 RNA level, and peripheral-blood lymphocyte subgroups) were completed at the screening visit, at study entry before treatment was begun, every 4 weeks until week 24, every 8 weeks until week 56, and then every 12 weeks from weeks 56 to 200. All adverse events were graded with the use of the Division of AIDS Toxicity Ta-

bles for Grading Severity of Pediatric Adverse Experiences (where a score of 0 indicates no adverse events, a score of 1 mild adverse events, a score of 2 moderate adverse events, a score of 3 severe adverse events, and a score of 4 potentially life-threatening adverse events).

The human-subjects committees at each clinical site approved these studies. Written informed consent was obtained from the children's legal guardians. Guidelines of the Department of Health and Human Services governing experimentation in humans were followed.

### LABORATORY ANALYSES

HIV-1 RNA levels were measured with the use of a PCR assay (Roche Ultrasensitive Amplicor assay, version 1.0). The children continued taking study drugs if their plasma HIV-1 RNA level was less than 1000 copies per milliliter by 16 weeks; they were then followed for up to 200 weeks. Children who discontinued drug therapy for any reason underwent clinical and laboratory evaluations every 3 months for 1 year after the discontinuation of therapy (median duration of follow-up, 48 weeks).

The number of copies of HIV-1 proviral DNA was serially quantified in CD4 T cells with the use of a modification of the Roche Amplicor HIV-1 test system, as previously described.<sup>8</sup> Genotyping of viruses obtained in baseline plasma specimens for mutations associated with antiretroviral resistance was performed with the use of the TruGene HIV-1 Genotyping kit (Visible Genetics).

### STATISTICAL ANALYSIS

The primary statistical analysis used an intention-to-treat approach in which treatment was considered successful if children were still taking their original treatment regimen and had an HIV-1 RNA level of less than 1000 copies per milliliter at week 16 or less than 400 copies per milliliter between weeks 48 and 200, whereas treatment was considered to have failed in all other cases. Chi-square analyses were used to evaluate whether treatment success was significantly associated with the regimen, age at baseline (three months or younger vs. older than three months), and the presence or absence of prior antiretroviral exposure. Post hoc comparisons between regimens were contingent on statistically significant results of chi-square testing among all three regimens and consisted of pairwise chi-square tests. We used t-tests to examine whether children classified as having successful treatment differed signif-

**Table 1. Baseline Characteristics of the Study Cohort.\***

Age Group and Regimen	Total No. of Children (N=52)	Sex		CDC Stage†	History of ART		Median Duration of Prior ART wk	Median Age at Initiation of ART mo	Median CD4 T Cells %	Median Plasma HIV-1 RNA log copies/ml
		Female (N=30)	Male (N=22)		Mother	Child				
		no. of children			no. of women	no. of children				
<b>≤3 Mo at initiation of therapy</b>										
ZDV, 3TC, and NVP	8	4	4	N, 7 A, 1	ZDV, 4 ZDV and 3TC, 2	ZDV, 6 ZDV and d4T, 1	5.5 (2.0–10.0)	2.4 (0.5–2.9)	34.0 (15.0–47.0)	5.2 (4.3–6.2)
ZDV, 3TC, NVP, and ABC	8	6	2	N, 6 A, 1 B, 1	ZDV, 4 ZDV, 3TC, and NLF, 1	ZDV, 6 ZDV and ddl, 1	4.5 (2.0–10.0)	2.1 (0.8–3.0)	45.0 (24.0–63.0)	5.2 (3.3–6.3)
D4T, 3TC, NVP, and NLF	9	5	4	N, 6 A, 2 B, 1	ZDV, 1 ZDV and ddl, 1 ZDV, 3TC, and IDV, 1	ZDV, 5	6.0 (4.0–7.0)	1.9 (1.4–2.6)	34.0 (19.0–55.0)	5.4 (5.2–5.8)
<b>Total</b>	<b>25</b>	<b>15</b>	<b>10</b>	<b>N, 19 A, 4 B, 2</b>	<b>ZDV, 9 ZDV and 3TC, 2 ZDV and ddl, 1 ZDV, 3TC, and NLF, 1 ZDV, 3TC, and IDV, 1</b>	<b>ZDV, 17 ZDV and d4T, 1 ZDV and ddl, 1</b>	<b>5.5 (2.0–10.0)</b>	<b>2.0 (0.5–3.0)</b>	<b>36.0 (15.0–63.0)</b>	<b>5.3 (3.3–6.3)</b>
<b>&gt;3 Mo at initiation of therapy</b>										
ZDV, 3TC, and NVP	9	6	3	N, 4 A, 4 C, 1	ZDV, 3 ZDV and ddC, 1 ZDV, 3TC, and d4T, 1	ZDV, 4	6.0 (5.0–8.0)	7.6 (3.6–24.0)	38.0 (14.0–48.0)	5.4 (4.3–6.2)
ZDV, 3TC, NVP, and ABC	9	5	4	N, 4 A, 4 B, 1	ZDV, 2 ZDV, 3TC, and IDV, 1	ZDV, 3 ZDV and ddl, 1	6.0 (4.0–6.0)	11.0 (3.5–20.8)	39.0 (27.0–49.0)	4.8 (4.2–5.8)
D4T, 3TC, NVP, and NLF	9	4	5	N, 4 A, 4 C, 1	ZDV, 1, ZDV and 3TC, 1	ZDV, 3 ZDV and 3TC, 1	6.0 (2.0–6.0)	4.9 (4.2–10.5)	31.0 (15.0–63.0)	5.9 (4.7–6.4)
<b>Total</b>	<b>27</b>	<b>15</b>	<b>12</b>	<b>N, 12 A, 12 B, 1 C, 2</b>	<b>ZDV, 6 ZDV and ddC, 1 ZDV and 3TC, 1 ZDV, 3TC and d4T, 1 ZDV, 3TC, and IDV, 1</b>	<b>ZDV, 10 ZDV and ddl, 1 ZDV and 3TC, 1</b>	<b>6.0 (2.0–8.0)</b>	<b>7.6 (3.5–24.0)</b>	<b>34.0 (14.0–63.0)</b>	<b>5.4 (4.2–6.4)</b>
<b>All children</b>	<b>52</b>	<b>30</b>	<b>22</b>	<b>N, 31 A, 16 B, 3 C, 2</b>	<b>ZDV, 15 ZDV and 3TC, 3 ZDV and ddl, 1 ZDV and ddC, 1 ZDV, 3TC and d4T, 1 ZDV, 3TC, and NLF, 1 ZDV, 3TC, and IDV, 2</b>	<b>ZDV, 27 ZDV and d4T, 1 ZDV and ddl, 2 ZDV and 3TC, 1</b>	<b>6.0 (2.0–10.0)</b>	<b>3.5 (0.5–24.0)</b>	<b>36.0 (14.0–63.0)</b>	<b>5.3 (3.3–6.4)</b>

\* CDC denotes the Centers for Disease Control and Prevention, ART antiretroviral therapy, ZDV zidovudine, 3TC lamivudine, NVP nevirapine, d4T stavudine, ABC abacavir, ddl didanosine, NLF nelfinavir, IDV indinavir, and ddC zalcitabine.

† The four CDC stages reflect the extent of disease progression, where N denotes no signs or symptoms and A, B, and C increasingly severe disease.

icantly from those in whom treatment was considered to have failed with respect to baseline values of viral RNA, proviral DNA, and the percentage of CD4 T cells. We used logistic regression to examine the extent to which each of the factors listed above predicted success or failure, with adjustment for the other factors. In these logistic models, the regimen of stavudine, lamivudine, nevirapine, and nelfinavir served as the reference group and the other regimens were represented by dummy variables. The correlation between HIV-1 RNA and proviral DNA was examined by Spearman's rank test. Differences between age groups with respect to baseline levels of proviral DNA were assessed with the use of a Mann-Whitney U test. Changes from baseline in proviral DNA levels were tested by means of a Wilcoxon matched-pairs signed-rank test. The effect of differences in the age group, height, and weight on the change in the CD4 T-cell count from baseline to week 200 was examined by means of a Wilcoxon rank-sum test.

## RESULTS

### PATIENT POPULATION

Fifty-two children were enrolled (Table 1). The age at the initiation of therapy ranged from 0.5 to 3.0 months (median, 2.0; interquartile range, 1.5 to 2.6) in the early-therapy cohort and 3.5 to 24 months (median, 7.6; interquartile range, 4.8 to 11.5) in the delayed-therapy cohort (Table 1). Twenty-four (46 percent) mothers had received antiretroviral therapy before or during pregnancy or at both times. Thirty-one of the 52 children (60 percent) had received antiretroviral therapy before study entry. Twenty-seven of these 31 children (87 percent) received zidovudine monotherapy as attempted prophylaxis against HIV transmission. Four additional children who had not received prophylaxis had received dual therapy with nucleoside reverse-transcriptase inhibitors for several weeks before enrollment (range, 4 to 10 weeks).

Three children withdrew from the study before completing 16 weeks of therapy. In one child, therapy with zidovudine, lamivudine, nevirapine, and abacavir was discontinued after two weeks because of fevers and rash, which were thought to represent hypersensitivity to one of the study medications. Another infant withdrew at her parents' request after two weeks of therapy with zidovudine, lamivudine, and nevirapine. A third child withdrew after 13 weeks of therapy with zidovudine, lamivudine,

nevirapine, and abacavir because of the guardian's difficulty in attending clinic visits.

### VIRAL SUPPRESSION

Baseline plasma HIV-1 RNA levels ranged from 3.3 to 6.4 log copies per milliliter (median, 5.3 log copies per milliliter). Plasma HIV-1 RNA levels fell to less than 1000 copies per milliliter at 16 weeks in 32 of the 52 children (62 percent) (Table 2). Plasma HIV-1 RNA levels were less than 400 copies per milliliter at 48 weeks in 26 children (50 percent), and this change occurred after a median of 10 weeks of therapy (range, 0.4 to 20). At 200 weeks of therapy, plasma HIV-1 RNA levels were less than 400 copies per milliliter in 23 of these 26 children (88 percent) and less than 50 copies per milliliter in 22 of these 26 children (85 percent).

The effect of differences in the level of viral suppression among the three treatment regimens at week 16 (assessed according to the number of children with less than 1000 copies of HIV-1 RNA per milliliter) and weeks 48 and 200 (assessed according to the number of children with less than 400 copies per milliliter) was determined with chi-square tests. There were no significant differences among the treatment regimens at week 16 ( $P=0.17$ ), but superior virologic responses were observed among the children receiving stavudine, lamivudine, nevirapine, and nelfinavir at week 48 ( $P=0.001$ ) and week 200 ( $P=0.01$ ). In post hoc pairwise comparisons, the regimen of stavudine, lamivudine, nevirapine, and nelfinavir was superior to each of the other two regimens at week 48 (response rate, 83 percent;  $P<0.001$  for the comparison with zidovudine, lamivudine, and nevirapine and  $P=0.001$  for the comparison with zidovudine, lamivudine, nevirapine, and abacavir) and week 200 (response rate, 72 percent;  $P=0.01$  for the comparison with each of the other regimens) (Table 2). There were no significant differences in viral suppression rates between the regimens of reverse-transcriptase inhibitors at either time point (week 48,  $P=0.27$ ; week 200,  $P=1.00$ ).

### FACTORS INFLUENCING VIRAL SUPPRESSION

Since the children were enrolled sequentially rather than randomly assigned to one of the three regimens, we evaluated the effects of a number of variables to determine whether these factors affected the success of treatment. These potential confounders included age at the initiation of therapy (three months or younger vs. older than three months), the presence or absence of prior antiretroviral

**Table 2. Rates of Viral Suppression According to Qualitative Predictors of the Risk of Treatment Failure.\***

Predictor	Total No. of Patients	Week 16		Week 48		Week 200	
		<1000 HIV-1 RNA Copies/ml <i>no. (%)</i>	P Value	<400 HIV-1 RNA Copies/ml <i>no. (%)</i>	P Value	<400 HIV-1 RNA Copies/ml <i>no. (%)</i>	P Value
<b>Regimen</b>							
ZDV, 3TC, and NVP	17	8 (47)	0.17	4 (24) <sup>†</sup>	0.001	5 (29) <sup>‡</sup>	0.01
ZDV, 3TC, NVP, and ABC	17	10 (59)		7 (41) <sup>§</sup>		5 (29) <sup>‡</sup>	
d4T, 3TC, NVP, and NLF	18	14 (78)		15 (83)		13 (72)	
<b>Age at initiation of therapy</b>							
≤3 Mo	25	16 (64)	0.73	15 (60)	0.16	15 (60)	0.03
>3 Mo	27	16 (59)		11 (41)		8 (30)	
<b>Prior antiretroviral treatment</b>							
Yes	31	17 (55)	0.23	16 (52)	0.78	13 (42)	0.69
No	21	15 (71)		10 (48)		10 (48)	

\* ZDV denotes zidovudine, 3TC lamivudine, NVP nevirapine, ABC abacavir, D4T stavudine, and NLF nelfinavir.

<sup>†</sup> P<0.001 for the comparison with stavudine, lamivudine, nevirapine, and nelfinavir.

<sup>‡</sup> P=0.01 for the comparison with stavudine, lamivudine, nevirapine, and nelfinavir.

<sup>§</sup> P=0.001 for the comparison with stavudine, lamivudine, nevirapine, and nelfinavir.

therapy, the percentage of CD4 T cells at baseline, baseline plasma HIV-1 RNA levels, and the baseline levels of proviral DNA in peripheral-blood mononuclear cells. There was no significant association between the age at the initiation of therapy or the presence or absence of prior antiretroviral treatment and the likelihood of viral suppression at week 16 or 48 (Table 2). Moreover, prior receipt of antiretroviral therapy did not predict viral suppression at week 200. However, age was a significant predictor of virologic success at week 200, with a lower rate of virologic response in the delayed-therapy group than in the early-therapy group (30 percent vs. 60 percent, P=0.03) (Table 2). Patients classified as having a virologic response at weeks 16, 48, and 200 did not differ significantly from those without a virologic response at those time points with respect to baseline percentage of CD4 T cells, baseline HIV-1 RNA levels, or baseline levels of proviral DNA (Table 3).

Tests for potential confounding differences among the three treatment regimens showed no significant differences with respect to age group (P=0.98), the presence or absence of prior antiretroviral treatment (P=0.59), or baseline percentage of CD4 T cells (P=0.51). A significant difference among the regimens in baseline HIV-1 RNA levels was observed (P=0.04). However, children who

were receiving the regimen of stavudine, lamivudine, nevirapine, and nelfinavir had the highest baseline levels of HIV-1 RNA (mean, 5.6 log copies per milliliter). Thus, the subsequent superiority of this regimen was apparent despite the higher baseline viral load.

A final test for potential confounding consisted of logistic-regression models, with viral suppression at weeks 16, 48, and 200 as outcome variables and predictors consisting of the treatment regimen and the potential confounders listed above. The baseline level of proviral DNA was excluded from these models, since it was not measured in children who were receiving the three-drug regimen and was not a significant predictor of virologic success for the other two regimens. With one exception, the results were consistent with those of the univariate analyses: none of the variables had statistically significant coefficients in the model predicting success at week 16; at weeks 48 and 200, the differences between the regimen of stavudine, lamivudine, nevirapine, and nelfinavir and each of the other two regimens remained significant, after adjustment for the other variables in the models; and the effect of age at the initiation of therapy, which emerged at week 200, remained significant after adjustment for the other variables. The one case in which the logistic-regression results differed from those of the

**Table 3. Quantitative Predictors of Virologic Success or Failure.\***

Predictor	Week 16			Week 48			Week 200		
	<1000 HIV-1 RNA Copies/ml	≥1000 HIV-1 RNA Copies/ml	P Value	<400 HIV-1 RNA Copies/ml	≥400 HIV-1 RNA Copies/ml	P Value	<400 HIV-1 RNA Copies/ml	≥400 HIV-1 RNA Copies/ml	P Value
	Baseline proviral DNA			0.59			0.76		
No. of patients	20	7		18	9		15	12	
Mean log copies/million CD4 T cells	3.62	3.45		3.55	3.64		3.51	3.67	
Baseline HIV-1 RNA			0.73			0.67			0.52
No. of patients	32	20		26	26		23	29	
Mean log copies/million CD4 T cells	5.22	5.29		5.21	5.29		5.18	5.30	
Baseline CD4 T cells			0.84			0.75			0.67
No. of patients	32	20		26	26		23	29	
Mean percentage	36.0	35.3		36.3	35.2		34.9	36.4	

\* P values were calculated with Student's t-test.

univariate analysis was that low baseline levels of HIV-1 RNA emerged as a significant predictor of virologic success at week 200. In the univariate analysis, this effect may have been masked by the significant difference among the three treatment groups in the baseline viral load.

#### PROVIRAL DNA LOAD

Baseline proviral DNA levels ranged from 60 to 52,138 copies per million CD4 T cells (median, 4667). Among the children who had not previously received therapy or who had not received therapy for more than six months before study entry, a positive correlation was observed between the baseline plasma HIV-1 RNA level and peripheral-blood mononuclear-cell levels of proviral DNA ( $r=0.59$ ,  $P=0.02$ ). Median baseline levels of proviral DNA in the early-therapy group did not differ significantly from those in the delayed-therapy group (3504 vs. 8273 copies per million CD4 T cells,  $P=0.08$ ).

In children with plasma HIV-1 RNA levels of less than 50 copies per milliliter at 48 weeks, the proviral DNA load in peripheral-blood mononuclear cells was significantly lower than the baseline level (median reduction, 1 log copy per milliliter;  $P<0.001$ ). The proviral load declined slightly thereafter but was not significantly different from the value at week 48 at subsequent time points ( $P>0.2$ ).

#### CD4 T-CELL COUNTS

At baseline, the median percentages of CD4 T cells were 36 percent in the early-therapy group and 34 percent in the delayed-therapy group. Only 4 of 25

children in the early-therapy group (16 percent) and 5 of 27 in the delayed-therapy group (19 percent) had CD4 T-cell percentages below 25 percent. The values increased to or remained at normal levels for age in all 23 children with plasma HIV-1 RNA levels of less than 400 copies per milliliter at week 200 but decreased over time in children in whom the suppression of viral replication was transient. However, the difference was not significant ( $P=0.19$ ).

#### CLINICAL STATUS

Although the primary objective of the study was to evaluate the suppression of viral replication, growth and HIV-1-associated symptoms were monitored. Changes from baseline in z scores for weight and height did not differ between children with and those without suppression at week 200 ( $P=0.16$  and  $P=0.63$ , respectively). Eight children who discontinued study treatment before week 200 had HIV-1-related disease progression (from Centers for Disease Control and Prevention [CDC] clinical stage N to stage A in seven and from CDC stage A to stage B in one). A change from CDC stage N to stage C was reported in one child in the early-therapy group who had a plasma HIV-1 RNA level of less than 400 at 200 weeks (because of developmental delay of uncertain cause at study week 176).

#### ADVERSE EVENTS

Twenty-six adverse clinical or laboratory events of moderate or greater severity (i.e., more than grade 2) that were probably or possibly related to therapy

**Table 4. Adverse Events Related or Possibly Related to Treatment.\***

Regimen and Age at Initiation of Therapy	Patient	Adverse Event	Grade	Week of Event	Last Week of Therapy
ZDV, 3TC, and NVP					
≤3 mo	1236	Rash	2	1	28
>3 mo	1213	Neutropenia	2	1, 2, and 17	66
		Anemia	2	2	
ZDV, 3TC, NVP, and ABC					
≤3 mo	1235	Rash	2	1	200
	1232	Neutropenia	3 and 4	3	200
	1238	Neutropenia	2–4	1, 4, 5, 7, 19, and 40	200
	1247	Neutropenia	2–4	1, 2, 8, 16, 20, 25, and 56	200
	1242	Rash	2 and 3	2	2
>3 mo	1254	Anemia	2	1, 2	40
d4T, 3TC, NVP, and NLF		None			

\* ZDV denotes zidovudine, 3TC lamivudine, NVP nevirapine, ABC abacavir, d4T stavudine, and NLF nelfinavir.

were reported in eight children (Table 4). All the adverse events were reported in infants who were receiving one of the two regimens of reverse-transcriptase inhibitors; no treatment-related adverse effects were reported among children who were receiving the regimen of stavudine, lamivudine, nevirapine, and nelfinavir.

#### RESISTANCE MUTATIONS

HIV-1 reverse-transcriptase and protease genes were sequenced from plasma specimens obtained before therapy from 47 of the children (90 percent). Children with viruses that could not be sequenced did not differ from children with viruses that could be sequenced with regard to baseline levels of plasma HIV-1 RNA or CD4 T cells or the presence or absence of prior antiretroviral therapy. Mutations associated with resistance to one or more reverse-transcriptase inhibitors were detected in pretherapy plasma samples from 4 of the 47 children (9 percent) (Table 5). All four children had mutations associated with at least one agent to which neither the children nor their mothers had been exposed.

Poor long-term suppression of viral replication was observed in three infants whose regimens included one or more agents to which resistance was detected at baseline (Table 5 and Fig. 1), despite reported adherence to therapy. Marked increases in the mean corpuscular volume of these infants' red cells, along with trough plasma nevirapine levels exceeding 1500 ng per milliliter at four weeks, also

suggested that there was good adherence to zidovudine and nevirapine therapy. By contrast, a child with baseline T215N/Y/S mutations who received stavudine, lamivudine, nevirapine, and nelfinavir had durable suppression of plasma HIV-1 RNA levels to less than 50 copies per milliliter through 200 weeks. Primary mutations associated with resistance to protease inhibitors were not detected in any children.

#### VIRAL REBOUND

Viral replication was initially controlled in two children who began therapy at three months of age or earlier (plasma HIV-1 RNA level, less than 50 copies per milliliter) but subsequently rebounded, exceeding  $10^4$  copies per milliliter in each child, in association with a decline in CD4 T-cell counts. These data suggest that residual virus in children with initial suppression of viral replication is fully replication competent and that a transient early reduction of viral load does not provide lasting benefit.

#### DISCUSSION

Our study addressed the safety and antiviral activity of three antiretroviral regimens in HIV-1-infected children. Treatment-related adverse clinical or laboratory events were infrequent, and only 1 of the 52 children discontinued therapy because of drug-related adverse effects. In intention-to-treat analyses, there were no significant differences among

**Table 5. Antiretroviral Resistance Mutations Detected in the Plasma of Four Children before Therapy.\***

Patient No.	Age <i>mo</i>	HIV-1 RNA <i>log copies/ml</i>	Antiretroviral Therapy			Resistance Mutations	
			Mother	Child	Regimen	Entry	Wk 24
1240	3.6	6.2	ZDV	ZDV for 6 wk	ZDV, 3TC, NVP	M41L, M184V, T215Y	V106A, M184V
1218	10	4.9	None	None	ZDV, 3TC, NVP	K70K/R, T215D/Y/S	K103R, V106A, V179D, M184V
1259	5.0	6.4	None	None	d4T, 3TC, NVP, NLF	T215N/Y/S	ND
1225	3.9	5.4	ZDV	ZDV for 6 wk	ZDV, 3TC, NVP	V108I	K101E, M184V, G190A, T215Y

\* M41L confers low-level resistance to zidovudine; K70K/R is a mixture of wild type and K70R, which causes low-level resistance to zidovudine; K101E confers low-level resistance to nevirapine; K103R does not cause resistance; V106A confers high-level resistance to nevirapine; V108I confers low-level resistance to nevirapine; V179D confers low-level resistance to nevirapine; M184V confers high-level resistance to lamivudine; G190A confers high-level resistance to nevirapine; T215Y confers intermediate resistance to zidovudine; T215D/Y/S is a mixture of mutations (T215Y, which confers intermediate resistance to zidovudine, and T215D/S, which are transitional mutations between wild type and the Y mutation and do not reduce drug susceptibility); and T215N/Y/S is a mixture of mutations (T215Y, which confers intermediate resistance to zidovudine, and T215S/N, which are transitional mutations between wild type and the Y mutation and do not reduce drug susceptibility). ZDV denotes zidovudine, 3TC lamivudine, NVP nevirapine, d4T stavudine, NLF nelfinavir, and ND not done (plasma HIV-1 RNA level, less than 50 copies per milliliter).

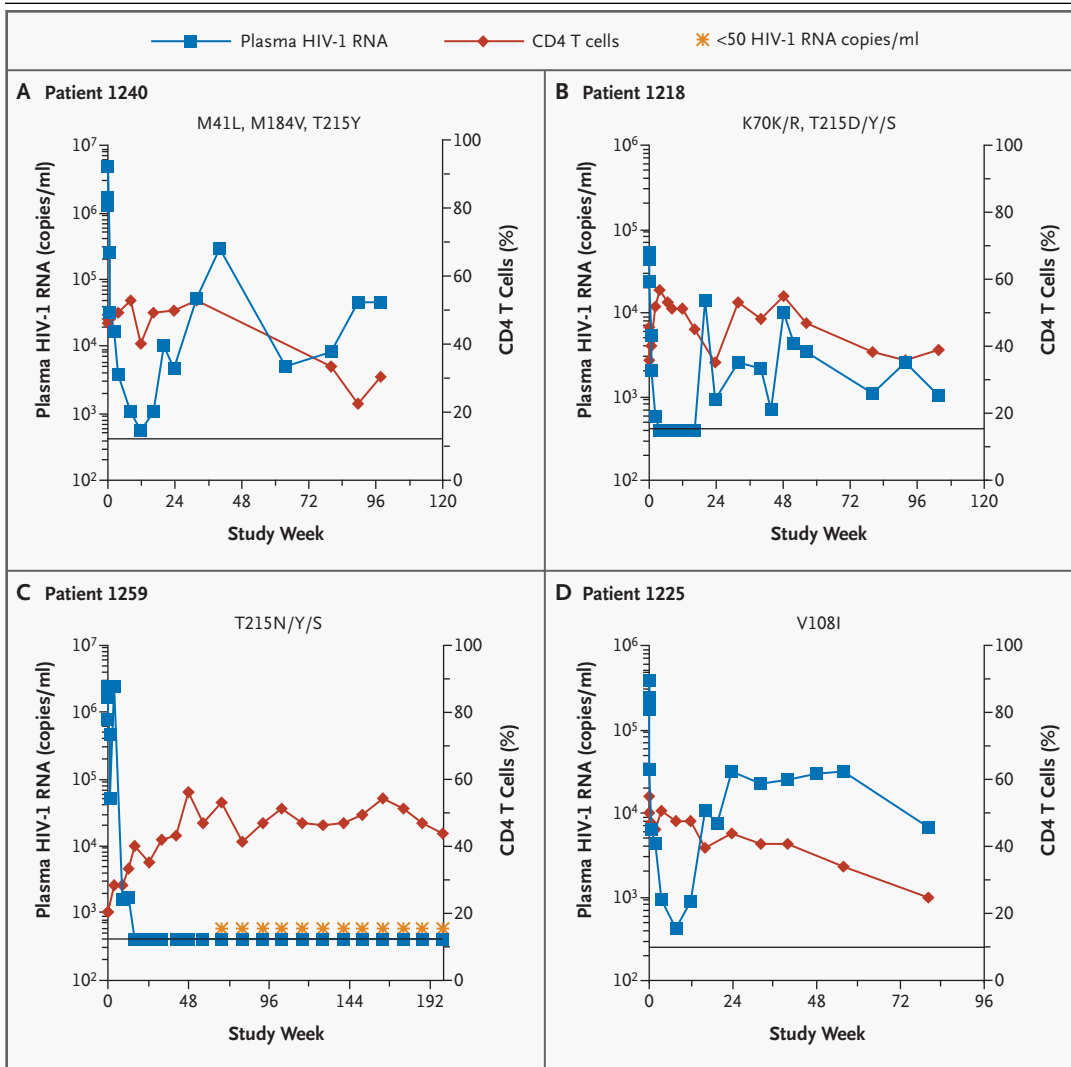
the treatment regimens in the rate of viral suppression at week 16, but the regimen of stavudine, lamivudine, nevirapine, and nelfinavir appeared to be superior to the two regimens of reverse-transcriptase inhibitors in suppressing viral replication at weeks 48 and 200. Early initiation of therapy (at three months of age or earlier) also appeared to be associated with improved long-term suppression of viral replication. In this small group of infants, detection of resistance mutations to reverse-transcriptase inhibitors before therapy was associated with a poor long-term response to the triple-drug (zidovudine, lamivudine, and nevirapine) regimen.

During the past few years, there has been a great deal of discussion about when to begin antiretroviral therapy. The recently revised U.S. guidelines for adults now recommend delaying therapy until symptoms develop,<sup>9</sup> CD4 T-cell counts fall below 350 per cubic millimeter, or plasma HIV-1 RNA levels exceed 55,000 copies per milliliter. However, disease progression occurs more rapidly in HIV-1-infected children than in adults.<sup>4,5,10</sup> Current U.S. pediatric guidelines thus recommend therapy for all infected infants less than 12 months of age who have symptoms or immune suppression and recommend consideration of therapy for asymptomatic infected infants under 12 months of age who have normal immune status.<sup>11</sup> European guidelines rec-

ommend the initiation of therapy in young infants only if they have any of the following findings: clinical acquired immunodeficiency syndrome, less than 20 percent CD4 T cells, or HIV-1 RNA levels that persistently exceed  $10^6$  copies per milliliter.<sup>12</sup>

Our data and others' provide increasing evidence that several regimens of antiretroviral therapy are safe, effective, and well tolerated during years of administration when started in infancy.<sup>13</sup> Although this trial was not randomized, our data suggest that an age of no more than three months at the initiation of therapy and treatment with stavudine, lamivudine, nevirapine, and nelfinavir are associated with improved long-term viral suppression. These findings have important implications and deserve further evaluation in large, randomized trials. However, since the tempo of disease progression is often accelerated in young HIV-1-infected children and since we lack reliable predictors of outcome in the first year of life,<sup>7</sup> it seems reasonable to consider early initiation of antiretroviral therapy when feasible until further data are available.<sup>3,4,6</sup>

In the United States and other developed countries, the use of perinatal antiretroviral regimens has markedly reduced mother-to-child transmission of HIV. Globally, however, more than 2000 children every day acquire HIV-1 infection from their mothers, 90 percent of whom reside in developing coun-



**Figure 1. Peripheral-Blood Viral Load and CD4 T-Cell Counts over Time in Infants with Antiretroviral-Therapy Resistance Mutations Detected at Baseline.**

M41L confers low-level resistance to zidovudine; K70K/R is a mixture of wild type and K70R, which causes low-level resistance to zidovudine; V108I confers low-level resistance to nevirapine; M184V confers high-level resistance to lamivudine; T215Y confers intermediate resistance to zidovudine; T215D/Y/S is a mixture of mutations (T215Y, which confers intermediate resistance to zidovudine, and T215D/S, which are transitional mutations between wild type and the Y mutation and do not reduce drug susceptibility); and T215N/Y/S is a mixture of mutations (T215Y, which confers intermediate resistance to zidovudine, and T215S/N, which are transitional mutations between wild type and the Y mutation and do not reduce drug susceptibility). The horizontal line in each graph represents the lower limit of detection of the assay for HIV-1 RNA.

tries. Current efforts focused on implementing antiretroviral therapy to prevent mother-to-child transmission in developing countries should be expanded to develop early diagnostic capabilities, as well as simplified, effective treatment strategies for HIV-1-infected women and their children. Some investigators have suggested that intensive treatment

during the first year of life alone may limit treatment costs and improve the outcome. However, our data demonstrating the rapid resumption of viral replication with subsequent depletion of CD4 T cells in children with initial suppression of viral replication suggest that this strategy will not be successful in the long term. The combination of early antiretrovi-

ral therapy with vaccination to generate and maintain HIV-1-specific immune responses may allow the development of more sparing antiretroviral therapy strategies in such patients.

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#### APPENDIX

The other PACTG 356 Investigators were R. Rutstein (Children's Hospital of Philadelphia), H. Gay (University of Mississippi), W. Borkowsky (New York University), R. van Dyke (Tulane), B. Stechenberg (Baystate Medical Center), K. McIntosh (Boston Children's Hospital), J. Farley (University of Maryland), S. Pelton (Boston Medical Center), A. Petru (Children's Hospital Oakland), S. Spector (University of California, San Diego), V. Bonagura (Schneider Children's Hospital), C. Cunningham (SUNY Syracuse), A. Deveikis (Miller Children's Hospital Long Beach), A. Ruff (Johns Hopkins), W. Shearer (Texas Children's Hospital Baylor), R. McKinney (Duke University), S. Bakshi (North Shore University Hospital), T. Rubio (Children's Hospital of King's Daughters), K. Rich (University of Illinois), S. Gaur (University of Medicine and Dentistry of New Jersey—Robert Wood Johnson Medical School), G. Johnson (Medical University of South Carolina), S. Rana (Howard University Hospital), J. Sleasman (University of Florida Gainesville), and I. Febo (University of Puerto Rico).

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