COMBINATION THERAPY WITH EFAVIRENZ, NELFINAVIR, AND NUCLEOSIDE REVERSE-TRANSCRIPTASE INHIBITORS IN CHILDREN INFECTED WITH HUMAN IMMUNODEFICIENCY VIRUS TYPE 1

STUART E. STARR, M.D., COURTNEY V. FLETCHER, PHARM.D., STEPHEN A. SPECTOR, M.D., FLORENCE H. YONG, M.S., TERENCE FENTON, ED.D., RICHARD C. BRUNDAGE, PHARM.D., PH.D., DOUGLAS MANION, M.D., NANCY M. RUIZ, M.D., MERRIL GERSTEN, M.D., MARK BECKER, PHARM.D., JAMES MCNAMARA, M.D., AND LYNNE M. MOFENSON, M.D., FOR THE PEDIATRIC AIDS CLINICAL TRIALS GROUP 382 TEAM*

ABSTRACT

Background Consistent long-term viral suppression has been difficult to achieve in children with human immunodeficiency virus type 1 (HIV-1) infection. We tested the safety and antiviral efficacy of a novel combination consisting of efavirenz, nelfinavir, and one or more nucleoside reverse-transcriptase inhibitors in 57 children previously treated with only nucleoside reverse-transcriptase inhibitors.

Methods The children were monitored for 48 weeks after the initiation of therapy. We assessed plasma concentrations of efavirenz and nelfinavir, plasma HIV-1 RNA levels, and lymphocyte subpopulations.

Results At base line, the 57 HIV-1–infected children (age range, 3.8 to 16.8 years) had a median of 699 CD4 cells per cubic millimeter and 10,000 copies of HIV-1 RNA per milliliter of plasma. The most common treatment-related effects of at least moderate severity were rash (in 30 percent of children), diarrhea (in 18 percent), neutropenia (in 12 percent), and biochemical abnormalities (in 12 percent). Serious side effects were uncommon. The mean values for the area under the curve for efavirenz and nelfinavir corresponded to expected values. In an intention-to-treat analysis, 76 percent of children had plasma HIV-1 RNA levels of less than 400 copies per milliliter after 48 weeks of therapy and 63 percent had levels of less than 50 copies per milliliter. A high plasma HIV-1 RNA level at base line significantly decreased the likelihood that plasma levels of HIV-1 RNA would become undetectable during treatment.

Conclusions In HIV-1–infected children who were previously treated with nucleoside reverse-transcriptase inhibitors, the combination of efavirenz, nelfinavir, and nucleoside reverse-transcriptase inhibitors was generally well tolerated and had a potent and sustained antiviral effect. (N Engl J Med 1999;341:1874-81.)

A MAJOR objective of antiretroviral therapy is to reduce the amount of human immunodeficiency virus type 1 (HIV-1) RNA in plasma to undetectable levels, because these levels appear to reflect the degree of viral replication in the body. Even low levels of replication may contribute to the emergence of resistant strains of HIV-1. Studies of highly active antiretroviral therapy have generally yielded less impressive results in HIV-infected children than in infected adults. In small studies of combinations that included a protease inhibitor in children who had previously been treated with nucleoside reverse-transcriptase inhibitors, plasma HIV-1 RNA levels decreased to undetectable levels (less than 400 copies per milliliter) in 39 to 78 percent, but subsequently rebounded in 20 to 44 percent.[1-5] In another study, only two of eight HIV-1–infected infants had undetectable plasma HIV-1 RNA levels after combination therapy with zidovudine, didanosine, and nevirapine.[6] Clearly, other promising antiretroviral combinations need to be tested in children.

Efavirenz, a new nonnucleoside reverse-transcriptase inhibitor, has good oral bioavailability and a long terminal half-life, permitting once-daily dosing.[7,8] We tested efavirenz in combination with a protease inhibitor in children, the majority of whom were receiving one or more nucleoside reverse-transcriptase inhibitors, in accordance with recommendations that at least two new antiretroviral drugs be added to an existing regimen.[9] Nelfinavir was selected as the protease inhibitor because it is available in a pediatric formulation, has approved dosing recommendations for children older than two years of age, and has no pharmacokinetic interactions with efavirenz.[10]

METHODS

Study Design

This open-label study was conducted at 18 Pediatric AIDS Clinical Trials Group (PACTG) sites. Eligibility criteria included

From Children's Hospital of Philadelphia, Philadelphia (S.E.S.); the University of Minnesota, Minneapolis (C.V.F., R.C.B.); the University of California at San Diego, San Diego (S.A.S.); Harvard School of Public Health, Boston (F.H.Y.); Frontier Science and Technology Research Foundation, Brookline, Mass. (T.F.); Dupont Pharmaceuticals Company, Wilmington, Del. (D.M., N.M.R.); Agouron Pharmaceuticals, La Jolla, Calif. (M.G., M.B.); the National Institute of Allergy and Infectious Diseases, Rockville, Md. (J.M.); and the National Institute of Child Health and Human Development, Rockville, Md. (L.M.M.). Address reprint requests to Dr. Starr at the Division of Immunologic and Infectious Diseases, Children's Hospital of Philadelphia, 34th St. and Civic Center Blvd., Philadelphia, PA 19104, or at starr@email.chop.edu.

Other authors were Lynette Purdue, Pharm.D., National Institute of Allergy and Infectious Diseases, Rockville, Md.; Suzanne Siminski, M.S., and Bobbie Graham, B.S., Frontier Science and Technology Research Foundation, Amherst, N.Y.; David M. Kornhauser, M.D., and William Fiske, Ph.D., Dupont Pharmaceuticals Company, Wilmington, Del.; Carol Vincent, C.R.N.P., Children's Hospital of Philadelphia, Philadelphia; Harold W. Lischner, M.D., St. Christopher's Hospital for Children, Philadelphia; Wayne M. Dankner, M.D., University of California at San Diego, San Diego; and Patricia M. Flynn, M.D., St. Jude Hospital for Children, Memphis, Tenn.

*Other participants in the study are listed in the Appendix.

an age of less than 16 years, a plasma HIV-1 RNA level of more than 400 copies per milliliter on measurement with a quantitative RNA reverse-transcriptase–polymerase chain reaction assay (Amplicor HIV-1 Monitor assay, Roche Diagnostic Systems, Branchburg, N.J.), no previous treatment with nonnucleoside reverse-transcriptase inhibitors or protease inhibitors, concomitant treatment with at least one nucleoside reverse-transcriptase inhibitor, and the ability to swallow capsules. The children were enrolled between October 27, 1997, and February 12, 1998.

Efavirenz (Sustiva, Dupont Pharmaceuticals, Wilmington, Del.) was provided by the manufacturer in 50-mg, 75-mg, and 100-mg capsules. Nelfinavir (Viracept, Agouron Pharmaceuticals, La Jolla, Calif.) was provided by the manufacturer in tablet (250 mg) and powder (50 mg per gram) form. The initial doses of efavirenz were allometrically scaled to body size according to the following formula: the dose (in milligrams per day) = (the weight of the child in kilograms ÷ 70)$^{0.7}$ × 600. The doses were rounded to the nearest 25-mg increment. Efavirenz was given daily in the morning. The initial dose of nelfinavir was the recommended pediatric dose of 20 to 30 mg per kilogram of body weight thrice daily. At entry, the children continued to take the same nucleoside reverse-transcriptase inhibitors or were switched to new ones, at the discretion of the site investigators.

The doses of efavirenz and nelfinavir were adjusted if the area under the curve was too small. The target value for the area under the curve from 0 to 24 hours was 190 to 380 μmol per liter · hour for efavirenz, representing the range from the 50th percentile to twice the 50th percentile of such values in adults receiving 600 mg per day of efavirenz. This target value was selected to help ensure that therapeutic levels would be achieved in this pediatric study. For nelfinavir, the target value for the area under the curve from zero to eight hours was 10 mg per liter · hour, the 10th percentile for adults receiving 750 mg of nelfinavir thrice daily. The areas under the curve for efavirenz and nelfinavir were determined at weeks 2 and 6 and again at week 10, if necessary. Blood samples were obtained before a dose and 2, 5, 6, 8, 12, and 24 hours after a dose. If target values for the area under the curve were not achieved, the doses of efavirenz, nelfinavir, or both were adjusted proportionally.

History taking and physical examinations were performed at base line; at weeks 2, 4, 6, and 8; and every four weeks thereafter. A complete blood count and differential count were obtained and blood chemical analysis and urinalysis were performed at base line and at weeks 2, 4, 8, 12, 16, 20, 24, 32, 40, and 48. Specimens for the determination of plasma HIV-1 RNA levels and lymphocyte subpopulations were obtained at base line and at weeks 2, 4, 8, 12, 20, 32, 40, and 48.

We used the Division of AIDS Toxicity Table to grade the severity of adverse effects. A grade of 0 indicates the absence of adverse effects; a grade of 1 indicates mild adverse effects, a grade of 2 moderate effects, a grade of 3 severe effects, and a grade of 4 life-threatening effects. Treatment was discontinued if a child had grade 4 effects, repeated grade 3 effects, or poor compliance, defined as consumption of less than 80 percent of the doses of efavirenz or nelfinavir over a one-month period. Virologic failure was defined as a reduction in plasma HIV-1 RNA levels of less than 1 log (on a logarithmic [base 10] scale) by week 12, HIV-1 RNA levels of more than 1000 copies per milliliter on two successive measurements in children whose levels had been less than 400 copies per milliliter, or HIV-1 RNA levels that were more than 0.75 log copies above the nadir value on two successive occasions in children whose plasma HIV-1 RNA levels had remained detectable.

The institutional review boards at each site approved the study. Written informed consent was obtained from the parents or guardians of all children.

Measurement of Plasma Efavirenz and Nelfinavir Concentrations

Concentrations of efavirenz and nelfinavir in plasma were measured by high-performance liquid chromatography. The total variability (expressed as a composite of within-day and day-to-day variability) of the efavirenz and nelfinavir assays was 1 to 4.5 percent and 1.7 to 10.3 percent, respectively.

Determination of Lymphocyte Subpopulations and Quantitation of Plasma HIV-1 RNA

The percentages and absolute numbers of CD4 cells were determined in PACTG-certified laboratories according to the PACTG consensus protocol for flow cytometry.[11] Plasma was stored at −70°C, and HIV-1 RNA was measured with a quantitative assay (Amplicor, Roche Diagnostic) with a lower limit of quantitation of 400 copies per milliliter. Samples containing less than 400 copies per milliliter were tested with an ultrasensitive assay with a lower limit of quantitation of 50 copies per milliliter. All RNA assays were performed in a single PACTG-certified laboratory.

Statistical Analysis

Primary efficacy was assessed on the basis of plasma HIV-1 RNA levels. For each child, a series of composite HIV-1 RNA measures was constructed as follows: the result of the quantitative assay was used, unless it was less than 400 copies per milliliter or was not available, in which case the result of the ultrasensitive assay was used. Log-transformed values were used for analyses.

Three types of analysis were used to evaluate primary efficacy: an analysis according to the intention to treat, in which all HIV-1 RNA levels were included, regardless of whether a child was still receiving drugs; an analysis in which only the HIV-1 RNA levels of children who were receiving treatment were included; and an analysis in which HIV-1 RNA levels were assigned a value of more than 400 copies per milliliter for all visits after the discontinuation of treatment (or a value of more than 50 copies per milliliter with the use of the ultrasensitive assay). Missing HIV-1 RNA values were assigned as a value of more than 400 copies per milliliter (or more than 50 copies per milliliter) if the first value preceding or following the missing value exceeded 400 (or 50) copies per milliliter. No value was imputed for a missing result if preceding and succeeding values were both less than 400 or less than 50 copies per milliliter.

We calculated exact 95 percent confidence intervals for the percentage of children with a virologic response at each visit, assuming a binomial distribution. We determined the median change in plasma HIV-1 RNA levels from base line and corresponding 95 percent confidence intervals according to the method of Brookmeyer and Crowley, in which HIV-1 RNA levels below the limit of detection were treated as censored observations.[12]

All other analyses used an intention-to-treat approach unless otherwise noted. The times to various outcomes were estimated according to the Kaplan–Meier method.[13] We used the Cox proportional-hazards regression model to estimate relative risks and corresponding 95 percent confidence intervals.[14] We used a stepwise proportional-hazards model to assess the relative importance of risk factors in predicting the likelihood that plasma HIV-1 RNA levels would become undetectable. Weight measurements were converted to z scores after adjustment for age and sex.[15] The effect of treatment on CD4 measures was examined with use of the Wilcoxon signed-rank test (pairwise comparison), and the one-sample application of the Wei–Johnson test, in which components of the global test statistic were weighted by reciprocals of variances of the test statistic at each time.[16] The 95 percent confidence intervals for the median changes in the absolute numbers and percentages of CD4 cells were based on order statistics.[17] The Wilcoxon rank-sum test was used to compare the duration of rash between children.[18] All reported P values are two-sided.

RESULTS

Base-Line Characteristics

The base-line characteristics of the 57 children who were enrolled in the study are shown in Table 1. Before enrollment, 55 children were receiving one or more nucleoside reverse-transcriptase inhibitors. Thir-

ty-four children (60 percent), including 2 who were not already receiving nucleoside reverse-transcriptase inhibitors, began to receive at least one new nucleoside reverse-transcriptase inhibitor within two weeks after study entry, 21 (37 percent) had no change in their regimen of nucleoside reverse-transcriptase inhibitors, and 2 (4 percent) had one nucleoside reverse-transcriptase inhibitor dropped from their regimen.

Adverse Effects

Twenty-six children (46 percent) had either no adverse effects or no more than mild effects, 25 (44 percent) had no more than moderate effects, 5 (9 percent) had severe effects, and 1 (2 percent) had a life-threatening adverse effect. The most common adverse effects of at least moderate severity were rash (17 children; 30 percent), diarrhea (10 children; 18 percent), neutropenia (7 children; 12 percent), and biochemical abnormalities (7 children; 12 percent). Most rashes (88 percent) appeared within 14 days after the initiation of study treatment (median, 9; range, 6 to 205) and lasted for a median of 6 days (range, 2 to 37). Most rashes were maculopapular and pruritic. One child had target lesions juxtaposed with a maculopapular rash, and two children had urticarial lesions. None of the children with a moderate rash were febrile at the time of the rash, and none had mucous-membrane involvement. Severe adverse effects consisted of neutropenia in two children and hepatic toxicity, diarrhea and neutropenia, and rash with fever (temperature, >39°C) in one child each. The only life-threatening adverse effect was neutropenia in a child with concurrent varicella. Central nervous system toxicity was uncommon. Eight children had mild dizziness or lightheadedness that resolved once efavirenz was given at bedtime rather than in the morning.

Treatment was discontinued in 14 children. One child was found to be ineligible owing to prior use of nevirapine. Another was unable to take efavirenz capsules. Treatment was discontinued at the request of the parent, guardian, or investigator in four children in whom a moderate rash developed and, as specified by the protocol, in one child who had a severe rash. None of these children resumed treatment after the rash resolved. The median duration of rash in the children who discontinued treatment was six days, as compared with nine days in those who continued treatment ($P=0.14$). Other reasons for discontinuation were virologic failure (six children) and noncompliance (one child).

Table 1. Base-Line Characteristics of the 57 Children in the Study.

Characteristic	Value
Sex — no. (%)	
Female	37 (65)
Male	20 (35)
Race or ethnic group — no. (%)	
Non-Hispanic white	9 (16)
Non-Hispanic black	33 (58)
Hispanic (all races)	15 (26)
HIV risk factors — no. (%)	
Maternal transmission	52 (91)
Transfusion before July 1985	2 (4)
Sexual partner with HIV	2 (4)
Sexual abuse	1 (2)
CDC class — no. (%)*	
Class N (asymptomatic)	4 (7)
Class A (mild)	32 (56)
Class B (moderate)	18 (32)
Class C (severe)	3 (5)
Age — yr	
Mean ±SD	8.5±3.3
Median	8.0
Range	3.8 to 16.8
CD4 cell count — per mm³	
Mean ±SD	841.1±513.8
Median	699
Range	4 to 2616
CD4 cell count — %	
Mean ±SD	30.6±10.7
Median	30
Range	1 to 56
Weight — kg	
Mean ±SD	27.9±14 2
Median	24.8
Range	13.2 to 96.4
Weight — z score†	
Mean ±SD	−0.25±1.11
Median	−0.34
Range	−2.47 to +3.21
Plasma HIV-1 RNA level — log copies/ml‡	
Mean ±SD	4.1±0.7
Median	4.0
Range	2.6 to 5.7

*CDC denotes Centers for Disease Control and Prevention.

†The z scores were adjusted for age and sex.

‡The quantitative assay was used. Two subjects had HIV-1 RNA levels of less than 400 copies per milliliter at entry, one of whom had an HIV-1 RNA level of 1180 copies per milliliter in the ultrasensitive assay.

Pharmacokinetic Evaluations

Pharmacokinetic evaluations of efavirenz and nelfinavir were performed on 50 children at weeks 2 and 6. At week 2, the mean dose of efavirenz was 310 mg per day (11.7 mg per kilogram per day), and the mean value for the area under the curve from 0 to 24 hours was 218 μmol per liter · hour. One child was excluded from the analysis because of apparently reduced clearance (a value of 1313 μmol per liter · hour for the 24-hour area under the curve). On the basis of the evaluations at week 2, increased doses of efavirenz were recommended for 22 children and decreased doses were recommended for 3 children. The average dose of efavirenz was 369 mg per day (14.2 mg per kilogram per day), and the average value for the 24-hour area under the curve was 244 μmol per liter · hour at week 6. On the basis of the evaluations at week 6, increased doses of efavirenz were recommended for nine children, and decreased doses were recommended for six children. An intention-to-treat

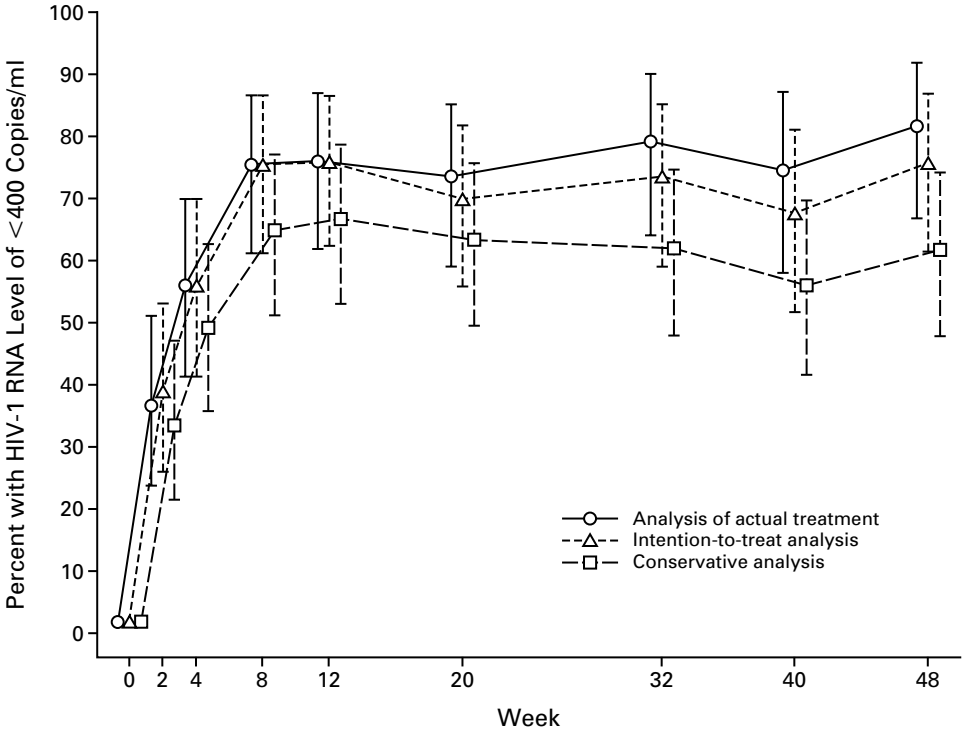

Figure 1. Percentage of Children with Plasma HIV-1 RNA Levels of Less Than 400 Copies per Milliliter, According to an Analysis in Which Only the HIV-1 RNA Levels of Children Who Were Receiving Treatment Were Included (Analysis of Actual Treatment), an Intention-to-Treat Analysis, and an Analysis in Which HIV-1 RNA Levels Were Assigned a Value of More Than 400 Copies per Milliliter for All Visits after the Discontinuation of Treatment (Conservative Analysis). Bars indicate 95 percent confidence intervals.

analysis showed that values for the 24-hour area under the curve were within the target range in 44 percent of children (22 of 50) at week 2 and 56 percent (28 of 50) at week 6.

The average doses of nelfinavir were 606 mg given three times a day (23.6 mg per kilogram) at week 2 and 631 mg given three times a day (24.6 mg per kilogram) at week 6. The corresponding mean values for the area under the curve from zero to eight hours were 21.6 mg per liter · hour at week 2 and 19.9 mg per liter · hour at week 6. On the basis of the evaluations at week 2 and week 6, the doses of nelfinavir were increased in two children and one child, respectively. Seventy-four percent of children (37 of 50) had values for the eight-hour area under the curve above the target range at week 2, and 80 percent (40 of 50) had such values at week 6. On the basis of evaluations performed at week 10 in 15 children, the doses of efavirenz were increased in 5 children and decreased in 1, and the dose of nelfinavir was increased in 1 child.

Effect of Treatment on Plasma HIV-1 RNA Levels

The percentage of children with plasma HIV-1 RNA levels of less than 400 copies per milliliter rose rapidly after the initiation of treatment and remained stable from week 8 to week 48 in the intention-to-treat analysis, the analysis in which only the HIV-1 RNA levels of children who were receiving treatment were included, and the analysis in which HIV-1 RNA levels were assigned a value of more than 400 copies per milliliter for all visits after the discontinuation of treatment (Fig. 1). After the exclusion of 2 children who discontinued treatment shortly after entry, we found that 50 of 55 children (91 percent) had decreases in plasma HIV-1 RNA levels to less than 400 copies per milliliter. Eight of these 50 children (16 percent) subsequently fulfilled the criteria for virologic failure. At week 48, the percentage of children with plasma HIV-1 RNA levels of less than 400 copies per milliliter was 81 percent in the analysis in which only the HIV-1 RNA levels of children who were receiving treatment were included, 76 percent in the intention-to-treat analysis, and 61 percent in the analysis in which HIV-1 RNA levels were assigned a value of more than 400 copies per milliliter for all visits after the discontinuation of treatment.

The proportion of children with plasma HIV-1 RNA levels of less than 50 copies per milliliter rose rapidly during the first 12 weeks of therapy and then more slowly (Fig. 2). At week 48, the percentage of children with plasma HIV-1 RNA levels of less than

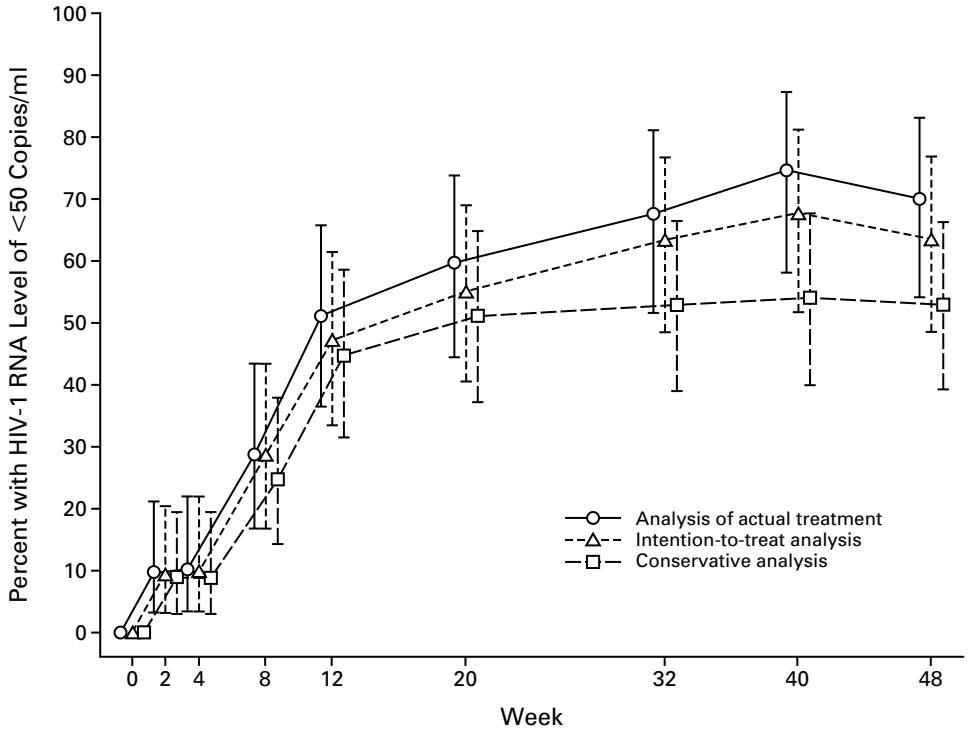

Figure 2. Percentage of Children with Plasma HIV-1 RNA Levels of Less Than 50 Copies per Milliliter, According to an Analysis in Which Only the HIV-1 RNA Levels of Children Who Were Receiving Treatment Were Included (Analysis of Actual Treatment), an Intention-to-Treat Analysis, and an Analysis in Which HIV-1 RNA Levels Were Assigned a Value of More Than 50 Copies per Milliliter for All Visits after the Discontinuation of Treatment (Conservative Analysis). Bars indicate 95 percent confidence intervals.

50 copies per milliliter was 70 percent in the analysis in which only the HIV-1 RNA levels of children who were receiving treatment were included, 63 percent in the intention-to-treat analysis, and 53 percent in the analysis in which HIV-1 RNA levels were assigned a value of more than 50 copies per milliliter for all visits after the discontinuation of treatment.

Plasma HIV-1 RNA levels fell dramatically during the first 20 weeks of therapy (Fig. 3). At both weeks 32 and 48, the upper 95 percent confidence bound for the median change was −2.7 log copies per milliliter; thus, there is a 95 percent probability that the median decrease was at least this amount at these points.

Predictors of a Decrease in Plasma HIV-1 RNA to Undetectable Levels

We used Cox proportional-hazards regression models to examine the effect of base-line characteristics on the likelihood that plasma HIV-1 RNA levels would decrease to less than 400 copies per milliliter and 50 copies per milliliter during treatment (Table 2). In univariate analysis, a higher percentage of CD4 cells at base line and a higher age- and sex-adjusted z score for weight significantly increased the likelihood that plasma HIV-1 RNA levels would drop below 400 copies per milliliter, whereas only the latter variable was associated with the likelihood of a drop in HIV-1 RNA levels to less than 50 copies per milliliter. Higher plasma HIV-1 RNA levels at base line significantly decreased the likelihood that plasma HIV-1 RNA levels would become undetectable, whereas changing the regimen of nucleoside reverse-transcriptase inhibitors within two weeks after entry into the study did not have a significant effect.

In models with multiple covariates, a high plasma HIV-1 RNA level at base line significantly decreased the likelihood that plasma HIV-1 RNA levels would drop below 400 copies per milliliter or 50 copies per milliliter, whereas the age- and sex-adjusted z score for weight was a significant risk factor for the failure of levels to drop below 400 copies per milliliter but not 50 copies per milliliter.

Effect of Treatment on CD4 Cells

At week 48, the CD4 cell count had increased by a median of 74 per cubic millimeter from base line (data not shown), and the absolute percentage of CD4 cells had increased by a median of 3 percent (Fig. 3). When all CD4 measurements during the 48-week period were combined, the increases in median percentage and number of CD4 cells were statistically

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