Is the Recommended Dose of Efavirenz Optimal in Young West African Human Immunodeficiency Virus-Infected Children?^{▽‡}

Déborah Hirt,^{1,2*} Saik Urien,^{1,2} Mathieu Olivier,³ Hélène Peyrière,³ Boubacar Nacro,⁴
Serge Diagbouga,⁵ Emmanuelle Zoure,^{4,5} François Rouet,^{5,6} Hervé Hien,^{5,6}
Philippe Msellati,⁶ Philippe Van De Perre,⁷ and Jean-Marc Tréluyer^{1,2,8}

*EA3620¹ and Service de Pharmacologie Clinique, AP-HP, Hôpital Cochin-Saint-Vincent-de-Paul,⁸ Université Paris–Descartes, and
Unité de Recherche Clinique, AP-HP, Hôpital Tarnier,² Paris, Service de Pharmacologie Médicale et Toxicologie, Hôpital Lapeyronie,
CHU de Montpellier,³ and Laboratoire de Bactériologie-Virologie, CHU de Montpellier, Hôpital Arnaud de Villeneuve,⁷
Montpellier, and UMR 145, IRD, Centre de Recherche Cultures Santé Sociétés/IFEHA, Université Paul Cézanne,
Aix en Provence,⁶ France, and Service de Pédiatrie, CHU Sourô Sanou,⁴ and Centre Muraz,⁵
Bobo Dioulasso, Burkina Faso*

Received 2 December 2008/Returned for modification 14 March 2009/Accepted 15 July 2009

We aimed in this study to describe efavirenz concentration-time courses in treatment-naïve children after once-daily administration to study the effects of age and body weight on efavirenz pharmacokinetics and to test relationships between doses, plasma concentrations, and efficacy. For this purpose, efavirenz concentrations in 48 children were measured after 2 weeks of didanosine-lamivudine-efavirenz treatment, and samples were available for 9/48 children between months 2 and 5 of treatment. Efavirenz concentrations in 200 plasma specimens were measured using a validated high-performance liquid chromatography method. A population pharmacokinetic model was developed with NONMEM. The influence of individual characteristics was tested using a likelihood ratio test. The estimated minimal and maximal concentrations of efavirenz in plasma ($C_{\min}$ and $C_{\max}$, respectively) and the area under the concentration-time curve (AUC) were correlated to the decrease in human immunodeficiency virus type 1 RNA levels after 3 months of treatment. The threshold $C_{\min}$ (and AUC) that improved efficacy was determined. The target minimal concentration of 4 mg/liter was considered for toxicity. An optimized dosing schedule that would place the highest percentage of children in the interval of effective and nontoxic concentrations was simulated. The pharmacokinetics of efavirenz was best described by a one-compartment model with first-order absorption and elimination. The mean apparent clearance and volume of distribution for efavirenz were 0.211 liter/h/kg and 4.48 liters/kg, respectively. Clearance decreased significantly with age. When the recommended doses were given to 46 of the 48 children, 19% (44% of children weighing less than 15 kg) had $C_{\min}$ s below 1 mg/liter. A significantly higher percentage of children with $C_{\min}$ s of >1.1 mg/liter or AUCs of >51 mg/liter · h than of children with lower values had viral load decreases greater than 2 log₁₀ copies/ml after 3 months of treatment. Therefore, to optimize the percentage of children with $C_{\min}$ s between 1.1 and 4 mg/liter, children should receive the following once-daily efavirenz doses: 25 mg/kg of body weight from 2 to 6 years, 15 mg/kg from 6 to 10 years, and 10 mg/kg from 10 to 15 years. These assumptions should be prospectively confirmed.

The combination of didanosine (ddI), lamivudine (3TC), and efavirenz (EFV) once daily has improved compliance for adults and shown good antiretroviral efficacy (12); moreover, the treatment could be better tolerated in the long term (5, 6, 13). For children, the efficacy and tolerance of this ddI-3TC-EFV combination have not been investigated. The aims of the BURKINAM (ANRS 12103) study, then, were to investigate the pharmacokinetics of EFV, ddI, and 3TC given once daily in children 30 months to 15 years old and to evaluate the efficacy and tolerance of this combination.

EFV is metabolized exclusively via CYP2B6 (cytochrome P450 isoenzyme) in the liver (20). Several factors (covariates), such as age (9), duration of treatment (7), or ethnicity (8), may affect EFV metabolism. In the present work, a population pharmacokinetic study was performed with African children in

order to describe the concentration-time courses of EFV and to study the influence of covariates on EFV pharmacokinetics.

Relationships between EFV antiretroviral efficacy/toxicity and plasma EFV concentrations have been established previously for adults (14). No or weak pharmacokinetic-pharmacodynamic relationships were reported in children. Moreover, pediatric studies suggested that the actual recommended EFV dosage produced insufficient concentrations of the drug in plasma (19, 21). In this study, the correlation between concentrations and efficacy was finally tested, the threshold area under the concentration-time curve (AUC) and minimum concentration of the drug in plasma ($C_{\min}$) that improved efficacy were determined, and an optimized dosing scheme was simulated.

MATERIALS AND METHODS

Patients. The BURKINAM–ANRS 12103 study was an open phase II trial evaluating the pharmacokinetics, efficacy, and toxicity of the ddI-3TC-EFV once-daily combination in human immunodeficiency virus (HIV)-infected children. It was conducted in Bobo-Dioulasso, Burkina Faso. The study was approved by the

* Corresponding author. Mailing address: Unité de Recherche Clinique, Hôpital Tarnier, 89 rue d'Assas, 75006 Paris, France. Phone: 33158412884. Fax: 33158411183. E-mail: deborah.hirt@univ-paris5.fr.

‡ This is an ANRS 12103 study.

[▽] Published ahead of print on 27 July 2009.

National Ethics Committee on AIDS of Burkina Faso and was registered in the NIH international database of clinical trials with the number NCT00122538.

The patients enrolled in this study included children 30 months to 15 years old, weighing at least 10 kg, infected with HIV type 1 (HIV-1), and naïve to all antiretroviral treatments (except a treatment preventing mother-to-child transmission). They were eligible if their HIV disease was classified, according to the Centers for Disease Control and Prevention (CDC), as either (i) clinical category C and/or $\leq 15\%$ CD4 cells for children ≤ 5 years old or a CD4 cell count of $\leq 200/\mu\text{l}$ for children > 5 years old or (ii) clinical category B, A, or N, a CD4 cell percentage of $\geq 15\%$ and $\leq 20\%$ for children ≤ 5 years old or a CD4 cell count of ≥ 200 and $\leq 350/\mu\text{l}$ for children > 5 years old, and a viral load greater than 100,000 copies/ml.

The following baseline laboratory values were required: a hemoglobin concentration of 7 g/dl or greater, a platelet count of at least 50,000/ μl , an amylase level less than 2.5 times the upper limit of normal, and aspartate aminotransferase and alanine aminotransferase levels lower than five times the upper limit of normal. The mother or the legal guardian provided a signed informed-consent form.

Clinical evaluations were carried out each month during the follow-up period. Consultations, hospitalizations, treatments, and tests were free of charge.

Virological and biochemical measurements were performed, beyond baseline, every trimester during the follow-up. Plasma HIV RNA levels were determined using real-time reverse transcriptase PCR targeted to the long terminal repeat of HIV-1. The detection threshold of this assay was 300 copies/ml using 0.2 ml of plasma (17). Biochemistry analysis were performed by using the Lisa 300 Plus machine (Hycel Diagnostics, Massy, France).

Treatments. Children were administered once-daily EFV, as 200-mg capsules, according to the body weight-dependent dose recommended for children. Children were also given a once-daily dose of 3TC (8 mg/kg of body weight) and 240 mg of ddI/m² of body surface area.

Sampling. The pharmacokinetic study was performed after 15 days of treatment; EFV was taken at 6:00 p.m. Forty-eight children had a pharmacokinetic sampling at week 2 of treatment, before and 1 and 3 h after EFV administration. Nine children had an additional pharmacokinetic sampling between months 2 and 5 of treatment, before and 1, 2, 3, 6, 12, and 24 h after intake. The time that elapsed between administration and sampling, age, body weight, and height were carefully recorded. Plasma was immediately isolated and frozen at -20°C until analysis.

Analytical method. EFV levels were determined by a previously published high-performance liquid chromatography (HPLC) method (3). Because little plasma was available, a small adaptation was required. Thus, 700 μl of deionized water was added to 300 μl of patient, quality control, and standard samples. Next, an unmodified protocol was followed: an equal volume of acetonitrile was dripped under agitation before the addition of 200 mg of potassium chloride. The demixing procedure was completed by a centrifugation step. Then the supernatant was diluted by half with water before direct injection. The chromatographic apparatus included a P1000XR pump, a UV6000 spectrophotometer, and an AS3000 autosampler piloted by ChromQuest software, version 4.1, all from Thermoseparation Products (Les Ulis, France). HPLC separation was performed on a LiChrospher 100 RP-8 (5 μm) column from Merck (Darmstadt, Germany). The isocratic mobile phase was unbuffered water-acetonitrile (52:48) at a flow rate of 1.5 ml/min. The wavelength of detection of EFV was set at 246 nm. A linear calibration curve was obtained from six dilutions in drug-free plasma (0.5, 1.0, 2.0, 4.0, 8.0, and 10 mg/liter) of an EFV stock solution (1 g/liter in dimethyl sulfoxide). Quality control sample levels (0.75, 4.50, and 9.00 mg/liter) were prepared to check accuracy. The lowest limit of quantification (LOQ) was 0.5 mg/liter. Several quality control samples were integrated in each batch of analysis, and the calibration curve was validated to determine whether the accuracy was in the acceptable range (85% to 115%). Only one analytical process was workable for each sample, but when possible a second determination was performed in a different batch to verify interbatch reproducibility. A 1:4 dilution with drug-free plasma was used when the EFV level exceeded 10 mg/liter.

Modeling strategy and population pharmacokinetic model. Data were analyzed using the nonlinear mixed-effect modeling program NONMEM (version VI, level 1.0) with the Digital Fortran Compiler (2). First-order conditional estimation was used. When a child had an EFV concentration below the LOQ, we set it to half of the LOQ (1). EFV data were analyzed according to a one-compartment and a two-compartment model. Different error models were investigated (i.e., multiplicative and additive error models) to describe residual variability. An exponential model was used for intersubject variability (ISV). Only significant ISVs in pharmacokinetics were kept, i.e., those producing a minimum decrease of 6.63 units using a likelihood ratio test in a backward elimination procedure. The effect of each patient covariate (age, body weight, size, duration

of treatment, creatinine clearance [CL]) was systematically tested via generalized additive modeling in the basic model; as an example, for CL, the equation $\text{CL} = \theta_{\text{CL}} \times [\text{CO}/\text{median}(\text{CO})]^{\beta_{\text{CO}}^{\text{CL}}}$ or $\text{CL} = \theta_{\text{CL}}/[\text{CO}/\text{median}(\text{CO})]^{\beta_{\text{CO}}^{\text{CL}}}$ was used, where θ_{CL} is the typical value of clearance for a patient with the median covariate value and $\beta_{\text{CO}}^{\text{CL}}$ is the estimated influential factor for the continuous covariate (CO). Ethnicity could not be tested, because the study included only black patients. All the covariates were tested via upward model building. A covariate was selected if (i) its effect was biologically plausible, (ii) it produced a minimum decrease of 6.63 units (by a chi-square test with 1 degree of freedom; $\alpha = 0.01$) in the objective function value, and (iii) it produced a reduction in the variability of the pharmacokinetic parameter, assessed by the associated ISV. Among the covariates tested with the base model, the most significant was added in an intermediate model. Then the other covariates were tested with this intermediate model, and the most significant covariate was retained. This process was repeated until no more covariates were significant (i.e., $P > 0.01$). For evaluation of the goodness of fit, the following graphs were plotted for the final model: observed and predicted concentrations versus time, observed concentrations versus population predictions, weighted residuals versus time, and weighted residuals versus predictions. Similar graphs using individual predictive post hoc estimation were displayed. Diagnostic graphics were obtained using RfN (<http://wfn.sourceforge.net/>) with the R program (10).

Evaluation and validation. (i) Bootstrap evaluation. The accuracy and robustness of the final population model were assessed using a bootstrap method, as previously described in detail (15). Briefly, from the original data set of n individuals, B bootstrap sets ($B = 1,000$) of n individuals are drawn with replacement (resampling). For each of the B bootstrap sets, the population pharmacokinetic parameters are estimated and the corresponding mean and standard-deviation are calculated. To validate the model, the parameters estimated from the bootstrap evaluation must be close to the estimates obtained from the original population set. The entire procedure was performed in an automated fashion using Wings for NONMEM (<http://wfn.sourceforge.net/wfninst.htm>). This procedure also provided statistics for the population parameters.

(ii) Visual predictive check validation. EFV concentration profiles were simulated and compared with the observed data to evaluate the predictive performance of the model. The vector of pharmacokinetic parameters from 1,000 patients was simulated using the final model. Each vector parameter was drawn in a log-normal distribution with a variance corresponding to the previously estimated ISV. A simulated residual error was added to each simulated concentration. All observed and simulated concentrations were standardized for a 250-mg EFV dose. The 5th, 50th, and 95th percentiles of the simulated concentrations at each time were then overlaid on the observed concentration data using RfN, and visual inspection was performed. The variability was reasonably estimated if the 90% confidence interval for the proportion of observed data outside the bounds included the theoretical value of 10%.

Concentration-effect relationships. For each patient, the maximum EFV concentration (C_{max}), C_{min} , and AUC were derived from the estimated individual pharmacokinetic parameters. Median values and ranges were calculated and compared to data from the literature. Efficacy was studied by monitoring the difference in viral loads after 3 months of treatment. The significance of the viral load decrease was first tested using a nonparametric Wilcoxon paired test. With respect to efficacy, the links among C_{min} , C_{max} , AUC, and the difference in HIV-1 RNA levels between the time of inclusion and month 3 of treatment were evaluated using Spearman correlation tests. The threshold AUC and C_{min} that significantly increase the percentage of children with viral load decreases higher than 2 log₁₀ copies/ml after 3 months of treatment (according to a Fisher exact test) were determined. Then the threshold C_{min} s increasing adverse events were chosen from studies of adults. The probability of obtaining the target C_{min} of 1.1 to 4 mg/liter in 48,000 children (1,000 simulations of our 48 children) was calculated for different once-daily doses (10, 15, 20, and 25 mg/kg) in six age groups (2 to 4, 4 to 6, 6 to 8, 8 to 10, 10 to 12, and 12 to 15 years).

RESULTS

Demographic data. Forty-eight children were available for pharmacokinetic evaluation. A total of 200 EFV concentrations were collected. Table 1 summarizes patient characteristics. Forty-six children received the recommended dose for their body weight; one received a lower dose, and one received a higher dose.

Population pharmacokinetics. No concentration was below the LOQ. A one-compartment model adequately described the

TABLE 1. Characteristics of the HIV-infected children ($n = 48$) enrolled in the pharmacokinetic study of the BURKINAME-ANRS 12103 trial

Covariate	Median (Minimum–maximum)
Age (yr).....	6.35 (2.77–14.70)
Body weight (kg).....	16.4 (11–37)
Size (cm).....	106 (85–150)
Serum creatinine concn ($\mu\text{mol/liter}$).....	63.0 (6.6–126.4)
Alanine aminotransferase concn (IU/liter).....	26 (7–92)
Aspartate aminotransferase concn (IU/liter).....	44 (12–200)
Total bilirubin concn ($\mu\text{mol/liter}$).....	6.5 (0–42.8)

data. The NONMEM subroutine ADVAN2 TRANS2 was used to describe the one-compartment model, and the parameters of the model were the apparent CL (CL/F), the apparent volume of distribution (V/F), and the absorption rate constant (k_a); F is the unknown bioavailability. Residual variability was best described by a multiplicative error model. The ISVs for CL/F and V/F , described by an exponential error model, were highly correlated ($r = 0.99$). We assumed a complete correlation between these ISVs, estimating a parameter K as follows: $CL/F = \theta_{CL/F} \times e^{\eta_{CL/F}}$ and $V/F = \theta_{V/F} \times e^{\eta_{CL/F} \times K}$, where $\eta_{CL/F}$ is the ISV for CL/F .

The linear body weight model was fixed. The effects of post-natal age, size, duration of treatment, and biological parameters (basal creatinine, alanine aminotransferase, aspartate aminotransferase, and total bilirubin levels) on CL/F and V/F were tested. The apparent elimination of EFV decreased significantly with age, resulting in a decrease of 11.57 units in the objective function value and a decrease in the ISV for CL from 66 to 61%. The final equations for CL and V were as follows: $CL/F = (\theta_{CL/F} \times BW)/[(\text{age}/6.35)^{0.535}]$, and $V/F = \theta_{V/F} \times BW$, where BW is body weight.

No more covariates responded to the three criteria of inclusion.

Figure 1 displays observed and predicted plasma EFV concentrations as a function of time. Table 2 summarizes the final

TABLE 2. Population pharmacokinetic parameters of EFV from the base model, the final model, and bootstrap evaluation for HIV-infected children ($n = 48$) enrolled in the BURKINAME-ANRS 12103 study

Parameter	Mean estimate (RSE [%]) ^a		
	Base model	Final model	Bootstrap
Structural model			
CL/F (liters/h/kg)	0.21 (10)	0.21 (9)	0.21 (9)
V/F (liters/kg)	4.44 (14)	4.48 (14)	4.47 (15)
$CL/F, \theta_{\text{age}}^b$		0.54 (28)	0.53 (32)
k_a (h^{-1})	0.45 (23)	0.45 (23)	0.45 (23)
Statistical model^c			
σ (%)	36 (12)	35 (13)	35 (7)
ω (CL/F) (%)	66 (20)	61 (19)	59 (10)
K^d	0.58 (18)	0.64 (10)	0.66 (14)

^a Mean estimates correspond to a median age of 6.35 years. RSE, relative standard error, calculated as (standard error of the estimate)/(estimate $\times$ 100). The base model and final model are calculated from the original data set. Bootstrap data are statistics from 1,000 bootstrap analyses.

^b θ_{age} , estimated influential factor for age on CL/F .

^c σ , residual variability estimate; ω , interindividual variability estimate.

^d Calculated as $\eta_V = e^{\eta_{CL} \times K}$ with the correlation $\omega_{CL/F} - \omega_{V/F} = 1$.

population pharmacokinetic estimates. Figure 2 displays CL/F as a function of age. The performance of the final model was assessed by comparing predicted population and predicted individual plasma EFV concentrations to observed plasma EFV concentrations and by comparing population weighted residuals versus predicted concentrations and versus time for EFV (data not shown).

Evaluation and validation. (i) Bootstrap evaluation. As shown in Table 2, the means and coefficients of variation of parameter estimates obtained from the bootstrap process (1,000 runs) were close to the estimates previously obtained with the original data set.

(ii) Visual predictive check. Figure 3 shows that the average prediction matches the observed concentration-time courses and that the variability is reasonably estimated. Thus, 178 of

FIG. 1. Observed EFV concentrations (circles) and predicted population EFV concentrations (curve) for a child with median body weight (16.4 kg) as a function of time.

FIG. 2. CL/F divided by body weight as a function of age. The line is drawn according to the equation used in the model.

200 observed points (89%) fell within the 90% prediction interval.

Concentration-effect relationships. Table 3 summarizes the EFV C_{max} , C_{min} , AUC, and CL/F for children in the present and previous studies. In previous pediatric studies, concentrations were compared to the target efficacy-toxicity intervals (determined from adult data): C_{min} s of >1 and <4 mg/liter and AUCs of >60 and <120 mg/liter $\cdot$ h (Table 4). With the EFV recommended dose administered to 46 out of 48 children of the study, 9 out of 48 children (19%) had C_{min} s below 1 mg/liter, and 8 of these 9 children weighed less than 15 kg. As well, 19 of 48 children (40%) had AUCs below 60 mg/liter $\cdot$ h, and 11 of the 19 were from the <15 -kg group. Children weigh-

ing less than 15 kg were more likely to have insufficient concentrations.

The target intervals for adults could not be related to toxicity and efficacy in previous pediatric studies. These links were now studied with our 48 children. For toxicity, although seven children had C_{min} s of >4 mg/liter, none of them had an adverse event related to EFV, so no relationship could be established. For efficacy, viral loads both at baseline and after 3 months of treatment were available for 47 of the 48 children. The viral load decreased significantly after 3 months of treatment, from a median (minimum to maximum) value of 5.5 (3.6 to 6.7) $\log_{10}$ copies/ml to 2.5 (2.5 to 6.0) $\log_{10}$ copies/ml ($P < 10^{-4}$). The C_{min} , C_{max} , and AUC could not be correlated to this significant

FIG. 3. Evaluation of the final model. Shown are comparisons between the 5th (bottom dashed line), 50th (solid line), and 95th (top dashed line) percentiles obtained from 1,000 simulations, standardized to a 250-mg dose for all the children, and the observed data (circles) for EFV concentrations.

TABLE 3. Comparison of EFV-derived pharmacokinetic parameters between our study and recently published studies of HIV-1-infected children taking EFV capsules

Study	No. of children	Age (yr)	Dose (QD) ^a	C _{min} (mg/liter)	C _{max} (mg/liter)	AUC _{0→τ} (mg/liter · h)	CL/F (liters/h/kg)	Reference
Fletcher et al.	50	7	275 mg (12 mg/kg)	1.45	4.09	60	0.19	7
Ren et al.	15	6.8	13.9 mg/kg	1.18				16
Wintergerst et al.	33	8.2	300 mg (13.3 mg/kg)				0.30	21
von Hentig et al.	11	4–10	10–15 mg/kg	1.29	5.52	63.6		19
Hirt et al.	48	6.5	250 mg (14.4 mg/kg)	1.64	3.71	65.2	0.21	This study

^a QD, once a day.

viral load decrease. However, a significantly higher percentage of children had viral load decreases greater than 2 log₁₀ copies/ml after 3 months of treatment when C_{min} was greater than 1.1 mg/liter (97% [33/34] versus 69% [9/13]; *P* = 0.02) or when AUC was greater than 51 mg/liter · h (97% [32/33] versus 71% [10/14]; *P* = 0.02) at 2 weeks of treatment.

Considering a target C_{min} of 1.1 mg/liter for efficacy (from our study) and a target C_{min} of 4 mg/liter for toxicity (from an adult study), the probabilities of obtaining the target C_{min} in 48,000 simulated children receiving the 10-, 15-, 20-, or 25-mg/kg EFV dose, as a function of age, are shown in Fig. 4. The highest percentage of children with C_{min}s between 1.1 and 4 mg/liter was obtained with a once-daily EFV dose of 25 mg/kg from 2 to 6 years, 15 mg/kg from 6 to 10 years, and 10 mg/kg from 10 to 15 years.

DISCUSSION

EFV concentrations were satisfactorily described by a one-compartment model with first-order elimination. This model has already been used for adults (4, 11) to describe the EFV concentration-time course. The following observations support the model's use.

(i) C_{min}s, C_{max}s, and AUCs were consistent with those of previous studies: 1.64 mg/liter, 3.71 mg/liter, and 65.2 mg/liter · h, respectively, in our study compared to 1.18 to 1.45 mg/liter, 4.09 to 5.52 mg/liter, and 60 to 63.6 mg/liter · h in previous studies (7, 16, 19) (Table 3).

(ii) The CL/F (0.21 liter/h/kg) was consistent with those of previous studies: 0.19 liter/h/kg for 50 children (7) and 0.30 liter/h/kg for 33 children (21).

(iii) The CL/F (per kilogram of body weight) decreases significantly with age, from 2.5 to 15 years. This is consistent with an increase in hepatic activity (including the metabolism of EFV by CYP2B6) between the ages of 1 and 4 years, exceeding adult levels (9).

Three different modeling approaches, i.e., fitted, fixed to an exponent of 0.75, and fixed to an exponent of 1 for body weight on CL/F, could have been used, since the same objective function values were obtained and the age effect was significant in all three cases. CL was finally handled as linearly related to body weight, mainly because using this general approach, the body weight exponent on CL was estimated as 1.13 (closer to 1 than to 0.75) and because doses are given linearly with body weight (in milligrams per kilogram of body weight).

Different relationships between concentrations and efficacy were used in order to adapt dosages for children. In the Pediatric AIDS Clinical Trials Group study (18), the dose of EFV was adjusted if the AUC from 0 to 24 h was out of the target interval of 190 to 380 μmol/liter · h (corresponding to 60 to 120 mg/liter · h), representing the range from the 50th percentile to twice the 50th percentile of such values in adults receiving 600 mg of EFV per day. Other studies adapt a criterion derived from the concentration-efficacy/toxicity relationship established for adults: for concentrations measured between 8 and 20 h after drug intake, virologic failure was significantly higher in patients with EFV concentrations of <1 mg/liter and central nervous system toxicity was three times more frequent in patients with EFV concentrations of >4 mg/liter (14). These concentration-effect relationships were not confirmed in children. Only one study showed a concentration-efficacy link in

TABLE 4. Percentages of children below, within, and above the target efficacy-toxicity intervals determined for adults in recent publications and in our study

Study	No. of children	Age (yr)	Target ^a	% of children with parameters:			Reference
				Below the target	Within the target interval	Above the target	
Fletcher et al.	50	7	60 < AUC < 120	44	50	6	7
Ren et al.	15	6.8	1 < C _{min} < 4	40	47	13	16
Wintergerst et al.	33	8.2	1 < plasma EFV concn < 4	36 ^b		42 ^b	21
von Hentig et al.	11	4–10	60 < AUC < 120	64	36	0	19
Hirt et al.	48	6.5	60 < AUC < 120	40 ^c	41	19	This study
			1 < C _{min} < 4	19 ^c	66	15	

^a AUC targets are measured in milligrams/liter · hour; plasma EFV concentration and C_{min} targets are measured in milligrams per liter.^b Multiple samples were collected from each child in the study of Wintergerst et al. (21); two-thirds of the samples were collected 8 to 20 h after the last dose. Data for that study are percentages of children for whom at least one sample was above or below the target. Nine percent of samples were below the target; 76% were within the target interval; and 15% were above the target.^c Percentages of AUCs and C_{min}s below the target were higher for children weighing <15 kg than for the whole cohort: 61% and 44%, respectively.

FIG. 4. Percentages of children with C_{min} s lower than 1.1 mg/liter (related to an efficacy decrease), between 1.1 and 4 mg/liter (target interval), and higher than 4 mg/liter (related to toxicity) in simulations of 48,000 children divided into six age groups.

children: 50% of children who had EFV AUC values less than 49 mg/liter $\cdot$ h reached less than 400 copies/ml of HIV RNA by week 8 compared with 86% of children who had AUC values greater than 49 mg/liter $\cdot$ h ($P = 0.01$) (7). In our study, a significantly higher percentage of children had viral load decreases greater than 2 $\log_{10}$ copies/ml after 3 months of treatment when the AUC was greater than 51 mg/liter $\cdot$ h (97% [32/33] versus 71% [10/14]; $P = 0.02$) at 2 weeks of treatment. This confirms that the EFV AUC that improves treatment efficacy is around 50 mg/liter $\cdot$ h. The target C_{min} could also be related to efficacy: 97% of children with a C_{min} of >1.1 mg/liter had a viral load decrease greater than 2 $\log_{10}$ copies/ml at month 3 compared to 69% of children with a C_{min} of <1.1 mg/liter ($P = 0.02$). The relationship between EFV plasma concentrations and efficacy did not exclude the influence of other factors (such as exposure to other antiretroviral drugs or the genotype of the virus). In this study, seven children had C_{min} s above 4 mg/liter, but none had adverse events related to EFV administration, so no relationship could be established between EFV concentrations and toxicity.

For adults and children, efficacy could be related to both AUC and C_{min} , and studies were not adequate to determine whether C_{min} or AUC is best correlated with efficacy. Theo-

retically, C_{min} may be more important to provide continuous suppression of viral replication; thus, we considered C_{min} the target pharmacokinetic parameter in our study. In all studies, a high percentage of children fell below the target concentrations (7, 16, 19, 21). Similarly, with the recommended dose of EFV administered to 46 out of 48 children in the present study, 19% of children had C_{min} s of <1 mg/liter. Among them, 89% weighed less than 15 kg, so we concluded that children weighing 11 to 15 kg were more likely to have insufficient EFV concentrations. In our study, all children weighing less than 15 kg received 200 mg of EFV, the recommended dose for children weighing 13 to 15 kg; there is no recommendation for children weighing less than 13 kg. Two strategies to increase C_{min} s in these youngest children could be considered: increase the once-daily dose or shorten the dose interval to every 12 h. The latter alternative was not considered, although it could avoid increasing EFV exposure, because the major aim of this trial was to test a once-daily combination to increase compliance. According to simulations, to optimize the percentage of children with C_{min} s between 1.1 and 4 mg/liter (related to efficacy and toxicity, respectively), children should receive the following once-daily EFV dose: 25 mg/kg from 2 to 6 years, 15 mg/kg from 6 to 10 years, and 10 mg/kg from 10 to 15 years.

These assumptions should be prospectively confirmed, because conclusions were drawn on the basis of only 48 children and no data on toxicity with these increased doses are available.

REFERENCES

1. Beal, S. L. 2001. Ways to fit a PK model with some data below the quantification limit. *J. Pharmacokinet. Pharmacodyn.* **28**:481–504.
2. Beal, S. L., and L. B. Sheiner. 1998. NONMEM user's guide. NONMEM Project Group, University of California, San Francisco.
3. Cociglio, M., D. Hillaire-Buys, H. Peyriere, and R. Alric. 2003. Performance analysis of a rapid HPLC determination with the solvent demixing extraction of HIV antiproteases and efavirenz in plasma. *J. Chromatogr. Sci.* **41**:80–86.
4. Csajka, C., C. Marzolini, K. Fattinger, L. A. Decosterd, J. Fellay, A. Telenti, J. Biollaz, and T. Buclin. 2003. Population pharmacokinetics and effects of efavirenz in patients with human immunodeficiency virus infection. *Clin. Pharmacol. Ther.* **73**:20–30.
5. Dybul, M., E. Nies-Kraske, R. Dewar, F. Maldarelli, C. W. Hallahan, M. Daucher, S. C. Piscitelli, L. Ehler, A. Weigand, S. Palmer, J. A. Metcalf, R. T. Davey, D. M. Rock Kress, A. Powers, I. Beck, L. Frenkel, M. Baseler, J. Coffin, and A. S. Fauci. 2004. A proof-of-concept study of short-cycle intermittent antiretroviral therapy with a once-daily regimen of didanosine, lamivudine, and efavirenz for the treatment of chronic HIV infection. *J. Infect. Dis.* **189**:1974–1982.
6. Ena, J., and F. Pasquau. 2003. Once-a-day highly active antiretroviral therapy: a systematic review. *Clin. Infect. Dis.* **36**:1186–1190.
7. Fletcher, C. V., R. C. Brundage, T. Fenton, C. G. Alvero, C. Powell, L. M. Mofenson, and S. A. Spector. 2008. Pharmacokinetics and pharmacodynamics of efavirenz and nelfinavir in HIV-infected children participating in an area-under-the-curve controlled trial. *Clin. Pharmacol. Ther.* **83**:300–306.
8. Haas, D. W., H. J. Ribaud, R. B. Kim, C. Tierney, G. R. Wilkinson, R. M. Gulick, D. B. Clifford, T. Hulan, C. Marzolini, and E. P. Acosta. 2004. Pharmacogenetics of efavirenz and central nervous system side effects: an Adult AIDS Clinical Trials Group study. *AIDS* **18**:2391–2400.
9. Hoody, D. W., and C. V. Fletcher. 2003. Pharmacology considerations for antiretroviral therapy in human immunodeficiency virus (HIV)-infected children. *Semin. Pediatr. Infect. Dis.* **14**:286–294.
10. Ihaka, R., and R. Gentleman. 1996. R: a language for data analysis and graphics. *J. Comput. Graph. Stat.* **5**:299.
11. Kappelhoff, B. S., F. van Leth, T. R. MacGregor, J. Lange, J. H. Beijnen, and A. D. Huitema. 2005. Nevirapine and efavirenz pharmacokinetics and covariate analysis in the 2NN study. *Antivir. Ther.* **10**:145–155.
12. Landman, R., R. Schiemann, S. Thiam, M. Vray, A. Canestri, S. Mboup, C. T. Kane, E. Delaporte, P. S. Sow, M. A. Faye, M. Gueye, G. Peytavin, C. Dalban, P. M. Girard, and I. Ndoye. 2003. Once-a-day highly active antiretroviral therapy in treatment-naïve HIV-1-infected adults in Senegal. *AIDS* **17**:1017–1022.
13. Maggiolo, F., D. Ripamonti, G. Gregis, G. Quinzan, A. Callegaro, C. Arici, L. Ravasio, and F. Suter. 2003. Once-a-day therapy for HIV infection: a controlled, randomized study in antiretroviral-naïve HIV-1-infected patients. *Antivir. Ther.* **8**:339–346.
14. Marzolini, C., A. Telenti, L. A. Decosterd, G. Greub, J. Biollaz, and T. Buclin. 2001. Efavirenz plasma levels can predict treatment failure and central nervous system side effects in HIV-1-infected patients. *AIDS* **15**:71–75.
15. Parke, J., N. H. Holford, and B. G. Charles. 1999. A procedure for generating bootstrap samples for the validation of nonlinear mixed-effects population models. *Comput. Methods Programs Biomed.* **59**:19–29.
16. Ren, Y., J. J. Nuttall, C. Egbers, B. S. Eley, T. M. Meyers, P. J. Smith, G. Maartens, and H. M. McIlleron. 2007. High prevalence of subtherapeutic plasma concentrations of efavirenz in children. *J. Acquir. Immune Defic. Syndr.* **45**:133–136.
17. Rouet, F., M. L. Chaix, E. Nerrienet, N. Ngo-Giang-Huong, J. C. Plantier, M. Burgard, M. Peeters, F. Damond, D. K. Ekouevi, P. Msellati, L. Ferradini, S. Rukobo, V. Marechal, N. Schvachsa, L. Wakrim, C. Rafalimanana, B. Rakotoambinina, J. P. Viard, J. M. Seigneurin, and C. Rouzioux. 2007. Impact of HIV-1 genetic diversity on plasma HIV-1 RNA quantification: usefulness of the Agence Nationale de Recherches sur le SIDA second-generation long terminal repeat-based real-time reverse transcriptase polymerase chain reaction test. *J. Acquir. Immune Defic. Syndr.* **45**:380–388.
18. Starr, S. E., C. V. Fletcher, S. A. Spector, F. H. Yong, T. Fenton, R. C. Brundage, D. Manion, N. Ruiz, M. Gersten, M. Becker, J. McNamara, and L. M. Mofenson for the Pediatric AIDS Clinical Trials Group 382 Team. 1999. Combination therapy with efavirenz, nelfinavir, and nucleoside reverse-transcriptase inhibitors in children infected with human immunodeficiency virus type 1. *N. Engl. J. Med.* **341**:1874–1881.
19. von Hentig, N., C. Koenigs, S. Elanjikal, R. Linde, D. Dunsch, W. Kreuz, and M. B. Funk. 2006. Need for therapeutic drug monitoring in HIV-1 infected children receiving efavirenz doses according to international guidelines. *Eur. J. Med. Res.* **11**:377–380.
20. Ward, B. A., J. C. Gorski, D. R. Jones, S. D. Hall, D. A. Flockhart, and Z. Desta. 2003. The cytochrome P450 2B6 (CYP2B6) is the main catalyst of efavirenz primary and secondary metabolism: implication for HIV/AIDS therapy and utility of efavirenz as a substrate marker of CYP2B6 catalytic activity. *J. Pharmacol. Exp. Ther.* **306**:287–300.
21. Wintergerst, U., F. Hoffmann, A. Jansson, G. Notheis, K. Huss, M. Kurowski, and D. Burger. 2008. Antiviral efficacy, tolerability and pharmacokinetics of efavirenz in an unselected cohort of HIV-infected children. *J. Antimicrob. Chemother.* **61**:1336–1339.