Population pharmacokinetics and exposure-response relationship of enfuvirtide in treatment-experienced human immunodeficiency virus type 1–infected patients

Objective: **Our objective was to characterize population pharmacokinetics of enfuvirtide, 90 mg twice daily injected subcutaneously, in treatment-experienced human immunodeficiency virus type 1 (HIV-1)–infected patients, as well as the relationship between exposure and antiviral effect.**

Methods: **Plasma concentrations of enfuvirtide and HIV-1 ribonucleic acid were obtained from 628 patients in 2 phase III studies. NONMEM software was used for population pharmacokinetic analysis and to assess the effects of age, gender, body weight, anti-gp41 antibodies, and concomitant drugs. Enfuvirtide exposure (area under the plasma concentration–12-hour time curve or steady-state trough concentration) was calculated from individual parameter estimates derived from the model. The decline in HIV-1 ribonucleic acid from baseline at week 2 or 24 was regressed against estimates of enfuvirtide exposure by a maximum effect model. The exposure-response relationship was examined in functional monotherapy (phenotypic sensitivity score of 0) and combination therapy (phenotypic sensitivity score ≥1).**

Results: **Enfuvirtide population pharmacokinetics was well described by a 1-compartment model with first-order absorption and elimination. Body weight and female gender were identified as affecting apparent clearance but not efficacy and safety. Concomitant medications had no significant effect on enfuvirtide pharmacokinetics. Antiviral response to enfuvirtide was independent of drug exposure, suggesting that the approved 90-mg twice-daily dose was in the plateau portion of the dose-response curve. For functional monotherapy (phenotypic sensitivity score of 0), approximately 66% of estimated maximal effect was achieved at week 2 and 73% at week 24, and for combination therapy, more than 92% was achieved at both weeks 2 and 24.**

Conclusions: **Body weight and gender affected enfuvirtide clearance, but changes in exposure did not affect efficacy or safety. Efficacy reached a plateau at the 90-mg twice-daily dosage in the exposure-response curve. (Clin Pharmacol Ther 2005;77:515-28.)**

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Enfuvirtide (Fuzeon; Hoffmann-La Roche Inc, Nutley, NJ) is a 36–amino acid synthetic peptide composed of naturally occurring L–amino acid residues. It is the first of a new class of antiretrovirals, the human immunodeficiency virus type 1 (HIV-1) fusion inhibitors. Unlike conventional antiretrovirals (nucleotide-nucleoside reverse transcriptase inhibitors, nonnucleoside reverse transcriptase inhibitors, and protease inhibitors), enfuvirtide is an extracellular inhibitor of gp41-mediated HIV-1 fusion to target cell membranes. By disrupting a conformational change in the gp41 surface protein of HIV-1 after binding of the virus to the CD4 receptor on the cell surface, enfuvirtide prevents virus entry and hence target cell infection.[1]

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Enfuvirtide pharmacokinetics has been well characterized in a 4-way crossover study in 12 HIV-1–infected patients.[2] Enfuvirtide exhibited high absolute bioavailability (84%), a small volume of distribution (5.5-6.6 L), low systemic clearance (1.4-1.7 L/h), and a short elimination half-life (1.69-1.91 h) when administered intravenously. It had complex absorption characteristics that were best described by an inverse Gaussian density input function. The antiviral activity of enfuvirtide was initially demonstrated in 2 short-term monotherapy (or functional monotherapy) studies (TRI-001[3] and TRI-003[4]) and 2 combination-therapy studies (T20-206[5] and T20-208[6]). In all of these studies, with limited numbers of subjects, enfuvirtide demonstrated a dose-related antiviral response and maximal antiviral effect at a 90-mg twice-daily dose (100 mg enfuvirtide in the vial [nominal dose] delivers a 90-mg dose [deliverable]). These results were subsequently confirmed in 2 large, pivotal phase III studies, TORO 1[7] (T20-301 versus Optimized Regimen Only) and TORO 2 (T20-302 versus Optimized Regimen Only).[8]

Although enfuvirtide has demonstrated a dose-related response in several phase I/II studies,[3-5] a full exploration of the exposure-response relationship was not previously possible for a number of reasons, including relatively small sample sizes, the use of different study populations, the use of different assay methods for the measurement of plasma HIV-1 ribonucleic acid (RNA), and differences in study design of the phase II studies T20-206 and T20-208. In contrast, TORO 1 and TORO 2 were similar in study design and population characteristics, there was centralized measurement of plasma HIV-1 RNA levels, and sparse pharmacokinetic plasma samples were collected from each patient.

The objectives of this study were, therefore, to use data from TORO 1 and TORO 2 to characterize the population pharmacokinetics of enfuvirtide, to evaluate the influence of patient factors and concomitant medications on the pharmacokinetic variability of enfuvirtide, and to explore the relationship between pharmacokinetic exposure and plasma HIV-1 RNA levels in treatment-experienced HIV-1–infected patients.

METHODS

Study design. TORO 1[7] and TORO 2[8] were phase III, randomized, open-label studies assessing the efficacy and safety of enfuvirtide, 90 mg twice daily injected subcutaneously, in combination with an optimized background antiretroviral regimen versus an optimized background antiretroviral regimen alone in HIV-1–infected patients with experience or prior documented resistance to each of the other 3 classes of approved oral antiretrovirals. All participants gave written informed consent to participate in the protocol, which was approved by the independent ethics committee or institutional review board at each of the study centers. The optimized background antiretroviral regimen consisted of 3 to 5 antiretrovirals selected on the basis of treatment history and the results of genotypic and phenotypic resistance tests. Genotypic testing detects drug resistance mutations that are present in relevant viral genes associated with antiretroviral agents, whereas phenotypic testing actually measures a virus's ability to grow in different concentrations of antiretroviral agents (ie, the sensitivity of the virus to the antiretroviral agents). Once the patient's optimized background regimen was chosen, each patient's background regimen was given a score, the phenotypic sensitivity score, defined as the total number of drugs in the optimized background regimen to which a patient's viral load demonstrated phenotypic sensitivity. If a patient is given drugs A, B, C, and D, but his type of virus is sensitive only to drugs A, B, and D, then his phenotypic sensitivity score or genotypic sensitivity score (depending on whether phenotypic or genotypic testing was used) would be 3. This score allowed analyses of responses with the number of active antiretrovirals in the patient's background regimen taken into account. The genotypic sensitivity score and phenotypic sensitivity score were used to stratify patients at randomization and to achieve balances across 2 studies. The treatment period was 48 weeks (plus an optional 48-week extension) with the primary efficacy measurement at week 24. After screening, patients who met the inclusion criteria were randomized in a 2:1 ratio to receive enfuvirtide plus optimized background regimen or optimized background regimen alone and continued this regimen at least until week 8, regardless of virologic response. Protocol-defined criteria for virologic failure are as follows:

1. Patients had a decrease of less than 0.5 $\log_{10}$ copies/mL from baseline either on 2 consecutive measurements at least 14 days apart or on 3 consecutive measurements with at least 14 days between the first and third measurements starting at week 8 or any time after week 8.
2. Patients had a decrease of less than 1.0 $\log_{10}$ copies/mL from baseline on consecutive measurements (as in criterion 1) starting from weeks 14 and 16 or anytime after week 16.
3. Patients achieved a decrease of 2.0 $\log_{10}$ copies/mL or greater from baseline on consecutive measurements but had HIV-1 RNA rebound from the aver-

age of the 2 lowest values (not necessarily consecutive) by greater than 1.0 $\log_{10}$ copies/mL on consecutive measurements starting from weeks 6 and 8 or anytime after week 8.

Six sparse blood samples for pharmacokinetic analysis were collected from patients who received enfuvirtide plus optimized background antiretroviral regimens, with 2 samples taken per visit at week 1 or week 2, week 8, and week 24 of the study treatment. These blood samples were drawn at least 1 to 2 hours apart in the following time slots relative to the morning enfuvirtide dose: 5 to 8 hours (week 1 or week 2), 3 hours before dosing to immediately before dosing (week 8), and 1 to 4 hours (week 24). Patients self-recorded the 3 previous doses before the blood sampling day, and this information was captured in the case report forms. Anti-gp41 antibody titer was measured from serum samples taken at baseline, week 8, and week 24. Because enfuvirtide is derived from a sequence of viral gp41, anti-gp41 antibodies cross react with enfuvirtide. Plasma samples for the measurement of HIV-1 RNA were taken at baseline, on day 1, and at weeks 1, 2, 4, 6, 8, 10, 12, 14, 16, 20, and 24.

Analytic methods. Concentrations of enfuvirtide in plasma were measured in the Bioanalytical Division at MDS Pharma Services, Lincoln, Neb, by use of validated liquid chromatography–tandem mass spectrometry.[9] The calibration range of standard curves was 5 to 2000 ng/mL, and the lower limit of quantitation was 10 ng/mL. Interassay precision for quality-control samples ranged from 5.7% to 14.4%. Overall accuracy (expressed as percentage relative error, which is defined as 100 × [Measured − Nominal]/Nominal) ranged from 1.3% to 9.7%.

Samples for measurement of plasma HIV-1 RNA were processed by Covance Central Laboratory Service Inc (Geneva, Switzerland; Sonic, Australia; and Indianapolis, Ind) by commercial methods. Plasma HIV-1 RNA levels were measured by use of a Roche Amplicor HIV-1 monitor (version 1.5; Roche Diagnostic Corp, Indianapolis, Ind) with ultrasensitive sample preparation (range, 50-75,000 copies/mL) or standard sample preparation (range, 400-750,000 copies/mL). Anti-gp41 antibody titer was measured by Trimeris Inc (Durham, NC). A validated indirect enzyme-linked immunosorbent assay was used for the quantitation of anti-gp41 serum antibodies. Known standards for the construction of a standard curve and quality control samples with high, medium, and low levels of anti-gp41 antibody were included in every assay. Samples were considered antibody-positive if they had 3 dilutions that fell on the standard curve and achieved a signal-to-noise ratio (enfuvirtide absorbance/negative control peptide T786 absorbance) greater than 3:1.

Population pharmacokinetic method. Patients from each phase III study were randomly assigned to 1 of 2 data sets—85% for inclusion in the model-building database and 15% for the validation database. Each database was created separately after the allocation of patients. Creatinine clearance was estimated via the method described by Cockcroft and Gault[10] with values capped at 150 mL/min.[11] Body surface area was estimated by use of the formula described by DuBois and Eugene.[12] Anti-gp41 reactive antibody status was defined by use of either group (where 1 is positive, 2 is negative, 3 is not quantifiable, and 0 is missing) or percentage change from baseline variables (where 1 is ≥30% decrease from baseline, 2 is <30% decrease from baseline to <30% increase from baseline, 3 is ≥30% increase from baseline, and 0 is negative or missing). Positive anti-gp41 reactive antibody status was defined by a measurable antibody titer at any assessment point. Urine protein was coded as 0 (absent), 1 (trace), or 2 (positive or strong positive). Race and acquired immunodeficiency syndrome (AIDS) wasting syndrome were also coded as categoric variables. Several commonly coadministered drugs used in the treatment of HIV-1 and associated comorbidities were evaluated for their effect on enfuvirtide clearance. Concomitant medication data indicated only the presence or absence of each medication; the dose of concomitant medications was not included in this analysis. All other covariates were assessed as continuous variables (Table I). The first-order conditional estimation method (FOCE) with interaction was used for all models tested during model development.[13,14]

The models used in this analysis were defined to represent covariate influences as shifts in the parameter of interest from the parameter value observed in a hypothetic reference patient. This patient was defined as a 42-year-old, white male patient weighing 70 kg, with a body surface area of 1.9 m^2, creatinine clearance of 120 mL/min, normal hepatic function (AST of 50 IU/L, ALT of 50 IU/L, and bilirubin of 0.50 mg/dL), serum albumin level of 50 g/L, no proteinuria, no AIDS wasting syndrome, and no liver cirrhosis.

Standard model-building approaches were used during model development.[15] Addition of a covariate was accepted only if it resulted in a reduction of the objective function of at least 10 points ($P < .001$) via the likelihood ratio test. The performance of the final model was evaluated by use of a maximum a posteriori evaluation of the internal validation data set. Consis-

Table I. Summary of baseline demographics of study population

Baseline characteristic	*Model-building data set (n = 534) [mean (SD)]*	*Validation data set (n = 94) [mean (SD)]*
Age (y)	42.3 (7.92)	42.4 (7.33)
Height (cm)	176 (8.21)	176 (8.07)
Weight (kg)	72.2 (12.7)	72.3 (13.0)
Body surface area (m^2)	1.87 (0.18)	1.88 (0.19)
Creatinine clearance (mL/min)	112 (26.6)	109 (24.9)
Urine protein	0 = 379	0 = 65
	1 = 127	1 = 24
	2 = 28	2 = 5
Antibody status*	0 = 178	0 = 29
	1 = 246	1 = 44
	2 = 6	2 = 0
	3 = 104	3 = 21
Antibody change from baseline†	0 = 332	0 = 60
	1 = 131	1 = 22
	2 = 54	2 = 11
	3 = 17	3 = 1
AST (IU/L)	46.0 (29.0)	41.9 (28.8)
ALT (IU/L)	47.8 (35.3)	43.7 (35.7)
Albumin (g/dL)	4.10 (0.45)	4.07 (0.44)
Serum creatinine (mL/dL)	0.88 (0.24)	0.91 (0.23)
Bilirubin (mg/dL)	0.48 (0.27)	0.45 (0.24)
Prothrombin time (s)	12.4 (1.61)	12.1 (0.66)
Gender (No.)	477 male, 57 female	86 male, 8 female
Race (No.)	476 white, 58 other	86 white, 8 other
Concomitant medication [patients taking drug (%)]		
Abacavir	28.5	34.0
Amprenavir	38.6	42.6
Didanosine	5.8	7.4
Efavirenz	42.9	44.7
Indinavir	16.1	16.0
Lamivudine	8.6	9.6
Lopinavir-ritonavir	43.1	44.7
Ritonavir	53.7	42.6
Saquinavir	53.7	42.6
Stavudine	2.4	0.0
Tenofovir	4.3	2.1
Zidovudine	77.7	73.4
Fluconazole	21.0	17.0
Sulfamethoxazole	47.9	41.5

*Antibody status (where 1 is positive, 2 is negative, 3 is not quantifiable, and 0 is missing).
†Antibody change from baseline (where 1 is ≥30% decrease from baseline, 2 is <30% decrease from baseline to <30% increase from baseline, 3 is ≥30% increase from baseline, and 0 is negative or missing).

tency between the observed and individual predicted concentrations in the validation data set obtained by use of the parameters estimated during model building was examined to confirm the estimated model parameters. Pharmacokinetic data were analyzed by use of NONMEM (version V, level 1.1; GloboMax, Hanover, Md).[16-19]

Exposure-response analysis method. Individual enfuvirtide exposure (area under the plasma concentration–12-hour time curve [AUC_{12}]) and steady-state trough concentration of enfuvirtide (C_{trough}) were estimated from the final population pharmacokinetic model by use of equations 1 and 2, respectively. All derived parameters were calculated for each occasion on which an individual underwent sampling. Because it was desirable to have 1 parameter estimate per individual, the mean of all values

for an individual was reported. Equations 1 and 2 are as follows:

$$AUC_{12} = \frac{F \cdot Dose}{CL} \quad (1)$$

$$C_{trough} = \frac{F \cdot Dose \cdot K_a}{V \cdot \left(K_a - \frac{CL}{V}\right)} \left[\left(\frac{1}{1 - e^{-\frac{CL}{V} \cdot \tau}} \right) e^{-\frac{CL}{V} \cdot \tau} - \left(\frac{1}{1 - e^{-K_a \cdot \tau}} \right) e^{-K_a \cdot \tau} \right] \quad (2)$$

in which F is the fraction absorbed (percentage), CL is total clearance (liters per hour), K_a is the absorption rate constant (per hour), V is the volume of distribution (liters), and τ is the dosing interval of 12 hours.

Change in plasma HIV-1 RNA (measured on a $\log_{10}$ scale) from baseline was used as a response or efficacy marker. The exposure-response analysis was performed for all patients with paired exposure and response data at week 2 and at week 24 (primary efficacy endpoint) by use of the same pharmacokinetic exposure parameters. Patients who met protocol-defined virologic failure criteria at week 8 (nonresponders) were excluded from the analysis at week 24, because the pharmacokinetic exposure of this group of patients was not different from that of patients who achieved a virologic response (data not shown) and inclusion of these patients might obscure the true exposure-response relationship in patients achieving an antiviral response. Additional subgroup analyses by phenotypic sensitivity score were also performed to measure the antiviral effect of enfuvirtide in a functional monotherapy setting (phenotypic sensitivity of 0), in which the antiviral effect was predominantly a result of enfuvirtide, and in a combination setting (phenotypic sensitivity ≥ 1), in which enfuvirtide and other active antiviral agents in the optimized background antiretroviral regimen contributed to the antiviral effect. An additional 20 patients from study TRI-003[4] were added to the database with a phenotypic sensitivity of 0 at week 2 to better describe the exposure-response relationship at lower concentrations because 9 patients received a 45-mg twice-daily dose in this study. Patients from TRI-003 were heavily treated, with high baseline HIV-1 RNA values, and were, therefore, grouped with the patients who had a phenotypic sensitivity score of 0 in the subgroup analysis by phenotypic sensitivity score. For these 20 patients, individual AUC_{12} and C_{trough} values were calculated from noncompartmental analysis.[4] During initial pharmacokinetic-pharmacodynamic modeling exercises, patients were grouped by phenotypic sensitivity scores 0, 1, 2, 3, 4, and 5. It was found that pharmacokinetic-pharmacodynamic relationships had no meaningful difference for all patients with a phenotypic sensitivity score of 1 or greater. Thus patients with a phenotypic sensitivity score of 1 or greater were grouped together and analyzed as a group for combination therapy.

The empiric 2-parameter maximum effect (E_{max}) model (equation 3) was considered appropriate to describe the exposure-response relationship of enfuvirtide. Uniform weighing was used for parameter estimates. Goodness of fit was judged by standard output for diagnosis in WinNonlin (Pharsight Corp, Mountain View, Calif) (residual plots, correlation coefficient for predicted versus observed, and Akaike criteria). The prediction of effect (E) for the doubling dose was done by use of the model parameters derived from the 90-mg twice-daily dose assuming a proportional increase in exposure based on a previous study.[2] Equations 3 and 4 were as follows:

$$E = -\frac{E_{max} \cdot AUC_{12}}{E(AUC_{12})_{50} + AUC_{12}} \quad (3)$$

$$E = -\frac{E_{max} \cdot C_{trough}}{E(C_{trough})_{50} + C_{trough}} \quad (4)$$

in which E is the enfuvirtide effect, defined as HIV-1 RNA decline from baseline at a given enfuvirtide exposure ($\log_{10}$ copies per milliliter); E_{max} is the maximal HIV-1 RNA decline from baseline achievable (based on the model) ($\log_{10}$ copies per milliliter) for either a combined regimen or enfuvirtide treatment alone; $E(AUC_{12})_{50}$ is the enfuvirtide AUC_{12} producing 50% of the maximum effect (hours times microgram per milliliter); and $E(C_{trough})_{50}$ is the enfuvirtide C_{trough} producing 50% of the maximum effect (micrograms per milliliter). Exposure-response data were analyzed by use of WinNonlin 4.1 (Pharsight Corp).

Exposure-safety analysis method. Exposure-safety analyses were performed to get a better understanding of pharmacokinetic changes with regard to drug safety. For these analyses, incidences of treatment-emergent adverse events, regardless of severity or causality, from patients who received enfuvirtide plus optimized background regimen were analyzed against the AUC_{12} values grouped by quartiles. Although these analyses were performed for all safety parameters, only those that are more frequent and major are listed in Table II.

Statistical analysis method. At week 2, the 3 variables of interest (AUC_{12}, C_{trough}, and HIV-1 viral load decline from baseline) were analyzed independently by a 1-way ANOVA. The factor tested was phenotypic sensitivity score. At week 24, the 3 variables of interest

Table II. Adverse events grouped by enfuvirtide exposure AUC_{12}

	Enfuvirtide exposure AUC_{12} (μg · h/mL)			
	≤40.9	*>40.9 and ≤50.8*	*>50.8 and ≤63.9*	*>63.9*
N	156	158	156	158
Adverse events (No. of patients and %)				
Total patients with ≥1 adverse event	147 (94.2)	148 (93.7)	145 (92.9)	147 (93.0)
Gastrointestinal disorders	79 (50.6)	90 (57.0)	88 (56.4)	94 (59.5)
Infections and infestations	83 (53.2)	83 (52.5)	88 (56.4)	93 (58.9)
Serious adverse events (No. of patients and %)				
Total patients with ≥1 adverse event	41 (26.3)	48 (30.4)	35 (22.4)	31 (19.6)
Gastrointestinal disorders	5 (3.2)	7 (4.4)	5 (3.2)	4 (2.5)
Infections and infestations	13 (8.3)	14 (8.9)	9 (5.8)	4 (2.5)
Adverse events by collapsed medical terms (No. of patients and %)				
All body systems	120 (76.9)	111 (70.3)	122 (78.2)	126 (79.7)
Nausea, vomiting, diarrhea, or gastroenteritis	60 (38.5)	61 (38.6)	64 (41.0)	77 (48.7)
Asthenic conditions	34 (21.8)	29 (18.4)	47 (30.1)	42 (26.6)
Hypersensitivity	31 (19.9)	27 (17.l)	30 (19.2)	36 (22.8)

AUC_{12}, Area under plasma concentration–12-hour time curve; N, number of patients from exposure-response database.

(AUC_{12}, C_{trough}, and HIV-1 viral load decline from baseline) were analyzed independently by a 2-way ANOVA with terms in the model for phenotypic sensitivity score and virologic failure (yes or no), as well as their interaction. At week 24, these 3 variables were analyzed independently by use of a 1-way ANOVA with factor gender. WinNonlin 4.1 software (Pharsight Corp) was used for statistical analysis.

RESULTS

Population pharmacokinetic database. A total of 2417 observations from 534 patients were included in the model-building database, and 446 observations from 94 patients were included in the validation database. Table I lists the baseline demographic characteristics of these patients and their concomitant medications. The baseline demographics for the validation and model-building databases were similar. All covariates listed in Table I were examined as potential predictors of enfuvirtide disposition.

Exposure-response database. A total of 648 patients were included in the exposure-response analysis: 534 patients from the model-building database, 94 patients from the model-validation database, and 20 patients from the early phase I/II study TRI-003.[4] The week 2 analysis included 629 patients (because 19 patients had missing values for plasma HIV-1 RNA data); of these patients, 198 were undergoing functional monotherapy (phenotypic sensitivity score of 0) and 431 were undergoing combination therapy (phenotypic sensitivity score ≥1). The week 24 analysis included 342 patients (because 286 patients met protocol-defined virologic failure by week 8 of the study, 123 had a phenotypic sensitivity score of 0, and 163 had a phenotypic sensitivity score ≥1); of these patients, 59 were undergoing functional monotherapy (phenotypic sensitivity score of 0) and 283 were undergoing combination therapy (phenotypic sensitivity score ≥1).

Enfuvirtide population pharmacokinetics. Several structural models to describe the time course of enfuvirtide concentrations were tested by use of FOCE with interaction. The final model selected was a 1-compartment model with first-order input and first-order elimination (ADVAN2 TRANS2)[20] and with total body weight and gender covariates on clearance. The model was parameterized for apparent clearance (CL/F), apparent volume of distribution (V/F), and absorption rate constant (K_a), with terms for interindividual variability in all parameters and a term for covariance between CL/F and V/F. The interindividual variability term for absorption was fixed at 20%. During model testing, the variance for K_a was estimated and was then fixed to that value to achieve a successful covariance step. Interoccasion variability was estimated for CL/F and V/F. The model used a

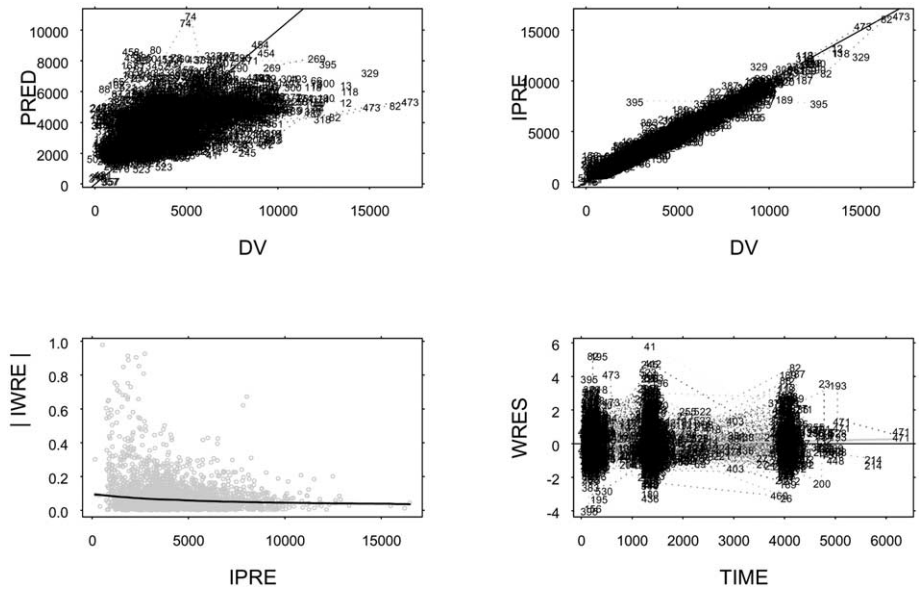

Fig 1. Diagnostic plots from model-building data set for final pharmacokinetic model. The *left top panel* shows the population predicted (PRED) versus observed (DV) enfuvirtide concentrations, and the *right top panel* shows the individual predicted (IPRD) versus observed (DV) concentrations. The line of unity is shown in both panels. The *bottom left panel* shows the absolute weight residuals (IWRE) versus individual predicted values (IPRE), and the *right bottom panel* shows the weighed residuals (WRES) versus time. The *line* in the *lower panels* is a loess smooth line.

combined constant coefficient of variation and additive residual error model.

Covariates identified as contributing to interindividual variability in CL/F included total body weight and gender. The final model parameterization is presented in equations 5, 6, and 7:

$$\frac{CL}{F} = \left(\theta_1 + \left(\frac{WT}{70}\right)\cdot\theta_4\right)\cdot\left(1 - SEX\cdot\theta_5\right) \quad (5)$$

$$\frac{V}{F} = \theta_2 \quad (6)$$

$$K_a = \theta_3 \quad (7)$$

in which θ_1 is the intercept value for mean apparent clearance (liters per hour), θ_2 is the mean apparent volume of distribution (liters), θ_3 is the mean absorption rate constant (per hour), θ_4 is the factor describing the effect of body weight (kilograms) normalized to 70 kg (WT, liters per hour) on CL/F, and θ_5 is the factor describing the effect of gender (0 for male and 1 for female) on CL/F.

The diagnostic plots for the final model are given in Fig 1. The plot of observed versus predicted concentrations of plasma enfuvirtide (left top panel) indicates that the model underpredicted concentrations at the higher concentration range. The plot of observed versus individual predicted concentrations (right top panel) shows that predicted and observed data were along the line of unity, and there was no overt bias at higher concentrations. The absolute weighed residuals versus individual predicted values are small and close to 0 (left bottom panel). The weighed residuals versus time are evenly scattered below and above 0 (right bottom panel). Diagnostic plots for the validation data set were as good as those for the model-building data set (Fig 2). The results of the maximum a posteriori Bayesian analysis of the validation data, with the use of the final model and population pharmacokinetic parameters from the model-building data set, showed good agreement between observed and predicted concentrations.

Parameter estimates for the final model are given in Table III. The mean θ_1 was 0.99 L/h (relative standard error for the estimate [SE], 22.1%), the mean V/F was 4.43 L (SE, 9.2%), and the mean K_a was 0.113 h^{-1} (SE, 5.8%). The θ_4 value for body weight effect was 0.883 (SE, 22.5%), and the θ_5 value for the effect of female gender was −2.03 (SE, 26.8%). The SEs of the parameter estimates were reasonable (≤26.8% for all param-

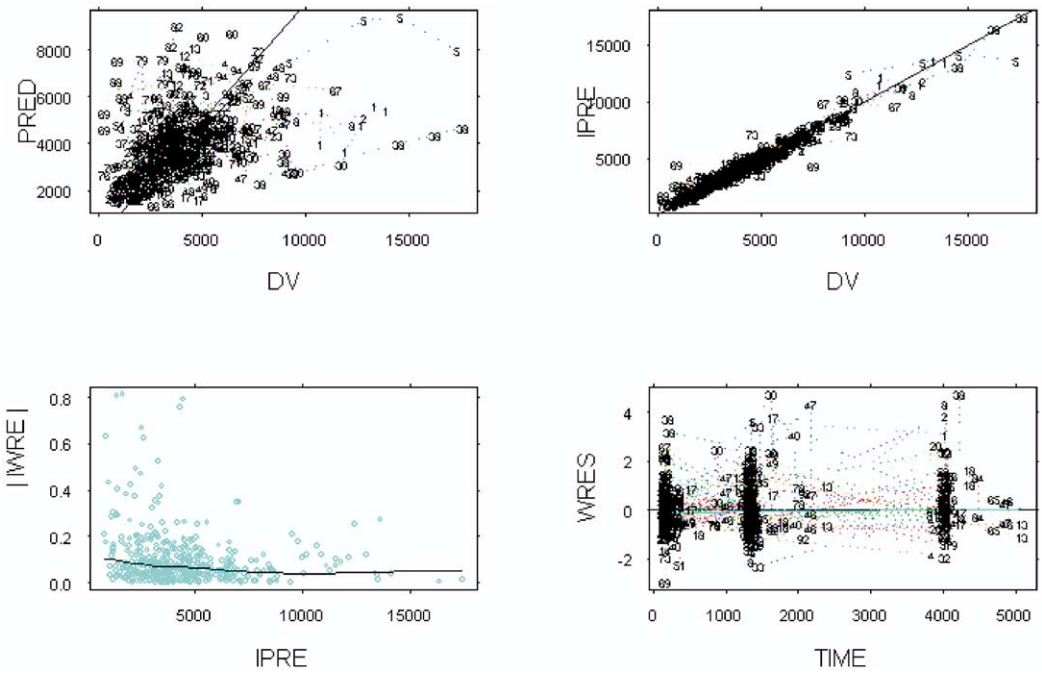

Fig 2. Diagnostic plots from model-validation data set by use of final pharmacokinetic model. The *left top panel* shows the population PRED versus DV enfuvirtide concentrations, and the *right top panel* shows the IPRD versus DV concentrations. The line of unity is shown in both panels. The *bottom left panel* shows the absolute IWRE versus IPRE values, and the *right bottom panel* shows WRES versus time. The *line* in the *lower panels* is a loess smooth line.

Table III. Pharmacokinetic parameter estimates from final population pharmacokinetic model

Pharmacokinetic parameter	*Population mean (SE %)*	*Interindividual variability (%)*	*Interoccasion variability (%)*
CL/F (L/h) (θ_1)	0.990 (22.1)	27.0	31.6
Effect of weight (L/h) (θ_4)	0.833 (25.5)		
Effect of female gender (θ_5)	−0.203 (26.8)		
V/F (L) (θ_2)	4.43 (9.20)	57.0	54.1
K_a (h^{-1}) (θ_3)	0.113 (5.8)	20.0 (fixed)	NE
Random residual CCV (variability as CV %)		16.2	
Random residual additive variability (ng/mL)		0.188	

SE, Relative standard error for estimate; CL/F, mean apparent clearance; V/F, mean apparent volume of distribution; K_a, mean absorption rate constant; NE, not evaluated; CCV, constant coefficient of variation; CV, coefficient of variation.

eters), and the random residual constant coefficient of variation was fairly low (16.2%). The additive portion of the residual error function was negligible (0.188 ng/mL). Interindividual variability (27.0%) and interoccasion variability (31.6%) for CL/F were acceptable, although both terms remained high for volume of distribution (57.0% and 54.1%, respectively). Covariates listed in Table I, other than weight and gender, were not found to affect the disposition of enfuvirtide. Creatinine clearance (>34.5 mL/min), markers of hepatic function (albumin, AST, ALT, total bilirubin, and prothrombin time), presence of protein in urine, presence of circulating anti-gp41 reactive antibodies, and concomitant medications did not contribute to the pharmacokinetic variability of enfuvirtide.

The effect of patient body weight and gender on apparent clearance is demonstrated in Fig 3. The mean apparent clearance (CL/F) was 1.82 L/h for a 70-kg male patient and 1.45 L/h for a 70-kg female patient. By use of the final model, the effects of

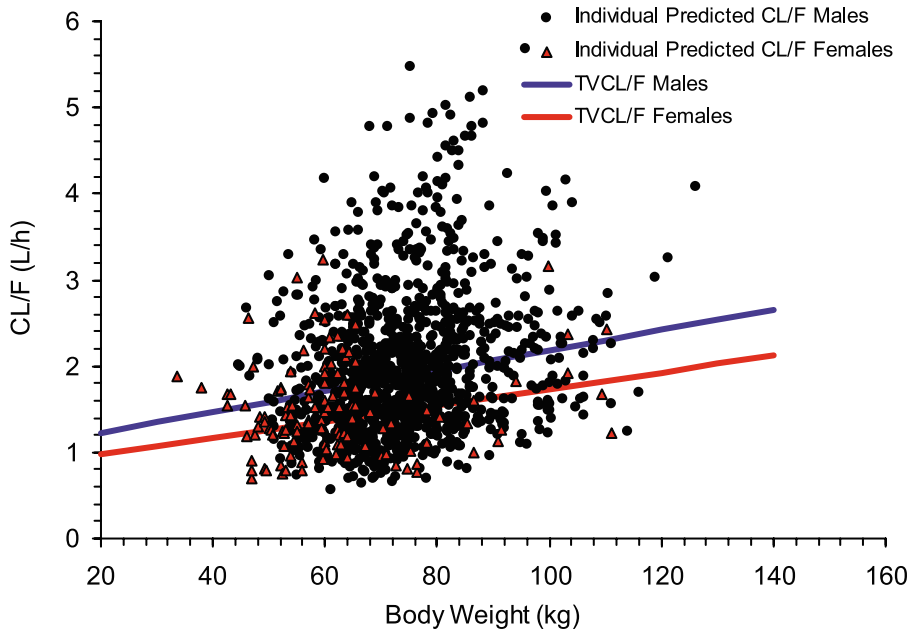

Fig 3. Individual and typical values of apparent clearance (CL/F) versus body weight by patient gender by use of final-model equation 5. TVCL/F, Population-typical value for apparent clearance.

Table IV. Pharmacokinetic and pharmacodynamic parameters

				Observed exposure and response (mean and SD)		
Week	*PSS*	*Responders*	*N*	*AUC_{12} (μg · h/mL) (mean and SD)*	*C_{trough} (μg/mL) (mean and SD)*	*Change in HIV-1 RNA from baseline (log_{10} copies/mL) (mean and SD)*
2	0	Yes	198	50.6 (19.2)*	2.78 (1.32)†	−1.32 (0.91)‡
2	≥1	Yes	431	54.3 (19.6)*	3.01 (1.27)†	−1.63 (0.68)‡
24	0	Yes	59	55.1 (18.7)	3.10 (1.29)	−2.33 (1.10)§
24	≥1	Yes	283	55.9 (20.9)	3.08 (1.33)	−2.68 (0.87)§
24	= 0	No	123	52.3 (18.0)	2.88 (1.25)	−0.32 (0.535)§
24	≥1	No	163	52.1 (17.7)	2.89 (1.20)	−0.43 (0.77)§

Data without footnote symbols indicate that there was no statistically significant difference at a level of 5%.
PSS, Phenotypic sensitivity score; C_{trough}, trough plasma concentration; HIV-1, human immunodeficiency virus type 1; RNA, ribonucleic acid.
*P = .017 for AUC_{12} for PSS of 0 versus PSS of 1 or greater.
†P = .025 for C_{trough} for PSS of 0 versus PSS of 1 or greater.
‡P < .001 for decline in HIV-1 RNA from baseline for PSS of 0 versus PSS of 1 or greater.
§Both PSS (P = .003) and virologic failure (P < .001) were statistically significant for HIV-1 RNA decline from baseline. No interaction was significant (P = .117).

gender and body weight on CL/F could be calculated for any body weight and gender. CL/F increased with increasing weight for both male and female patients. Male patients with a low body weight (40-50 kg) had an approximately 15% to 20% lower CL/F than a male patient with a 70-kg body weight. Similarly, male patients with higher body weights (110 kg) had a 26% higher CL/F. Female patients with a low body weight (40-50 kg) had clearance values that were approximately 35% lower than the value in the reference 70-kg male patient. The CL/F values for female patients were approximately 20% lower than those observed in male patients of the same weight.

Enfuvirtide exposure-response relationship. Mean AUC_{12}, C_{trough}, and HIV-1 plasma RNA decline from baseline are summarized in Table IV, and E_{max} model–estimated parameters at week 2 and week 24 by phenotypic sensitivity score are summarized in Table V.

The exposure parameters (AUC_{12} and C_{trough}) in patients who had protocol-defined virologic failure

Table V. E_{max} model–estimated parameters at week 2 and week 24

				AUC_{12} as exposure			*C_{trough} as exposure*		
Week	*PSS*	*N*	*Responders*	*E_{max} (log_{10} copies/mL)*	*EC_{50} (μg · h/mL)*	*R*	*E_{max} (log_{10} copies/mL)*	*EC_{50} (μg/mL)*	*R*
2	0	198	Yes	−2.07 ± 0.45	26.5 ± 16.5	0.211	−1.74 ± 0.27	0.76 ± 0.50	0.177
2	≥1	431	Yes	−1.80 ± 0.12	5.3 ± 3.5	0.073	−1.75 ± 0.10	0.20 ± 0.15	0.072
24	0	59	Yes	−3.28 ± 0.87	20.8 ± 19.3	0.071	−3.09 ± 0.66	0.90 ± 0.79	0.061
24	≥1	283	Yes	−2.88 ± 0.1	3.76 ± 3.26	0.205	−2.83 ± 0.15	0.14 ± 0.14	0.201
24	= 0	123	No	Not estimable					
24	≥1	163	No	Not estimable					

E_{max}, Maximum effect; EC_{50}, exposure as AUC_{12} or C_{trough} that produces 50% of maximal response; R, correlation coefficient (predicted, observed).

(n = 286) were similar to those observed in patients who achieved a protocol-defined virologic response, regardless of phenotypic sensitivity scores, indicating that virologic failure was not related to low enfuvirtide exposure (Table IV).

In those patients in whom a virologic response was achieved, the viral load decline was higher in those with a phenotypic sensitivity score of 1 or greater than in those with a phenotypic sensitivity score of 0 at week 2, as well as week 24, as expected. In addition, regardless of phenotypic sensitivity scores, the viral load decline was higher at week 24 than at week 2 (Table IV).

Fig 4 displays scatter plots and E_{max} modeling of the data in patients with a phenotypic sensitivity score of 0 and patients with a phenotypic sensitivity score of 1 or greater. The following general observations and inferences were made from the scatter plots in Fig 4: (1) Appreciable variability was seen in both exposure and response observations; (2) the antiviral response appeared to be almost independent of pharmacokinetic exposure in patients with a phenotypic sensitivity score of 1 or greater, which would be expected if the 90-mg dose represented the maximally effective dose and the resultant exposure and response data described the plateau of the dose-response curve; (3) the strength of all relationships, as measured by correlation coefficient *R* (0.06-0.21), was low, probably because of variability in the data and lack of antiviral data at the lower end of pharmacokinetic exposure; (4) the E_{max} model with AUC_{12} used as exposure (equation 3) predicted that the 90-mg twice-daily dose achieved 66% (week 2) and 73% (week 24) of maximal effect for patients undergoing monotherapy and 92% (week 2) and 93% (week 24) of maximal effect for patients undergoing combination therapy; and (5) the E_{max} model with C_{trough} used as exposure (equation 3) predicted similar results, with 78% (week 2 and 24) of maximal effect for patients undergoing monotherapy and 91% (week 2) and 94% (week 24) of maximal effect for patients undergoing combination therapy. These results suggest that the 90-mg twice-daily dose of enfuvirtide if combined with 1 or more other active antiretroviral drugs provides a better chance of therapeutic effectiveness.

Table VI summarizes the effect of gender and body weight on the enfuvirtide exposure and antiviral response. No difference in the efficacy of treatment was observed between male and female patients, even though female patients had an approximately 18% lower body weight and 28% higher AUC_{12} than male patients (Table VI).

Enfuvirtide exposure-safety analysis. The results for the exposure-safety analyses from 628 patients receiving enfuvirtide plus optimized background regimen are shown in Table II. These analyses indicated that the incidence of frequencies of adverse events and serious

Fig 4. Population pharmacokinetic model–estimated area under plasma concentration–12-hour time curve (AUC_{12}) (**A**, **C**, **E**, and **G**) and steady-state trough concentration of enfuvirtide (C_{trough}) (**B**, **D**, **F**, and **H**) values versus observed change in human immunodeficiency virus type 1 (HIV-1) ribonucleic acid (RNA) viral load from baseline (BL) for patients undergoing monotherapy (phenotypic sensitivity score [PSS] of 0) at week 2 (**A** and **B**) and week 24 (**E** and **F**) and those undergoing combination therapy (PSS ≥1) at week 2 (**C** and **D**) and week 24 (**G** and **H**). The *solid line* is the maximum effect (E_{max}) model–predicted HIV-1 RNA viral load change from baseline.

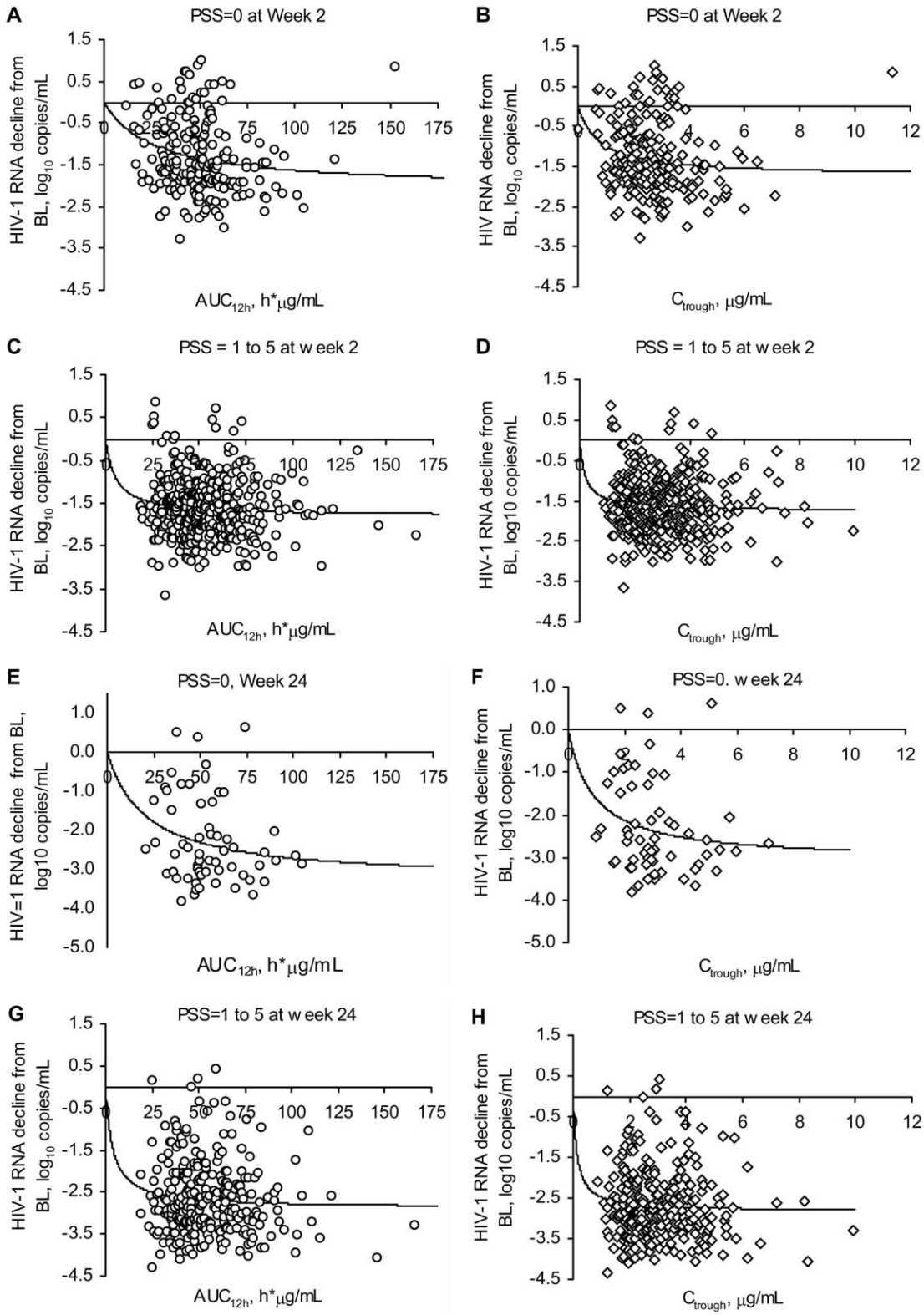
A
PSS=0 at Week 2
HIV-1 RNA decline from BL, log10 copies/mL
AUC12h, h*µg/mL
B
PSS=0 at Week 2
HIV RNA decline from BL, log10 copies/mL
Ctrough, µg/mL
C
PSS = 1 to 5 at w eek 2
HIV-1 RNA decline from BL, log10 copies/mL
AUC12h, h*µg/mL
D
PSS = 1 to 5 at w eek 2
HIV-1 RNA decline from BL, log10 copies/mL
Ctrough, µg/mL
E
PSS=0, Week 24
HIV=1 RNA decline from BL, log10 copies/mL
AUC12h, h*µg/mL
F
PSS=0. w eek 24
HIV-1 RNA decline from BL, log10 copies/mL
Ctrough, µg/mL
G
PSS=1 to 5 at w eek 24
HIV-1 RNA decline from BL, log10 copies/mL
AUC12h, h*µg/mL
H
PSS=1 to 5 at w eek 24
HIV-1 RNA decline from BL, log10 copies/mL
Ctrough, µg/mL

Table VI. Mean enfuvirtide exposure and antiviral response grouped by gender and body weight

Gender	*N*	*Age (y) (mean ± SD and range)*	*Body weight (kg) (mean ± SD and range)*	*AUC_{12} (μg · h/mL) (mean ± SD and range)*	*Change in HIV-1 RNA viral load from baseline at wk 24 (log_{10} copies/mL) (mean ± SD and range)*
Male	564	42.8 ± 7.8 (21 to 67)	73.8 ± 12.0 (46 to 123)	52.6 ± 18.4* (19.2 to 153)	−1.61 ± 1.37† (−4.54 to 1.20)
Female	64	38.4 ± 7.3 (16 to 62)	60.7 ± 14.1 (32.7 to 111)	67.3 ± 22.8* (32.4 to 167)	−1.54 ± 1.48† (−4.10 to 2.21)
All	628	42.3 ± 7.8 (16 to 67)	72.5 ± 12.8 (32.7 to 123)	54.1 ± 19.4 (19.2 to 167)	−1.60 ± 1.38 (−4.54 to 2.21)

*$P < .0001$ for AUC_{12} for male patients versus female patients.
†$P = .7042$ for change in HIV-1 RNA viral load from baseline for male patients versus female patients.

adverse events of enfuvirtide remains unchanged over the exposure range examined in this study.

DISCUSSION

In the early stages of developing the population pharmacokinetic model, several structural models were investigated, including the inverse Gaussian absorption model linked with a 2-compartment disposition mode, which was identified as the best model for a well-controlled, 4-way, single-dose crossover study.[2] They were rejected on the basis of high objective function values, poor residual plots, or unidentifiable parameters. During the model development process, data from a previous intensive pharmacokinetic study[2] were added to the database to improve the performance of an inverse Gaussian density absorption model, but this was also rejected. The poor performance of the inverse Gaussian density function was related to the fact that there were insufficient data in the absorption phase from the sparse pharmacokinetic sampling schedule used for this analysis. However, the estimated CL/F values from this study (1.82 L/h for male patients and 1.45 L/h for female patients) were very close to values obtained previously with the inverse Gaussian density absorption model (1.48 L/h),[2] indicating that there was no potential issue from misspecification of the absorption model. V/F is low because enfuvirtide is not expected to penetrate intracellularly. The V/F value of 4.43 L from this analysis is very close to the true volume of distribution at steady state (V_{ss} = 5.48 L) after intravenous administration if divided by bioavailability (84.3%).[2]

Despite an observed difference between AUC_{12} for male and female patients, there was no difference in treatment efficacy between the genders (Table VI) and AUC_{12} was not related to the frequency of adverse events and serious adverse events (Table II). Therefore the effect of body weight and gender on CL/F does not appear to be clinically relevant.

No relationship between renal function and enfuvirtide pharmacokinetics was identified. This may be because phase III studies excluded patients with creatinine clearance lower than 30 mL/min. Although the data do not support the need for dose adjustment in patients with mild or moderate renal impairment (creatinine clearance of 30-80 mL/min), it is not possible to draw conclusions about enfuvirtide pharmacokinetics in patients with severe renal impairment or end-stage renal disease. Likewise, none of the tested markers of hepatic function (albumin, AST, ALT, total bilirubin, and prothrombin time) contributed to interindividual variability in enfuvirtide pharmacokinetics. Although the presence of cirrhosis was a planned covariate for population analysis, this data set did not include any patients with cirrhosis. Therefore no conclusions can be drawn regarding the pharmacokinetics of enfuvirtide in patients with hepatic cirrhosis. No significant effect of concomitant drugs on enfuvirtide CL/F was identified in this analysis. These results are consistent with several independent drug-drug interaction studies.[21-23]

Enfuvirtide was approved for subcutaneous injection in combination with other antiretroviral agents for the treatment of HIV-1 infection in treatment-experienced patients with evidence of HIV-1 replication despite ongoing antiretroviral therapy. The current treatment guideline calls for the use of combinations of 3 or more active antiretroviral drugs to achieve and maintain a viral load decline to a clinically significant level over a long period of time.[24] For those patients in whom HIV is insensitive to the currently available antiretroviral drugs, the only treatment option is either an investigational agent or a newly approved antiretroviral agent. These patients will have virologic failure, although often caused by problems with adherence, suboptimal pharmacokinetics, or other reasons, which is eventually associated with development of resistance to the antiretrovirals in the patients' regimen, whereas continued treatment in the face of ongoing viral replication can

lead to cross-resistance to other drugs within a class. Treatment-experienced patients thus face decreasing options of finding active drugs to which their virus is sensitive or adding newly approved or investigational agents. Including only 1 fully active antiretroviral (it may be one not previously used or a new or investigational agent), when combined with other recycled drugs, represents functional monotherapy. Patients undergoing functional monotherapy will generally have only short-term benefit (typically 2-4 weeks) with these newer drugs because 1 active drug in the regimen is inadequate to exert the selective pressure to fully suppress the virus and the virus will mutate and become insensitive. In the 2 phase III studies (TORO 1 and TORO 2), at week 2, 198 patients (31.5% of total patients) had HIV that was insensitive to the currently available drugs and the only active drug in their regimen was enfuvirtide; thus enfuvirtide was used as functional monotherapy. Resistant virus developed at week 24 for this reason in 123 (62%) of these 198 patients. At week 24, for the same reason, 163 of 431 patients (37.8%) with a phenotypic sensitivity score of 1 or greater (ie, using enfuvirtide with $\geq$1 other active antiretroviral) also met the protocol-defined virologic failure. The pharmacokinetic exposure in patients meeting virologic failure criteria was not different from that in those with a virologic response ($P > .05$), thus suggesting that the virologic failure was not a result of enfuvirtide pharmacokinetics but was most likely a result of other reasons, such as changes in viral dynamics, change in the immune status of patients, compliance with the antiviral drug regimen, and so on. Although it may be argued that exclusion of these patients will inflate the magnitude of an antiviral response, it is also true that a dose- or exposure-response relationship is difficult to achieve in patients who do not respond to a drug therapy for reasons other than those related to dose or pharmacokinetics. Therefore the present exposure-response relationship was studied at 2 time points—at week 2, which included all patients, and at week 24, which included only patients who did not meet the protocol-defined virologic failure criteria. The relationships at these time points are generally quite similar both qualitatively and quantitatively, supporting the approach and conclusion of these analyses.

In 4 previous monotherapy studies, doses lower than 90 mg twice daily were found to be inferior in achieving the antiretroviral effect.[3-6] Although a theoretic argument can be made that the 90-mg twice-daily dose may be appropriate for patients with a phenotypic sensitivity score of 0 and a dose lower than 90 mg twice daily may be sufficient for patients with a phenotypic sensitivity score of 1 or greater, there are no clinical data to support a lower dose. Higher doses or exposures are preferred in dosing because no exposure-related adverse events have been noted with enfuvirtide.

Because no clinical data are available for enfuvirtide doses higher than 90 mg twice daily, it is important to confirm the adequacy of the selected dose with regard to its ability to suppress viral replication. The exposure-response analyses indicated that the 90-mg twice-daily dose was appropriate for patients with a phenotypic sensitivity score of 1 or greater and suggested that some additional benefit might be achieved from higher drug exposure in patients with a phenotypic sensitivity score of 0. However, the E_{max} model also suggested that there was only a limited benefit (additional decline of <0.3 $\log_{10}$ copies/mL) of doubling the daily dose in patients undergoing functional monotherapy.

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References

1. Veronese FD, DeVico AL, Copeland TD, Oroszlan S, Gallo RC, Sarngadharan MG. Characterization of gp41 as the transmembrane protein coded by the HTLV-III/LAV envelope gene. Science 1985;229:1402-5.
2. Zhang X, Nieforth K, Lang JM, Rouzier-Panis R, Reynes J, Dorr A, et al. Pharmacokinetics of plasma enfuvirtide after subcutaneous administration to patients with human immunodeficiency virus: inverse Gaussian density absorption and 2-compartment disposition. Clin Pharmacol Ther 2002;72:10-9.
3. Kilby JM, Hopkins S, Venetta TM, DiMassimo B, Cloud GA, Lee JY, et al. Potent suppression of HIV-1 replication in humans by T-20, a peptide inhibitor of gp41-mediated virus entry. Nat Med 1998;4:1302-7.
4. Kilby JM, Lalezari JP, Eron JJ, Carlson M, Cohen C, Arduino RC, et al. The safety, plasma pharmacokinetics, and antiviral activity of subcutaneous enfuvirtide (T-20), a peptide inhibitor of gp41-mediated virus fusion, in HIV-infected adults. AIDS Res Hum Retroviruses 2002; 18:685-93.
5. Lalezari JP, DeJesus E, Northfelt DW, Richmond G, Wolfe P, Haubrich R, et al. A controlled phase II trial assessing three doses of enfuvirtide (T-20) in combination with abacavir, amprenavir, ritonavir, and efavirenz in non-nucleoside reverse transcriptase inhibitor-naive HIV-infected adults. Antivir Ther 2003;8:279-87.
6. Wheeler DA, Lalezari JP, Kilby JM, Wheat J, Delehanty J, DeMasi R, et al. Safety, tolerability, and plasma pharmacokinetics of high-strength formulations of enfuvirtide

(T-20) in treatment-experienced HIV-1-infected patients. J Clin Virol 2004;30:183-90.
7. Fletcher CV. Enfuvirtide, a new drug for HIV infection. Lancet 2003;361:1577-8.
8. Lalezari JP, Henry K, O'Hearn M, Montaner JS, Piliero PJ, Trottier B, et al. Enfuvirtide, an HIV-1 fusion inhibitor, for drug-resistant HIV infection in North and South America. N Engl J Med 2003;348:2175-85.
9. Chang D, Kolis SJ, Linderholm KH, Julian TF, Nachi R, Dzerk AM, et al. Bioanalytical method development and validation for a large peptide HIV fusion inhibitor (enfuvirtide, T-20) and its metabolite in human plasma using LC-MS/MS. J Pharm Biomed Anal. In press 2005.
10. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. Nephron 1976;16:31-41.
11. Kirkpatrick CM, Duffull SB, Begg EJ. Pharmacokinetics of gentamicin in 957 patients with varying renal function dosed once daily. Br J Clin Pharmacol 1999;47:637-43.
12. DuBois DD, Eugene F. A formula to estimate the approximate surface area. Arch Intern Med 1916;17:863-71.
13. Gobburu JV, Lawrence J. Application of resampling techniques to estimate exact significance levels for covariate selection during nonlinear mixed effects model building: some inferences. Pharm Res 2002;19:92-8.
14. Wahlby U, Jonsson EN, Karlsson MO. Assessment of actual significance levels for covariate effects in NONMEM. J Pharmacokinet Pharmacodyn 2001;28: 231-52.
15. Mandema JW, Verotta D, Sheiner LB. Building population pharmacokinetic–pharmacodynamic models. I. Models for covariate effects. J Pharmacokinet Biopharm 1992;20:511-28.
16. Beal SL, Sheiner LB. The NONMEM system. Am Stat 1980;34:118-229.
17. Sheiner LB, Rosenberg B, Marathe VV. Estimation of population characteristics of pharmacokinetic parameters from routine clinical data. J Pharmacokinet Biopharm 1977;5:445-79.
18. Sheiner LB, Beal S, Rosenberg B, Marathe VV. Forecasting individual pharmacokinetics. Clin Pharmacol Ther 1979;26:294-305.
19. Sheiner LB, Beal SL. Bayesian individualization of pharmacokinetics: simple implementation and comparison with non-Bayesian methods. J Pharm Sci 1982;71: 1344-8.
20. Boeckmann AJ, Sheiner LB, Beal SL. NONMEM users guide. Part V. Introductory guide. San Francisco (CA): NONMEM Project Group, University of California, San Francisco; 1994. p. 149.
21. Zhang X, Lalezari JP, Badley AD, Dorr A, Kolis SJ, Kinchelow T, et al. Assessment of drug-drug interaction potential of enfuvirtide in human immunodeficiency virus type 1-infected patients. Clin Pharmacol Ther 2004; 75:558-68.
22. Ruxrungtham K, Boyd M, Bellibas SE, Zhang X, Dorr A, Kolis S, et al. Lack of interaction between enfuvirtide and ritonavir or ritonavir-boosted saquinavir in HIV-1-infected patients. J Clin Pharmacol 2004;44:793-803.
23. Boyd MA, Zhang X, Dorr A, Ruxrungtham K, Kolis S, Nieforth K, et al. Lack of enzyme-inducing effect of rifampicin on the pharmacokinetics of enfuvirtide. J Clin Pharmacol 2003;43:1382-91.
24. Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents. Developed by the Panel on Clinical Practices for Treatment of HIV Infection convened by the Department of Health and Human Services (DHHS). Oct. 29, 2004. Available from: http://AIDSinfo.nih.gov. Accessed April 18, 2005.