CENTER FOR DRUG EVALUATION AND RESEARCH

Application Number 21-481

CLINICAL PHARMACOLOGY and BIOPHARMACEUTICS REVIEW(S)

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW

NDA:	21-481 1P
Submission Dates:	07/30, 08/13, 08/22, 09/10/2002 (Rolling Submission); 09/13, 10/23, 11/12, 11/15, 11/20, 12/05/2002; 01/22, 03/10/2003
Brand Name:	Fuzeon™
Generic Name:	Enfuvirtide
Indication:	Treatment of HIV-1 infection
Applicant:	Hoffmann-La Roche
Formulation:	Lyophilized powder for injection, 90 mg
Pharmacometrics Reviewer:	Jenny J. Zheng, Ph.D.
Reviewer:	Robert O. Kumi, Ph.D.
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OCPB Division:	DPE III
ORM Division:	Division of Antiviral Drug Products
Draft Review Dates:	12/31/2002, 01/13/03, 02/24/2003

I. Executive Summary

Introduction and Background

Enfuvirtide (T-20) is a linear 36-amino acid synthetic peptide, composed of naturally occurring L-amino acid residues. T-20 is an inhibitor of HIV-1 gp41 mediated fusion and has potent activity against HIV. T-20 is proposed for the treatment of HIV-1 in treatment experienced subjects. The compound is a member of a new class of compounds for HIV treatment, known as the fusion inhibitors.

Two Phase III clinical (efficacy and safety) trials and 14 pharmacokinetic (PK) studies were conducted in support of the enfuvirtide application, NDA 21-481. In addition, a population PK analysis and *in vitro* studies were included in the NDA submission. All studies were conducted in HIV-infected subjects because of a theoretical concern for development of antibodies to T-20 that might make non-HIV infected subjects test positive for HIV. The pivotal efficacy trials, Studies T20-301 (NV 16054) and T20-302 (BV 16052), were conducted in treatment experienced subjects on optimized antiretroviral background regimens. According to the Medical Reviewer, the proposed T-20 dose of 90 mg twice daily given subcutaneously (SC) showed safety and effectiveness (decrease in HIV viral load) in both Phase III trials. The most common adverse event was injection site reaction. An exposure-response relationship was not validated for T-20; however, in two short-term pilot trials T-20 (monotherapy) exhibited a dose-response relationship and was more effective than placebo.

In all PK studies, T-20 was well absorbed following SC administration of the proposed 90 mg twice daily dose. PK of T-20 were adequately evaluated with respect to intrinsic and extrinsic factors including, gender, weight, race, concomitant medications and injection site. The population pharmacokinetic analysis indicated that weight and gender were the most critical intrinsic factors affecting T-20 clearance. The main limitation of most of the pharmacokinetic analyses was that patient compliance was not well-monitored.

Clinical Considerations in T-20 Development: Subcutaneous Dosing and Drug Reconstitution

Fuzeon™ (enfuvirtide or T-20) will be given by SC injection. The subcutaneous route is one of the most viable routes for delivery of peptides, as peptides are highly labile when administered orally. However, this administration route may present challenges to the patient population. A

major challenge will be to ensure that T-20 is administered consistently and correctly during therapy. Subjects are instructed to self-administer T-20 with a half-inch needle at a 45° angle and rotate injection sites. Conceivably, subjects will run out of SC sites if severe and long-lasting injection site reactions occur; consequently, subjects might administer T-20 to non-SC sites or be reluctant to administer T-20.

T-20 is available as a lyophilized powder that requires reconstitution; proper reconstitution may present a challenge to patient use. Specific reconstitution and storage procedures have to be followed to maintain good formulation properties; these procedures include avoiding the formation of air bubbles, waiting for powder to dissolve completely (may take up to 45 minutes), and limited storage time (once reconstituted, the refrigerated solution is stable for 24 hours). The clinical division and applicant are working closely together to provide appropriate patient education to enhance correct administration of T-20 during HIV therapy.

A. Recommendations

The Office of Clinical Pharmacology and Biopharmaceutics (OCPB) reviewed the information submitted to NDA 21-481. Overall, the information provided by the applicant adequately addresses the requirements of 21 CFR Part 320 and is sufficient to make labeling recommendations related to clinical pharmacology, biopharmaceutics and pharmacokinetics. Based on the review of the clinical pharmacology information the following recommendations can be made regarding Fuzeon administration:

- The dose of enfuvirtide in treatment-experienced HIV-1 infected adults is 90 mg subcutaneously given twice daily
- Enfuvirtide can be administered at the proposed dosage with commonly prescribed antiretroviral agents and agents used to treat coexisting conditions.
- The dose of enfuvirtide in HIV-infected pediatric subjects between of 6 and 16 years (inclusive) of age is 2.0 mg/kg subcutaneously given twice daily up to a maximum dose of 90 mg twice daily.

B. Proposed Phase IV Commitments

1. Evaluate the effect of impaired renal function (creatinine clearance < 35 mL/min) on enfuvirtide pharmacokinetics.
2. Provide additional pharmacokinetic data in children less than six years old

[redacted]

Robert O. Kumi, Ph.D.
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II. Table of Contents

III. Summary of Clinical Pharmacology and Biopharmaceutics Findings

Clinical Pharmacology and Biopharmaceutics Program

A dose of 90 mg enfuvirtide twice daily is proposed for the treatment of HIV-1 infection. The following clinical pharmacology and biopharmaceutics studies were included in NDA 21-481:

Study Identifier	Brief Description/Title
Evaluation of Pharmacokinetics and T-20 Formulations	
NP16220/T20-501	Absolute bioavailability and dose proportionality assessment
NP16370/T20-506	Influence of subcutaneous site on T-20 absorption
NV16059/T20-208	Comparison of formulations: relative bioavailability
Metabolism and Drug-Drug Interactions	
6131-321	Metabolism of T-20 in hepatocytes
100 3497	Metabolism of T-20 in microsomes
[redacted]64C-07482-006	*In vitro* metabolic inhibition
NP16221/T20-502	Drug-drug interaction: common CYP substrates and T-20
NP16324/T20-503	Drug-drug interaction: ritonavir/saquinavir and T-20
NV16325/T20-504	Drug-drug interaction: ritonavir and T-20
NP16334/T20-505	Drug-drug interaction: rifampin and T-20
Special Populations and Evaluation of Intrinsic Factors	
NV16056/T20-310	Pediatric pharmacokinetics: multiple dose using mg/kg scheme
NV16054/T20-301	Population PK: effect of intrinsic and extrinsic factors on T-20 clearance
BV16052/T20-302	Population PK: effect of intrinsic and extrinsic factors on T-20 clearance
Miscellaneous Studies: Protein Binding and Supportive Pharmacokinetic Studies	
[redacted]03	Plasma protein binding and blood distribution
D01034	Plasma protein binding and displacement effects of coincubated medications
NV16060/T20-204	Pediatric pharmacokinetics: dose finding study using mg/m^2 dosing scheme
T20-206	Dose-proportionality and dose-response of T-20 in presence of antiretrovirals
T20-205	Pharmacokinetics of low dose T-20 in the presence of antiretroviral agents
[redacted]001	Dose-proportionality and dose-response for IV dosing
[redacted]002	Subcutaneous Dosing: continuous infusion (study terminated)
[redacted]003	Subcutaneous Dosing: continuous infusion and injection multiple dose

This section summarizes the Key Clinical Pharmacology and Biopharmaceutics findings related to T-20 for the treatment of HIV infection. At the proposed dose (90 mg twice daily), the mean

PK measures for T-20 in adults are: AUC ≈ 44 μg·hr/mL, C_{max} ≈ 5 μg/mL, C_{min} ≈ 3 μg/mL, and median T_{max} ≈ 4 hr.

- Absorption: Approximately 80 % of T-20 is absorbed following subcutaneous (SC) administration of T-20 (45 to 180 mg single dose). Maximal T-20 concentrations were obtained between 5 and 7 hours post dose, followed by a monoexponential decline in drug concentrations. T-20 appears to exhibit flip-flop kinetics ($t_{1/2}$ IV < apparent $t_{1/2}$ SC). Absorption was independent of SC administration site (arm, thigh and abdomen).

- Dose proportionality: Exposure of T-20 increased in an approximately dose proportional manner following single dose administration over the 45 to 180 mg dose range. The data provided were insufficient to make a definitive conclusion regarding dose-proportionality following multiple dose administration.

- Accumulation: Following multiple dose administration at the proposed clinical dose, approximately 30 % accumulation occurs.

- Distribution: T-20 is greater than 90 % bound to plasma proteins, particularly albumin. The volume of distribution was approximately 5 L following a 90 mg intravenous dose of T-20.

- Demographic Characteristics (Race, Gender and Body Weight): The clearance of T-20 is affected by gender and body weight; T-20 clearance is not affected by race. The T-20 clearance in female subjects is 20 % lower than in male subjects having the same body weight. T-20 clearance decreases with decreased body weight, irrespective of gender.

- Metabolism: T-20 is expected to undergo catabolism rather than metabolism. *In vitro* studies (microsomes and hepatocytes) indicate that T-20 is broken down into three degradation products (M1, M2, and M3). Only the deamidated metabolite, M3 or Ro 50-6343, has been characterized in clinical pharmacology studies. M3 accounts for less than 20 % of total T-20 exposure.

- Elimination: T-20 has a low systemic clearance (after IV administration) of approximately 1.5 L/h, and has a short elimination half-life, $t_{1/2}$ < 4 hr.

- Drug-drug interactions: Based on *in vitro* and *in vivo* metabolism data, T-20 has a low potential to undergo cytochrome P-450 (CYP) based drug-drug interactions at clinically relevant concentrations. *In vitro* studies indicate that T-20 does not inhibit the major CYP enzymes. *In vivo* studies suggest that T-20 does not alter the pharmacokinetics of common CYP substrates. However, T-20 exposure was increased (7 – 26 %) in the presence of ritonavir; the mechanism of the interaction is not known. The influence of transporters on T-20 pharmacokinetics has not been characterized.

- Pediatric subjects: Pediatric subjects between 5 and 17 years old receiving 2.0 mg/kg enfuvirtide achieve T-20 exposure that is comparable to that in adults receiving the 90 mg dose; however, data in pediatric subjects are more variable than in adult subjects. Data provided are insufficient to make dosing recommendations for children < 6 years old.

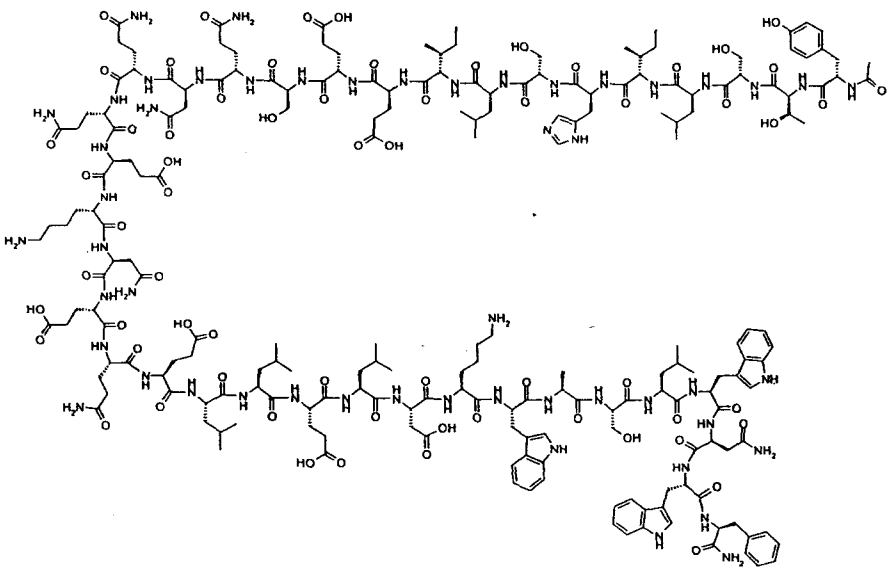
IV. QUESTION-BASED REVIEW

1. What are the general attributes of enfuvirtide and enfuvirtide formulations?

1.1 Physico-chemical characteristics

The empirical formula of enfuvirtide or T-20 is $C_{204}H_{301}N_{51}O_{64}$, and the molecular weight is 4,492 Dalton. Enfuvirtide has the following primary amino acid sequence (n = 36):

Ac-Tyr-Thr-Ser-Leu-Ile-His-Ser-Leu-Ile-Glu-Glu-Ser-Gln-Asn-Gln-Gln-Glu-Lys-Asn-Glu-Gln-Glu-Leu-Leu-Glu-Leu-Asp-Lys-Trp-Ala-Ser-Leu-Trp-Asn-Trp-Phe-NH_2



Physicochemical characteristics of enfuvirtide include:

- Physical Form- white to off-white, amorphous solid
- Melting Point- T-20 does not melt, but decomposes upon heating; decomposition begins at approximately 189°C
- Solubility- insoluble to slightly soluble in most organic solvents, apart from [redacted] and [redacted], negligible solubility in pure water; fairly soluble in buffers with pH 7.5
- pH solubility profile- insoluble at low pH but solubility increases with increasing pH; major inflection in solubility occurs at approximately pH 6.1; predicted solubility at pH 11.0 is 296 mg/mL
- Isoelectric point: occurs at pH 4.8

The trade name for enfuvirtide is Fuzeon. Fuzeon is a white to off-white, sterile, lyophilized powder containing enfuvirtide, sodium carbonate, mannitol, sodium hydroxide, and hydrochloric acid. The powder is reconstituted with 1.1 mL sterile water for injection to yield a 90 mg/mL solution of enfuvirtide and provides a 90 mg deliverable dose (see section 2.3.1). The drug product is supplied in single use 3 mL vials for subcutaneous (SC) administration.

The proposed enfuvirtide dose in HIV-1 infected individuals is as follows:

- Adults: 90 mg SC twice daily (BID)
- Children: 2 mg/kg (maximum of 90 mg) in HIV infected children aged 6 to 16 years old.

1.2 Proposed mechanism of drug action and therapeutic indication

Enfuvirtide inhibits the structural arrangement of HIV-1 gp41 by binding extracellularly to the virus protein; consequently, the HIV virus is prevented from entering the cell. The antiviral activity of enfuvirtide results from its association with a heptad-repeat motif (HR1) within native gp41 on the viral surface. Enfuvirtide is proposed for the treatment of HIV-infection in treatment experienced subjects.

1.3 Efficacy and safety information that contribute to the assessment of clinical pharmacology and biopharmaceutic study data

Efficacy and safety information obtained in the two pivotal efficacy trials, T-20-301 (n = 491) and T-20-302 (n = 504), suggest that the 90 mg twice daily enfuvirtide dose is safe and effective when combined with an optimized background (OB) antiretroviral regimen. The effectiveness of enfuvirtide was based on a comparison of enfuvirtide + OB vs. OB. The most frequent adverse event associated with T-20 administration is local injection site reactions. Pharmacokinetic data were obtained in both studies and evaluated as a part of the population pharmacokinetic analyses. An exposure (dose)-response relationship was not evaluated formally.

2. What are the general clinical pharmacology characteristics of enfuvirtide?

2.1 Selection and measurement of surrogate endpoints

The primary surrogate endpoints for HIV-1 infection are plasma HIV viral load and CD4 cell counts. The viral load tends to be more predictive of the progression of HIV infection than CD4 cell counts. As indicated previously (Mechanism of Action, section 1.2), enfuvirtide exhibits its antiretroviral activity by inhibiting cell fusion. Therefore, it is expected that the prevention of viral fusion will lead to decreased viral load. Twenty-four week viral load data and CD4 cell count data were included in the NDA submission.

2.2 Determination of PK measures for assessment of exposure-response relationships

Enfuvirtide was adequately measured in the plasma; therefore, one could estimate relevant enfuvirtide measures and parameters, such as AUC, C_{max}, C_{min} and apparent clearance (CL/F). Enfuvirtide is the active moiety in Fuzeon. The active metabolite, Ro 50-6343, was also adequately measured in plasma in some studies. Refer to Bioanalytical section of the QBR for further details on how enfuvirtide concentrations were determined.

2.3 Enfuvirtide exposure-response relationships

Enfuvirtide exposure-response relationships have not been formally established. However, in two short-term monotherapy studies and in combination therapy studies there was some indication that a dose-response relationship exists. The 90 mg SC dose was the most active of all SC doses evaluated (range of doses 45 to 90 mg), and was as safe as all other tested doses; thus this dose was chosen for the pivotal clinical studies. The Phase III clinical findings indicated that T-20 at the 90 mg dose was effective in causing viral load suppression. At week 24, there was a greater

than 1.0 log drop in the viral load. This decline in viral load is significant for subjects who have evidence of intolerance, resistance or previous experience with nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (nNRTIs), or protease inhibitors (PIs). In some studies the applicant indicated that a target minimum concentration or C_{trough} of 1 μg/mL, which corresponds to the mean *in vitro* IC_{50} for HIV suppression, is required for *in vivo* activity. However, no clear exposure-response existed for the C_{trough} exposure measure.

2.3.1 Delivered dose vs. nominal dose

The applicant indicates that there was some discrepancy between nominal doses and delivered doses in several Phase I and Phase II trials (Table I):

Table I: Nominal vs. Delivered T-20 Doses in Early Phase I and II Trials

Nominal Dose	Fill Volume/vial	Delivered Dose (mg)	Studies
25 mg carbonate	28.75	Testing not performed	—001 T-20-206
50 mg carbonate	53	45	T20-205 T20-208
50 mg carbonate	57.5	48.7*	—001 T20-205 T-20-206 T20-208
100 mg carbonate	106	90	T20-208

All other Phase II and III studies and protocols indicate that the deliverable dose is 90 mg. In the applicant's reports, both nominal and delivered doses were recorded, which made data interpretation difficult at times. The doses reported in this review will primarily refer to actual doses delivered, particularly for information that will be included in the label.

2.3.2 Monotherapy

In study —001, a two week pilot study (n =12) over the 3 to 100 mg IV (twice daily) dose range, there was a dose-response relationship (Figure 1a) between enfuvirtide dose and nadir viral load. The relationship between dose and CD4 cell counts was unclear (not shown). In study —003, at the nominal 50 and 100 mg SC (twice daily) dose levels, the 100 mg dose achieved higher suppression in viral load than the 50 mg dose (Figure 1b) over the four-week study period. Viral load began to return to baseline during monotherapy (Study —003) as has been shown with some classes of antiretrovirals.

Figure 1a: Study —001

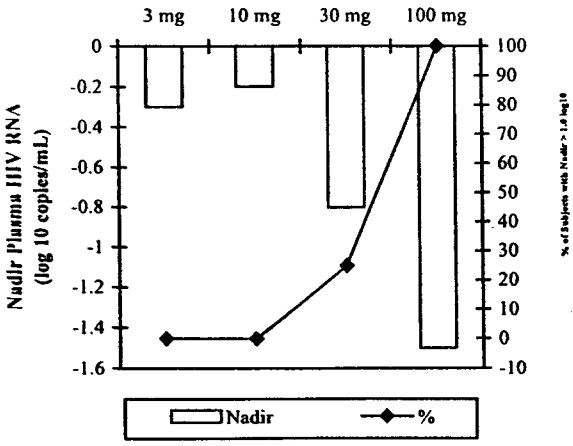
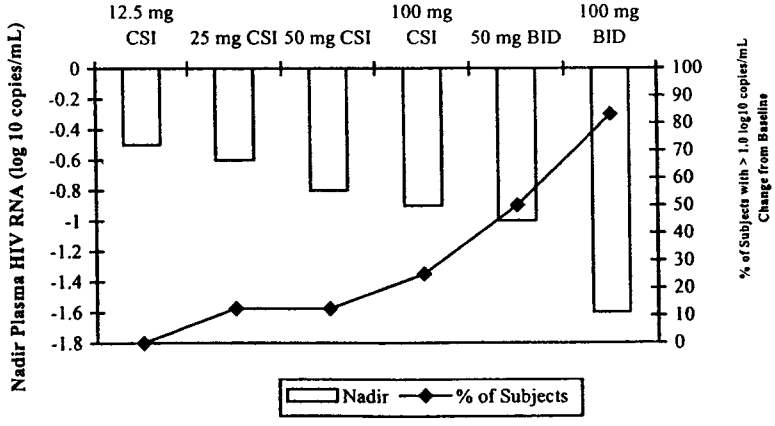


Figure 1b: Study —003



In figure 1b, CSI refers to the continuous subcutaneous infusion group and other subjects received enfuvirtide SC (non-CSI group).

2.3.3 Combination therapy

In two phase II studies, Studies 206 and 208, the degree of viral load suppression increased with increasing T-20 dose in the presence of a background regimen. In both studies, subjects were randomized to treatment groups that received a background regimen of ARV agents with or without T-20. These ARV agents were administered mainly at their recommended doses. Agents coadministered with T-20 in study 206 were amprenavir, efavirenz, abacavir and ritonavir (booster dose of 200 mg BID). In study 206, viral load suppression was greater in the 100 mg group than the 50 mg dose group at Week 16. In study 208, the efficacy of a placebo group (no T-20) was compared to different T-20 dose groups (nominal doses 50, 75 and 100 mg twice daily SC) at Weeks 2 and 16. In study 208 each subject received an optimized background regimen with T-20. The 100 mg twice daily dose achieved the greatest viral suppression among all the groups evaluated. The applicant indicates that the difference in viral suppression between the 75 mg BID (intent-to-treat: mean change from baseline = -2.62) and 100 mg BID (intent-to-treat: mean change from baseline = -2.39) dose was small and suggested that the 100 mg BID dose is approaching the upper portion of the enfuvirtide dose-response curve. This interpretation seems appropriate, based on the information provided even though the studies were not adequately powered. Collectively, the findings from studies 206 and 208 support the existence of a T-20 dose-response relationship, even though the other ARVs also contribute to the overall response. According to the applicant, safety of all T-20 doses was comparable, and there did not seem to be a dose-response relationship for any safety parameter in these studies. The Medical Reviewer agrees with the sponsor's interpretation of these study results.

2.4 Dose proportionality of enfuvirtide exposure/dose dependency/time dependency/ diurnal variation of enfuvirtide pharmacokinetics and

2.4.1 Dose dependency and dose proportionality

The pharmacokinetics of enfuvirtide exhibited dose independence, as shown by the relatively constant clearance, volume of distribution and half life (surrogate for elimination rate constant, particularly for IV) over the clinical dose range.

Table II: Mean ± SD IV Pharmacokinetic Measures Obtained after First and Seventh Dose of T-20 (Study ——01)

	3 mg	10 mg	30 mg	100 mg
Measure	N=4	N=5	N=4	N=4
$t_{½}$ (hr)				
First Dose	3.36 ± 2.99	2.27 ± 0.438	2.43 ± 0.551	2.73 ± 0.309
Seventh Dose	2.80 ± 1.71	2.29 ± 0.292	2.53 ± 0.532	2.65 ± 0.128
CL (L/hr)				
First Dose	2.34 ± 1.46	2.15 ± 0.668	1.86 ± 0.757	1.68 ± 0.325
Seventh Dose	2.27 ± 1.13	2.22 ± 1.190	1.70 ± 0.711	1.36 ± 0.267
V_d (L)				
First Dose	6.99 ± 2.39	7.16 ± 2.58	6.12 ± 1.49	6.61 ± 1.49
Seventh Dose	7.13 ± 1.61	7.04 ± 3.14	5.79 ± 1.56	5.23 ± 1.21

Table III: Mean (%CV) PK Parameters Following administration of IV and SC single dose (n= 12 Study [redacted]16220)

Treatment Groups	$t_{1/2}$ (h)	CL[a] (L/h)	V_{ss}[b] (L)
IV			
90 mg	3.16 [redacted]	1.40 [redacted]	5.48 [redacted]
SC			
45 mg	3.46 [redacted]	1.85 [redacted]	9.30 [redacted]
90 mg	3.80 [redacted]	1.68 [redacted]	9.42 [redacted]
180 mg	4.35 [redacted]	1.65 [redacted]	10.3 [redacted]

[a] CL/F reported for SC treatment groups (B, C, and D)
[b] V_z/F reported for SC treatment groups (B, C, and D)

Table IV: Mean (SE) Pharmacokinetic Parameters Following administration of T-20 SC [redacted] 003)

T-20 Dose	50 mg BID		100 mg BID	
Parameter	Day 0 (n = 12)	Day 14 (n =10)	Day 0 (n = 12)	Day 14 (n = 10)
CL/F	2.89 (0.45)	4.27 (0.51)	2.96 (0.47)	3.65 (0.49)

The differences in parameter values across studies are likely due to the different assays used (see Bioanalytical section): [redacted]001 ([redacted] no final validation report); [redacted] 003 ([redacted] validated); T20-501 ([redacted] validated).

Following single dose administration, the enfuvirtide exposure approximated dose proportionality over the 45 to 180 mg SC administration dose range (Table V).

Table V: Mean (%CV) T-20/Ro 29-9800 PK Measures Following SC Administration (n =12, Study [redacted]16220)

Treatment Groups	C_{max} (µg/mL)	t_{max} (h)	AUC_{inf} (h*µg/mL)	C_{24h} (µg/mL)
45 mg	2.48 [redacted]	5.38 [redacted]	25.8 [redacted]	0.120 [redacted]
90 mg	4.59 [redacted]	6.92 [redacted]	55.8 [redacted]	0.425 [redacted]
180 mg	8.05 [redacted]	6.50 [redacted]	113 [redacted]	1.25 [redacted]

[a] CL/F reported for SC treatment groups (B, C, and D)
[b] V_z/F reported for SC treatment groups (B, C, and D)

In the multiple dose study, dose-proportionality could not be definitively shown; the 50 mg and 100 mg dose appeared to exhibit dose-proportionality, but the 75 mg dose had higher than expected increase in exposure (Table VI). The reason for this finding is unclear.

Table VI: Mean (CV%) T-20 PK Parameters following 50, 75 or 100 mg BID for 28 days (Study T20-206)

Regimen	N	C_{max} (µg/mL)	C_{trough} (µg/mL)	$AUC_{0\text{-}12hr}$ (µg·hr/mL)	CL/F (L/hr)
50 mg BID	12	2.58 [redacted]	1.04 [redacted]	23.1 [redacted]	2.36 [redacted]
75 mg BID	12	4.63 [redacted]	2.22 [redacted]	42.8 [redacted]	1.89 [redacted]
100 mg BID	9	4.99 [redacted]	2.60 [redacted]	48.6 [redacted]	2.11 [redacted]

2.4.2 Time dependency

No formal studies were conducted to determine the pharmacokinetics of enfuvirtide over extended periods of time, however, based on examination of C_{trough} over 48 weeks (T20-205 and -206) and CL information in short-term multiple dose studies ([redacted]001 and 003), enfuvirtide kinetics appeared time-independent. The C_{trough}s at steady state were relatively constant over the 48-week period and suggests that the PK of enfuvirtide do not change with time (long-term).

Table VII: Mean T-20 trough concentrations following SC administration of T-20 (Study 206)

T-20 Dose	Week 4	Week 16	Week 32	Week 48
50 mg BID	0.972 (n = 4)	1.05 (n = 14)	1.14 (n = 12)	1.28 (n = 11)
75 mg BID	2.75 (n = 7)	2.27 (n = 15)	2.63 (n = 14)	2.19 (n = 10)
100 mg BID	3.63 (n = 3)	2.10 (n = 8)	2.72 (n = 6)	2.03 (n = 6)

(n) number of subjects

Table VIII: Mean ± SD (range) Trough T-20 plasma concentrations at the 50 mg BID dose (Study 205)

Study Week	N	C_{trough} (μg/mL)
Week 16	50	1.10 ± 0.71
Week 24	41	1.09 ± 0.58
Week 48	30	1.04 ± 0.66
Week 72	28	1.22 ± 0.69
Week 96	20	1.05 ± 0.85

(N) number of subjects

In two studies, the CL after the first dose (100 mg) was comparable to the CL after multiple doses (see Tables II and IV), indicating that T-20 PK were not time dependent (short-term).

2.4.3 Diurnal variation

T-20 pharmacokinetics did not appear to exhibit diurnal variation; although morning concentrations were numerically greater than evening concentrations. Due to the high variability in predose concentrations, the numerical difference in morning and evening concentrations is unlikely to be statistically significant (analysis not conducted). The mean morning and evening concentrations exceed the target minimum concentration (C_{trough}) value (1μg/mL) that the applicant indicates is required for activity. The clinical significance of achieving the target C_{trough} is unclear.

Table IX: Mean (CV%) Pre-dose Plasma Concentrations of T-20 (μg/mL) on Day 7 (Study NP16370)

Injection site	Morning	Evening
Abdomen (n = 12)	2.70	2.35
Thigh (n = 12)	3.34	2.65
Arm (n = 12)	3.69	3.41

2.5 Basic pharmacokinetics of enfuvirtide

2.5.1 Pharmacokinetics of enfuvirtide in healthy subjects

No PK studies were conducted in healthy subjects (non-HIV infected) due to the theoretical concern of development of antibodies to T-20 that may result in a positive reaction to HIV testing.

2.5.2 Absolute bioavailability

The absolute bioavailability (BA) of T-20 is approximately 80 %. Bioavailability assessments were made in different studies, but the most reliable BA estimates were obtained in Study 16220. In Study 16220 subjects received a single dose of 90 mg IV T-20 and one of three different SC T-20 dose levels (45, 90 or 180 mg) in a crossover fashion. Absorption was comparable at all dose levels.

Table XII: Mean (CV) Absolute Bioavailability of T-20 (n =12)

T-20 SC dose	Absolute bioavailability (%)
45 mg	77.3 —
90 mg	84.3 —
180 mg	85.6 —

2.5.3 Influence of SC administration site

Comparable T-20 exposure (Table IX) was obtained following administration of T-20 (90 mg twice daily) into the SC tissue of the arm, abdomen or thigh. Due to the comparability of exposure amongst the three sites, SC injection sites may be rotated during HIV therapy. The exposure comparison was made using the abdomen as the reference site; this site was chosen because it is the most frequently used SC injection site.

Table XII: Injection Site Comparisons- Geometric Mean Ratios (GMR) and 90% Confidence Intervals (CI) for T-20 (n = 12)

PK Parameter	GMR (%)	90 % CI
Thigh/Abdomen		
C_{max}	88.2	71.9 - 108
AUC_{12h}	101	82.2 - 125
Arm/Abdomen		
C_{max}	102	83.5 - 126
AUC_{12h}	117	95.0 - 144

2.5.4 Distribution, protein binding, and displacement effects of coincubated drugs

2.5.4.1 Volume of distribution

Enfuvirtide has a relatively small volume of distribution (following IV administration), approximately 5 L (see Tables II and III in Section 2), which suggests that enfuvirtide is mainly confined to the blood volume.

2.5.4.2 Protein binding and partitioning into red blood cells

Enfuvirtide is highly plasma protein bound in HIV+ and HIV- plasma (greater than 90 % bound) over the concentration range, . —— µg T-20/g plasma. The plasma protein binding of T-20 is mainly due to serum human albumin (> 90 % bound). At clinically relevant concentrations, T-20 partitioning into red blood cells was approximately 70 %.

2.5.4.3 *In vitro* displacement effects

In vitro, T-20 did not alter the protein binding of any of the following drugs: warfarin, midazolam, itraconazole, lopinavir, amprenavir, saquinavir, nelfinavir, efavirenz, or nevirapine. Similarly, none of the drugs listed altered T-20 plasma protein binding.

2.6 Elimination and metabolism of enfuvirtide

The applicant indicates that because T-20 is a peptide it is expected to undergo catabolism and metabolism. Peptidases, proteinases and enzymes located in various tissues and organs are expected to contribute to the catabolism of T-20. No data were provided to support these statements; however, this mechanism appears reasonable in the context of typical protein and peptide degradation. The literature indicates the liver and kidneys are the most important organs for elimination of peptides, such as T-20.

2.6.1 Mass balance

According to the applicant, a mass balance study was not conducted due to technical and safety concerns. The applicant indicated that it would be difficult technically to place the radiolabel on a metabolically stable part of the molecule that is not subject to exchange; additionally, measurement and identification of all the metabolic fragments would be difficult. These technical challenges appear reasonable in this reviewer's opinion. Safety concerns center on the fact that as T-20 is catabolized into its constituent amino acids, the labeled amino acid may become incorporated (via protein synthesis) into the body and remain in the body for an indeterminate length of time. In rats, complete radioactive (^{3}H-label) recovery was obtained in 7 days with the following distribution of radioactivity: 35 % in urine, feces and expired air and 65 % in the carcass (homogenized). The applicant's safety concerns have merit and are supported by the findings in animals.

Reviewer's Note

It should be noted that mass balance studies have been conducted with peptides in the past, and the FDA considers mass balance studies with peptides as reasonably safe. The applicant will not be asked to conduct a mass balance study, because the information is unlikely to alter the clinical use of T-20 even though it may generate useful PK information. However, due to the absence of mass balance information it is unclear if the hepatic and/or renal pathway is the major route of elimination.

2.6.2 *In vivo* metabolism of T-20

The T-20 metabolite, Ro 50 –6343 (M3) has activity (*in vitro)* that is 20 % that of T-20. Plasma concentrations of M3 are ———— of T-20 (range ———— , suggesting that its potential influence on total T-20 activity is minimal.

Table X: Mean (CV%) Steady-State T-20 PK Parameters Following SC Administration in Three SC sites (n = 12)

T-20 metabolite	**Abdomen**	**Thigh**	**Arm**
C_{max}, µg/mL	0.784 ——	0.800 ——	0.816 ——
C_{trough}, µg/mL	0.371 ——	0.444 ——	0.475 ——
AUC_{12h}, hr·µg/mL	6.44 (——)	7.03 ——	7.46 ——
Metabolite/parent			
AUC_{12h} Ratio	15.2% (——)	16.4% ——	14.4% ——

2.7 Pharmacokinetic modeling

2.7.1 Compartmental modeling

The applicant proposed the following PK models for describing T-20 plasma concentrations:

- For SC Administration: two compartment model with absorption input (inverse Gaussian density function) with first order elimination from the central compartment
- For IV Administration: two compartment model with bolus input and first order elimination from central compartment

The two models proposed by the applicant are acceptable, as they adequately describe T-20 pharmacokinetics. PK results obtained by these models were comparable to those obtained via non-compartmental analyses for the respective administration routes.

2.7.2 Population pharmacokinetic modeling: Predicted exposure parameters based on population PK analyses

The applicant indicates that the PK exposure predicted by the population model was comparable to that observed in other T-20 clinical trials. The PM reviewer indicates that the applicant's assessment is acceptable.

Table XIII: Mean AUC and C_{min} for T-20: Population Prediction vs. PK Studies

Exposure Measure	Population PK Prediction	Obtained in PK studies
C_{min} (μg/mL)	3.00	2.96*
AUC (μg·hr/mL)	54.0	49.1^

* data from Studies T20-208, NP16370 and NP16221

^ Applicant-pooled data- sources not indicated

Reviewer Comment

In the population PK modeling, the applicant used a simple input function, a first order absorption model, rather than the previously described inverse Gaussian function. The PM reviewer indicates that the data available for the population PK analyses precluded the use of the inverse Gaussian function; therefore use of the first order absorption model was acceptable.

3. What intrinsic factors (renal and hepatic function, age, race, weight, gender) affect enfuvirtide exposure?

Weight and gender were the only evaluable intrinsic factors that affected enfuvirtide exposure. Based on the findings from the applicant's population pharmacokinetic analyses, race (Black/White), renal function and hepatic function did not affect T-20 clearance in adults. The effect of age in the adult population could not be adequately evaluated because the PK of T-20 have not been studied in subjects over 65 years old. Pediatric subjects (6 to 16 years old) receiving a 2 mg/kg twice daily dose achieved comparable exposure to adults receiving 90 mg BID.

3.1 Population pharmacokinetics for analyses of intrinsic factors (PM Consult)

3.1.1 Renal and hepatic impairment

Formal studies were not conducted to assess the effect of renal or hepatic function on T-20 clearance. There was no clear signal from clinical or non-clinical studies, or mechanistic basis to suggest that T-20 PK will be significantly altered in subjects with impaired hepatic or renal function. However, the applicant will be asked to address the effect of Cl_{cr} < 35 mL/min on T-20 clearance as part of the Phase IV commitments. The applicant partially addressed the effect of hepatic and renal impairment on T-20 exposure in the population PK analyses. The applicant's analyses did not show any relationship between T-20 clearance and the markers of hepatic function (assessed independently) or degree of renal function (> 35 mL/min).

[ ]

[ ]

3.1.2 Weight, gender and race

Weight and Gender

Two highlights of the applicant's PK population analysis related to weight and gender were:

- Clearance of T-20 is 20 % lower in females than males; when matched for weight
- Relative to a 70 kg male reference subject: 1) the T-20 clearance is 26 % higher in a 110 kg male subject and 20 % lower in a 40 kg male subject; whereas 2) the T-20 clearance is the same in a 110 kg female subject and the clearance is 36 % lower in a 40 kg female subject

The Pharmacometrics reviewer agrees with the applicant's analysis. The relationship derived by the applicant to describe the apparent clearance with respect to gender and weight is

$$CL/F = [(0.99 + (\text{Body weight})/70)^* 0.833)^*(1 - 0.20\ GF)]$$

where, GF is a categorical gender factor (0 for male and 1 for female)

By appropriate substitution into the equation above, the typical value of clearance for a male patient weighing 70 kg is 1.82 L/h. A 40 kg male will have a CL of 1.47 and a 100 kg male will have a clearance of 2.18 L/h. The applicant indicates that the differences in T-20 exposure based on body weight do not require dose adjustment. The applicant provided justification for this recommendation by conducting an exposure-response analysis. In the analysis body weight served as a "surrogate" for T-20 exposure.

The applicant's exposure-response analysis showed a poor correlation (linear relationship, data not shown) between the change in baseline viral load vs. body weight ($R^2 = 0.0101$; $p = 0.011$). The range of body weights was 33 to 123 kg ($n = 628$), with the majority of patients weighing between 50 and 100 kg. Efficacy data were highly variable and overlapped considerably over the range of weights. For example, subjects with body weight below 50 kg had viral load changes ranging from + 0.5 to – 4 log copies/mL; these viral load changes were comparable for subjects weighing between 50 and 100 kg. The applicant's analyses of the safety data did not show any correlation between adverse events (incidence and/or severity) and body weight. Potentially, female subjects with body weight less than 50 kg would have very high T-20 exposures; therefore, the PM reviewer suggested that a subpopulation analyses be conducted to evaluate the effect of low body weight (< 50 kg) and gender on T-20 safety. The Medical Reviewer indicated that it was not feasible to evaluate the impact of low body weight and female gender on T-20 safety due to insufficient number of patients meeting the criteria.

Overall, the data and analyses provided support the applicant's labeling recommendation regarding body weight and gender in relation to T-20 dosage. However, there are insufficient data to make a definitive conclusion regarding the lack of clinical significance of the effect of body weight and/or gender. This reviewer proposes that the label indicate that dosage adjustment based on body weight or gender is not recommended, but the clinical significance of low body weight and gender is unknown. Following internal discussions and communications with the applicant it was agreed that the label will indicate that there is no dose adjustment required based on body weight and/or gender.

Race

The population PK findings related to weight are supported by information in Asian and non-Asian subjects.

Table I: Multiple Dose PK of T-20 in Drug Interaction Studies and other PK Studies: Asian vs. Non-Asian Subjects

Reference (Study)	White	Asian	Female/ Male	Age (years)	Weight (kg)	AUC_{12h} [a] (hr·µg/mL)	CL/F (L/hr)	Dose (mg/kg)	Adjusted AUC_{12h} (hr·µg/mL)
NP 16325	0 %	100 %	6/6	34± 6	58 ±7.6	82 ± 18		1.67 ± 0.24	39
NP 16324	0 %	100 %	6/6	34 ± 6	58 ± 10	61 ± 10		1.60 ± 0.29	45
NP16334	0%	100%	8/4	32±6	56±10	74 ± 17	1.3 ± 0.4	1.65 ± 0.29	45
NP16370[b]	92%	0%	0/12	45±6	78±11	43 ± 11	2.2 ± 0.6	1.17 ± 0.16	37
T20-208	82%	0%	0/11	42±8	76±11	49 ± 19	2.4 ± 1.0	1.21 ± 0.16	40
NP16220	100%	0%	3/9	38±8	68±10	56 ± 12	1.7 ± 0.4	1.35 ± 0.22	41

[a] Adjusted AUC_{12h}=observed AUC_{12h}/(90mg/body weight)

It is noted that the weight variable appears nested within race and gender, as members of certain races tend to have lower weight than others and women tend to weigh less than men. According to the applicant's population PK analyses, the clearance of T-20 in Black subjects (n = 44) was comparable to that in White (n = 476) subjects; however, the clearance of T-20 in Asian (n = 36) subjects was lower than in White subjects. When clearance is adjusted based on a subject's body weight, there does not appear to be a difference between T-20 clearance in White and Asian subjects. Race differences have been observed in terms of drug delivery with some transdermal systems that may be applicable to SC administration. However, the clinical efficacy data suggest that T-20 was effective in all races studied.

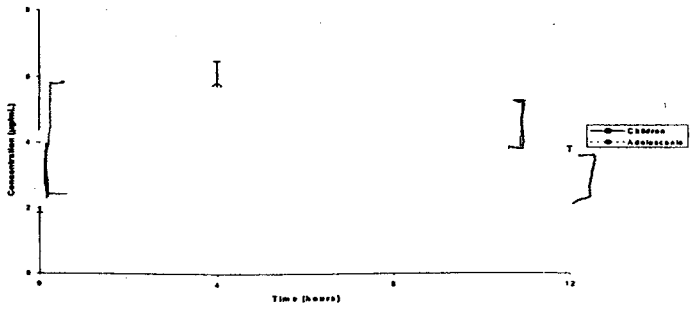
3.1.3 Role of antibody

The applicant's population pharmacokinetic analysis indicated that the presence of gp41 antibodies that cross-react with T-20 do not contribute to the PK variability of T-20. The gp41 antibody exists in most HIV subjects. Theoretically, this antibody could sequester T-20 in an immune response leading to alteration of T-20 PK. The Pharmacometrics reviewer agrees with the applicant's assessment.

3.2 Pediatric population

A dose of 2 mg/kg twice daily in pediatric subjects (5 to 16 years old) achieved similar concentrations to those obtained in adult subjects receiving 90 mg twice daily. The PK of T-20 in pediatric subjects was evaluated in approximately 40 subjects in two studies, a pilot study (Study 204; mg/m^2 dosing) and a long-term ongoing study (Study 310; mg/kg dosing). In study 310, the age distribution was 5 to < 12 years (n = 7) and > 12 to 16 (n = 13). In study 204, all children were between the ages of 3 and 12 (n =12 for Part A and n = 14 part B). The most reliable information was obtained in Study 310, which included a multiple dose PK substudy and the LC/MS/MS assay was used to determine plasma drug concentrations. Plasma concentrations of T-20 exhibited relatively high inter-individual variability at each blood sampling time, with CV% values ranging from ———— The mean steady-state T-20 plasma concentration-time profiles obtained from children and adolescents were comparable (figure 1).

Figure 1: T-20 Plasma Concentration time-profile in children receiving 2 mg/kg T-20



Although PK data in pediatric subjects were more variable than in adult subjects, the exposures for the two pediatric groups were generally comparable to each other and to adults. Consequently, the PK data from this study support the proposed pediatric dosage. Approximate clearance values (mL/h/kg) were 30 and 40 in adults and children, respectively.

Table II: T-20 Mean ± SD Steady-State PK Parameters (Study T20-310)

	Age Groups		**All Subjects**
PK Parameter	Children 5 to < 12 years (n=7)*	Adolescents ≥ 12 to < 17 years (n=13)	(n=20)
C_{max}, µg/mL	5.68 ± 1.30	5.88 ± 2.81	5.81 ± 2.35
T_{max}, hr	3.98 ± 0.06	5.05 ± 2.67	4.68 ± 2.19
C_{trough}, µg/mL	2.51 ± 1.04	2.98 ± 1.66	2.82 ± 1.46
AUC_{12h}, µg•hr/mL	49.1 ± 11.3	52.7 ± 27.4	51.4 ± 22.8

Table III: Mean ± SD or (CV%) Steady-State T-20 PK Measures after SC Administration: Children vs. Adults

	Study 16370 (Adults, n =12)			**Study 310**
PK Measure	**Abdomen**	**Thigh**	**Arm**	**Children (n = 20)***
C_{max}, µg/mL	5.35	4.71	5.63	5.81 ± 2.35
T_{max}, hr	3.30	2.93	5.03	4.68 ± 2.19
C_{trough}, µg/mL	2.67	3.00	3.66	2.82 ± 1.46
AUC_{12h}, hr·µg/mL	43.3	43.7	53.1	51.4 ± 22.8

* injection sites (abdomen, thigh and arm) were rotated and drug administered on an outpatient basis

The applicant conducted additional exploratory analyses to determine if the AUC obtained with 2.0 mg/kg T-20 dosing was affected by Tanner stage, gender, age, body weight, or body surface area. No trend was apparent between any of the listed covariates and AUC. However, the analyses showed that PK data in children over 11 years old, weighing more than 40 kg or with BSA above 1.2 m^2 were more variable than in children < 11 years old, weighing less than 40 kg or with BSA < 1.2 m^2. It is difficult to identify the source(s) of variability in the study. However, the potential sources include:

- Incorrect dosing amount and/or frequency
- Inherent interpatient PK variability (absorption/elimination)
- Drug-drug interactions (role of concomitant medications)
- Number of subjects

Insufficient data are available to confidently evaluate these potential sources of variability. Interestingly, the children between 5 and 8 years old, inclusive (youngest children, n = 4) with the fewest number of subjects had the lowest degree of variability; generally, variability is inversely related to sample size. These young subjects were likely more closely monitored than the older subjects were; additionally these young subjects probably received more accurate and consistent dosing than the other subjects as the young subjects would not self-administer the drug. It is noted that the study was conducted on an outpatient basis, so patient compliance may not have been adequately controlled. Because adherence to the dosage regimen impacts PK, it is unclear if the results obtained in this trial accurately reflect T-20 PK in the pediatric population.

Reviewer Comment

It is well recognized that it is often difficult to recruit pediatric subjects for PK trials, therefore certain allowances in the protocol are required to facilitate the conducting of such studies. These allowances include limited blood sampling, allowing concomitant medications and limiting the

amount of time spent at the clinical facility. However, the limited control of extrinsic factors, such as concomitant medications should be documented and considered for labeling purposes.

4. What extrinsic factors affect enfuvirtide pharmacokinetics?

The only extrinsic factor that influenced T-20 exposure was concomitant medications, particularly, drug combinations including low-dose ritonavir. Other extrinsic factors such as diet, smoking, alcohol use were not formally evaluated, but these factors are not expected to influence T-20 exposure because of the route of drug administration. Drugs administered by the SC route are systemically absorbed and not subject to most effects associated with absorption following oral administration, including hepatic first past effect and food-drug interaction.

4.1 *In vitro* metabolism

4.1.1 *In vitro* metabolism studies in microsomes and hepatocytes

The *in vitro* metabolism studies in microsomes and hepatocytes indicated that T-20 undergoes some degree of metabolism. In the *in vitro* microsome study, the Ro 50-6343 metabolite (M3) was identified; this metabolite was formed by hydrolysis of the amide group of the phenylalanine group (C-terminus) to the carboxylic acid derivative. The mechanism and site of the conversion are unclear; the reaction required viable microsomes but was not NADPH-dependent. In the hepatocyte study, three enfuvirtide metabolites were detected, M1, M2, and M3. The structural identities of M1, M2, and M3 were not elucidated, however, M3 had the same HPLC retention time as Ro 50-6343.

4.1.2 Inhibition of common CYP enzymes

In vitro, T-20 did not appear to be an inhibitor of common CYP enzymes at clinically relevant concentrations (Table I); this finding suggests that T-20 will not inhibit the metabolism of CYP substrates. Typical *in vivo* T-20 concentrations are less than 10 μM.

Relative to the vehicle control, T-20 coincubation with CYP enzymes resulted in two statistically significant changes ($p \leq 0.05$) in enzyme activity:

1) At a concentration of 100 μM, there was a 21 % decrease in CYP2C19 activity ⇒ inhibition
2) At a concentration of 10 μM, there was a 59 % increase in CYP2D6 activity

The observed inhibition of CYP2C19 is not likely to be of clinical significance because the 100 μM concentration greatly exceeds *in vivo* T-20 concentrations (≤ 1 μM) following the proposed drug dosage (90 mg twice daily dosing). Although an increase in CYP2D6 activity was observed at the 10 μM concentration, no such increase was observed at the 100 μM. The reason for the discrepancy in results at the two concentrations is not clear.

Overall, the findings from this *in vitro* study are consistent with the Pittsburgh cocktail study (see Section 4.2.1.1) and the population PK study (see Section 4.1.2.4). However, the applicability of this *in vitro* testing system to T-20, a compound with a high molecular weight (> 4000 Dalton), is unknown.

Table I: Mean ± SD Activity* of human hepatic microsomal CYP450 isozymes.

Enzyme	Control	10 μM T-20	100 μM T-20
CYP1A2	0.98 ± 0.04	0.98 ± 0.04	0.98 ± 0.05
CYP2A6	0.40 ± 0.09	0.45 ± 0.03	0.35 ± 0.04
CYP2B6	51.6 ± 18.2	59.9 ± 7.5	45.0 ± 8.8
CYP2C8	0.06 ± 0.00	0.07 ± 0.02	0.07 ± 0.00
CYP2C9	0.144 ± 0.014	0.139 ± 0.010	0.146 ± 0.017
CYP2C19	59.8 ± 3.5	54.0 ± 5.5	47.2 ± 3.4^
CYP2D6	110.0 ± 27.7	175.0 ± 26.7^	118.3 ± 11.3
CYP2E1	1.68 ± 0.11	1.88 ± 0.29	1.43 ± 0.32
CYP3A4	2.27 ± 0.15	2.24 ± 0.21	1.84 ± 0.26
CYP4A11	0.589 ± 0.105	0.569 ± 0.182	0.642 ± 0.089

* Activity expressed in concentration of metabolite/mg protein/min

^ Significantly different from control ($p \leq 0.05$)

4.2 *In vivo* drug-drug interaction studies

4.2.1 Effect of T-20 on concomitant dedications

4.2.1.1 Substrates of common CYP pathways (Pittsburgh cocktail)

The Pittsburgh cocktail study demonstrated that T-20 did not affect the mean phenotypic index parameters (PIP), a measure of exposure, (based on geometric mean ratio and confidence intervals) of common CYP substrates; however, data from this study were highly variable and may have been confounded by the presence of concomitant medications. Changes in mean PIP of probe substrates were used to assess the induction and inhibition potential of T-20.

Table II: Phenotypic Index Parameters (Mean ± SD; n = 12*)

Isozyme	Substrate	No T-20 (Day –15)	With T-20 (Day 6)	Geometric Mean Ratio (90 % CI)	Arithmetic Mean ± SD Change (range %)
CYP1A2, C_{8h}	Caffeine	0.81 ± 0.55	0.76 ± 0.53	0.94 (0.71 – 1.17)	2.0 ± 42 (-50 – 102)
CYP2E1, C_{4h}	Chlorzoxazone	1.2 ± 0.3	1.3 ± 0.4	1.08 (0.87 – 1.29)	15 ± 48 (-43 – 115)
CYP2D6 (DeRR)	Debrisoquine[1]	0.70 ± 0.20	0.69 ± 0.23	1.02 (0.97 – 1.06)	1.0 ± 8.9 (-15 – 14)
CYP2C19, Urine recovery*	Mephenytoin	81.8 ± 60.6	93 ± 60	1.13 (0.98 – 1.28)	21.3 ± 40.2 (-33 – 104)
CYP3A4 (DaRR)	Dapsone[2]	0.34 ± 0.15	0.33 ± 0.16	0.99 (0.88 – 1.09)	-2.1 ± 16.4 (-34 – 16)
N-Acetyltransferase, C_{8h}	Dapsone	0.39 ± 0.27	0.35 ± 0.23	0.90 (0.82 – 0.98)	-8.5 ± 15 (-42 – 16)

[1] Total amount of free and conjugated drug

DeRR- total amount of 4-OH-debrisoquine/(total amount of 4-OH-debrisoquine + total amount of debrisoquine

DaRR- total amount of dapsone hydroxylamine/(total amount of dapsone hydroxylamine + total amount of dapsone

n = 9

Overall, mean changes in PIPs based on standard exposure comparisons (geometric mean ratios and 90 % confidence intervals) were less than 20 %, suggesting a lack of a significant interaction. However, examination of individual data showed a wide range of effects for the same substrate. The sources of variability are unclear but are likely to include the small number of subjects and the unknown impact of concomitant medications. The wide interindividual variability for a given substrate emphasizes the importance of cautiously interpreting the results of this type of study, particularly for a limited number of subjects and in a patient population receiving concomitant medications.

4.2.1.2 Antiretroviral agents (abacavir, amprenavir, ritonavir, efavirenz)

The potential for T-20 to affect the pharmacokinetics of concomitant medications was not evaluated quantitatively in any PK studies. However, qualitatively T-20 did not have a significant effect on any of the coadministered drugs. In study 206, the concentrations of concomitant antiretrovirals did not appear to be affected by different T-20 doses (50, 75 and 100 mg). This conclusion is based on comparisons of plasma concentration-time profiles of the antiretrovirals in the presence and absence of T-20. The major limitation of this comparison is the fact that the time coordinate was based on the time of T-20 administration, rather than the time of administration of the antiretroviral.

4.2.2 Effect of concomitant medications on T-20 PK

4.2.2.1 Ritonavir-based interaction (a potent CYP inhibitor with putative PGP activity as a PGP substrate and inhibitor)

Two studies (NP 16325 and NP 16324) demonstrated that T-20 exposure was increased in the presence of ritonavir (Tables III and IV, respectively). The changes in T-20 exposure do not appear to be clinically significant. The lack of apparent clinical significance is supported by the fact that > 80 % of the subjects in the clinical trials received RTV and T-20, yet the safety profile of these subjects was acceptable according to the Medical Reviewer. The T-20 dosage in both studies was 90 mg BID; the dosage of RTV in Study 16325 was 200 mg BID and the dosage of RTV/SQV in Study 16324 was 100 mg/1000 mg BID.

Table III: T-20 Mean ± SD Steady State Pharmacokinetic Parameters (Study 16325, n = 12)

PK Parameter	No RTV (Day 3)	With Ritonavir (Day 7)	Geometric Mean Ratio (%)	90% Confidence Interval
AUC_{12h}, h·μg/mL	61.3 ± 9.73	75.8 ± 19.7	122	108-137
C_{max}, μg/mL	6.73 ± 0.88	8.51 ± 2.34	124	109-141
C_{trough}, μg/mL	3.34 ± 0.81	3.83 ± 1.04	114	102-128
T_{max}, h	5.11 ±1.18	4.09 ± 0.67	-	-
$t_{1/2}$, h	4.46 ± 0.52	4.80 ± 0.62	-	-
K_{el}, h^{-1}	0.158 ± 0.021	0.147 ± 0.018	-	-

It is unclear if the magnitude of the interaction is dependent on the RTV dose. Based on the cross study comparison (Study 16325 vs. 16324), the magnitude of interaction appeared to be dependent on the RTV dose. If the magnitude is dose-dependent, potentially subjects could have T-20 exposures increased beyond 20 % in the presence of RTV when RTV is given at doses above 200 mg or when coadministered drugs increase RTV exposure. It is unclear currently if these higher T-20 exposures will pose additional safety concerns.

Table IV: T-20 and T-20 Metabolite Mean (±SD) Steady-State PK Parameters (Study 16324, n = 12)

PK Parameter	No RTV/SQV	With RTV/SQV	Geometric Mean Ratio (%)	90% Confidence Interval
AUC_{12h}, hr·μg/mL	82.3 ± 18.1	94.0 ± 19.9	107	94.3 – 121
C_{max}, μg/mL	9.83 ± 2.09	10.6 ± 2.49	126	117 – 135
C_{trough}, μg/mL	4.12 ± 0.93	5.17 ± 1.17	114	115 – 124
T_{max}, hr	5.26 ± 1.43	5.5 ± 1.51	-	-
$T_{1/2}$, hr	4.49 ± 0.45	5.06 ± 0.74	-	-
K_{el}, hr^{-1}	0.156 ± 0.015	0.139 ± 0.017	-	-

The mechanism of the RTV-T-20 interaction is unknown, but does not appear to be CYP-based. Potentially, the RTV-T-20 drug-drug interaction is transporter-based; however, no data are currently available to evaluate this hypothesis. Some scientific literature indicates that PGP affects the transport of proteins and peptides. Ritonavir and other protease inhibitors are PGP substrates and/or inhibitors. Because T-20's structure is based on a section of a peptide molecule (gp41), it is feasible that a compound associated with the PGP transporter will affect the transport and subsequent exposure of T-20.

4.2.2.2 Rifampicin (rifampin) study

The rifampin study indicated that T-20 AUC and C_{max} was not affected by the presence of rifampin. The T-20 C_{min} was decreased by approximately 15 % in the presence of rifampin, but this decrease in C_{min} is unlikely to be clinically significant. A Phase II study indicated that the nominal 50 mg and 75 mg dose produced appreciable efficacy, even though the C_{min} at these two doses was lower than that of the proposed 90 mg dose.

Table V: T-20 and T-20 Metabolite Mean (±SD) Steady-State PK Parameters in Rifampin Study (Study 16334, n =12))

Drug	PK Parameter	No Rifampin	With Rifampin	GMR (%)	90 % CI
T-20	C_{max}, µg/mL	8.12 ± 1.89	8.26 ± 1.60	103	92.9 - 114
	C_{trough}, µg/mL	4.06 ± 1.08	3.47 ± 1.02	84.9	77.8 - 92.8
	AUC_{12h}, hr·µg/mL	74.2 ± 17.3	72.2± 16.3	97.5	89.3 - 106
	T_{max}, hr	5.26 ±1.67	3.93 ± 0.79		
	$T_{1/2}$, hr	4.74 ± 0.77	4.64 ± 0.82		
	K_{el}, hr^{-1}	0.150 ± 0.023	0.153 ± 0.022		
T-20 Metabolite	C_{max}, µg/mL	0.178 ± 0.054	0.198 ± 0.052		
	C_{trough}, µg/mL	0.139 ± 0.041	0.128 ± 0.032		
	AUC_{12h}, hr·µg/mL	1.76± 0.49	1.90 ± 0.51		
T-20 Metabolite/Parent	AUC_{12h} Ratio,%	2.42 ± 0.58	2.67 ± 0.57		

The findings from the rifampin study suggest that T-20 is unlikely to be a substrate of CYP 2C9, 2C19 or 3A4 enzymes.

4.2.2.3 Cross-study comparisons: Effect of antiretroviral agents

The applicant pooled PK data (Table VI) from six studies to evaluate the effect of concomitant antiretroviral medications on T-20 exposure; however, this reviewer considered data from only three studies. The data presented in the table below met the following two criteria: (1) similar patient populations and (2) same assay.

Table VI: Effect of antiretroviral agents on the AUC of T-20

Study	PI and NNRTI Status	AUC_{0-12h}* Mean ± SD	Range	CV %
T20-208	Allowed	42 ± 19.2	24.0 – 75.5	
NP 16370	Allowed	37.5 ± 10.5	26.5 – 61.9	
NP 16221	Not Allowed	42.7 ± 17.3	31.3 – 75.8	

* AUC is weight normalized: AUC_{0-12h}/dose/body weight

The mean steady state data from this limited number of studies suggest that the presence or absence of antiretrovirals does not impact T-20 exposure. However, it should be noted that these data were not obtained from controlled drug-drug interaction studies; in a controlled drug-drug interaction study, T-20 exposure was significantly altered by the presence of RTV.

Reviewer comment
The applicant should consider reevaluating the potential drug-drug interaction between T-20 and some specific antiretrovirals in light of the observed T-20-RTV interaction.

4.2.2.4 Population pharmacokinetic analyses results

The applicant's population analyses indicated that CYP-450 inducers or inhibitors commonly administered to this patient population do not alter enfuvirtide PK. According to the applicant, in all cases there was a less than 15 % change in enfuvirtide clearance in the presence of concomitant medications identified as CYP inducers or inhibitors, primarily renally eliminated or other pathway. The finding of a lack of a CYP interaction is consistent with the rifampin drug interaction study results, but is inconsistent with the RTV (potent CYP inhibitor) study results. It is noteworthy that RTV interacts with other pathways (e.g. putative PGP inhibitor and/or substrate), therefore the population PK results can be reconciled to the RTV interaction results. The major shortcoming of the population drug-drug interaction results is the absence of an adequate control (T-20 alone) and the overlapping effects (mitigating effects of inducers and inhibitors of the same enzymes)of concomitant medications. Practically, it is difficult to obtain an adequate control in the context of antiretroviral (ARV) therapy because T-20 monotherapy is not an acceptable form of ARV treatment. In summary, the applicant's population PK analysis of drug-drug interactions is a useful exploratory tool, but the analysis can not be used to make definitive conclusions regarding T-20 drug interactions.

5. What are the general biopharmaceutical characteristics of enfuvirtide formulations?

Different T-20 formulations were evaluated in the various clinical trials, but the formulations were generally comparable with proportional increases in the formulation components. The main differences between the formulations existed in the amount of T-20 per vial (fill volume), but the amount of drug delivered upon reconstitution was quantified. Batch sizes for the powder ranged in size from approximately ———— units. Most of the batch sizes used in the pivotal clinical trials had ———— units.

5.1 To-be-marketed formulation-———— carbonate buffered formulation

The to-be-marketed formulation, ———— carbonate formulation was used in the pivotal clinical trials (301 and 302); therefore, a pivotal bioequivalence study was not required or conducted. Blood samples were collected in the pivotal trials and the concentrations obtained in the trials were consistent with data from other PK studies.

Table I: Composition of Clinical Formulation

Component	Nominal Weight per vial	% weight/volume	Function
Enfuvirtide			
———— Sodium carbonate			
Mannitol			
Sodium Hydroxide			
Hydrochloric acid			
Water for injection			

This 100 mg/mL carbonate formulation was also used in the primary pharmacokinetic studies reported in the label: Studies NP 16220, 16221, 16370, 16325, and 16334. The applicant also

provided chemistry manufacturing and control information to show comparability of the formulations. The Chemistry Reviewer indicates that the formulations are acceptable.

5.2 Other T-20 formulations: 50 mg/mL* carbonate and [redacted] * TRIS

In the pediatric studies, both the proposed [redacted] formulation and a 50 mg/mL carbonate formulation were used. According to the applicant, the 50 mg/mL formulation was used to reduce the potential for dosing errors in the pediatric population as dosing was weight-based and required close dose titration. The 50 mg/mL formulation was also used in Phase II studies (TRI-001, T20-206, T20-208, T20-204/Part B), but was replaced with the [redacted] formulation to reduce the number of injections required to deliver the proposed dose. It should be noted that the applicant does not intend to market the 50 mg/mL carbonate formulation.

In Study 208, the applicant compared the [redacted] formulations to the 50 mg/mL formulation. The [redacted] carbonate formulation B provided comparable exposure (was bioequivalent) to the 50 mg/mL formulation A (Table II). On the other hand, the [redacted] TRIS formulation C was not bioequivalent to the 50 mg/mL carbonate formulation (data not shown). The [redacted] formulation achieved lower concentrations than the carbonate formulation.

* The deliverable dose of the 100 mg/mL carbonate formulation was 90 mg and that of the 50 mg/mL formulation was 45 mg. See Section 2 for discussion on nominal vs. delivered dose

Table II: Mean (CV%) T-20 PK Parameters Following SC Administration of 75 or 100 mg T-20 Formulations

Cohort I, 100 mg (N=11)	**Formulation A**	**Formulation B**
C_{max} (µg/mL)	4.77 [redacted]	5.00 [redacted]
T_{max} (h)[a]	4.00	4.07
AUC_{12h} (µg.h/mL)	46.2 [redacted]	48.7 [redacted]
CL/F (L/h)	2.33 [redacted]	2.39 [redacted]
V_z/F (L)	25.4 [redacted]	25.4 [redacted]
C_{12h} (µg/mL)	2.66 [redacted]	3.44 [redacted]
Cohort II, 75 mg (N=8)	**Formulation A**	**Formulation B**
C_{max} (µg/mL)	3.85 [redacted]	3.70 [redacted]
T_{max} (h)[a]	5.13	4.13
AUC_{12h} (µg.h/mL)	37.0 [redacted]	34.4 [redacted]
CL/F (L/h)	2.43 [redacted]	2.77 [redacted]
V_z/F (L)	17.[redacted]	20.8 [redacted]
C_{12h} (µg/mL)	2.24 [redacted]	2.38 [redacted]
Cohort III, 100 mg (N=7)	**Formulation A**	**Formulation C**
C_{max} (µg/mL)	5.17 [redacted]	3.51 [redacted]
T_{max} (h)[a]	4.00	4.00
AUC_{12h} (µg.h/mL)	44.7 [redacted]	35.0 [redacted]
CL/F (L/h)	2.45 [redacted]	3.15 [redacted]
V_z/F (L)	18.4 [redacted]	29.5 [redacted]
C_{12h} (µg/mL)	2.00 [redacted]	2.41 [redacted]

[a] Median value reported for T_{max}

Study [redacted]-001 employed a [redacted] formulation to deliver IV doses of T-20. Bioequivalence of this [redacted] formulation to the powders was not established.

6. What bioanalytical methods were used to quantify enfuvirtide concentrations in plasma?
Two validated methods were determine T-20 concentrations in plasma samples:

- [redacted]
- [redacted]

6.1 General assay information (Performance characteristics)
An [redacted] method was used in Study [redacted]001, but the applicant did not provide a final validation report for this method. The final method chosen for T-20 assays was the [redacted] method, which allowed quantitation of the T-20 metabolite, Ro 50-6343. The [redacted] method allowed quantitation of T-20. For the [redacted] assay the standard curve range was [redacted] ng/mL and for the [redacted] method the standard curve range was [redacted] ng/mL for T-20 and [redacted] ng/mL for the metabolite. The [redacted] assay was not designed to measure the metabolite; however, the assay is specific for T-20. Other assay performance characteristics- accuracy, precision, and sensitivity were acceptable for both methods. Typical assay performance characteristics for the [redacted] assay were as follows:
Study NP16370- lower limit of quantitation = [redacted] ng/mL; coefficient of variation for QC samples (measure of precision) = [redacted] for T-20 and [redacted] for T-20 metabolite; relative error (measure of bias) = [redacted] % for T-20 and [redacted] for T-20 metabolite.

6.2 Assay cross validation: [redacted] vs. [redacted]
A cross validation was conducted between the [redacted] and [redacted] and this validation indicated that T-20 concentrations were not generally comparable between the methods. However, the applicant indicates that the assays are comparable. For this cross-validation, the applicant analyzed 171 samples from three subjects from one clinical study (Study 16220) by both the [redacted] and [redacted] methods (see Appendix for data). The applicant indicated that "for the majority of samples the percent difference in enfuvirtide concentration between the two methods was ≤ 40 %, and there was no apparent bias between the methods". Inspection of the data indicates that the applicant's interpretation is acceptable. It should be noted that approximately 10 % of all samples analyzed had differences greater than 40 % between the two assays. In this reviewer's opinion these large range of concentration differences ([redacted]) demonstrate a significant difference in the two methods. However, the fact that there was no apparent systematic bias makes it difficult to propose and make specific adjustments to the data obtained by either method. The applicant notes that samples containing T-20 concentrations [redacted] ng/mL tended to have higher T-20 concentrations with the [redacted] method compared to the [redacted] The percent differences were generally above 40 % for these low concentration samples.

6.2.1 Labeling impact of using two different assays
It is difficult to compare results across studies, particularly for labeling purposes, because the [redacted], and [redacted] assays perform differently. The following studies used the [redacted] method: TRI-002, TRI-003, T20-205, T20-206, T20-208, T20-501. [redacted] was used in the following studies: T20-208, T20-501, T-20-502, T-20-503, T-20-504, T-20-505, T-20-302, T20-301 [redacted] and T-20-506. Studies T-20-208 (NV 16059) and T-20-501 (NP 16220) potentially provide data for comparison of the two assays. Based on the cross-assay validation report (Study NP 16220), this reviewer does not recommend that quantitative cross-assay information (combining or interchanging) be included in the label, because the [redacted] and

assay are not comparable. This reviewer recommends that quantitative information based on only the be included in the label. The assay selection is based on the fact that the assay is stability-indicating (detects and quantifies metabolite) and the assay was used in the major PK and clinical studies, including the pivotal efficacy trials. In general, the proposed label includes quantitative information based on the assay. Information obtained using the two assays can be considered in a qualitative context (for review purposes) because each method was validated individually.

6.3 Sample stability

Plasma samples containing T-20 and its metabolite were stable and there was less than 12 % reduction in enfuvirtide concentrations under a variety of conditions. These conditions included

[ ]

The least stable conditions were 88-96 % of control for metabolite under heat treatment and 89 – 108 % for enfuvirtide in long-term storage. Overall, the stability of the samples was acceptable, and would provide accurate concentration determinations. In most studies, samples were sent to the analytical facility under long term storage conditions (packed with dry ice) and analyzed at room temperature. Samples were analyzed in a timely manner once they were received at the analytical site; thus, information obtained by analysis of these samples should be acceptable.

APPENDICES

Title:	An open-label, randomized crossover study to characterize the PK of T-20 following single dose administration by subcutaneous and intra-venous routes, to HIV-infected patients
Study	T20-501 (NP16220)
Investigators/Centers:	[ ]
Study Period:	01/01 – 04/01

Objectives

- To determine pharmacokinetic parameters of T-20 after IV administration
- To determine pharmacokinetic parameters and bioavailability of T-20 after SC administration
- To assess the dose-proportionality in pharmacokinetics of T-20 after SC administration
- To evaluate tolerability of T-20 in HIV patients.

An additional exploratory objective of the study was to determine an appropriate structural model to describe T-20 disposition following IV and SC administration to patients with HIV infection.

Study Design

This was an open-label, single dose, randomized, 4-way crossover study conducted in 12 patients with HIV infection. The patients received the following four single dose treatments of T-20 in a crossover fashion separated by a washout period of at least one week:
Treatment A: single IV bolus 90 mg dose
Treatment B: single SC 45 mg dose (45 mg/0.5 mL)
Treatment C: single SC 90 mg dose (90 mg/1.0 mL)
Treatment D: single SC 180 mg dose (180 mg/2.0 mL)
The four doses were given in a randomized fashion using one of four treatment sequences (ABCD, BCDA, CDAB, and DABC; 3 subjects per sequence). The doses were given at nominal weekly intervals. Doses were given in the morning. The SC site was the abdominal wall. During the in-house phase (the night before the dose to 24 hours after each dose administration), meals were given at normal scheduled times in the morning, noon, and evening. Water, tea, or coffee were allowed *ad libitum* during the in-house phase

Concomitant Medications

All patients in the study had previous or concomitant treatments. These medications included antiretroviral (ARV) agents, mild analgesics, and antirheumatic and anti-inflammatory agents, sedatives and hypnotics. The non-ARVs were allowed for treatment of adverse events.

Reviewer's Comment on Concomitant Medications

The use of concomitant medications for a PK assessment is not ideal; however, due to severity and advanced nature of the subjects' disease it is unethical to withhold all concomitant medications.

Formulation

T-20/Ro 29-9800 was supplied in a glass vial. Batch number C200730-008 was used for both the IV and SC doses. Each vial delivered up to 90 mg when reconstituted.

Antibody Assessment: T-20 antibody (at screening and follow-up)
Blood samples for T-20 antibodies were taken and shipped at screening and follow-up visits only. T-20 antibody samples were sent to [redacted] and were analyzed using [redacted] for the measurement of antigen-specific serum antibody levels.

Blood Sampling
Blood samples for determination of plasma T-20 concentrations were collected prior to dosing and at the following time points:

- IV dosing: 5, 15, 30 minutes, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, and 48 hours post-dose
- SC dosing: 30 minutes, 1, 2, 3, 4, 6, 8, 12, 18, 24, 30, 36, and 48 hours post-dose

Assay
The pharmacokinetic samples were analyzed by [redacted].
Plasma T-20 concentrations were determined using a validated [redacted] method with a lower limit of quantification of [redacted] ng/mL. The $R^2 > 0.994$ for standard curve. The inter-assay precision was assessed as mean CV- (6 – 12 %) and the inter-assay accuracy was assessed by the relative error (0.2 – 2.6 %). The assay performance was acceptable.

Pharmacokinetic Parameters
The following pharmacokinetic parameters of T-20 were determined:

- After IV administration: Cmax, AUC_{inf}, CL, V_{ss}, $t_{1/2}$, and K_{el}.
- After SC administration: Cmax, t_{max}, AUC_{inf}, F, $t_{1/2}$, K_{el}, and CL/F.

The calculation of computed parameters and analyses of variance was performed using WinNonlin version 3.0 . Pharmacokinetic data collected in this study were analyzed by both non-compartmental and compartmental modeling methods.

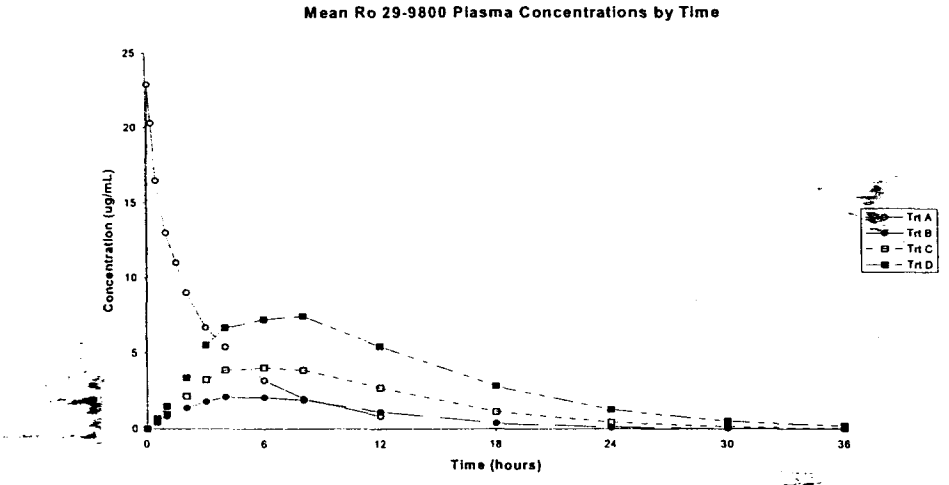
Safety Parameters
Safety parameters in this study included vital signs, ECG, injection site reaction, laboratory tests, and adverse events.

Statistical Model
Standard pharmacokinetic-statistical analyses were conducted to evaluate dose-proportionality and assess bioavailability for the various treatments. These analyses were based on non-compartmental analysis results.

Results
Disposition of Patients
A total of 12 patients were enrolled in the study and randomized to one of the four treatment sequences. All 12 patients who entered the study completed all four study periods. Most subjects were male (75%) and all subjects were Caucasian. The mean age was 38.4 years. Additional demographic data and baseline characteristics are presented in the appendix.

Pharmacokinetic Results
Non-compartmental Analysis Results
Mean plasma T-20/Ro 29-9800 concentration-time profiles for all treatment groups are presented in figure 1.

Mean Plasma T-20/Ro 29-9800 Concentration-Time Profiles in HIV Infected Patients (N=12) Following IV Bolus Administration of 90 mg T-20 (Treatment A) and SC Administration of 45 mg (Treatment B), 90 mg (Treatment C), and 180 mg (Treatment D) T-20



The mean (CV) PK parameters of T-20 following IV and SC Administration of T-20 are summarized in Table I.

Table I: Mean (%CV) T-20/Ro 29-9800 Pharmacokinetic Parameters Following IV and SC Administration

Treatment Groups	C_{max} (µg/mL)	t_{max} (h)	AUC_{inf} (h*µg/mL)	$t_{1/2}$ (h)	CL[a] (L/h)	V_{ss}[b] (L)	F (%)	C_{24h} (µg/mL)
IV								
A (90 mg)	24.2 (23)	--	67.4 (26)	3.16 (13)	1.40 (20)	5.48 (21)	--	0.0614 (62)
SC								
B (45 mg)	2.48 (33)	5.38 (44)	25.8 (26)	3.46 (12)	1.85 (24)	9.30 (29)	77.3 (17)	0.120 (63)
C (90 mg)	4.59 (32)	6.92 (36)	55.8 (22)	3.80 (15)	1.68 (22)	9.42 (35)	84.3 (18)	0.425 (66)
D (180 mg)	8.05 (23)	6.50 (27)	113 (20)	4.35 (11)	1.65 (20)	10.3 (24)	85.6 (15)	1.25 (53)

[a] CL/F reported for SC treatment groups (B, C, and D)
[b] V_z/F reported for SC treatment groups (B, C, and D)

- IV Administration

Following a 90 mg IV dose, plasma T-20 concentrations declined in a bi-exponential fashion with a mean terminal elimination half-life of 3.16 h. T-20 plasma concentrations were below the detection limit of the assay (— ng/mL) in most patients by the 36 h time point. The mean systemic clearance (CL) was 1.40 L/h and the mean volume of distribution at steady state (V_{ss}) was 5.48 L, which approximates total blood volume.

- SC Administration

After SC administration, the absorption of T-20 was sustained over a 6-hour period. Mean C_{max} occurred between 5 and 7 hours after SC dosing at the three dose levels. The mean terminal elimination half-life ranged from 3.46 to 4.35 h, which was numerically slightly longer than that observed after IV dosing. This finding suggests that prolonged absorption of T-20 occurs following SC administration, suggesting that a flip-flop in kinetics may occur. The inter-patient variability in C_{max} and AUC_{inf} was relatively low, ranging from 23%-33% for C_{max} and 20%-26% for AUC_{inf}.

- Absolute Bioavailability of SC Administration

Overall the estimated absolute bioavailability (BA) values were similar regardless of the dose size. The absolute BA of T-20 after a single subcutaneous injection to the abdomen was 84% for the 90 mg SC dose group. The BA values for the 45 mg SC dose group and the 180 mg SC dose group were comparable to the 90 mg dose group (proposed T-20 dose).

- Dose Proportionality of SC Doses

T-20 C_{max} and AUC_{inf} increased in an approximately dose-proportional fashion over the 45 to 180 mg SC dose range. However, statistically significant deviations from true dose proportionality were noted for both C_{max} (p=0.036) and AUC_{inf} (p=0.043). These deviations from dose-proportionality are not currently relevant since the maximum dose is 90 mg and no dose reduction is required for this drug. It is noted that these deviations are relatively small for both parameters.

Compartmental Modeling Results

A summary of model-derived pharmacokinetic parameters is presented in Table II.

Table II: Model-Derived Mean (CV%) Pharmacokinetic Parameter Estimates

Trt* Data	V_c (L)	CL (L/h)	CL_d (L/h)	V_t (L)	F	MAT (h)	NV^2
A	3.85 (23)	1.47 (20)	2.01 (49)	1.66 (29)	—	—	—
A+B	3.75 (23)	1.46 (22)	2.18 (51)	1.54 (29)	83 (17)	7.00 (37)	1.07 (30)
A+C	3.79 (23)	1.48 (21)	2.35 (51)	1.60 (29)	92.6 (19)	7.80 (32)	0.92 (58)
A+D	3.80 (22)	1.46 (22)	2.31 (50)	1.67 (30)	92.2 (15)	10.5 (32)	1.31 (65)
A+B+C+D	3.80 (22)	1.44 (21)	2.32 (47)	1.73 (37)	89 (13)	7.26[a] (42) 8.65[b] (38) 9.79[c] (31)	1.16 (56)

Trt* - treatments A, B, C, and D are as previously defined (see Study Design)
MAT – mean absorption time, and NV^2 - Normalized variance of the input time distribution
[a] MAT = MAT45; [b] MAT = MAT90; [c] MAT = MAT180
A — Fit 90 mg IV data to the two-compartment open model
A+B or A+C or A+D — Fit 90 mg IV data and 45 mg or 90 mg or 180 mg SC data simultaneously to final model
A+B+C+D — Fit 90 mg IV data and 45, 90, and 180 mg SC data simultaneously to final model
Final model — Two-compartment open model with inverse Gaussian input function

The applicant's compartmental analyses demonstrated that T-20 plasma concentration-time data were well described using a two-compartment open model with first-order elimination from the central compartment. The PK parameters for the compartmental approach were comparable to that of the noncompartmental analysis (NCA) approach. For example, the apparent total volume of distribution was 5.51 L ($V_c + V_t$) is similar to the volume of distribution estimated using NC methods (V_{ss} = 5.48 L). Also, the mean clearance, CL, (1.47 L/h) was similar to the total clearance estimated by non-compartmental methods (1.40 L/h).

For SC administration, the applicant proposed a two-compartment open model with elimination from the central compartment and an inverse Gaussian density absorption function to describe the plasma concentration-time curves. The data are fit reasonably well (data not shown) by the proposed model, although this reviewer considers the input (absorption) function overly complex. Key findings from the applicant's compartmental analyses were as follows:

- Values for the disposition parameters (V_c, CL, CL_d, and V_t) are consistent with the data obtained from IV dose data modeling.

- Bioavailability, F, ranged from 83 to 92 %
- The mean absorption time (MAT) increased with increasing dose (p=0.0027).

Table III: Mean T-20/Ro 29-9800 Parameters (CV%) from Various Modeling Strategies

Model	Trt Data	F (%)	CL (L/h)	CL_d (L/h)	V_c (L)	V_t (L)	V_{ss} (L)
M-D	A, B, C, D	89 (12.7)	1.44 (21)	2.32 (47)	3.80 (22)	1.73 (37)	–
M-I	A	–	1.40 (20)	–	–	–	5.48 (21)
M-I	B	77.3 (17)	1.43 (24)[a]	–	–	–	–
M-I	C	84.3 (22)	1.42 (22)[a]	–	–	–	–
M-I	D	85.6 (15)	1.41 (20)[a]	–	–	–	–

M-D — Inverse-Gaussian density input function with two-compartment open model
M-I — Non-compartmental analysis
Trt A: 90 mg IV
Trt B: 45 mg SC
Trt C: 90 mg SC
Trt D: 180 mg SC
– — Not calculated
[a] Calculated by apparent clearance value (CL/F) multiplied by F

In summary, compartmental and noncompartmental modeling yielded comparable results. Collectively, the models indicate that T-20 is well absorbed following SC administration. The PK data obtained from the various modeling strategies conducted by the applicant are summarized in Table III.

T-20 Antibody Serum Concentrations

T-20 antibody serum concentrations were drawn at screening and follow-up. The range of antibody concentrations was: at screening BLQ to —— and at follow-up BLQ to ——. It is noted that for two patients (Patients 28797/0006 and 28798/0008), T-20 antibody serum concentrations at follow-up increased by more than 30% from screening values. There was no clear relationship between antibody concentration and T-20 PK suggesting that the presence of antibody does not affect T-20 PK. Theoretically, antibodies could sequester T-20 thereby altering T-20 PK.

Safety Results (applicant's summary)

Eight subjects reported a total of 20 adverse events during the study. Adverse events were slightly more common for patients in treatment groups B and D. The most common adverse event was injection site reaction. There were no deaths reported during this study. There were no premature withdrawals due to adverse events during this study.

Conclusions

- The absolute bioavailability of T-20 after single subcutaneous injection to the abdominal wall of 45 mg, 90 mg, and 180 mg T-20/Ro 29-9800 was approximately 80%.
- T-20/Ro 29-9800 C_{max} and AUC_{inf} increased in a roughly dose proportional fashion following single SC doses of 45 mg, 90 mg, and 180 mg.
- The pharmacokinetics of T-20 following IV injection can be described by a two-compartment model with elimination occurring from the central compartment.
- The pharmacokinetics of T-20/Ro-29-9800 following SC administration can be described by an absorption and a two-compartment disposition model.

Appendix

Table I: Demographics for all study Treatments

	ALL PERIODS N = 12
Sex	
MALE	9 (75%)
FEMALE	3 (25%)
n	12
Race	
CAUCASIAN	12 (100%)
BLACK	-
ORIENTAL	-
OTHER	-
n	12
Age	
Mean	38.4
SD	8.34
SEM	2.41
Median	35.0
Min-Max	28 - 53
n	12
Weight in kg	
Mean	68.01
SD	10.475
SEM	3.024
Median	70.40
Min-Max	52.5 - 83.0
n	12
Height in cm	
Mean	171.6
SD	7.72
SEM	2.23
Median	172.0
Min-Max	156 - 183
n	12

n represents number of subjects contributing to summary statistics.
Percentages are based on n (number of valid values). Percentages not calculated if n < 10.

Table II: T-20 Antibody Serum Concentrations

CRTN / Patient No.	Screening (in µg/mL)	Follow-up (in µg/mL)
28797 / 0001		
28797 / 0002		
28797 / 0003		
28797 / 0004		
28797 / 0005		
28797 / 0006		
28798 / 0007		
28798 / 0008		
28798 / 0009		
28798 / 0010		
28798 / 0011		
28798 / 0012		

BLQ = Below limit of quantitation
NOR = Signal to noise ratio < 3:1

APPEARS THIS WAY
ON ORIGINAL

Title:	Study to determine the influence of subcutaneous (SC) injection site on the steady state pharmacokinetics of T-20 (Ro 29-9800) in HIV-1 infected patients
Study	T20-506 (NP16370)
Investigators/Centers:	
Study Period:	07/01 – 11/01

Rationale for the Study and Study Design

In the clinical studies of T-20, HIV-1-infected subjects administered T-20 as a subcutaneous (SC) injection to one of three anatomical sites, (A) Abdomen, (B) Thigh or (C) Arm, and could rotate the injection site from one SC site to another (could also rotate within a given anatomical site). Administration of T-20 at different sites could increase pharmacokinetic variability, if the absorption of the drug was injection site dependent. Therefore, this study was designed to assess the steady-state pharmacokinetics (PK) of T-20 after administering a minimum of 5 doses of T-20 (90 mg twice daily or BID) at each of the three injection sites.

Objectives

- To determine steady-state PK parameters of T-20 and its metabolite (Ro 50-6343) after SC administration at each of three injection sites: abdomen (A), thigh (B) and arm (C).
- To assess the relative bioavailability of T-20 from the arm and thigh relative to the reference injection site, the abdomen.

Study Design

This was an open-label, single-center, randomized, sequential, crossover study. Twelve HIV-1-infected subjects received T-20 at 90 mg BID by the SC route at three different injection sites: abdomen (A), thigh (B) and arm (C). Subjects were randomly assigned to one of the three sequences (ABC, BCA, and CAB) with four patients per sequence. The study consisted of three consecutive treatment periods of approximately 7 days each. The first 4 (± 1) days the patients injected T-20 at their preferred injection site, and the next 3 days T-20 was administered at the specified injection site (A, B or C) by the study unit staff using a q12h (± 1h) schedule. The applicant indicates that study unit staff injected the last 5 doses of each period to minimize the intra-patient variability related to SC injection by patients and to ensure that the steady-state was reached (adherence). Subjects rotated to the next period of approximately 7 days after completing the study-specific procedures of the preceding period. During the in-house phase at the study unit, meals were given at normal scheduled times in the morning, noon and evening. Water, tea or coffee were allowed *ad libitum* during the in-house phase.

Concomitant Medication/Treatment

Patients were to maintain their background antiretroviral medications and other drug regimen for the duration of the study.

Reviewer Comment

This is one of the only studies in which drug administration was strictly controlled, thus, results from this study are likely to be the most accurate with respect to determination of PK. However, a potential shortcoming of this study as in all T-20 PK studies is the unknown impact of concomitant medications on T-20 PK. Regulation of diet is not critical for SC administration because diet is not expected to affect drug exposure.

Formulation
T-20 was supplied as a lyophilized powder for injection. Each vial delivered up to 90 mg of T-20 when reconstituted. Batch numbers in this study were TTJ007 and ADP0425.

Blood Sample Collection for PK Analysis
Blood samples for determination of plasma T-20 and T-20 metabolite concentrations were collected prior to dosing, and at 30 minutes, 1 h, 1.5 h, 2 h, 3 h, 4 h, 6 h, 8 h, 10 h and 12 h after the morning SC dose. This collection took place after the 5th dose was administered by study staff.

Assay
PK samples were analyzed by [redacted] Plasma T-20 and T-20 metabolite concentrations were determined using a validated [redacted] method with a lower limit of quantification of [redacted] ng/mL. The inter-assay precision was assessed by CV: ranged from 5.5% to 15.5% for T-20 and 10.7% to 13.5% for T-20 metabolite. The accuracy was assessed by relative error: ranged from -4.7% to 4.4% for T-20 and -1.3% to 4.0% for T-20 metabolite. The assay performance was acceptable.

Pharmacokinetic Parameters
The following PK parameters were determined for T-20 and T-20 metabolite: C_{max}, C_{trough} and AUC_{12h}. The value of t_{max} was determined only for T-20. Additionally the AUC_{12h} ratio of T-20 metabolite/T-20 was determined.

Local Injection Site Reactions (ISRs)
Local ISRs at the specified site of administration of a minimum of five T-20 doses were recorded approximately 6 hours following the administration of the morning dose.

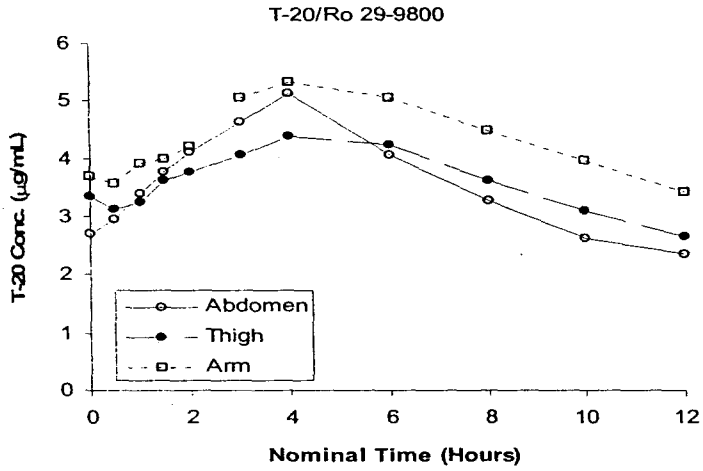
Statistical Model
An analysis of variance with factors sequence, patient within sequence, period and treatment was applied to natural log transformed values of C_{max}, C_{trough} and AUC_{12h} for T-20 and T-20 metabolite. Standard pharmacokinetic-statistical methods were used for exposure comparisons. The abdomen was used as the reference SC injection site. The calculation of computed parameters and analyses of variance was performed using WinNonlin (Pro version 3.2).

Results
Disposition of Patients
The 12 patients enrolled in this study were recruited from the ongoing T-20 Phase II trials, T20-205 (25%), T20-206 (17%) and T20-208 (58%). All 12 patients who entered the study completed all 3 study periods. All 12 patients in this study were males. Eleven patients were Caucasian and one was Other (Egyptian). The mean age was 44.6 years. See appendix for additional details on demographic data.

Pharmacokinetic Results
- T-20

Mean steady-state T-20 plasma concentration-time profiles for the three injection sites are shown in figure 1.

Figure 1: Mean Steady-State T-20 Plasma Concentration-Time Profiles Following SC Administration (T-20 90 mg BID) to Abdomen, Thigh and Arm



Based on inspection of the plots above and analysis of the mean exposure data (Table I), the order of exposure was arm > abdomen > thigh. Although exposure data were variable, the trend for individual data (figure 2) was similar to that reflected in mean data. The apparently increased exposure for the arm relative to the abdomen may be expected due to blood flow differences.

Figure 2: Individual T-20 AUC_{12h} Values by Injection Site

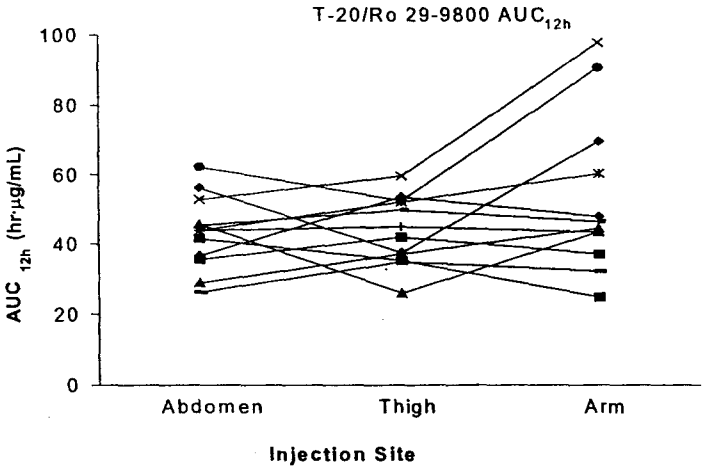


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Table I: Mean (CV%) Steady-State Pharmacokinetic Parameters Following SC Administration to Abdomen, Thigh and Arm

PK Parameter	Abdomen	Thigh	Arm
T-20			
C_{max}, µg/mL	5.35	4.71	5.63
T_{max}, hr	3.30	2.93	5.03
C_{trough}, µg/mL	2.67	3.00	3.66
AUC_{12h}, hr·µg/mL	43.3	43.7	53.1
T-20 metabolite			
C_{max}, µg/mL	0.784	0.800	0.816
C_{trough}, µg/mL	0.371	0.444	0.475
AUC_{12h}, hr·µg/mL	6.44	7.03	7.46
Metabolite/parent			
AUC_{12h} Ratio	15.2%	16.4%	14.4%

Results of ANOVA test for relative bioavailability of T-20 from thigh and arm compared with abdomen are shown in Table II.

Table II: Injection Site Comparisons- Geometric Mean Ratios (GMR) and 90% Confidence Intervals (CI) for T-20

PK Parameter	GMR (%)	90 % CI
Thigh/Abdomen		
C_{max}	88.2	71.9 - 108
AUC_{12h}	101	82.2 - 125
Arm/Abdomen		
C_{max}	102	83.5 - 126
AUC_{12h}	117	95.0 - 144

The bioequivalence-based exposure comparisons (GMR) indicate the following:

- For Thigh, the C_{max} is lower than the abdomen but AUC is comparable to the abdomen
- For Arm, C_{max} is comparable to the abdomen, but AUC is greater than that of the abdomen

For both the arm and thigh, these apparent exposure differences compared to the abdomen are not statistically significant because the 90 % CI includes 100 %.

- **T-20 metabolite/Ro 50-6343**

Mean steady-state T-20 metabolite plasma concentration-time profiles (not shown) and exposure measures were comparable for all three injection sites. The mean metabolite/parent ratios of AUC_{12h} were between 14 and 17 % for all sites (Table I), suggesting that the T-20 metabolite was formed to a similar extent following administration at these different SC sites, while remaining a minor species in plasma.

Comparison of Study Results with Other T-20 Studies

The PK parameters C_{max}, T_{max}, C_{trough} and AUC_{12h} of T-20 are in reasonable agreement with the previously reported values in studies T20-206 and T20-208 (dose-finding studies).

Table III: Comparison of PK Parameters

Reference	Injection site	C_{max} (μg/mL)	T_{max} (hr)	C_{trough} (μg/mL)	AUC_{12h} (hr·μg/mL)
Present study	Abdomen	5.35	3.30	2.67	43.3
	Thigh	4.71	2.93	3.00	43.7
	Arm	5.63	5.03	3.66	53.1
T20-208	Abdomen	5.00	4.07	3.44	48.7
T20-206	Abdomen	4.99	4.64	2.60	48.6

Variability in PK as a function of SC injection site

Inter-patient variability in T-20 PK parameters (exposure) was relatively low, ranging from 24 to 32 % in the abdomen, 23% to 25% in the thigh and 37% to 42% in the arm. The variability tended to be higher in the Arm than the other two SC sites. Inspection of the individual data showed that the higher inter-patient variability in PK parameters from the arm was primarily driven by two patients (#4 and #6). Both patients had higher values of C_{max} (>9 μg/mL), AUC_{12h} (>90 hr·μg/mL) and C_{trough} (>4.8 μg/mL) in comparison with the corresponding values found for the remaining patients. The source of the varied exposure in patients 4 and 6 is unclear, especially because dosing was well controlled. It is noted that individual blood flow affects drug absorption following SC administration.

Assessment of diurnal variation and attainment of steady state (trough measurement)

Morning and evening predose T-20 plasma concentrations determined on Day 6 and Day 7 are summarized below.

Table IV: Mean (CV%) Pre-dose Plasma Concentrations of T-20 (μg/mL)

Injection site	Day 6		Day 7	
	Morning	Evening	Morning	Evening
Abdomen	2.74 (41.5)	2.88 (38.2)	2.70 (39.4)	2.35 (42.7)
Thigh	3.23 (29.0)	2.80 (29.9)	3.34 (27.2)	2.65 (40.0)
Arm	4.15 (47.3)	3.40 (37.6)	3.69 (41.9)	3.41 (51.9)

Due to the similarity in predose concentrations on Days 6 and 7 (same time of day), the data suggest that steady state was achieved by Day 6. Based on mean data the following trends were observed for Days 6 and 7:

- Morning concentrations > evening concentrations for all SC sites, except Day 6 morning concentrations in the abdomen
- Predose concentrations: Arm > Thigh > Abdomen

The numerical differences in morning and evening predose concentrations for a given site do not appear to be statistically significant (analyses not conducted).

Signs and Symptoms of ISRs (applicant's summary)
The percentage of patients experiencing each of the signs and symptoms of an ISR varied by injection site. Nodules and cysts were experienced by patients on the abdomen (66.7%), thigh (33.3%) and arm (16.7%). Patients experienced erythema when T-20 was injected in abdomen (41.7%), thigh (8.3%) and arm (16.7).

Conclusions
- Absorption of T-20 is comparable from abdomen, thigh and arm following 90 mg BID T-20.
- The metabolism of T-20 to the T-20 metabolite is comparable in the SC tissue of the arm, thigh and abdomen

Recommendation
Because of the comparability in absorption of T-20 from the three different SC injection sites, HIV-1-infected patients can rotate the T-20 injection site among three anatomical sites: abdomen, thigh and arm.

Appendix

Summary of Demographic Data by Trial Treatment, All Patients

	ALL TREATMENTS ALL PERIODS N = 12
Sex	
MALE	12 (100%)
FEMALE	-
n	12
Race	
CAUCASIAN	11 (92%)
BLACK	-
ORIENTAL	-
OTHER	1 (8%)
n	12
Age	
Mean	44.6
SD	6.08
SEM	1.76
Median	45.0
Min-Max	37 - 56
n	12
Weight in kg	
Mean	77.93
SD	10.600
SEM	3.060
Median	74.95
Min-Max	65.8 - 93.0
n	12
Height in cm	
Mean	175.683
SD	6.8356
SEM	1.9733
Median	177.800
Min-Max	162.56 - 185.42
n	12

n represents number of patients contributing to summary statistics.
Percentages are based on n (number of valid values). Percentages not calculated if n < 10.

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Title:	Tolerance and pharmacokinetics of ———— Buffer High Strength (——) Formulations of T-20/Ro 29-9800 (HIV-1 Fusion Inhibitor) compared to the current ————Formulation (50 mg/mL), each at doses of 75 and 100 mg BID, in treatment experienced HIV-infected patients
Study:	T-20-208 (NV16059)
Investigators and sites:	multiple investigators and sites within the US
Study Period:	02/2000-05/2001

Study Objectives

- To evaluate the safety, tolerability, and pharmacokinetics (PK) of two high strength 100 mg/mL formulations of T-20 and of the current formulation (50 mg/mL) at doses of 100 mg BID and 75 mg BID in treatment-experienced HIV infected patients
- To evaluate the safety and tolerability of two doses of 100 mg/mL carbonate formulation of T-20, 100 mg BID and 75 mg BID, with the 100 mg/mL TRIS formulation at 100 mg BID.

Study Design

This was a controlled, multi-center, cross over study (48 week duration). The PK substudy took place over the first four weeks. Subjects took T-20 in combination with an optimized background (OB) regimen of ARVs. The two variables in the study were dose and formulation. There were three cohorts in the study as shown in Table I. Subjects were accrued sequentially into each of the cohorts, with the first 24 subjects entering Cohort I, the next 12 subjects into Cohort II, and the next 12 subjects into Cohort III. Once entered into a cohort, each subject remained on that dose to which he or she was initially assigned while sequencing through the various formulations and strengths.

Table I: Study Design and Plan

Study Period	Study Dosing Days*	Study Week	Cohort I 100 mg CO_3 BID	Cohort II 75 mg CO_3 BID	Cohort III 100 mg CO_3 or TRIS BID
1	1-14	1,2	B (100mg/mL CO_3)	B (100mg/mL CO_3)	C (100mg/mL TRIS)
2	15-28	4	A (50mg/mL CO_3)	A (50mg/mL CO_3)	A (50mg/mL CO_3)
3	>28	>4	B (100mg/mL CO_3)	B (100mg/mL CO_3)	C (100mg/mL TRIS)

*Study dosing days do not correspond to the same window used for safety and efficacy evaluations.

PK and safety analyses for each cohort were performed throughout the trial. T-20 was administered by SC injection twice daily except on Days 14 and 28. The evening T-20 dose was skipped on Days 14 and 28 to enable analysis of T-20 half-life and elimination rate. The next T-20 dose was given at the clinic on the following day (Day 15 or 29) after the 24-hour PK sample was obtained. Subjects injected the appropriate volume of reconstituted T-20 drug product into the SC tissue of the abdomen, deltoid, or anterior aspect of the thigh. Throughout the study, subjects self-administered the appropriate dose on an outpatient basis. Injection volumes varied for each dosing cohort depending on the dose required and formulation used.

Prior and Concomitant Therapy

Drugs known to have a major effect on plasma protein binding and drug transport were to be avoided wherever possible. Subjects were permitted to continue to take the following medications during the study:

- prophylactic medications for *P. carinii* pneumonia and for *M. avium*, including azithromycin (in accordance with current Centers for Disease Control recommendations).
- antibiotics for bacterial infections

- medications for symptomatic treatment such as antipyretics, analgesics, and antiemetics.

Comment
The prior and concomitant drugs listed above are not expected to alter T-20 exposure, because these agents do not affect T-20 ADME. Therefore, their use is acceptable and will not affect the study conclusions.

Formulations
Formulations and lot numbers of the study medication used in this trial are listed in Table II.

Table II: Study Medication Lot Numbers

T-20	Strength (formulation)
ADP0407	50 mg/vial (CO3)
ADP0412	100 mg/vial (CO3)
ADP0413	100 mg/vial (CO3)
800426	50 mg/vial (CO3)
ADP0411	[redacted]
ADP0414	[redacted]
ADP0410	100 mg/vial (CO3)
ADP0409	100 mg/vial (CO3)
ADP0418	100 mg/vial (CO3)

According to the applicant, the nominal 100 mg vials used in T20-208 contained 106 mg of T-20 (for the [redacted] formulation), providing a 90 mg deliverable dose. The sponsor did not specify the deliverable doses for the 50 mg carbonate and [redacted] formulation in the study report; however, based on information from other studies one can surmise the following: the nominal 50, 70 and 100 mg [redacted] doses delivered 45, 67.5 and 90 mg T-20, respectively.

Blood Samples
- Trough samples were obtained on study Days 14 and 28 before the morning dose of T-20
- Intensive samples were obtained on Days 14 and 28 at 1, 2, 4, 6, 8, 10, 12, 16, 20, and 24 hours post-dose.
- At Week 48, a single blood sample for the determination of T-20 trough concentration was collected.

Assay
Concentrations of T-20 in plasma were determined by a sensitive and specific [redacted] conducted by [redacted]

Standard curve range: [redacted] and lower limit of quantitation (LLOQ) of [redacted]. The correlation coefficient for the standard curve regression was [redacted] Interassay precision was measured by CV: range was [redacted] The interassay accuracy was measured by relative error: range was between [redacted]

Comment
The assay performance was acceptable. The final assay method adopted by the applicant is [redacted] The applicant indicates that cross-validation was not conducted, but some samples

in this study were reanalyzed by the — method. The applicant indicated that the results were comparable for both assays.

Pharmacokinetic Parameters

PK parameters were calculated from plasma T-20 concentration-time data (intensive PK Day 14 and Day 28) using standard non-compartmental PK methods and WinNonlin Professional, Version 3.1. Estimated parameters included the following: C_{max}, C_{12h}, T_{max}, $AUC_{0\text{-}12h}$, CL/F, Vz/F, and C_{trough} (Week 48) at 12±4 hours following administration of T-20.

Pharmacokinetic Analyses

Standard pharmacokinetic-statistical analyses were performed to assess bioavailability of the various formulations. The reference formulation was the carbonate 50 mg/mL (Formulation A). It is noted that a BA analysis was not originally planned in the protocol.

Results

Disposition of Subjects

Forty-six subjects were enrolled in the study and received at least one dose of study medication: 22 in Cohort I (T-20 100 mg BID CO_3), 12 in Cohort II (T-20 75 mg BID CO_3), and 12 in Cohort III (T-20 100 mg BID TRIS). Of the 46 subjects enrolled who received at least one dose of study medication, 3 subjects discontinued T-20 before Week 48. Only one of these withdrawals was due to an adverse event. It is noted that no subjects from Cohort II (T-20 75 mg BID CO_3) discontinued prematurely.

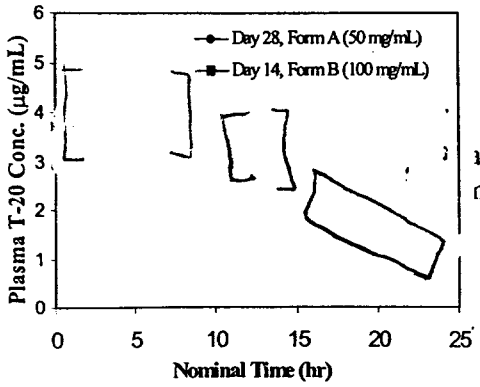
Demographics and Baseline Characteristics

The population across cohorts was comprised predominately of male subjects (96%). Additionally, most subjects were Caucasian. Mean age across all cohorts was 40 years. Other baseline characteristics were comparable in all cohorts. For additional information on subject demographics see appendix. The use of concomitant medications in the study population was frequent in many cases and represented treatment for many types of infections or conditions. In most cases, the distribution of medications was balanced among treatment groups. The list of concomitant medications is extensive; these concomitant medications will not be discussed further in this review. However, the role of concomitant medications on T-20 PK and vice versa has been addressed in other studies.

Pharmacokinetics of T-20

Mean T-20 plasma concentration-time profiles (steady-state) for the formulations tested in this trial are depicted in the following figures.

100 mg BID T-20 as Carbonate Formulations Cohort I (N=11).



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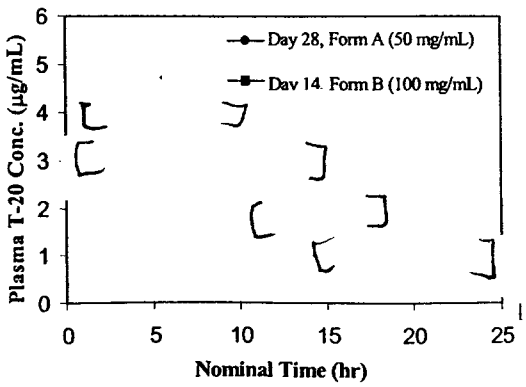
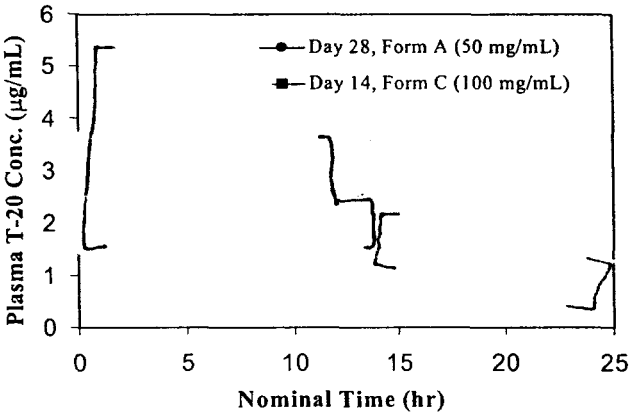
100 mg BID T-20 as Carbonate Formulations Cohort II (N = 8)



100 mg BID T-20 as [redacted] and [redacted] Formulation C: Cohort III (N=7).

At the 100 mg dose, formulations B and A had similar profile shapes; however, formulation B had higher mean plasma concentrations over the 24-hour period (left panel). At the 75 mg dose level, both carbonate formulations have profiles that overlap significantly (right panel).



The [redacted] formulation profile was different from that of the reference [redacted] formulation, particularly during the absorption phase. In general, T-20 concentrations from the [redacted] formulation were lower than that of the [redacted] formulation until 10 hours post dose, where the T-20 [redacted] concentrations exceeded those of the [redacted] formulation.

The mean PK parameters for 26 subjects in Cohort I, Cohort II, and Cohort III are summarized Table III.

Table III: Mean (CV%) T-20 PK Parameters Following SC Administration of 75 or 100 mg T-20 Formulations

Cohort I, 100 mg (N=11)	**Formulation A**	**Formulation B**
C_{max} (µg/mL)	4.77 [redacted]	5.00 [redacted]
T_{max} (h)[a]	4.00	4.07
AUC_{12h} (µg.h/mL)	46.2 [redacted]	48.7 [redacted]
CL/F (L/h)	2.33 [redacted]	2.39 [redacted]
V_z/F (L)	25.4 [redacted]	25.4 [redacted]
C_{12h} (µg/mL)	2.66 [redacted]	3.44 (46)
Cohort II, 75 mg (N=8)	**Formulation A**	**Formulation B**
C_{max} (µg/mL)	3.85 [redacted]	3.70 [redacted]
T_{max} (h)[a]	5.13	4.13
AUC_{12h} (µg.h/mL)	37.0 [redacted]	34.4 [redacted]
CL/F (L/h)	2.43 [redacted]	2.77 [redacted]
V_z/F (L)	17.4 [redacted]	20.8 [redacted]
C_{12h} (µg/mL)	2.24 [redacted]	2.38 [redacted]
Cohort III, 100 mg (N=7)	**Formulation A**	**Formulation C**
C_{max} (µg/mL)	5.17 [redacted]	3.51 [redacted]
T_{max} (h)[a]	4.00	4.00
AUC_{12h} (µg.h/mL)	44.7 [redacted]	35.0 [redacted]
CL/F (L/h)	2.45 [redacted]	3.15 [redacted]
V_z/F (L)	18.4 [redacted]	29.5 [redacted]
C_{12h} (µg/mL)	2.00 [redacted]	2.41 [redacted]

[a] Median value reported for T_{max}

For the carbonate formulations (Cohorts I and II), T-20 exposure was comparable at the same dose, suggesting that the formulations were equally bioavailable (see Table IV). On the other hand, the PK exposure parameters C_{max}, AUC_{12h}, and C_{12h} for Formulation C (TRIS buffer) were lower than the corresponding parameters for Formulation B (carbonate buffer) at the same 100 mg dose level. The exposure achieved by the TRIS formulation at the 100 mg dose was comparable to the exposure achieved by the carbonate buffer formulation at the 75 mg dose level. The reasons for the difference in exposure between the two 100 mg formulations are unclear. Based on these results and other factors, such as poor tolerability and poor efficacy, the applicant decided to discontinue development of the TRIS formulation.

Reviewer Comment

The applicant's reason for discontinuing development of the TRIS formulation appears reasonable. The applicant has conducted a variety of physicochemical analyses and was unable to detected differences in the structure or physical properties of T-20 in the two formulations. In vitro antiviral analyses also have not shown differences. The applicant indicates that plasma samples were assayed by both the ——— and ——— method to determine if the ——— method was not sensitive for T-20 TRIS samples. The analysis indicated that the two assay methods provided similar results for T-20 TRIS samples.

The PK exposure parameters C_{max}, AUC_{12h}, and C_{12h} increased proportionally as dose increased from 75 mg (Cohort II) to 100 mg (Cohort I) for the carbonate-based formulations.

Bioequivalence Assessment

As shown in table IV, the carbonate formulations were bioequivalent.

Table IV: Results of ANOVA: Analyzing Formulation Interaction for C_{max} and AUC_{12h} in PK Evaluable Population[a]

		90% Confidence Interval (B/A)[b]	
Parameter	**Ratio (B/A)**	**Lower Bound**	**Upper Bound**
C_{max}	98.0	86.7	111
AUC_{12h}	95.6	85.0	107

[a] ANOVA test was based on the nominal dose adjusted C_{max} and AUC_{12h}.
[b] Formulation A (=carbonate 50 mg/mL) / Formulation B (=carbonate 100 mg/mL).

Because ANOVA results showed that the cohort by formulation interaction was not statistically significant for C_{max} and AUC_{12h} ($p > 0.40$) and exposure was dose proportional, the formulation comparison could be made by combining results from Cohorts I and II.

The C_{trough} values at Week 48 were similar to the C_{12h} on Day 14 and Day 28 within the same cohort.

Table V Summary of Steady-State Mean (CV %) C_{trough} Values at Week 4 vs. Week 43- 48

Dose	N	Formulation (concentration)		PK Time (hr)	C_{trough}[1] (µg/mL)	C_{trough}[2] (µg/mL)
100 mg BID	17	B (100 mg/mL, CO_3)	Mean (CV%)	12.95 (9.0)	2.615 (72.6)	3.44 (46)
			Min-Max	———	———	

75 mg BID	9	B (100 mg/mL, CO_3)	Mean (CV%)	12.82 (12.3)	1.650 (69)	2.38 (56)
			Min-Max	[redacted]	[redacted]	
100 mg BID	6	C (100 mg/mL, [redacted])	Mean (CV%)	12.31 (11.9)	2.15 (51.8)	2.41 (59)
			Min-Max	[redacted]	[redacted]	

[1] C_{trough} obtained at Week 4 from pooled data (Cohorts I, II, or III when applicable)
[2] C_{trough} obtained at Week 43-48

The C_{trough} data suggest that BID dosing of T-20 results in sustained, dose proportional exposure over the 48-week treatment period. However, the data were highly variable. It is noted that patient compliance could have influenced the study results.

Safety Conclusions (applicant's analyses)

The safety results demonstrated that all formulations and all doses of T-20 were well tolerated in this mostly heavily treated HIV-1-infected population treated for 48 weeks. All subjects experienced at least one ISR during the study. During the first 4 weeks, there was no obvious pattern observed in Cohorts I and II when switching from Formulation B to Formulation A. In Cohort III, all ISR parameters tended to improve after switching from Formulation C to Formulation A. No deaths occurred during the study. No subjects discontinued treatment with T-20 due to an ISR. There was only one tolerability failure (100 mg BID CO_3; abdominal distension); it was considered by the investigator to be unrelated to the use of T-20. The overall tolerability and safety profiles observed in this study population support the use of the 100 mg/mL carbonate formulation (two injections per day) for the Phase III studies.

Conclusions

The 100 mg/mL T-20 carbonate formulation was bioequivalent to the 50 mg/mL carbonate formulation whereas the 100 mg/mL TRIS formulation was not (by inspection of exposure data; analysis was not conducted).

Table 1 **Subject Characteristics at Screening and Calculated Baseline Averages of HIV-1 RNA and CD4 by Cohort: Intent-To-Treat Subjects**

Characteristic	Cohort I 100 mg BID CO_3 (N=22)		Cohort II 75 mg BID CO_3 (N=12)		Cohort III 100 mg BID TRIS (N=12)		Total N=46	
	n	%	n	%	n	%	n	%
Gender N (%)								
Male	22	100	11	92	11	92	44	96
Female	0	0	1	8	1	8	2	4
Ethnic Origin N (%)								
Caucasian	18	82	11	92	9	75	38	83
Black	2	9	1	8	3	25	6	13
Asian	1	5	0	0	0	0	1	2
Hispanic	1	5	0	0	0	0	1	2
Age (years), N	22		12		12		46	
Mean (SE)*	44 (1.7)		42 (2.3)		39 (1.6)		43 (1.1)	
Height (cm), N	22		12		12		46	
Mean (SE)*	178 (1.5)		178 (2.6)		180 (2.8)		178 (1.2)	
Weight (kg), N	20		11		12		43	
Mean (SE)*	78 (2.0)		73 (3.6)		85 (5.9)		79 (2.2)	
Calculated BL[a] Plasma HIV-1 RNA (log_{10} copies/mL), N	22		12		12		46	
Mean (SE)	5.17 (0.11)		5.39 (0.11)		5.46 (0.08)		5.30 (0.07)	
Median**	5.12		5.44		5.50		5.37	
Derived BL[b] CD4 Cell Count (cells/mm^3), N	22		12		12		46	
Mean (SE)	121 (27)		20 (6)		43 (15)		74 (15)	
Median***	98		12		23		24	

Cross reference: Statistical Tables 5, 6, and 7.

[a] Calculated baseline was the average of log_{10} transformed Screening and Baseline Visit HIV-1 RNA values.

[b] Baseline was the value corresponding to the last measurement prior to the first dose of study medication.

*Mean and SE were rounded to the nearest whole number and tenth, respectively.

** Mean, SE, and median were rounded to nearest hundredth.

*** Mean, SE, and median were rounded to nearest whole number.

BL = Baseline

Study Title: *In vitro* metabolism of ^{3}[H] T-20 by rat, monkey, and human hepatocytes
Study Number: 6131-321
Report Date: September 4, 2001
Site: [redacted]

Method

The applicant used standard procedures for evaluating drug metabolism by hepatocytes. Only results for the human hepatocytes will be reported. Hepatocytes from three individual donors were incubated with T-20 for 0, 30, 60, 120 and 240 minutes. T-20 concentrations of 1 or 10 µM were added to hepatocytes plated at a density of 1 x 10^{6}/mL and incubated at 37°C in a high oxygen atmosphere (95 % O_2/ 5 % CO_2). The suspension medium was [redacted] - The metabolism of control samples without hepatocytes was also evaluated. The reactions were stopped with [redacted]. Supernatants were analyzed for T-20 and metabolites by [redacted] and [redacted] detection.

Results

T-20 was rapidly and almost completely biotransformed to metabolites. There were three metabolites formed:

- The M1 metabolite, a highly polar band that eluted (within five minutes) near the column void volume, and accounted for > 90 % of the radioactivity
- The M2 metabolite eluted between seven and nine minutes and accounted for 2-7 % of the radioactivity
- The M3 metabolite, which was less polar than T-20 and eluted just after T-20; the amount of this metabolite was ≤ T-20 (< 5 % of radioactivity)

The applicant did not identify the structures of the M1, M2, and M3 metabolites; however, the M3 metabolite had the same retention time as the previously identified metabolite (Ro 50-6343) that is formed by deamidation.

Percent of Radioactivity in incubation samples of primary human hepatocytes with ^{1}H-T-20 at 1 µM for different incubation periods

	Donor 1*			Donor 2*			Donor 3			
Time	M1	T-20	M3	M1	T-20	M3	M1	M2	T-20	M3
0	0.4	88.4	ND	ND	90.2	ND	1.2	ND	80.1	ND
30	15.9	55.2	20.2	25.0	47.2	18.4	18.1	1.7	46.2	20.1
60	27.5	47.1	15.9	35.9	38.3	17.3	22.3	2.8	39.8	21.5
120	45.4	28.0	17.6	62.0	20.5	10.5	48.7	5.3	17.6	18.5
240	74.4	11.1	6.4	92.0	3.9	2.4	69.8	7.1	6.9	9.1

* The M2 metabolite was not detectable in the donor's samples

At the 10 µM T-20 concentration without hepatocytes (control), T-20 was the predominant radioactive [redacted] species, no M2 or M3 (not detectable) was formed and low or no radioactivity was associated with the M1 metabolite [redacted]%). These findings indicate that T-20 is relatively stable to metabolism in the absence of hepatocytes. Results at the 1 µM T-20 concentration (results not shown) were comparable to that obtained at the 10 µM concentration.

Discussion and Conclusion

Because the identity of the highly polar metabolites was not identified it is not possible to make definitive conclusions about the metabolic products. The M1 and M2 metabolites were more polar than T-20 and eluted fairly rapidly suggesting that they were of a low molecular weight (molecular weight:

metabolites << T-20). The applicant reports that potentially these metabolites correspond to small peptide fragments bearing the [redacted] Any of these proposed components or a combination of these components may contribute to the M1 or M2 peaks. The applicant's assessment of the M1 and M2 species appears reasonable. In conclusion, incubation of T-20 with hepatocytes produced three metabolites, M1, M2, M3; however, the structures of these metabolites were not identified.

Study Title: Identification of a metabolite of T-20 found in rat plasma and (rat and human) liver microsomal incubations.
Report Number: 1003497
Report Date: January 30, 2001
Site: Hoffmann-La Roche Inc., Nutley, NJ USA

Method

The applicant used standard procedures for evaluating drug metabolism by microsomes. Only results for the human microsomes will be reported. Gender pooled human liver microsomes) were preincubated for 5 minutes with a 20 μM T-20 solution. The incubation media contained buffer (pH 7.4), and . The reactions were initiated with and proceeded for 30 minutes. The reactions were stopped with . Control experiments were conducted in the absence of Another set of experiments was conducted with heat-treated microsomes. Metabolites were assayed by

Results and Discussion

A metabolite was formed with or without (control) added. Conversely, the metabolite was not formed with the heat-treated microsomes. The microsomal metabolite had the same retention time as a previously identified metabolite (Ro 50-6343) that is formed by deamination. Synthetically produced Ro 50-6343 and the metabolite obtained in this study were identical by According to the applicant's analysis, this metabolite's molecular weight is one Dalton less than that of T-20 and this difference in weight occurs within the first two amino acids from the C-terminus. Furthermore, based on the structures of the terminal amino acids (tryptophan and phenylalanine), the only possible hydrolysis position is the amide group of the phenylalanine. The applicant's interpretation appears reasonable. Overall, the findings from this study suggest that Ro 50-6343 is produced in the presence of microsomes. is a necessary cofactor in many xenobiotic reactions, including cytochrome P-450 (CYP) reactions, and is required for measurement of oxidase activity catalyzed by CYP enzymes. In this study did not affect the metabolism of T-20 suggesting that T-20 is not a CYP substrate.

Conclusion

Incubation of T-20 with microsomes produces the previously identified T-20 metabolite, Ro-50-6343; this reaction is not dependent on

Title: Assessment of the inhibition of the activities of human hepatic microsomal cytochrome P450 isozymes by T-20 *in vitro*.

Study Site: [redacted]

Protocol [redacted]/64C-07482-006

Objective: To determine the potential of T-20 to inhibit human cytochrome P450 activity

Study Design

The methodology adopted in this study was the typical design for *in vitro* metabolism studies. A pooled sample of human liver microsomes (lot number [redacted] 260) was obtained from [redacted] The metabolic status with respect to poor or extensive metabolizers was not available. T-20 (batch number 711013) was obtained from [redacted] Enzymes, model substrates, metabolites monitored and controls used in this study were acceptable. The vehicle control contained carbonate and mannitol. It is noted that the applicant did not include any positive controls. These positive controls would have been useful to provide assurance that the test system was functioning appropriately. However, the applicant indicates that the activities of the pooled human microsomes were in reasonable agreement with the activities reported by the supplier of the microsomes. Enzyme activity was determined at two T-20 concentrations, 10 and 100 μM. These concentrations are not clinically relevant as the maximum T-20 concentration obtained *in vivo* is less than or equal to 1 μM. Ideally, the 1 μM concentration should have been studied.

Enzyme Systems used to assess inhibitory potential of T-20

Enzyme	Enzyme Substrate	Marker Activity	Concentrations
CYP1A2	Acetanilide	Acetanilide-4 hydroxylation	2 μM
CYP2A6	coumarin	Coumarin-7 hydroxylation	5 μM
CYP2B6	(S)- mephenytoin	(S)-4-mephenytoin demethylation	1 mM
CYP2C8	Taxol	Taxol 6-α hydroxylation	20 μM
CYP2C9	tolbutamide	Tolbutamide hydroxylation	2 mM
CYP2C19	(S)- mephenytoin	(S)-4-mephenytoin hydroxylation	1 mM
CYP2D6	Dextromethorphan HBr	Dextromethorphan O-demethylation	1 mM
CYP2E1	p-nitrophenol	p-nitrophenol hydroxylation	0.1 M
CYP3A4	Testosterone	6β-hydroxytestosterone hydroxylation	125 nM
CYP4A11	[redacted]		

Reviewer Comment on Methodology

Overall the applicant used the preferred substrates described in the Draft MaPP for in vitro studies (FDA In Vitro Metabolism Working Group) and EUFEPS for the evaluated enzymes. The two cited groups do not provide any information on the CYP4A11 enzyme; however, some literature information is available on the CYP4A11 enzyme.

Results and Discussion

The *in vitro* activities of human hepatic microsomal P450 systems in the presence of 10 and 100 μM T-20 and vehicle control are summarized in Table II. Generally, T-20 did not inhibit the activity of any of the P450 enzymes tested. Relative to the vehicle control, T-20 presence resulted in two statistically significant differences ($p \leq 0.05$) in enzyme activity:

1. At a concentration of 100 μM, there was a 21 % decrease in CYP2C19 activity ⇒ inhibition

2. At a concentration of 10 μM, there was a 59 % increase in CYP2D6 activity

The observed inhibition of CYP2C19 is not likely to be of clinical significance because the 100 μM concentration far exceeds *in vivo* T-20 concentrations (≤ 1 μM) following the proposed drug dosage (90 mg twice daily dosing). Although an increase in CYP2D6 activity was observed at the 10 μM concentration, no such increase was observed at the 100 μM. The reason for discrepancy in results at the two concentrations is not clear.

Table 2: Mean ± SD Activity* of human hepatic microsomal CYP450 Isozymes.

Enzyme	Control	10 μM T-20	100 μM T-20
CYP1A2	0.98 ± 0.04	0.98 ± 0.04	0.98 ± 0.05
CYP2A6	0.40 ± 0.09	0.45 ± 0.03	0.35 ± 0.04
CYP2B6	51.6 ± 18.2	59.9 ± 7.5	45.0 ± 8.8
CYP2C8	0.06 ± 0.00	0.07 ± 0.02	0.07 ± 0.00
CYP2C9	0.144 ± 0.014	0.139 ± 0.010	0.146 ± 0.017
CYP2C19	59.8 ± 3.5	54.0 ± 5.5	47.2 ± 3.4^
CYP2D6	110.0 ± 27.7	175.0 ± 26.7^	118.3 ± 11.3
CYP2E1	1.68 ± 0.11	1.88 ± 0.29	1.43 ± 0.32
CYP3A4	2.27 ± 0.15	2.24 ± 0.21	1.84 ± 0.26
CYP4A11	0.589 ± 0.105	0.569 ± 0.182	0.642 ± 0.089

* Activity expressed in concentration of metabolite/mg protein/min

^ Significantly different from control ($p \leq 0.05$)

Conclusion

Based on the *in vitro* findings in this study, it is unlikely that T-20 will inhibit the activity of any of the following CYP450 enzymes *in vivo* at clinically relevant concentrations: CYP1A2, CYP2A6, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4, and CYP4A11. However, the applicability of this *in vitro* testing system to T-20, a compound with a high molecular weight (> 4000 Dalton), is unknown. Consequently, *in vivo* studies should be conducted to confirm the *in vitro* findings, particularly for the most common CYP pathway (CYP3A4).

Title:	Study to investigate the influence of ritonavir (Norvir®) on the pharmacokinetics of T20/Ro 29-9800 in HIV-infected patient volunteers
Study:	T20-504 (NP16325)
Investigators/Sites	
Study Period:	12/2001- 03/2002

Study Rationale

Ritonavir (RTV) is the most potent CYP3A4 enzyme inhibitor among all approved antiretroviral (ARV) agents. Consequently, RTV is frequently used in assessing drug-drug interactions with ARV candidates with respect to CYP3A4 interactions. In HIV therapy RTV is used at a full dose (600 mg) for therapeutic benefit or at lower booster doses (< 300 mg) to increase the exposure of concurrently given protease inhibitors (PIs). T-20 is a peptide and is expected to be eliminated by catabolism, rather than CYP-based mechanisms; however, it is important to assess the potential for a drug-drug interaction with drugs, such as RTV, since T-20 is likely to be coadministered with RTV and other compounds with similar inhibition properties as RTV.

Objective

To determine the metabolic inhibitory effect of ritonavir (Norvir®) on the pharmacokinetics of T-20 in HIV-1 infected patient volunteers.

Study Design

This was an open-label, sequential, crossover study. T-20 90 mg was given subcutaneously (SC) every 12 hours on study days 1-7, except for the evening doses on days 3 and 7. The evening dose of T-20 on day 3 and day 7 was omitted in order to characterize the plasma concentration-time profile of T-20 over 24 hours. On study days 4-7 patients also received ritonavir (RTV) 200 mg orally (po) every 12 hours, in addition to T-20. Study staff administered all study medication. T-20 was administered into the SC tissue of the abdomen. Abdominal injections sites were rotated in an effort to prevent or lessen the occurrence of injection site reactions (ISRs.) RTV was administered orally with a full glass of water, every 12 hours, with a meal. On study days 4 and 7, ritonavir was to be given within 5 minutes of the T-20 dose.

Concomitant Medication/Treatment

All 12 patients reported taking at least one medication within 3 months of screening. The medications taken most frequently were ARVs (at least one, 10 patients) and sulfamethoxazole/trimethoprim (5 patients). The sponsor indicates that none of the medications taken during the study constituted a protocol violation and none had the potential to be a confounding factor. The sponsor's conclusion is acceptable. Prohibited medications included: anti-fungal treatment (e.g. ketoconazole, amphotericin, miconazole etc.) and herbal remedies (e.g. St John's wort, Ginko Biloaba and traditional Chinese medicines). No enzyme inhibitors or inducers were to be taken as concomitant therapy during the study.

Dose Selection Rationale

The duration of T-20 dosing was sufficient to achieve steady state levels of T-20 and was kept to a minimum to minimize the potential for development of resistance to T-20 monotherapy. The dose of ritonavir (200 mg po q12h) is one of the doses routinely used as a booster dose in the treatment of HIV; therefore this dose was selected for this study. It should be noted that the