

**CENTER FOR DRUG EVALUATION AND RESEARCH**

**Application Number** 21-481

**PHARMACOLOGY REVIEW(S)**

**PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION**

NDA number: 21-481 Serial 000

Sponsor Letter Date: November 19, 2002, CDER Stamp Date: November 20, 2002

Sponsor and/or agent: Hoffmann La-Roche, Roche Laboratories, Inc., Nutley, NJ

Manufacturer of drug substance: Roche, Basel, Switzerland.

Manufacturer of drug product: \_\_\_\_\_ and F. Hoffmann-La Roche Ltd., Basel, Switzerland.

Reviewer name: William H. Taylor

Division name: Division of Anti-Viral Drug Products, HFD 530

Review completion date: November 25, 2002

## Drug:

Trade name: Fuzeon

Generic name: enfuvirtide, T-20, T-20 Peptide, T-20 (Ro 29-9800)

Molecular formula: N-Acetyl-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-CONH<sub>2</sub>Empirical Formula: C<sub>204</sub>H<sub>301</sub>N<sub>51</sub>O<sub>64</sub>, Molecular Weight: 4,492 u.Relevant INDs/NDAs/DMFs: INDs \_\_\_\_\_, DMF \_\_\_\_\_  
DMF \_\_\_\_\_

Drug class: Anti-viral, anti-HIV, HIV-1 inhibitor

Indication: Treatment of HIV-1 infected adults and children.

T-20 is a new molecular entity derived from naturally occurring sequences within a transmembrane glycoprotein (gp41) of HIV-1. It consists of a linear chain of 36 amino acids. It was designed to interfere with the interaction of the DP-107 and DP-178 regions of gp41 in the HIV-1 virus, and thereby interfere with the virus's ability to bind with CD4 T-lymphocytes.

This submission contains responses to two questions faxed to the sponsor concerning the \_\_\_\_\_ study \_\_\_\_\_ 005/002348/SS, *T-20: Skin sensitization to the guinea-pig (\_\_\_\_\_ method)*. The study is included as Reference 3601 to the NDA and was previously submitted to IND \_\_\_\_\_ Serial 056 and 211. The questions were about what drug solutions were used on the study. The sponsor adequately responded to the questions. This submission requires no further action.

William H. Taylor  
Pharmacologist/ToxicologistConcurrences:  
HFD-530/JFarrelly**APPEARS THIS WAY  
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/s/

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William H. Taylor  
12/2/02 11:16:30 AM  
PHARMACOLOGIST

James Farrelly  
12/2/02 01:29:46 PM  
PHARMACOLOGIST

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## PHARMACOLOGY/TOXICOLOGY COVER SHEET

**NDA number:** 21-481

**Sequence number/date/type of submission:** 000/8-30-02/Original NDA

**Sponsor and/or agent:** Hoffmann-La Roche Inc., 340 Kingsland St, Nutley, NJ 07110-1199

**Manufacturer for drug substance:** Roche Colorado Corporation, 2075 N 55<sup>th</sup> St, Boulder, CO 80301

**Reviewer name:** William H. Taylor, PhD, DABT

**Division name:** Division of Antiviral Drug Products

**HFD #:** 530

**Review completion date:** January 3, 2003

**Drug:**

**Trade name:** Fuzeon

**Generic names:** enfuvirtide, T-20

**Code name:** Ro 29-9800

**Chemical name:** (36-L-amino acid polypeptide)

**CAS registry number:** 159519-65-0

**Molecular formula/molecular weight:** C<sub>204</sub>H<sub>301</sub>N<sub>51</sub>O<sub>64</sub>, 4,492 u

**Structure:** CH<sub>3</sub>CO-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-CONH<sub>2</sub>

**Relevant INDs/NDAs/DMFs:** IND \_\_\_\_\_ IND \_\_\_\_\_ IND \_\_\_\_\_ DMF \_\_\_\_\_  
DMF \_\_\_\_\_

**Drug class:** Antiviral, anti-HIV-1

**Indication:** Treatment of persons infected with HIV-1 virus.

**Clinical formulation:** White to off-white amorphous lyophilized powder, 90 mg deliverable vial. Other components supplied in a separate vial are sodium carbonate, anhydrous, mannitol, sodium hydroxide/hydrochloric acid for pH adjustment, and water for injection.

**Route of administration:** Subcutaneous injection, twice daily (b.i.d.).

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## **Executive Summary**

### **I. Recommendations**

#### **A. Recommendation on Approvability**

There are no pharmacology issues that preclude approval of enfuvirtide.

#### **B. Recommendation for Nonclinical Studies**

Due to the possible increase in incidence of bacterial respiratory infections in enfuvirtide-treated patients (enfuvirtide plus optimized background) versus patients on optimized background in Phase III clinical trials, please conduct nonclinical immune function studies to investigate whether enfuvirtide causes immunosuppression. Such studies could help to elucidate the mechanism of immunosuppression, provide information for risk evaluation, and possibly indicate biomarkers for monitoring clinical status. Please conduct a general-purpose immune suppression screening assay such as a T-cell dependent antibody-forming assay and a more specific host resistance assay using an appropriate animal model for upper-respiratory bacterial infections. Please complete these studies within one year.

#### **C. Recommendations on Labeling**

This drug should be used during pregnancy only if clearly needed.  
Mothers should be instructed not to breast-feed if they are receiving FUZEON.

### **II. Summary of Nonclinical Findings**

#### **A. Brief Overview of Nonclinical Findings**

The principal nonclinical findings are injection site reactions and antibody production. Injection site reactions were observed in rats, guinea pigs, minipigs, and cynomolgus monkeys. The injection site reactions ranged from minor tissue discoloration to granulomatous inflammation, hemorrhage, fibrosis, edema and necrosis (including both dermal and muscle tissues). Changes in hematology and clinical chemistry parameters that are consistent with injection site reactions were observed in the rat and monkey. In addition, microscopic changes in the spleen and thymus that are consistent with injection site hypersensitivity reactions were observed in the monkey. Antibody titers to enfuvirtide were measured in the rat, minipig, and monkey. Skin sensitization (delayed contact hypersensitivity) from intradermal injections and direct skin application of enfuvirtide was demonstrated in the guinea pig.

#### **B. Pharmacologic Activity**

In in vitro studies, enfuvirtide and some of its metabolites have been shown to interfere with the entry of HIV-1 into target cells (CD4 T-lymphocytes and

macrophages). Enfuvirtide has not been tested for pharmacologic activity against HIV-1 virus in animal models.

**C. Nonclinical Safety Issues Relevant to Clinical Use**

The principal relevant clinical safety issues derived from nonclinical studies are systemic and localized (injection site) hypersensitivity reactions.

**III. Administrative**

**A. Reviewer signature:** \_\_\_\_\_

**B. Supervisor signature:**   Concurrence - \_\_\_\_\_

  Non-Concurrence - \_\_\_\_\_  
  (see memo attached)

**C. cc: list:**

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## PHARMACOLOGY/TOXICOLOGY REVIEW

[Numbers in square brackets in this document refer to individual studies as listed in Appendix X. Individual study reviews are included in Appendix XI. The sequence is the same as presented in the Master Index submitted by the sponsor to the NDA.]

### I. PHARMACOLOGY:

**Primary pharmacodynamics and mechanism of action:** Enfuvirtide is derived from a naturally occurring amino acid sequence within the HIV-1 gp41 transmembrane glycoprotein. By binding to the DP-107 and DP-178 regions of gp41, enfuvirtide interferes with the virus's ability to infuse its genomic RNA into CD4+ T-lymphocytes and macrophages.

**Drug activity related to proposed indication:** Interference with infusion of viral RNA into CD4+ T-lymphocytes and macrophages inhibits viral replication.

The sponsor has not submitted studies demonstrating anti-HIV-1 activity of enfuvirtide in animals.

### II. SAFETY PHARMACOLOGY:

**Neurological effects:** Enfuvirtide administered subcutaneously at doses up to 50 mg/kg did not affect the spontaneous locomotor activity of mice [55] and did not cause Irwin scale changes in behavior or physiological state in mice [56].

**Cardiovascular effects:** Enfuvirtide administered intravenously at doses of 15 and 50 mg/kg produced slight transient changes in blood pressure, heart rate, respiration rate and tidal volume in three female anesthetized beagle dogs but not in the single male anesthetized dog [57]. Normal electrocardiograms were obtained throughout the experiments and there were no marked changes in the QTc interval in any animal during this study. There was a slight transient decrease in mean PR interval and in QRS amplitude that coincided with the slight heart rate increase in the females after administration of enfuvirtide at 50 mg/kg. One female exhibited a transient reduction in QRS amplitude accompanied by ST-elevation after administration of 50 mg/kg. The ECG waveform of the male animal was unaffected by the administration of enfuvirtide or placebo. The \_\_\_\_\_ attributed all of the gradual changes over the course of the experiment to the anesthetic [57, 58].

**Pulmonary effects:** Rats administered enfuvirtide in a single intravenous bolus dose at 20, 50, and 100 mg/kg had dark red lesions involving all lobes of the lungs. Five of six rats receiving 100 mg/kg died following drug administration [25]. Microscopically, the lesions exhibited dose-related, non-specific (multi-focal to diffuse) congestion, hemorrhage, and edema (mainly perivascularly around large pulmonary vessels) and included over 50% of alveolar tissue. No clinical signs were observed for animals treated with T-20 at 50 mg/kg or lower or for animals given T-20 at 100 mg/kg by the intraperitoneal route in the same study. The \_\_\_\_\_ concluded that the intravenous injection resulted in a protein bolus that presented as a significant first pass "protein shock" in the lung, and the effect was exacerbated by slow clearance of the test material

from the lung [25]. In intravenous injection repeat-dose studies of enfuvirtide in rats at doses up to 10 mg/kg/day for 28 days, microgranulomas occurred more frequently in the lungs of rats in the high dose group (13/20) than in the controls (3/20). (Tissues in the two low-dose enfuvirtide groups were not examined microscopically.) The pathologist stated that the microgranulomas often had foreign material at their centers, which suggested to him that hair had entered the circulation during dose administration. The sponsor did not attribute any effects to enfuvirtide [27]. In nonclinical subcutaneous injection studies of enfuvirtide, pulmonary effects were not observed.

**Renal effects:** There were no significant renal effects noted in nonclinical studies of enfuvirtide [29, 38].

**Gastrointestinal effects:** There were no significant gastrointestinal effects noted in nonclinical studies of enfuvirtide [29, 38].

**Abuse liability:** The abuse liability of enfuvirtide is negligible.

**Other:** Microscopic changes in the spleen and thymus that are consistent with injection site hypersensitivity reactions were observed in the monkey [38]. The spleens of monkeys at the high and middle doses had increased prominence of follicular germinal centers and increased lymphocytic proliferative activity. Thymic cortical lymphocyte depletion was observed in animals following 3 and 9 months of dosing, although there was not an obvious dose-related response. Statistically significantly smaller mean weights of female spleens and thymuses at the 9-month necropsy compared with controls were attributed to the relatively large body weights of female monkeys in the control group.

Antibody titers to enfuvirtide were measured in the rat, minipig, and monkey. Skin sensitization (delayed contact hypersensitivity) from intradermal injections and direct skin application of enfuvirtide was demonstrated in the guinea pig.

**Safety pharmacology summary:** The principal findings in nonclinical studies of enfuvirtide are injection site reactions and antibody production.

**Safety pharmacology conclusions:** Patients who elect to take enfuvirtide should be informed of the risks for hypersensitivity reactions and injection site reactions and what to do about them.

### III. PHARMACOKINETICS/TOXICOKINETICS:

**PK parameters:** The sponsor has provided measurements of plasma concentrations of enfuvirtide from multiple studies in both rats and monkeys, and from one study each in rabbits and dogs. The studies include various designs with several formulations, doses, dosages, routes of administration, schedules for blood collection, and with various pharmacokinetic parameters calculated. The principal metabolite of enfuvirtide (Ro 50-6343) was measured in some of the studies. Plasma enfuvirtide levels and pharmacokinetic parameters varied sometimes by 30% or more within and between studies for both the rat and monkey model. Confounding factors, such as differences in study design, differences in formulations, dosing with T-1249 prior to dosing

with enfuvirtide, and pregnancy, limit the comparison of pharmacokinetic results across studies. In the summary that follows, pharmacokinetic trends and ranges are assumed to be more important than the values of specific pharmacokinetic parameters. Results are primarily drawn from studies where enfuvirtide was administered most similarly to the way it is administered in humans: in carbonate-mannitol buffer vehicle, via subcutaneous injection, and twice daily in repeat-dose studies for evaluation of long-term and steady state exposures.

In both rats and monkeys, there was a dose-proportional increase in systemic exposures (C<sub>max</sub> and AUC) below the clinical range of doses, but there was a less than dose-proportional increase in systemic exposures with increasing doses at and above the clinical range. Across three monkey studies [10, 11, 40] C<sub>max</sub> and AUC values both increased at approximately 75% of the administered dose over the dose range from 0.5 mg/kg to 10 mg/kg. There were no obvious differences in exposures with sex or with day of measurement (day 1, day 4, end of month 3, and end of month 9) in monkeys. T<sub>max</sub> values ranged from 1.7 to 4 hours. Across four rat studies [2, 30, 32, 35], the same pharmacokinetic trends were evident, but the dose range was higher (up to 125 mg/kg/dose) and there was a significant increase in exposures between those measured on day 1 and those measured at the end of week 1. This was particularly evident for doses greater than or equal to 15 mg/kg. After one week, there was no further obvious increase in C<sub>max</sub> and AUC for a given dose in rats.

**Absorption:** When enfuvirtide was administered via continuous subcutaneous infusion in rats and monkeys, it was poorly absorbed and caused more severe local adverse effects than when enfuvirtide was administered by subcutaneous injection. Difficulties in continuous subcutaneous infusion studies included catheter occlusion, kinking, and dislocation. Targeted plasma concentrations of enfuvirtide were generally not reached except at very low administered doses, and the sponsor abandoned this route of administration in favor of administration by subcutaneous injection.

After subcutaneous injection, enfuvirtide was absorbed systemically, with typical T<sub>max</sub> values at approximately 3 hours in monkeys and 2 hours in rats. Compare these with human T<sub>max</sub> values of 5 to 7 hours following a single subcutaneous injection of enfuvirtide in (clinical protocol NP16220).

**Distribution:** Tritiated enfuvirtide (<sup>3</sup>H-T-20, <sup>3</sup>H-Ro 29-9800) was administered to rats by intravenous or subcutaneous injection in six rat studies to explore tissue distribution, metabolism and excretion of enfuvirtide. In the autoluminography study [23] it was noted that the quantity of enfuvirtide in plasma following a single subcutaneous injection of enfuvirtide (222 mg/kg, 577 μCi/kg), as measured by HPLC, was detectable at 4 hours post dosing but undetectable at the end of eight hours post dosing. Compare this dose with the clinical dose per injection to humans of 90 mg or 1.5 mg/kg for a 60-kg human. Thus, enfuvirtide was rapidly metabolized, diluted, distributed, or excreted in the rat. However, radioactivity was detectable in all tissues and fluids assayed at all time points in these studies. The relationship between the quantity of radioactivity measured in tissues and the quantity of enfuvirtide remaining, as a function of time, is unknown.

Forty-eight hours after an intravenous injection of tritiated enfuvirtide [18], the rat muscle contained approximately 50% of the recovered radioactivity, based on tissue sampling and the assumption that skeletal muscle is 45.5% of body weight. The tissue with the next highest quantity at 48 hours, the liver, contained 8% of the recovered radioactivity.

After a single subcutaneous injection of tritiated enfuvirtide to pregnant rats in a placental transfer study [24], radioactivity was measured at 8 time points to 48 hours post dosing in the brain, heart, kidney, liver, lung, mammary gland, ovaries, placenta, uterus, dam carcass, fetuses, blood, plasma, and amniotic fluid. The highest radioactivity attained in any tissue at any measurement was in the dam carcass (77% of recovered dose at 0.5 hours post dosing), but the highest overall radioactivity was in the liver (14% of the total recovered radioactivity added over all tissues and all eight time measurements). The amniotic fluid had the least radioactivity (2% maximum radioactivity recovered at a single measurement and 2% overall) followed by the brain (3% maximum and overall). The whole blood had higher levels of radioactivity associated with it (10% recovered at a single measurement and 8% overall) than the plasma (5% maximum and 3% overall). Fetuses had 9% maximum recovered radioactivity at a single time point and 7% overall recovered radioactivity. None of these data were presented relative to tissue weight.

After a single subcutaneous injection of tritiated enfuvirtide to nursing dams in a milk secretion study [24], radioactivity in the blood, plasma, milk and carcasses was measured at 8 time points to 48 hours post dosing. The milk had the highest percentage of radioactivity measured at any single time point (27% at 8 hours post dose), but the carcass had the overall highest radioactivity recovered (32%) at the end of 48 hours.

**Metabolism:** Rates of in vitro metabolism of tritiated enfuvirtide incubated with rat, monkey and human hepatocytes were highest by rat and lowest by monkey hepatocytes. At 1  $\mu\text{M}$ , enfuvirtide was essentially all metabolized by rat hepatocytes at 30 minutes, and was essentially all metabolized by human or monkey hepatocytes at 4 hours. At 10  $\mu\text{M}$ , enfuvirtide was essentially all metabolized by rat hepatocytes at 4 hours, but was not fully metabolized with the human or monkey hepatocytes at 4 hours. With human hepatocytes, metabolism was initially rapid then decreased after 30 minutes and was linear to 120 minutes [17].

The sponsor identified one principal metabolite, Ro 50-6343, which is an acid hydrolysis product of enfuvirtide resulting from deamidation of the C-terminal phenylalanine residue. Ro 50-6343 appeared in the plasma following either intravenous or subcutaneous injection of enfuvirtide in the rat, or after subcutaneous injection in the monkey or rabbit. After a single subcutaneous injection of enfuvirtide in rats [31], plasma concentrations of Ro 50-6343 peaked at 4 to 6 hours post dosing, which was 3 to 5 hours after plasma concentrations of enfuvirtide peaked.  $C_{\text{max}}$  values of Ro 50-6343 were 3- to 5-fold lower than  $C_{\text{max}}$  values of enfuvirtide, and AUC values of Ro 50-6343 were 2- to 3-fold lower than AUC values of enfuvirtide. When enfuvirtide was administered twice daily to rats for 7 days [30],  $C_{\text{max}}$  and AUC values were similar for enfuvirtide and Ro 50-6343. Variability among these data may be due to problems with the detection method, which were evident in several studies.

Other metabolites of enfuvirtide are evident from \_\_\_\_\_ and the \_\_\_\_\_ studies discussed earlier, but were not identified in the pharmacology-toxicology studies submitted.

Plasma half-life ( $t_{1/2}$ ) values of enfuvirtide in repeat-dose subcutaneous injection studies were consistently between 1.5 and 2.5 hours in rats and between 2.5 and 3.5 hours in monkeys. In humans (Protocol NP16220), terminal elimination half-life values following a single subcutaneous injection were between 3.5 and 4.5 hours.

**Excretion:** After rats were administered a single intravenous injection of tritiated enfuvirtide ( $^3\text{H-T-20}$ ,  $^3\text{H-Ro 29-9800}$ ) in a week long study [20], males excreted 10% of the administered radioactivity in urine, 9% in feces, and 9% in expired air, and females excreted 17% of administered radioactivity in urine, 11% in feces, and 11% in expired air. In another one-week study [22], the recovery of administered radioactivity in urine, feces, expired air, and cage wash water (2 rats/sex) was 7%, 14%, 12%, and 2%, respectively. The remainder of the radioactivity, at the end of one week (50% to 60% for females and 60% to 75% for males) was associated with the carcass. HPLC analysis of rat urine (for samples collected 0-6 hours, 6-24 hours, and 24-48 hours) in the latter study revealed two peaks comprising 3 or more compounds, none of which was the parent enfuvirtide. Lyophilization of urine samples revealed that 25% of the radioactivity was associated with water in the 6-24 hour sample, and 69% was associated with water in the 72-96 hour (day 4) sample.

In the milk secretion study [24] the sponsor estimated that, following subcutaneous injection, 3% of the total radioactivity administered was recovered in the milk after collection for 48 hours. The ——— did not detect enfuvirtide in milk by HPLC, but reported difficulties with the method that renders the result indeterminate.

**Other studies:** Pharmacokinetic data were collected in a pilot rabbit study [46] designed to provide exposure information for a teratology study. Enfuvirtide was administered by subcutaneous injection to pregnant rabbits for 6 days during the period of gestation at doses of 10, 30, 60, and 100 mg/kg. Both greater-than dose-proportional and less-than dose-proportional increases in AUC values and  $C_{\text{max}}$  values were observed on the first and last days of dosing over this dose range. In a reproductive study in rats [45] using a similar dosing regimen but higher doses, some very high AUC values were attained. Pregnancy apparently alters the pharmacokinetics of enfuvirtide but these studies do not provide sufficient data to characterize those changes. The data from these studies are difficult to interpret, in part because exposures in nonpregnant rodents change substantially during the first week of dosing. Until more data become available, the pharmacokinetics of enfuvirtide in nonpregnant animals should not be considered representative of the pharmacokinetics in pregnant animals.

Pharmacokinetic data in dogs were collected [58] to correlate with data obtained from a cardiovascular and respiratory study in dogs [57].  $C_{\text{max}}$  values were high compared to rats. AUC values were not calculated because escalating doses were administered sequentially in the same animals.

In *in vitro* studies, enfuvirtide was greater than 97% bound to human plasma in concentrations from ——— greater than 94% bound to human serum albumin in concentrations from ——— and greater than 36% bound to human  $\alpha$ -1 acid glycoprotein in concentrations from ———. Commingled drugs (saquinavir, nelfinavir, efavirenz, and nevirapine) had no obvious effect on binding of enfuvirtide to plasma protein. Enfuvirtide was 82% to 49% associated with human blood cells in concentrations from ——— [12]. In *in vitro* binding time-to-equilibrium of enfuvirtide with human plasma was 20 hours, and was slightly more bound (95%) in healthy human plasma compared with HIV-infected plasma (92%) [13]. Compare the *in vitro* protein binding time-to-equilibrium of enfuvirtide in human plasma (20 hours) with the *in vivo* plasma half-life of enfuvirtide in humans of 3.5 to 4.5 hours.

**PK/TK summary:** Enfuvirtide was absorbed systemically in rats, monkeys, dogs, and rabbits following subcutaneous injection. The increase in systemic exposures (C<sub>max</sub> and AUC) was less than dose-proportional with increasing doses within and above the clinical range of doses. In distribution studies in the rat using radio-labeled enfuvirtide, radioactivity quantities following subcutaneous injection were highest in muscle and liver. However, radioactivity was detected in all tissues and fluids assayed, including brain, amniotic fluid, and the milk of nursing dams. The temporal relationship between amount of radioactivity measured and quantity of parent enfuvirtide is not known. The sponsor identified one major metabolite with HPLC methods, though a few others were noted in \_\_\_\_\_ and many others (smaller peptides and amino acids) are presumed to exist. Metabolism was rapid, with enfuvirtide plasma half-life values between 1.5 and 2.5 hours in the rat and 2.5 and 3.5 hours in the monkey. Following a single subcutaneous injection, measurable quantities of enfuvirtide were detected in rat plasma at four hours but not at eight hours post dosing. One in vitro hepatocyte study suggests humans metabolize enfuvirtide slower than rats but slightly faster than monkeys; however, clinical data indicate humans metabolize enfuvirtide slower than both rats and monkeys. In week-long mass-balance studies in rats, following a single intravenous injection of radio-labeled enfuvirtide, radioactivity recovered was approximately 10% each in urine, feces, and expired air, though radioactivity recovered in excretions from females was slightly greater than from males. The proportion of radioactivity in urine that was associated with water increased with time. However, no parent enfuvirtide was detected in urine by HPLC in any of the studies at any time points.

**PK/TK conclusions:** Enfuvirtide is absorbed, distributed, metabolized and excreted faster and with greater variability in rats than in monkeys, and faster in monkeys than in humans. Rat and rabbit studies suggest that variations in absorption, distribution, metabolism, and excretion of enfuvirtide may occur with pregnancy.

Nonclinical pharmacokinetics were important in the earlier development of this drug. Applications of pharmacokinetic/toxicokinetic data included determining the relative safety of different formulations, the relative usefulness of different routes of administration, and identifying unexpected characteristics in the absorption, distribution, metabolism, and excretion of enfuvirtide. Should the sponsor change the formulation of the drug product, the generation of further nonclinical pharmacokinetic data may be necessary. At this time, there appears to be sufficient adult human pharmacokinetic data from clinical studies to address safety and efficacy questions necessary for evaluating this NDA. The sponsor is currently still collecting and submitting human pediatrics pharmacokinetic data to the agency.

#### IV. GENERAL TOXICOLOGY:

The sponsor submitted nine rat toxicology studies [25, 27-35] and four cynomolgus monkey toxicology studies [26, 36-40]. The pivotal long-term nonclinical studies are a 6-month repeat-dose rat study [29] and a 9-month repeat-dose monkey study [38]. These two studies are summarized below.

**Study title:** A 6-month toxicity study of T-20 peptide administered twice per day via subcutaneous injections in the rat with a 4-week recovery period [29].

**Key study findings:** The primary toxic effects from treatment with enfuvirtide were injection site abnormalities (subdermal hemorrhages, subdermal chronic inflammation/fibrosis, subdermal edema, and degeneration of the cutaneous muscle). Minor changes observed in hematology and clinical chemistry values were consistent with injection site reactions.

**Study no:** Study 98-2579

**Volume #s:** 11-14

**Conducting laboratory and location:** \_\_\_\_\_

**Date of study initiation:** April 15, 1998.

**GLP compliance:** Yes, except that analyses of dosing solutions and toxicokinetics samples were not performed under GLP compliance.

**QA report:** yes ( x ) no ( )

**Drug, lot #, radiolabel, and % purity:** \_\_\_\_\_ batch numbers 709016, 708010, 711013, and 800123. No radiolabeled drug was used in this study.

**Formulation/vehicle:** Enfuvirtide was provided as a lyophilized powder. The placebo was a mannitol and carbonate buffer, and was also provided as a lyophilized powder. The diluent was sterile water for injection.

**Dosing:**

**Species/strain:** CrI:CD (SD)IGS BR Albino rats (outbred) VAF/Plus

**#/sex/group or time point (main study):** 30 rats/sex/group in Groups I and IV, and 25 rats/sex/group in Groups II and III. Ten animals/sex/group were sacrificed at Day 29, 15/sex/group were sacrificed at 6 months (Days 184 and 185), and 5 rats/sex/group (Groups I and IV) were sacrificed at 7 months (end of treatment-free Recovery, on Day 211).

**Satellite groups used for toxicokinetics or recovery:** Toxicokinetics were evaluated on main study and recovery rats. Recovery comprised 5 rats/sex/group (Groups I and IV only).

**Age:** 55 days at initiation of dosing.

**Weight:** Males: 297.7 g (range 271 – 326 g); females: 188.3 g (range 172 – 206 g).

**Doses in administered units:** Days 1 – 28: 0, 2.76, 10.35, 34.5 mg/kg/day (Groups I, II, III, IV, respectively). Days 29 – 183: 0, 2.4, 9, 30 mg/kg/day (Groups I, II, III, IV, respectively).

**Route, form, volume, and infusion rate:** Subcutaneous injection, bid, approximately half the daily dose, approximately 12 hours apart, 1 mL/kg/dose.

**Observations and times:**

**Clinical signs:** Twice daily for mortality and severe adverse effects; physical exams twice pretest and weekly throughout the study.

**Body weights:** Twice pretest and weekly throughout the study, and at termination.

**Food consumption:** Weekly beginning one week prior to treatment.

**Ophthalmoscopy:** Pretest, and end of Month 1, Month 6, and Month 7 (Recovery).

**EKG:** Not done.

**Hematology:** End of Months 1, 3, 6, and 7.

**Clinical chemistry:** End of Months 1, 3, 6, and 7.

**Urinalysis:** Not collected.

**Gross pathology:** End of Months 1, 6, and 7.

**Organs weighed:** End of Months 1, 6, and 7.

**Histopathology:** End of Months 1, 6, and 7.

**Toxicokinetics:** Day 1, and end of Months 1, 2, 3, 4, 5, and 6 at a single timepoint, 1.5 hours post-dosing from 5 rats/sex/group. Days 7 and 36 at six timepoints from 3 rats/sex/group.

**Other:** Serum samples for antibody measurements from 5 rats/sex/group at pre-dose, end of Week 1, and Months 1 through 7.

**Results:**

**Mortality:** All rats survived until their scheduled sacrifice.

**Clinical signs:** There were no clinical findings attributed to treatment.

**Body weights:** High dose males gained less weight than controls, high dose females gained more weight than controls. These differences were not statistically significant.

**Food consumption:** High dose rats (males and females) consumed more food than controls. Some differences for males were statistically significant.

**Ophthalmoscopy:** There were no ophthalmoscopic findings attributed to treatment.

**Electrocardiography:** N/A.

**Hematology:** Mean hematologic values of enfuvirtide-dosed rats were mostly similar to controls. Some elevations in neutrophil counts and lymphocyte counts (maximum = +64% compared with controls) do not suggest a trend, but are consistent with injection site reactions.

**Clinical chemistry:** Mean clinical chemistry values of enfuvirtide-treated rats were mostly similar to control rats. In females, some aspartate aminotransferase (AST) and alanine aminotransferase (ALT) values were higher than controls, but none of the differences were statistically significant.

**Urinalysis:** N/A.

**Organ weights:** Mean organ weights of enfuvirtide-treated rats were mostly similar to controls. There was an increase (+30% to +33%) in male adrenal weights (high-dose group, absolute and relative to body weights and brain weights) at recovery sacrifice compared with controls. This was the only statistically significant organ weight difference. However, the rat adrenal weights are small (<0.1 g), and the biological significance of this observation is not known.

**Gross pathology:** There were higher incidences of discoloration at injection sites in animals treated with enfuvirtide than with controls.

**Histopathology:** Histopathology findings were consistent with the macroscopic findings. Incidence of injection site abnormalities (subdermal hemorrhages, subdermal chronic inflammation/fibrosis, subdermal edema, and degeneration of the cutaneous muscle) outnumbered other findings.

**Toxicokinetics:** Enfuvirtide administered to rats by subcutaneous injection produced less than dose proportional increases in enfuvirtide plasma concentrations. Peak enfuvirtide plasma concentrations diminished slightly and occurred at later times on study Day 36 compared with study Day 7, indicating diminished absorption.

**Serum antibody measurements:** There were no measurable serum antibody titers in blood samples from Group I (placebo) or Group II (low enfuvirtide-dosed) rats. Among 85 Group III and Group IV rats (middle and high enfuvirtide doses), 8 rats had single blood samples with antibody titers of 1:100 or 1:400 in samples collected at the end of Months 1, 2, 4, 5, or 6.

**Summary of individual study findings:** The primary toxic effects from treatment with enfuvirtide were injection site abnormalities (subdermal hemorrhages, subdermal chronic inflammation/fibrosis, subdermal edema, and degeneration of the cutaneous muscle).

Minor changes in hematology and clinical chemistry values are consistent with injection site reactions.

**Study title:** A 9-month toxicity study of T-20 peptide administered twice per day via subcutaneous injection in the non-human primate [38].

**Key study findings:** The primary toxic effects from treatment with enfuvirtide were injection site abnormalities (subdermal hemorrhages, inflammation, fibrosis, edema, necrosis and degeneration of the cutaneous muscle). Changes observed in hematology and clinical chemistry values, presence of antibody titers, and microscopic changes in the spleen and thymus were consistent with injection site hypersensitivity reactions.

**Study no:** Study 98-3371

**Volume #s:** 21-22

**Conducting laboratory and location:** \_\_\_\_\_

**Date of study initiation:** August 5, 1998.

**GLP compliance:** Yes, except that analyses of dosing solutions, toxicokinetics samples, and serum antibody samples were not performed under GLP compliance.

**QA report:** yes (x) no ( )

**Drug, lot #, radiolabel, and % purity:** \_\_\_\_\_ batch numbers 800247, X800329, and X800349. No radiolabeled drug was used in this study.

**Formulation/vehicle:** Enfuvirtide was provided as a lyophilized powder. The placebo and vehicle was a mannitol and carbonate buffer, and was also provided as a lyophilized powder. The diluent was sterile water for injection.

**Dosing:**

**Species/strain:** Cynomolgus monkeys (*Macaca fascicularis*).

**#/sex/group or time point (main study):** 6 monkeys/sex/group in Group I (placebo control), and 8 monkeys/sex/group in Groups II and III (low and high enfuvirtide doses). Two Group I monkeys/sex/group and three Group II and Group III monkeys/sex/group were sacrificed at the end of Month 3. Three Group I monkeys/sex/group and four Group II and Group III monkeys/sex/group were sacrificed at the end of Month 9. One monkey/sex/group (all Groups) were sacrificed at the end of Month 10 (end of treatment-free Recovery).

**Satellite groups used for toxicokinetics or recovery:** Toxicokinetics were evaluated on main study and recovery monkeys. Recovery comprised 1 monkey/sex/group (all Groups).

**Age:** Young adults, approximately 3 years old.

**Weight:** Males: 3.7 kg (range 3.1 – 5.3 kg); females: 3.0 kg (range 2.7 – 3.3 kg).

**Doses in administered units:** Group I: 0 (mannitol/carbonate placebo), Group II: enfuvirtide 10 mg/kg/day, Group III: enfuvirtide 20 mg/kg/day.

**Route, form, volume, and infusion rate:** Subcutaneous injection, bid, approximately half the daily dose, approximately 12 hours apart, 1 mL/kg/dose.

**Observations and times:**

**Clinical signs:** Twice daily for mortality and severe adverse effects; physical exams twice pretest and weekly throughout the study.

**Body weights:** Twice pretest and weekly throughout the study, and at termination.

**Food consumption:** Weekly beginning one week prior to treatment.

**Ophthalmoscopy:** Pretest, and end of Month 3 and Month 9.

**EKG:** Twice during pretest (all monkeys), once at the end of Month 3 (2/sex in Group I and 3/sex/group in Groups II and III), and once at the end of Month 9 (all monkeys).

**Hematology:** Pretest, end of Months 3, 5, 6, 7, 8, 9 and 10.

**Clinical chemistry:** Pretest, end of Months 3, 5, 6, 7, 8, 9 and 10.

**Urinalysis:** Not collected.

**Gross pathology:** End of Months 1, 6, and 7.

**Organs weighed:** End of Months 1, 6, and 7.

**Histopathology:** End of Months 1, 6, and 7.

**Toxicokinetics:** Day 1, and end of Months 3 and 9, at 8 timepoints each from 3 monkeys/sex/group/day.

**Other:** Blood for serum antibody determination was collected on Day 1, end of Week 1 and at the end of every month through Month 10.

#### Results:

**Mortality:** All monkeys survived until their scheduled sacrifice.

**Clinical signs:** The only clinical findings attributed to treatment were swelling and edema at injection sites beginning in Week 17. No dose-relation was evident, but the incidence was slightly higher in males than in females. No edema was observed during the recovery period.

**Body weights:** High dose males gained more weight (+8% at Week 39) than controls, high dose females gained less weight (-19% at Week 39) than controls. These differences were not statistically significant.

**Food consumption:** The — reported that there were no changes in food consumption related to the administration of T-20 and that values for controls and treated animals were similar. However, the — did not present numerical analyses or graphs of the food consumption data.

**Ophthalmoscopy:** There were no ophthalmoscopic findings attributed to treatment.

**Electrocardiography:** N/A.

**Hematology:** Enfuvirtide administration was associated with elevated numbers of eosinophils in most ( $\geq 70\%$ ) treated animals compared with controls. Several monthly increases in eosinophil counts were 4 to 16 times the values of the control means, except in Month 9, when the maximum increase was nearly 70 times the control mean (probably due to an exceptionally low control value). One of the four animals held over to 10 months (recovery period) had an elevated eosinophil count at necropsy (approximately twice the eosinophil count as the control). Effects were higher in males than in females but were not dose-related. The increases in eosinophil counts were reflected in elevated total leukocyte counts. WBC values, when increased, were generally +20% to +50% above control values, but were increased approximately +100% in months 7 and 8 in low-dose males perhaps because the control mean values were low in those months.

**Clinical chemistry:** A few treated animals had elevated alanine aminotransferase (ALT) values compared with controls at every month of measure (Months 0, 3, 5, 6, 7, 8, 9).

One low-dose female always had ALT values 3 to 10 times the control mean. But these increases were also reflected in the pretest values (before dosing) so they were probably not drug-related. Also, there was no correlation between elevated ALT values and histopathological findings in the livers or spleens of the enfuvirtide-dosed animals and elevated ALT values did not correlate well with elevated eosinophil values.

**Urinalysis:** N/A.

**Organ weights:** There were no obvious effects of the administration of enfuvirtide on organ weights. Statistically significantly smaller weights of female heart, spleen, and thymus at the 9-month necropsy compared with controls were likely due to the relatively large body weights of female monkeys in the control group.

**Gross pathology:** The most frequent macroscopic observation was discoloration at injection sites. At terminal sacrifice the injection sites of enfuvirtide administration showed discoloration, thickening, and cysts. There was some discoloration at placebo control injection sites but the incidence and severity was much less than at enfuvirtide injection sites. At recovery sacrifice, discoloration was still apparent where enfuvirtide had been injected, but not at placebo injection sites.

**Histopathology:** Histopathology findings were consistent with the macroscopic findings. Microscopic observations of enfuvirtide injection sites at the end of 3 months revealed varying degrees of subcutaneous hemorrhage, edema, inflammatory infiltrate, focal necrosis and fibrosis of the subcutaneous tissue, as well as degeneration of the fibers of the *panniculus carnosus* muscle. At the end of nine months of dosing, the incidence and severity of subcutaneous hemorrhage, edema and inflammatory infiltrate was markedly greater at the sites of enfuvirtide dosed animals compared with controls. The inflammatory infiltrate was predominantly lymphocytic and frequently formed lymphoid follicles. There was also a significant plasma cell and eosinophilic polymorphonuclear component to the infiltrate, suggesting a hypersensitivity reaction. A high proportion of the enfuvirtide injection sites also showed fibrosis, and in some animals, cystic spaces, focal necrosis and abscess formation were also present in subcutaneous tissue. There was no obvious dose relationship in the severity of the findings between the two enfuvirtide dose groups.

Microscopic changes in the spleen were attributed to enfuvirtide dosing. Following three months dosing, the spleens of enfuvirtide-dosed animals showed increased prominence of follicular germinal centers in all animals receiving 20 mg/kg/day and in one female and one male receiving 10 mg/kg/day. The increased prominence of germinal centers was characterized by increased size and increased lymphocytic proliferative activity. Following nine months dosing there was a smaller increase in the incidence and severity of this finding compared with the finding at three months.

Thymic cortical lymphocyte depletion was observed in many enfuvirtide-treated animals following 3 and 9 months of dosing. No animal showed this at the 10-month recovery necropsy. There was not an obvious dose-related response.

**Toxicokinetics:** At Day 1 and Month 3, enfuvirtide exhibited linear pharmacokinetics within the range of doses tested. A directly proportional increase in  $C_{max}$  and AUC were demonstrated with dose. At Month 9 there was a 1.3-fold difference in  $C_{max}$  between the two dose groups, and no difference with AUC. The sponsor suggested that tissue damage and the production of anti-enfuvirtide serum antibodies might have contributed to the decreased rate of enfuvirtide absorption and to the apparent loss in dose response relationship.

**Serum antibody measurements:** No anti-enfuvirtide antibodies were detected in placebo-dosed monkeys or pre-dose, Day 1, or Week 1 sera from enfuvirtide-treated monkeys. All low-dose enfuvirtide animals developed enfuvirtide antibodies by Day 29 and 8 of 10 high dose animals showed anti-enfuvirtide antibodies by Day 29 (the first test

day that any titers were measurable). Maximum titers at Month 9 were \_\_\_\_\_ and \_\_\_\_\_ from low- and high-enfuvirtide dosed animals, respectively.

**Summary of individual study findings:** The primary toxic effects from treatment with enfuvirtide were injection site abnormalities (subdermal hemorrhages, inflammation, fibrosis, edema, necrosis and degeneration of the cutaneous muscle). Changes observed in hematology and clinical chemistry values, presence of antibody titers, and microscopic changes in the spleen and thymus are consistent with injection site hypersensitivity reactions.

**Toxicology summary:** The primary toxic effects from treatment with enfuvirtide in long-term repeat-dose rat and monkey studies were injection site reactions. Changes in hematology and clinical chemistry values consistent with injection site reactions were observed in both the rat and monkey. In addition, the cynomolgus monkey model exhibited measurable antibody titers, and microscopic changes in the spleen and thymus that are consistent with injection site hypersensitivity reactions.

**Toxicology conclusions:** The primary risks from repeated dosing of enfuvirtide by subcutaneous injection are systemic and localized (injection site) hypersensitivity reactions.

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**Histopathology Inventory for NDA # 21-481**

Study	98-2579	98-3371
	6-month	9-month
Species	rat	monkey
Adrenals	X, *	X, *
Aorta	X	X
Bone Marrow smear	X	X
Bone (femur)	X	X
Brain	X, *	X, *
Cecum	X	X
Cervix	X	X
Colon	X	X
Duodenum	X	X
Epididymis	X, *	X, *
Esophagus	X	X
Eye	X	X
Fallopian tube		
Gall bladder		X
Gross lesions	X	X
Harderian gland		
Heart	X	X, *
Ileum	X	X
Injection site	X	X
Jejunum	X	X
Kidneys	X, *	X, *
Lachrymal gland	X	
Larynx		
Liver	X, *	X, *
Lungs	X	X
Lymph nodes, cervical		
Lymph nodes mandibular		
Lymph nodes, mesenteric	X	X

Study	98-2579	98-3371
	6-month	9-month
Species	rat	monkey
Mammary Gland	X	X
Nasal cavity		
Optic nerves	X	X
Ovaries	X, *	X
Pancreas	X	X
Parathyroid	X	X
Peripheral nerve		
Pharynx		
Pituitary	X	X
Prostate	X	X
Rectum	X	X
Salivary gland	X	X
Sciatic nerve	X	X
Seminal vesicles	X	X
Skeletal muscle	X	X
Skin		X
Spinal cord	X	X
Spleen	X, *	X, *
Sternum	X	
Stomach	X	X
Testes	X, *	X, *
Thymus	X, *	X, *
Thyroid	X	X
Tongue		
Trachea	X	X
Urinary bladder	X	X
Uterus	X	X
Vagina	X	X
Zymbal gland	X	
Standard List		

X = histopathology performed; \* = organ weight obtained

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ON ORIGINAL

## V. GENETIC TOXICOLOGY:

**Key findings:** Enfuvirtide was not mutagenic in either an Ames/Salmonella-E. coli reverse mutation assay [48] or an \_\_\_\_\_ mammalian cell forward gene mutation assay [49], and it was non-clastogenic in an in vivo micronucleus test in mouse bone marrow erythropoietic cells [50].

### Labeling recommendations:

#### Mutagenesis

Enfuvirtide was neither mutagenic nor clastogenic in a series of in vivo and in vitro assays including the Ames bacterial reverse mutation assay, a mammalian cell forward gene mutation assay in AS52 Chinese Hamster ovary cells, or an in vivo mouse micronucleus assay.

## VI. CARCINOGENICITY:

**Key findings:** Long-term carcinogenicity studies of enfuvirtide have not been conducted.

**Summary of status:** The sponsor requested a waiver from conducting carcinogenicity studies of enfuvirtide in a submission to IND \_\_\_\_\_ dated September 6, 2000. On April 17, 2001, the Division presented the sponsor's request before the CDER Executive Carcinogenicity Assessment Committee (Executive CAC) for comment. On April 27, 2001, we held a teleconference with employees of Hoffmann-La Roche and Trimeris, Inc. to ask them questions. After consideration of the sponsor's arguments and responses and the discussion with the CDER Executive CAC, the Division granted the sponsor's request to waive carcinogenicity studies of enfuvirtide (letter dated May 22, 2001). For more details, see Appendix XI.

### Labeling recommendations:

#### Carcinogenesis

Long-term animal carcinogenicity studies of enfuvirtide have not been conducted.

## VII. REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY:

**Key findings:** Reproduction studies of enfuvirtide in rats and rabbits have not shown a risk to the fetus.

**Summary of individual study findings:** In three rats studies [41, 42, 47] and two rabbit studies [43, 44] enfuvirtide was administered by subcutaneous injections up to 30 mg/kg/day and no statistically significant differences from controls were seen in indices of mating, fertility, gestation, fetal development or fetal toxicology. However, this high dose did not result in frank toxicity to the dams and is too low for the studies to be considered conclusive. In one rat study [42] and one rabbit study [44] there were slight increases in the numbers of early (rat) or late (rabbit) resorptions. The increase in resorptions in rats was dose-related, but the increase in

resorptions in rabbits was not. Rabbit fetuses also exhibited a higher number of discolored (reddened) thymuses compared with controls [44]. The Division was concerned whether these observations would have been statistically significant if the enfuvirtide doses had been higher. The sponsor conducted an additional rat reproduction study and increased the dose range to 500 mg/kg/day [45]. This dose is at or near the maximum injectable dose and is considered acceptable. Dams were treated by subcutaneous injection from Gestation Day 6 to 11 for toxicokinetic evaluations (n=12/group), and from Gestation Day 6 to 17 for teratologic evaluations (n=22/group). There were no statistically significant differences between enfuvirtide-treated dams/litters and controls in numbers of resorptions, resorption rates, litter size, numbers of viable male fetuses, or body weights of fetuses. Convoluted and/or dilated ureters and renal pelvises were seen in visceral tissues. Skeletal findings included short or misshapen ribs, vertebrae or sternums and ossified metacarpals and phalanges. The mean numbers of treated litters and fetuses with findings of any type were never greater than controls, and were occasionally lower than controls [45].

**Reproductive and developmental toxicology summary:** Reproduction studies of enfuvirtide in rats and rabbits have not shown a risk to the developing fetus. Reproduction studies of effects on fertility are inconclusive.

**Reproductive and developmental toxicology conclusions:** Nonclinical reproduction studies do not indicate that enfuvirtide poses a risk to the developing fetus.

**Labeling recommendations:**

**Impairment of Fertility**

Enfuvirtide produced no adverse effects on fertility in male or female rats at doses up to 30 mg/kg/day administered by subcutaneous injection (1.6 times the maximum recommended adult human daily dose on a m<sup>2</sup> basis).

**Pregnancy**

Pregnancy Category B. Reproduction studies have been performed in rats and rabbits at doses up to 27 times and 3.2 times the adult human dose on a m<sup>2</sup> basis. The animal studies revealed no evidence of harm to the fetus from enfuvirtide. There are no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

**VIII. SPECIAL TOXICOLOGY STUDIES:**

**Milk secretion and placental transfer study:** The sponsor submitted one report of studies of milk secretion and placental transfer of enfuvirtide in rats [24]. In the milk secretion study (n = 24), <sup>3</sup>H-enfuvirtide was administered once by subcutaneous injection (197 mg/kg) at approximately Day 14 post-parturition and blood and milk samples were collected from 3 rats each at eight times over the subsequent 48 hours. \_\_\_\_\_ was then used to measure radioactivity in blood, plasma, milk, and carcasses, and HPLC was used to measure the concentration of enfuvirtide in plasma and milk. In the placental transfer study (n = 24), <sup>3</sup>H-enfuvirtide was administered once by subcutaneous injection (196 mg/kg) on approximately Day 18 of gestation and amniotic fluid, brain, heart, kidneys, liver, lungs, mammary glands, ovaries,

placenta, and uterus were collected from 3 rats each at eight times over the subsequent 48 hours. Also, the number of fetuses was recorded and the fetuses of each litter were pooled. \_\_\_\_\_ was used to measure radioactivity of blood, plasma, each tissue, the pooled fetuses, and the dam residual carcass, and HPLC was used to measure the concentration of enfuvirtide in amniotic fluid and placental homogenate. In both studies, radioactivity was initially highest in the carcass and began to diminish from the carcass after 4 hours. Radioactivity peaked in blood, plasma, and milk between 4 and 8 hours post-dosing but was much higher in milk (by at least 30%) during this period than in either whole blood or plasma. Radioactivity in most other organs and tissues increased rapidly with the 2-hour samples and reached their maximum values at 8 hours or 24 hours post-dosing. The only tissues that contained 5% or more of the radioactivity administered at any time during the study were the carcass, the fetuses, and the liver. The amniotic fluid, the brain, and the plasma had the lowest levels of radioactivity on a per-weight basis throughout the study.

The sponsor estimated that 3% of the total radioactively administered was detected in milk (in the milk secretion study) during the 48-hour sample collection period and (in the placental transfer study) >10% of the total radioactivity administered was detected in the fetuses. HPLC analysis of plasma samples from the milk secretion study revealed the presence of parent enfuvirtide only in plasma collected up to 1 hour post dosing. HPLC \_\_\_\_\_ of milk, amniotic fluid, and placental homogenate (samples from both studies) revealed only a single metabolite of enfuvirtide. These results are consistent with the rapid metabolism of enfuvirtide and the subsequent widespread distribution of amino acids throughout the body. These results are consistent with earlier mass distribution studies of enfuvirtide except that in Study No. D00111 (approximately the same dose administered) enfuvirtide was detected in sera samples collected up to 4 hours post-dosing. In this report the \_\_\_\_\_ stated that the extraction or analysis methods may have been "inefficient" or "suboptimal," and therefore the absence of enfuvirtide in the \_\_\_\_\_ is not conclusive evidence that parent compound was not present in the samples. Radioactivity levels in the fetuses and milk do not conclusively settle whether the rat fetuses were exposed to enfuvirtide in utero or whether the nursing pups were exposed to enfuvirtide in milk.

**Summary of study findings:** In the milk secretion study in rats, enfuvirtide was not detected in milk but a major metabolite of enfuvirtide was detected. In the placental transfer study in rats, enfuvirtide was not detected in amniotic fluid or placental homogenate, but a major metabolite of enfuvirtide was detected. The \_\_\_\_\_ stated that the extraction processes were suboptimal and the results do not conclusively settle whether the rat fetuses were exposed to enfuvirtide in utero or whether the nursing pups were exposed to enfuvirtide in milk.

#### **Labeling recommendations:**

##### **Nursing Mothers**

**The Centers for Disease Control and Prevention recommends that HIV-infected mothers not breast-feed their infants to avoid the risk of postnatal transmission of HIV. It is not known whether enfuvirtide is excreted in human milk. Because of both the potential for HIV transmission and the potential for serious adverse reactions in nursing infants, mothers should be instructed not to breast-feed if they are receiving FUZEON.**

Studies where radio-labeled <sup>3</sup>H-enfuvirtide was administered to lactating rats indicated that radioactivity was present in the milk. It is not known whether the radioactivity in the milk

was from radio-labeled enfuvirtide or from radio-labeled metabolites of enfuvirtide (i.e., amino acids and peptide fragments).

**Antigenicity studies:** The sponsor submitted two skin sensitization studies in guinea pigs using the \_\_\_\_\_ method (also called the \_\_\_\_\_ test) [51, 52]. In the first study, during the induction phase, enfuvirtide was administered to the animals in sterile water (50 mg/mL) or enfuvirtide in sterile water combined with Freund's Adjuvant (50 mg/mL in a 50:50 mixture). The challenge phase consisted of enfuvirtide at concentrations of 25 mg/mL or 50 mg/mL. The assay resulted in a dose-dependent incidence of slight erythema that is indicative of a delayed contact hypersensitivity reaction [51].

In the second assay, enfuvirtide was administered in solution concentrations of 100 mg/mL in carbonate buffer or 100 mg/mL in Tris buffer (in the induction phase) and \_\_\_\_\_ or \_\_\_\_\_ in carbonate buffer or Tris buffer in the challenge phase. Both enfuvirtide formulations resulted in positive results indicative of a delayed contact hypersensitivity reaction. Enfuvirtide in the Tris buffer formulation produced a higher incidence and a stronger reaction (thickening, dryness, edema, and sloughing of the epidermis) than the carbonate formulation. [52].

In February 2001 the sponsor indicated that the enfuvirtide Tris formulation would be discontinued clinically.

**Summary of study findings:** In the \_\_\_\_\_ assays, enfuvirtide caused delayed contact hypersensitivity reactions when administered in sterile water, carbonate buffer or Tris buffer vehicles.

**Injection site studies:** The sponsor submitted two 14-day repeat-dose studies of enfuvirtide administered by subcutaneous injection to minipigs to study injection site reactions [53, 54]. In the first study enfuvirtide was dissolved in mannitol buffer at 50 or 100 mg/mL and in the second study, enfuvirtide was dissolved in carbonate buffer at 50 or 100 mg/mL or in Tris buffer at 100 mg/mL. Full-thickness skin biopsies were taken from each animal at each treatment site and from untreated sites periodically during the 14-day recovery periods. In both studies, subcutaneous masses ranging from 1 to 5 cm at one or more test material injection sites were noted for each animal beginning on Day 8. The masses dissipated upon cessation of treatment and were generally not apparent by the end of the recovery periods. In the first study, skin biopsies revealed minimal to moderate inflammation, edema, hemorrhage, congestion, necrosis, and degenerated collagen in both enfuvirtide-treated and vehicle-treated sites [53]. In the second study, one or more treatments were suspended based on observations of dose sites. Subcutaneous masses were observed at the enfuvirtide-treated sites beginning on Day 4 before becoming palpable on Day 8 and well-defined granulomas were evident at enfuvirtide-treated injection sites as early as Day 8. In addition, a cloudy white discharge from an enfuvirtide-Tris buffer injection site was noted for one animal on Day 25. Enfuvirtide with Tris appeared to induce the most severe granulomas and the most number of granulomas compared with the enfuvirtide carbonate formulations. The Tris site granulomas were characterized by a necrotic center with amorphous eosinophilic, proteinaceous debris, and surrounded by multinucleated giant cells and foamy macrophages with distended cytoplasm. Positive staining for enfuvirtide was noted only in biopsy sections with granulomas [54].

**Summary of study findings:** Fourteen-day repeat-dose studies of enfuvirtide administered by subcutaneous injection to minipigs resulted in palpable subcutaneous masses with varying degrees of tissue inflammation and destruction.

**Locomotor activity study:** The sponsor submitted one locomotor activity study [55] in which a single subcutaneous dose of enfuvirtide was administered to mice (10/group) at doses of 5, 15, or 50 mg/kg. Control groups received either vehicle (mannitol and carbonate buffer) or 5 mg/kg diazepam (positive control). Fifteen minutes following the test article administration, the mice were placed individually into cages on an activity meter. The mobility of the animals was recorded at 10-minute intervals over the following one-hour period. The positive control produced statistically significant decreases in activity counts. There were no statistical differences in activity counts between the enfuvirtide-treated mice and the vehicle controls, however, the maximum enfuvirtide dose administered appears to be too small [55].

**Summary of study findings:** A single dose of enfuvirtide did not cause a change in the activity level of mice under the conditions of this study.

**Irwin general behavior study:** The sponsor submitted one Irwin general behavior study [56] in which a single subcutaneous dose of enfuvirtide was administered to mice (4/group) at doses of 5, 15, and 50 mg/kg. A control group received vehicle (mannitol and carbonate buffer). At 5, 15, 30, 60, and 120 minutes and 24 hours after dosing, the behavior of enfuvirtide-dosed mice was scored to assess changes in behavior or physiological state. There were no abnormalities recorded for any animal in any group at any observation time during this study, however the study did not appear to be conducted under GLP conditions and the maximum dose may have been too low [56].

**Summary of study findings:** A single dose of enfuvirtide to mice did not cause changes in Irwin general behavior parameters under the conditions of this study.

**Cardiovascular and respiratory studies:** The sponsor submitted two cardiovascular-respiratory studies in beagle dogs [57, 58]. In the first study, numerous catheters were introduced into 4 dogs (3 female, 1 male) to administer anesthetic and test article, and to record cardiovascular and respiratory parameters. All four beagles received placebo and enfuvirtide intravenous injections at 0, 5, 15, and 50 mg/kg, in that order, with each succeeding enfuvirtide dose occurred 30 minutes after the previous dosing. Enfuvirtide administration at 15 and 50 mg/kg produced a slight mean transient decrease in diastolic pressure and a slight mean increase in systolic pressure, heart rate, tidal volume, respiration rate, and mean minute volume in female anesthetized beagles, but no change in the one male beagle. Normal electrocardiograms were obtained throughout the experiments. There were no marked changes in the QTc interval in any animal during this study. Two female animals exhibited transient reductions in QRS amplitude after administration of enfuvirtide at 15 and 50 mg/kg. A third female exhibited a transient reduction in QRS amplitude accompanied by ST-elevation after administration of 50 mg/kg only. The ECG waveform of the male animal was unaffected by administration of enfuvirtide or placebo. The — attributed gradual changes in females over the course of the experiment to the anesthetic and concluded that intravenous administration of enfuvirtide or placebo did not produce any overt cardiorespiratory effects in beagles under the conditions of this study [57]. The second study [58] was a toxicokinetics study to measure plasma concentrations in beagles dosed intravenously at the same levels and at the same time intervals as in the previous beagle

study. However, in the second study, anesthetic was not administered and cardio-respiratory parameters were not measured. There were no marked gender differences in the measured plasma levels of enfuvirtide. C<sub>max</sub> values were 0, 108, 396, and 1240 µg/mL, for administered enfuvirtide doses of 0, 5, 15, and 50 mg/kg, respectively. AUC and half-life values were not calculated since doses were administered sequentially in the same animals [58].

The sponsor notes that in clinical trial T20-501/NP16220, the mean peak plasma concentration (C<sub>max</sub>) after subcutaneous injection of the recommended human clinical dose (90 mg) was approximately 5 µg/mL. The highest plasma concentration attained in the second dog study from the 5 mg/kg administered dose was 108 µg/mL. Therefore, the NOAEL dose in dogs (5 mg/kg) in the nonclinical cardiovascular and respiratory study resulted in a plasma concentration of enfuvirtide that was greater than 20 times the plasma concentration in humans following the standard clinical dose.

**Summary of study findings:** Intravenous administration of enfuvirtide to beagle dogs at doses resulting in plasma concentrations that exceed by more than 20-fold the human plasma concentrations when enfuvirtide is administered by subcutaneous injection at the recommended clinical dose, resulted in no cardiovascular or respiratory effects to the beagles.

## IX. DETAILED CONCLUSIONS AND RECOMMENDATIONS:

**Conclusions:** From a pharmacology-toxicology viewpoint, enfuvirtide is approvable. The primary adverse effects seen in animal studies were also seen in studies in humans.

**General Toxicology Issues:** The important toxicology issues that were demonstrated in nonclinical studies are injection site reactions and hypersensitivity reactions.

**Recommendations:** Due to the possible increase in incidence of bacterial infections in enfuvirtide-treated patients (enfuvirtide plus optimized background) versus patients on optimized background in Phase III clinical trials, the sponsor should conduct nonclinical immune function studies to investigate whether enfuvirtide causes immunosuppression. Such studies could help to elucidate the mechanism of immunosuppression, provide information for risk evaluation, and possibly indicate biomarkers for monitoring clinical status.

Labeling recommendations include the following: This drug should be used during pregnancy only if clearly needed. Mothers should be instructed not to breast-feed if they are receiving FUZEON.

### Labeling:

#### Mutagenesis

Enfuvirtide was neither mutagenic nor clastogenic in a series of in vivo and in vitro assays including the Ames bacterial reverse mutation assay, a mammalian cell forward gene mutation assay in AS52 Chinese Hamster ovary cells, or an in vivo mouse micronucleus assay.

**Carcinogenesis**

Long-term animal carcinogenicity studies of enfuvirtide have not been conducted.

**Impairment of Fertility**

Enfuvirtide produced no adverse effects on fertility in male or female rats at doses up to 30 mg/kg/day administered by subcutaneous injection (1.6 times the maximum recommended adult human daily dose on a m<sup>2</sup> basis).

**Pregnancy**

Pregnancy Category B. Reproduction studies have been performed in rats and rabbits at doses up to 27 times and 3.2 times the adult human dose on a m<sup>2</sup> basis. The animal studies revealed no evidence of harm to the fetus from enfuvirtide. There are no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

**Nursing Mothers**

The Centers for Disease Control and Prevention recommends that HIV-infected mothers not breast-feed their infants to avoid the risk of postnatal transmission of HIV. It is not known whether enfuvirtide is excreted in human milk. Because of both the potential for HIV transmission and the potential for serious adverse reactions in nursing infants, **mothers should be instructed not to breast-feed if they are receiving FUZEON.**

Studies where radio-labeled <sup>3</sup>H-enfuvirtide was administered to lactating rats indicated that radioactivity was present in the milk. It is not known whether the radioactivity in the milk was from radio-labeled enfuvirtide or from radio-labeled metabolites of enfuvirtide (i.e., amino acids and peptide fragments).

**X. APPENDIX/ATTACHMENTS:**

**Addendum to review:** Addenda to this report include the appendices described below.

**Other relevant materials (Studies not reviewed, appended consults, etc.):** The following appendices are attached: A summary review of the sponsor's request for a waiver from conducting carcinogenicity studies of enfuvirtide; the sponsor's Master Index of pharmacology-toxicology studies submitted to this NDA, and reviews of all individual nonclinical pharmacology-toxicology studies submitted to this NDA in the same order as in the sponsor's Master Index. All nonclinical pharmacology-toxicology studies submitted to this NDA have been reviewed, and all the reviews are included in this report.

**Any compliance issues:** There are no compliance issues.

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## XI. CARCINOGENICITY STUDIES WAIVER DISCUSSION

This appendix is a summary of discussions concerning the sponsor's request for a waiver from conducting carcinogenicity studies of enfuvirtide (T-20). On September 6, 2000, Hoffmann-La Roche submitted a 10-page position paper presenting arguments why enfuvirtide is unlikely to be a carcinogen and therefore why carcinogenicity testing is not necessary. On April 17, 2001, the Division presented Hoffmann-La Roche's request before the CDER Executive Carcinogenicity Assessment Committee (Executive CAC) for comment. On April 27, 2001, the Division held a conference call with employees of Hoffmann-La Roche and Trimeris, Inc. to ask them questions. This appendix summarizes CDER's review of the sponsor's request.

### HOFFMANN-LA ROCHE POSITION

Hoffmann-La Roche, Inc. discussed criteria from the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) S1A and S6A guidance documents in support of its request for a waiver. Its arguments are presented below with comments. Some arguments are paraphrased. In this section, reviewer comments are indented and preceded by the word, "Comment." Agreement with Hoffmann-La Roche is implied where arguments are presented without comment.

#### 1. Concerns related to product class.

T-20 is a novel retroviral agent with respect to biological and chemical characteristics and therefore there is no concern based on carcinogenicity of other antiretroviral agents

**Comment:** Insofar as T-20 is a novel retroviral agent (and there are no other products in its class), the argument is irrelevant whether other products in its class have not demonstrated carcinogenic activity. CDER does not share the opinion that there is no concern of carcinogenicity, based on this argument, but recognizes that there may be no evidence to the contrary. This point was made by the Executive CAC chair.

The unique mechanism of action of T-20 involves a specific protein-protein interaction with the extracellular portion of the transmembrane glycoprotein gp41 of the HIV virus, thus blocking viral fusion with the T-cell membrane.

**Comment:** The chair of the Executive CAC asked whether there was evidence for T-20-tissue (protein-protein) interactions (that might suggest that T-20 stimulates cell division, or blocks cell apoptosis). We responded that we did not know of any such evidence, but we would ask Hoffmann-La Roche. During our conference call, Hoffmann-La Roche personnel said they did not know of any studies that looked at T-20-tissue interactions except that NIH had conducted a study of T-20 that produced ambiguous or difficult-to-interpret data.

Polypeptides are not known to be carcinogenic.

**Comment:** There are polypeptides that are known to be carcinogenic. All those that we located are hormones or ones that demonstrated hormonal activity. Hoffmann-La Roche indicated during our conference call that they made efforts to identify examples of

polypeptides that are known carcinogens and only succeeded in identifying some hormones.

2. Structure-activity relationships.

T-20 is expected to be metabolized into smaller peptides and amino acids. As such, there are no classical structural alerts suggesting a carcinogenic risk.

3. Concerns from repeated-dose toxicity studies.

In repeat dose studies of T-20 (6 months in rats, 9 months in monkeys), hyperplasia and preneoplastic lesions have not been observed.

**Comment:** In a 28-day repeat-dose intravenous injection study of T-20 in monkeys (Series 003), splenic hyperplasia was observed in most of the animals (all of the high-dose animals), and the severity of splenic hyperplasia was greater in T-20-injected animals than in control animals. One animal in the high-dose group had a spleen that was greater than 3% of its body weight. This hyperplasia likely resulted from an immune reaction to T-20. However, we do not believe it is correct to state that there is no evidence of treatment-related hyperplasia.

4. Long-term retention of parent compound or metabolite.

Pharmacokinetic studies in rats after intravenous injection revealed a 2.4 hour half-life and low distribution (26.3 mL) suggesting that there is little long-term retention of T-20 in rodents.

**Comment:** Studies where T-20 was administered by subcutaneous injection to rats or monkeys indicated poor absorption at the injection site in some instances, which in turn suggests long-term retention of T-20 is possible under some circumstances.

Studies of the rates of elimination of radioactivity in feces, urine, and air after a single intravenous injection of tritiated T-20 in the rat suggest catabolism of T-20 into amino acids and reincorporation of radiolabel into body tissue proteins. The highest amount of radiolabel appeared in skeletal muscle 48 hours after T-20 administration. No systematic degeneration of muscle tissue was noted after chronic T-20 administration.

5. Genotoxicity.

There was no evidence of genotoxicity in the standard recommended genotoxicity test battery: bacteria reverse mutation (Ames) tests, forward gene mutations in mammalian (Chinese hamster ovary AS52) cells, and mouse bone marrow micronuclei tests.

**Comment:** Not all carcinogens are genotoxic.

6. Compounds producing chronic irritation/inflammation.

Repeated twice-daily injections of T-20 to rats or monkeys resulted in various degrees of inflammation and irritation at the injection sites. In the 6-month rat study, the incidence and severity of lesions was comparable to that observed in vehicle-treated animals. Evidence of an injection site reaction in primates was not common until week 17. Injection site reactions have been observed in humans, which vary in severity and rarely cause treatment

discontinuation. Patients are instructed to rotate sites of injection to avoid chronic irritation/inflammation at a single site.

**Comment:** We consider that early studies of T-20 (e.g., rat 6-month repeat-dose study) were inconclusive as to whether injection site inflammation was solely a result of the procedure or the drug or some combination of the two. In reviewing the data retrospectively, it appears likely that inflammation is, at least in part, a reaction to the drug. It is irrelevant that microscopic evidence of an injection site reaction in primate was not common until week 17. In the same (9-month primate) study, increased eosinophils (compared with controls) were measured at 3 months (the earliest post-dosing hematological measurement) and inflammatory responses were evident in the spleens (increased germinal centers and lymphocytic proliferation) and thymuses (cortical lymphocytic depletion) at 3 months. In the 28-day, repeat-dose primate study (Series 003), antibodies to T-20 were detected at day 7. Thus, inflammation, while not clearly evident macroscopically until week 17 in the 9-month study, was microscopically evident earlier on.

Observations that injection site reactions in T-20 patients rarely cause treatment discontinuation, and that "patients are instructed to rotate sites of injection, thus avoiding chronic irritation/inflammation at a single site" are irrelevant to the carcinogenic risk assessment but important to the potential carcinogenic risk management.

7. Patient population.

T-20 is intended for patients with advanced HIV infection, and will be tested in patients who have experienced all three classes of marketed antiviral drugs. These patients have limited treatment options.

**Comment:** It is true that HIV infection is a life-threatening disease and that patients with advanced systemic HIV infection face limited treatment options. However, there are treatment options available to some of the patients. Since the advent of HAART regimens and the consequential decrease in mortality and increase in longevity of these individuals, carcinogenic risk from anti-HIV drugs is a concern, especially for children. The ICH guidance S1A states that where life expectancy in the indicated population is short (i.e., less than 2 to 3 years) long-term carcinogenicity studies may not be required. Many HIV-infected individuals are now living longer than 3 years, and so this argument does not apply to all HIV-infected individuals who might receive T-20.

It is expected that patients may continue treatment of T-20 beyond 6 months, the treatment duration limit beyond which carcinogenicity studies for conventional pharmaceutical agents are generally needed. However, HIV patients have limited treatment options and the benefit:risk ratio for T-20 is considered extremely high, irrespective of the outcome of animal carcinogenicity studies.

**Comment:** Treatment with T-20 beyond 6 months is a reason for conducting carcinogenicity testing. It is no longer true that carcinogenic risk is not a concern in the treatment of HIV-infected individuals.

8. Relevance of carcinogenicity testing of polypeptides.

Standard carcinogenicity bioassays may be inappropriate for compounds such as large molecular weight proteins and polypeptides.

**Comment:** Large molecular weight proteins are not mentioned in the ICH guidance documents referenced.

## SUMMARY

The strongest arguments presented by Hoffmann-La Roche for NOT conducting carcinogenicity testing of T-20 are as follows:

- a) T-20 did not induce tumors in 6-month repeat-dose studies in rats or 9-month repeat-dose studies in monkeys.
- b) T-20 is a polypeptide comprised of naturally occurring L-amino acids. There are data indicating that T-20 is metabolized to amino acids and they, in turn, are incorporated into body tissues.
- c) T-20 was not mutagenic in the standard battery of mutagenicity tests.
- d) The only examples that we could find of proteins that are carcinogenic are proteins that demonstrated hormonal action. As far as we know, T-20 does not have hormonal action.

Reasons to support conducting carcinogenicity testing of T-20 are as follows:

- a) In some cases T-20 administration was shown to cause chronic irritation and inflammation at injection sites when administered by subcutaneous injection or infusion. In some cases, injection of T-20 caused hard masses at injection sites, suggesting that T-20 was not well-absorbed.
- b) Administration of T-20 is expected to exceed 6 months in some patients.
- c) T-20 is a new molecular substance.

## DISCUSSION:

Executive CAC members commented that it is difficult to conduct a two-year subcutaneous injection study in rats. Rats have limited body surface area for subcutaneous injection sites and the procedure harms the rats. Unless there is a compelling reason to think T-20 might be carcinogenic, the Executive CAC thought that it is reasonable to grant the requested waiver.

Probably the most compelling reason to be concerned about the carcinogenic potential of T-20 is that the subcutaneous administration of T-20 causes chronic irritation and inflammation. However, as noted above, this adverse effect in humans can be monitored and managed. Other evidence suggests that T-20 is not a carcinogen.

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**CONCLUSION:**

The Division agreed to grant Hoffmann-La Roche a waiver from conducting carcinogenic studies of enfuvirtide. A letter to this effect dated May 22, 2001 was sent to the sponsor.

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## XII. NONCLINICAL PHARMACOLOGY AND TOXICOLOGY MASTER INDEX

The sponsor included a Master Index with the NDA. The list that follows is the portion of the Master Index pertaining to nonclinical pharmacology and toxicology studies. Each study in the list is reviewed in the appendix that follows the list.

### ADME/Nonclinical PK Study Reports

#### Single Dose P/K

##### Rodent

1. NDA 21-481 Reference 2000  
Study 98-3647 – 2000 - Mandella R. A pilot pharmacokinetic study of T-20 peptide administered subcutaneously via continuous infusion or injection in the rat. 2002.
2. NDA 21-481 Reference 2001  
Study 98-3648 – 2001 - Mandella R and Robison RL. Final Study Report - A 72-hour study of T-20 peptide administered subcutaneously via continuous infusion and injection in the rat. 2002.
3. NDA 21-481 Reference 2002  
Study 60777#1T-20 – 2002 - Final Study Report - Venetta TM. Pharmacokinetic analyses from single dose intravenous administration of T-20 in rats. 2000.
4. NDA 21-481 Reference 2003  
Study 60777T-20#2 / 60777T-20LY – 2003 - Final Study Report - Venetta TM.  
Pharmacokinetic analyses from pharmacokinetics of T-20 in plasma and lymph following intravenous administration of a single-dose in rats. 2000.
5. NDA 21-481 Reference 2004  
Study 07399 - 2004 - Tang J, et al. Final Study Report - Ro 29-9800 (T20): A single dose subcutaneous pharmacokinetic study of two formulations in fed male rats. RR 1004781. 2002.
6. NDA 21-481 Reference 2005  
Study 07431 – 2005 - Tang JP, et al. Final Study Report - Ro 29-9800 (T-20 Fusion Inhibitor): A single dose subcutaneous or intravenous pharmacokinetic study in fed male rats. RR 1006412. 2001.

##### Non-rodent

7. NDA 21-481 Reference 2006  
Report 20912 – 2006 - Loveday K. Final Study Report - A pharmacokinetic study of T20 peptide in cynomolgus monkeys. 1995.
8. NDA 21-481 Reference 2007  
Report 31501 – 2007 - Loveday K. Final Study Report - A pharmacokinetic study of T20 peptide in three male cynomolgus monkeys. 1996.
9. NDA 21-481 Reference 2008  
Study 98-3646 – 2008 - Mandella RC. Final Study Report - A pharmacokinetic study of T-20 peptide in male and female cynomolgus monkeys. 2002.
10. NDA 21-481 Reference 2009  
Study No. EHAW-114 - 2009 - Venetta TM. Final Study Report – Pharmacokinetic analyses from bioequivalence testing of T-20 formulations following subcutaneous administration to cynomolgus monkeys. 2000.
11. NDA 21-481 Reference 2010  
Study No. EHAW-116 – 2010 - Venetta TM. Pharmacokinetic analyses from bioequivalence testing of T20 formulations following subcutaneous administration to cynomolgus monkeys. 2000.

##### Red Cell Partitioning and Protein Binding

12. NDA 21-481 Reference 2200  
Study 103 – 2200 - Cole R. Final Study Report - Plasma protein binding and blood distribution studies with 3H-T-20. 2000.
13. NDA 21-481 Reference 2201  
Study D01034 – 2201 - Colter S. Final Study Report - No. 29-9800: The in vitro binding of Ro 29-9800 (T20) to human plasma protein in healthy volunteers and patients with human deficiency virus (HIV+), and displacement effects of concomitant medications in healthy volunteers. RR 1007511. 2002.

##### Metabolic Studies

##### In vivo

14. NDA 21-481 Reference 2202  
Study Rt99-1 – 2202 - Venetta TM. Final Study Report - An in vivo study of enzyme induction by T-20. Protocol No 729. Report 64C-07482-003. 2000.

**In vitro**

15. NDA 21-481 Reference 2203  
Report 64C-07482-006 -- 2203 - Venetta TM. Final Study Report - Assessment of the inhibition of the activities of human hepatic microsomal cytochrome P450 by T-20 in vitro. 2000.
16. NDA 21-481 Reference 2204  
Report 1003497 -- 2204 - Chang D, et al. Final Study Report - Ro 29-9800: Identification of a metabolite of T-20 found in rat plasma and (rat and human) liver microsomal incubations. 2001.
17. NDA 21-481 Reference 2205  
Study 6131-321 -- 2205 - Moore D. Final Study Report - In vitro metabolism of [3H]T-20 by rat, monkey, and human hepatocytes (Study No. 6131-321). RR 1006292. 2002.

**-Distribution**

18. NDA 21-481 Reference 2300  
Study 01 -- 2300 - Cole R. Final Study Report - Preliminary metabolism, distribution and pharmacokinetic study of 3H-T-20 following intravenous administration to Sprague-Dawley Rats. 2000.
19. NDA 21-481 Reference 2301  
Study 02 -- 2301 - Cole RJ. Final Study Report - Metabolism and excretion study of 3H-T-20 following intravenous administration to Sprague-Dawley rats. 2001.
20. NDA 21-481 Reference 2302  
Study 04 -- 2302 - Cole RJ. Final Study Report - Mass balance study of 3H-T-20 following intravenous administration to Sprague-Dawley rats. 2001.
21. NDA 21-481 Reference 2303  
Study 07461 -- 2303 - Final Study Report - Ro 29-9800/000 (T-20, fusion inhibitor): A single dose (subcutaneous injection) exploratory study to evaluate distribution of T-20 fusion inhibitor in lymph nodes of male rats. RR 1004433. 2001.
22. NDA 21-481 Reference 2305  
Study D01021 -- 2305 - Cotler S, Tang J-P. Final Study Report - Ro 29-9800: Mass balance study of 3H Ro 29-9800 (3H T20) Following subcutaneous administration to male Long Evans rats. RR 1004809. 2001.
23. NDA 21-481 Reference 2306  
Study D00111 -- 2306 - Cotler S and Fulton J. Final Study Report - Ro 29-9800: Mass balance and in rat following subcutaneous administration of 3H Ro 29-9800. RR 1005251. 2002.
24. NDA 21-481 Reference 2307  
Study D01022 -- 2307 - Cotler S. Final Study Report - Ro 29-9800: Milk secretion and placental transfer studies with 3H-Ro 29-9800 (3H-T20) Following Subcutaneous Administration to Female Rats. RR 1007715. 2002.

**Toxicology Study Reports****Acute (single dose) Toxicology Studies**

25. NDA 21-481 Reference 3000  
Study 680 -- 3000 - Atkinson JE. Final Study Report - A single dose toxicity study of T-20 in the albino rat. 1996.
26. NDA 21-481 Reference 3001  
Study 3-B44 -- 3001 - Loveday KS. Final Study Report - Single dose toxicity study of a test article (T-20) administered intravenously to cynomolgus monkeys. 1996.

**Repeat Dose (subchronic & chronic) Studies****Rat**

27. NDA 21-481 Reference 3100  
Study 682 -- 3100 - Atkinson J.E. Final Study Report - A 28-day repeat dose toxicity study of T-20 in the albino rat followed by a 2-week recovery period. 1996.
28. NDA 21-481 Reference 3101  
Study 793 -- 3101 - Atkinson J. Final Study Report - A maximum tolerated dose (MTD) determination and a 7-day intravenous toxicity study of two batches of T-20 in rats. 1996.
29. NDA 21-481 Reference 3102  
Study 98-2579 -- 3102 - Auletta C. Final Study Report - A 6-month toxicity study of T-20 peptide administered twice per day via subcutaneous injections in the rat with a 4-week recovery period. 2002.
30. NDA 21-481 Reference 3103  
Study 07496 -- 3103 - Pignatello M, et al. Final Study Report - Ro 29-9800/000 (T-20 Fusion Inhibitor): A seven-day subcutaneous (b.i.d.) toxicity, local tolerance and toxicokinetic study in male rats. RR 1005285. 2001.

31. NDA 21-481 Reference 3111  
Study 07595 – 3111 - Rusin G, et al. Final Study Report - Ro 29-9800 [T-20] (Fusion Inhibitor): A pilot toxicokinetic study of Ro 29-9800 administered subcutaneously to female CrI-CD BR VAF/plus rats. RR 1005341. 2002.
  32. NDA 21-481 Reference 3112  
Study 07678 – 3112 - Downing JC, Final Study Report - Ro 29-9800 (T20, Fusion Inhibitor): A four-week b.i.d. subcutaneous (injection) toxicity and toxicokinetics study in rats with T-20 and impurities. RR 1006069. 2002.
  33. NDA 21-481 Reference 3113  
Study 98-3645 – 3113 - Hui J and Rusnak PP. Final Study Report - A 28-day toxicity study of T-20 peptide administered subcutaneously via continuous infusion in the rat. 2002.
  34. NDA 21-481 Reference 3114  
Report EDS107.R01-00 - 3114 - Stuelke E. Final Study Report - A report on serum antibody assessments from a 6-month toxicity study of T-20 peptide administered twice per day via subcutaneous injections in the rat with a 4-week recovery period (Study 98-2579). 2002.
  35. NDA 21-481 Reference 3115  
3115 - DiMassimo B and Venetta TM. Final Study Report - Toxicokinetic analyses from A 6-month study of T-20 peptide administered twice per day via subcutaneous injections in the rat with a 4-week recovery period. 1999.
- Monkey**
36. NDA 21-481 Reference 3106  
Study 3845 – 3106 - Lilja HS. Final Study Report - Twenty-eight day repeated dose toxicity study of T-20 peptide administered intravenously to cynomolgus monkeys. 1996.
  37. NDA 21-481 Reference 3107  
Study 98-3373 – 3107 - Blanset D and Robison RL. T-20 route 1 and T-20 route 2: A 28-day bridging study of two formulations of T-20 administered via twice daily subcutaneous injections in the cynomolgus monkey. 2002.
  38. NDA 21-481 Reference 3109  
Study 98-3371 – 3109 - Auletta CS and Creasy D. A 9-month toxicity study of T-20 peptide administered twice per day via subcutaneous injection in the non-human primate. 2002.
  39. NDA 21-481 Reference 3110  
Study 07447 – 3110 - Catala AR and Steele TD. Ro 29-9801 and Ro 29-9800 (T-1249 and T-20): A 28-day toxicity study with T-1249 and T-20 in cynomolgus monkeys (Study No. 6131-323, Roche Study No. 07447). RR 1002995. 2002.
  40. NDA 21-481 Reference 3116  
3116 – Toxicokinetic analyses and serum antibody assessments from a 9-month toxicity study of T-20 peptide administered twice per day via subcutaneous injection in the non-human primate. 2002.
- Reproductive Toxicology Studies**
- Segment I Fertility**
41. NDA 21-481 Reference 3200  
Study 99-4162 – 3200 - Thornton SR. Final Study Report - Study of the effect of T-20 peptide on fertility and early embryonic development to implantation in the rat via subcutaneous administration. 2002.
- Segment II Teratogenicity**
42. NDA 21-481 Reference 3300  
Study 99-4163 – 3300 - Thornton SR. Final Study Report - The effects of T-20 peptide on embryo-fetal development in rats via subcutaneous administration. 2002.
  43. NDA 21-481 Reference 3301  
Study 99-4164 – 3301 - Thornton SR. Final Study Report - A range-finding study of the effects of T-20 on embryo-fetal development in rabbits via subcutaneous administration. 1999.
  44. NDA 21-481 Reference 3302  
Study 99-4165 – 3302 - Thornton SR. The effects of subcutaneous administration of T-20 peptide on embryo-fetal development in rabbits. 1999.
  45. NDA 21-481 Reference 3303  
Study 07666 – 3303 - Rusin G. Final Study Report - Ro 29-9800 (T20; Fusion Inhibitor): Teratology and Plasma Concentration Study of Ro 29-9800 Administered Subcutaneously (b.i.d.) to Pregnant CrI:CD (R) (SD) BR Rats. RR 1006929. 2002.
  46. NDA 21-481 Reference 3304  
Study 07487 – 3304 - Staudner H, et al. Final Study Report - Ro 29-9800 [T-20] (Fusion Inhibitor): A pilot plasma concentration study of Ro 29-9800 administered subcutaneously (b.i.d.) to pregnant New Zealand white rabbits. RR 1004950. 2002.

**Segment III Peri Post Natal**

47. NDA 21-481 Reference 3400  
Study 99-4176 - 3400 - Rodwell DE. Final Study Report - The Effects of T-20 Peptide on Pre- and Postnatal Development, including Maternal Function, in Rats. 2002.

**Genotoxicity Studies****-Genetic Toxicology**

48. NDA 21-481 Reference 3500  
Study 0301FT14.001 - 3500 - Stankowski L. Final Study Report - Ames/Salmonella -E. Coli reverse mutation assay on test article T-20. 1996.
49. NDA 21-481 Reference 3501  
Study 0314FT14.001 - 3501 - Stankowski L. Final Study Report - mammalian cell forward gene mutation assay on T-20. 1997.
50. NDA 21-481 Reference 3502  
Study 0309FT14.001 - 3502 - Stankowski L. Final Study Report - In vivo micronucleus test with T-20 in mouse bone marrow erythropoietic cells. 1996.

**-Antigenicity Studies**

51. NDA 21-481 Reference 3600  
Study 001 - 3600 - Coleman D. Final Study Report - Skin Sensitization in the guinea-pig. Report 001/982397/SS. 1998.
52. NDA 21-481 Reference 3601  
Study 005 - 3601 - Coleman D. Final Study Report - Skin Sensitization to the guinea-pig (Report 005/002348/SS. 2000).

**-Special Toxicity Studies**

53. NDA 21-481 Reference 3703  
Study 07385 - 3703 - Downing JC. Final Study Report - Exploratory assessment of injection site lesions after subcutaneous administration of T-20 fusion inhibitor in minipigs. RR 1003491. 2001.
54. NDA 21-481 Reference 3704  
Study 07421 - 3704 - Downing JC. Final Study Report - Assessment of injection site lesions in minipigs after subcutaneous administration of three formulations of T-20 fusion inhibitor. RR 1003492. 2001.

**Other Nonclinical Toxicology**

55. NDA 21-481 Reference 3700  
Study 002/994088 - 3700 - Williams C. Final Study Report - Effect of subcutaneously administered T-20 on locomotor activity in mice. Report 002/994088. 2000.
56. NDA 21-481 Reference 3701  
Study 003/994087 - 3701 - Williams C. Final Study Report - Irwin general behaviour analysis in mice following subcutaneous administration of T-20. Report 003/994087. 2000.
57. NDA 21-481 Reference 3702  
Study 004/994526 - 3702 - Jordan S. Final Study Report - Effect of intravenously administered T-20 on cardiovascular and respiratory function in the anaesthetised dog. Report 004 994526. 2000.
58. NDA 21-481 Reference 3705  
Study 07660 - 3705 - Geng W. Final Study Report - Ro 29-9800/000 (T20, Fusion Inhibitor): A Pharmacokinetic Study with Escalating Single Intravenous Doses Administered to Fed Dogs. RR 1007599. 2002.

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