XIII. REVIEWS OF PHARMACOLOGY AND TOXICOLOGY STUDIES

ADME/NONCLINICAL PK STUDY REPORTS

Single Dose PK – Rodent

1. NDA 21-481 Reference 2000, Volume 2. IND ——Serial 047, 333.
A pilot pharmacokinetic study of T-20 peptide administered subcutaneously via continuous infusion or injection in the rat. ——, Study 98-3647. Final Report, November 18, 1999, amended May 9, 2002. Trimeris report document EDS103.R01-00, March 18, 2002, amended April 30, 2002.

——, conducted the in-life portion of this non-GLP study during March 1998 and sent pharmacokinetic samples to Trimeris for analysis.

Four rats/sex/group were administered a single subcutaneous bolus dose of T-20 at 12.5 mg/kg and blood was collected at eight timepoints out to 6 hours post dosing.

Four additional rats/sex/group were administered T-20 at a dose of 13 mg/kg in a continuous subcutaneous infusion at 0.02 mL/hour for 6 days, except that infusion was discontinued in one animal (a female) after 3 days due to infusion line occlusion. Blood samples for pharmacokinetic analysis were collected from continuous infusion rats at a single timepoint on Days 2, 4, and 7, except that the last blood sample from the female that experienced continual infusion difficulties was collected on Day 3.

Swelling at the infusion site was observed in 7/8 rats receiving T-20 by subcutaneous continuous infusion. By the end of the infusion period, yellow caseous exudate was evident in or at the end of the catheter in 2 males and 3 females. Between Day 3 and the termination of the study, there were large variations in the actual volumes delivered compared with theoretical volumes.

Pharmacokinetic results are presented below.

Plasma pharmacokinetic parameters of T-20 in rats after a single subcutaneous bolus dose (12.5 mg/kg).

Parameter	Males	Females	Mean
Cmax (µg/mL)	4.6	7.6	6.1 ± 2.1
t_{max} (h)	1	1	1
$AUC_{(0-6h)}$ (µg-h/mL)	15.4	17.2	16.3 ± 1.3
$AUMC_{(0-6h)}$ (µg-h^2/mL)	36.2	35.3	35.8 ± 0.7
t ½ (h)	1.4	1.9	1.7 ± 0.3
Range (h)	2 – 6	2 – 6	
$AUC_{(0-\infty)}$ (µg-h/mL)	16.5	19.6	18.1 ± 2.2
$AUMC_{(0-\infty)}$ (µg-h^2/mL)	45.1	56.9	51.0 ± 8.4
MRT (h)	2.7	2.9	2.8 ± 0.1
CL/F (L/h)	0.76	0.64	0.70 ± 0.09

All parameters were calculated from composite plasma concentrations for each sex; i.e., n = 2. AUMC = area under the first moment curve. MRT = mean residence time = ($AUMC_{(0-\infty)}$ / $AUC_{(0-\infty)}$) x 100%.

Female rats had slightly higher Cmax and $AUC_{(0-6h)}$ values than male rats after a single subcutaneous injection of T-20.

Mean and standard deviation plasma T-20 concentrations of males (n = 4) and females (n = 4) administered T-20 by continuous subcutaneous infusion (13 mg/kg/day x 6 days) were 2.5 ± 1.5 µg/mL and 2.9 ± 0.8 µg/mL, respectively, on Day 2 (48 hours post initial infusion). Most of the continuous infusion plasma samples collected on Days 3, 4 and 7 had no detectable T-20 levels. Three exceptions were as follows in one rat/group on Days 3, 4, and 7: Day 3 (female), 1.1 µg/mL; Day 4 (male), 1.1 µg/mL; Day 7 (male), 1.3 µg/mL.

Trimeris concluded that continuous infusion delivery of T-20 in rats produced plasma concentrations that were below targeted levels and that it is doubtful that this method of delivery would be practical.

2. NDA 21-481 Reference 2001, Volumes 2-3. IND ——— Serial 047, 333.
A 72-hour study of T-20 peptide administered subcutaneously via continuous infusion and injection in the rat. ——— Study 98-3648. Final Report, November 18, 1999, amended May 16, 2002.

——— conducted this GLP study during April 1998 to measure delivery of T-20 when administered to rats subcutaneously via continuous infusion and as intermittent injections over a period of 72 hours. The sponsor intended to use results from this study in selecting a dosing regimen for a chronic study of T-20 when administered via subcutaneous injection. ——— provided the blood samples to Trimeris for analysis. The original final report was amended to note that Trimeris did not assay T-20 concentrations in dosing solutions or in plasma samples in compliance with GLPs.

The rats for this study were Albino Rats (Outbred) VAF/Plus, Crl:CD (SDE)IGS BR from (———

There were 12 groups of 6 rats/sex/group in this study, divided into three dosing regimens: Twice per day intermittent subcutaneous injection (b.i.d. ISI, 2.3 and 30 mg/kg/dose), three times per day ISI (20 mg/kg/dose, t.i.d.), and continuous subcutaneous infusion (CSI, 1, 4, and 13 mg/kg/day). Each dosing regimen included both a saline control group and a placebo (———, buffer) control group.

Physical observations, body weights and food consumption measurements were performed on all animals predosing and at necropsy. Hematology and clinical chemistry evaluations were performed on all animals at necropsy. Blood for toxicokinetic evaluations was collected from all injection animals and from selected infusion animals at selected timepoints during the treatment period.

After at least 72 hours of treatment, animals were necropsied, 7 selected organs were weighed, 36 selected tissues and organs were collected and examined macroscopically, and tissues from injection or infusion sites (only) were examined microscopically.

Results:
There were no mortalities on this study. One placebo control male from the continuous infusion study was "electively sacrificed" one day early, but no reason was provided.

Discoloration at the injection sites was noted often in rats on both the repeat injection and continuous infusion portions of the study.

In animals treated via continuous subcutaneous infusion there was slight to moderate swelling at the dose sites seen in all dose groups including the saline and placebo controls. The incidence was greatest in the high-dose (13 mg/kg/day) group (4/6 males and 4/6 females), but the severity of swelling was not dose-dependent.

In the ISI groups, microscopic subacute inflammation at injection sites occurred more frequently in the T-20 groups than in the controls.

Incidence of Subacute Inflammation at Injection Sites				
	Saline control N=12	Placebo control* N=12	4.6 mg/kg/day N=12	60 mg/kg/day N=12
2x ISI	0	1	2	5
3x ISI	0	0	-	1

* buffer - No low-dose cohort in the t.i.d. study.

In the ISI groups, chromodacryorrhea (bloody tears) occurred 9 times, in animals representing nearly all the groups. This was probably because blood was collected from the eyes. Alopecia occurred once in each of the three T-20 groups in the continuous infusion study and twice in control animals in the b.i.d. ISI study. These were the only general findings that occurred with some frequency, apart from those related to injection/infusion sites.

There were no obvious effects of T-20 on body weight, body weight gain or food consumption in any of the animal groups during the study.

There were increases (+29%, +29%, +28%) in the thymus weights (absolute and relative to body weights, and relative to brain weights, respectively) of males in the high-dose b.i.d. ISI group compared with the saline control means. But the males in the high-dose t.i.d. ISI group had smaller thymus weights than the control means.

There was a statistically significant increase in kidney weights for females in the high dose CSI group compared with saline controls (+12%, +11%, and +15%, absolute, relative to body weight, relative to brain weight, respectively).

In the CSI middle-dose group (4 mg/kg/day), females exhibited statistically significant decreases in absolute lymphocyte counts (–35%) and white blood cell counts (–28%) compared with the saline control means, but these differences were not dose-proportional.

Mean aspartate aminotransferase (AST) values were increased in the high-dose ISI group compared with saline control means. In the males, the differences were +51% and +21% (b.i.d.

and t.i.d. ISI groups) and in the females, +59% and +41% (b.i.d. and t.i.d ISI groups, respectively). Increases in mean AST values were smaller in other groups.

—— noted that mean AST values in all the CSI groups were elevated compared with the mean AST values from the ISI saline control groups (<1% to 11%) and states that increases in AST without concurrent increases in ALT values suggest an effect on skeletal muscle. —— noted that AST effects in the CSI groups are consistent with the implanted catheter lying near the dorsal muscles.

Reviewer comment:
The differences between AST means in the CSI groups compared with the CSI saline control are small and none are statistically significant. The ——s explanation that elevated AST values are related to the indwelling catheter and not to T-20 does not explain the larger AST differences compared with saline controls in the two ISI studies. The increases in three of four high-dose ISI mean AST values are statistically significant compared with the saline control AST values in those two ISI studies.

Inflammation and subcutaneous hemorrhage were reported from the histological examinations of injection sites in both ISI groups. In the b.i.d. ISI group there was also one case each of subcutaneous edema and muscle necrosis/coagulation. However, the infusion sites of CSI study animals exhibited many more instances and types of pathology than the ISI animals, including thrombosis, fibrosis, abscess, and granulation tissue. Subacute and chronic inflammation were reported the most number of times, and seem to be unrelated to dose group. Animals with abscesses and granulation tissue were only from the high-dose groups.

In-life study conclusion:
—— concludes that there were no observed effects of T-20 in the ISI studies, but there were histomorphological findings at 13 mg/kg/day in the CSI study. Therefore, the no observed effect level for T-20 administered by subcutaneous injection 2 or 3 times daily in the rat is 60 mg/kg/day, and the no effect level for T-20 administered for three days by continuous subcutaneous infusion in the rat is 4 mg/kg/day (the middle dose).

Reviewer comments:
In both of the ISI studies, microscopic subacute inflammation occurred more frequently at the injection sites in the high-dose (60 mg/kg/day) group than in the controls, although the numbers are small. Also in these studies, AST values are significantly elevated in the high-dose groups compared with the saline controls. Other findings in the two ISI studies were more ambiguous.

In the CSI study, the incidence of swelling at injection sites was greatest in the high-dose (13 mg/kg/day) group, but it occurred in the two lower dose groups and both placebo groups, as well. Other effects at injection sites (i.e., tissue granulation and abscess) occurred only in T-20 dosed rats. The frequency and variety of microscopic findings at injection sites in the CSI study suggest that catheter effects may be masking host responses to T-20.

Thus, the data do not strongly support a different conclusion from that presented in the report, but it is likely that the NOAELs presented by —— are overstated (too high) because this study does not distinguish between the toxicologic effects of T-20 and the administration procedure.

Toxicokinetic data are presented below.

Dosing solutions for the subcutaneous infusion arms of the study assayed at 94% to 101% of nominal values. Concentrations of dosing solutions for continuous subcutaneous infusion were not determined. Dose volumes delivered during the continuous subcutaneous infusion arms of the study varied between –60.5% and +31.9% of nominal values.

Calculated pharmacokinetic parameters from the subcutaneous injection arms of the study on study Day 1 are as follows:

Parameter	Group 3 (2.3 mg/kg b.i.d.)	Group 4 (30 mg/kg b.i.d.)	Group 7 (20 mg/kg t.i.d.)
Cmax (µg/mL)	2.9 ± 0.0	6.9 ± 0.4	7.1 ± 0.5
t_{max} (h)	2.0 ± 0.0	1.5 ± 0.7	2.0 ± 0.0
$AUC_{(0\text{-}8h)}$ (µg-h/mL)	12.7 ± 2.1	22.9 ± 0.8	24.4 ± 4.2
$AUC_{(0\text{-}12h)}$ (µg-h/mL)	14.2 ± 2.3	24.7 ± 2.0	26.3 ± 3.7
$AUC_{(0\text{-}24h)}$ (µg-h/mL)	14.8 ± 2.1	25.9 ± 3.4	26.7 ± 3.5
t ½ (h)	2.5 ± 0.6	2.8 ± 1.4	1.9 ± 0.3
Range (h)	4 - 8	4 – 8	2 - 8
$AUC_{(0-\infty)}$ (µg-h/mL)	14.8 ± 2.1	26.1 ± 3.6	26.7 ± 3.5
CL/F (L/h/kg)	0.16 ± 0.02	1.16 ± 0.16	0.75 ± 0.10
Vz/F (L)	0.57 ± 0.20	4.5 ± 1.7	2.1 ± 0.6

Calculated pharmacokinetic parameters from the subcutaneous injection arms of the study on study Day 3 are as follows:

Parameter	Group 3 (2.3 mg/kg b.i.d.)	Group 4 (30 mg/kg b.i.d.)	Group 7 (20 mg/kg t.i.d.)
Cmax (µg/mL)	7.6 ± 0.4	27.4 ± 0.6	13.0 ± 0.5
t_{max} (h)	2.0 ± 0.0	2.0 ± 0.0	1.5 ± 0.7
$AUC_{(0-8h)}$ (µg-h/mL)	30.9 ± 6.1	110.1 ± 2.2	44.5 ± 16.3
$AUC_{(0-12h)}$ (µg-h/mL)	33.0 ± 6.1	138.4 ± 12.8	
$AUC_{(0-24h)}$ (µg-h/mL)	65.9 ± 12.2	276.8 ± 25.6	133.3 ± 49.0
t ½ (h)	2.0 ± 0.4	3.9 ± 1.8	
Range (h)	4 - 8	2 – 8	
CL/F (L/h/kg)	0.07 ± 0.01	0.22 ± 0.2	0.48 ± 0.18

Calculated pharmacokinetic parameters from the continuous subcutaneous infusion arms of the study on study Day 1 are as follows:

Parameter	Group 10 (1 mg/kg/day)	Group 11 (4 mg/kg/day)	Group 12 (13 mg/kg/day)
Cmax (µg/mL)	1.0 ± 0.1	3.6 ± 1.4	8.7 ± 0.3
t_{max} (h)	7.0 ± 1.4	5.0 ± 1.4	5.0 ± 1.4
$AUC_{(0-12h)}$ (µg-h/mL)	7.8 ± 0.7	26.3 ± 7.7	78.0 ± 2.9
Css, ave (µg/mL)	0.65 ± 0.06	2.2 ± 0.6	6.5 ± 0.2
CL/F (L/h/kg)	0.07 ± 0.01	0.08 ± 0.02	0.08 ± 0.00

Less than dose-proportional increases in mean $AUC_{(0-24)}$ and Cmax values were observed with rats receiving intermittent subcutaneous injections. Since Groups 4 and 7 received the same daily dose (60 mg/kg/day) similar $AUC_{(0-24)}$ values were expected, but not observed. The mean $AUC_{(0-24)}$ for Group 7 was less than 50% of the mean value for Group 4 on Day 3 and the trend is apparent even when anomalistic (low) plasma concentrations in Group 7 are disregarded. Additionally, the Group 3 mean AUC values were higher than what was expected from the other Group mean AUC values.

In the continuous subcutaneous infusion portion of the study, mean Cmax, Css,ave and $AUC_{(0-12)}$ values increased roughly in proportion to increased dose. Values of tmax were in the range of 5 to 7 hours, irrespective of dose, and CL/F values were relatively independent of dose. However, plasma samples collected at single timepoints 48 hours after initiation of infusion indicate that systemic levels of T-20 were lower than levels observed in the first 12 hours.

The sponsor did not speculate as to the reason for the decrease in the magnitude of some pharmacokinetic parameters with administered dose, frequency of dose, or time.

3. NDA 21-481 Reference 2002, Volume 4. IND [redacted] Serial 079.
Pharmacokinetic analyses from "Single dose intravenous administration of T-20 in rats."
[redacted] Study 6077#1 T-20. Final Report, July 31, 2000.

This non-GLP study was designed to determine the plasma pharmacokinetics of T-20 in albino Sprague-Dawley rats following administration of a single dose of T-20 by intravenous injection. The animal portion of the study was conducted in January 1995. Plasma samples and remaining test solution were sent to Trimeris, Inc. for analysis.

Five adult male albino Sprague-Dawley Crl:CD®(SD)BR rats were obtained from [redacted] for this study. Trimeris, Inc. prepared the solution of T-20 in [redacted] saline for [redacted] All five rats were administered 4 mg/kg intravenously in a dose volume of 0.8 mL/kg.

Blood was collected at predose, 5, 15, and 30 minutes, 1, 2, 4, 6, and 24 hours post dosing from an [redacted] Plasma was isolated from blood by centrifugation, and [redacted] extracts of plasma were collected for shipment to Trimeris for analysis. Trimeris measured T-20 in samples using an [redacted]) with a [redacted]

[redacted] also collected 24-hour urine samples from all animals during this study and sent them to Trimeris.

Results:
There are no clinical data provided in this report. There are also no data verifying the T-20 dosing solution concentrations and no urine data in this report.

T-20 exhibited biphasic pharmacokinetics in all animals following intravenous administration (4 mg/kg, single injection). Plasma T-20 levels were measurable at all timepoints through 6 hours, but not at 24 hours.

T-20 in plasma:

Parameter	Mean (N=5)	SD
T_{max} (hours)*	0.083	0.0
C_{max} (µg/mL)	40.3	4.3
$t_{1/2, terminal}$ (hours)	2.42	0.81
$AUC_{(0-6)}$ (µg-h/mL)	45.0	5.1
$AUC_{(0-\infty)}$ (µg-h/mL)	50.6	5.2

* Represents time of first blood collection.

Comments:
There are no unusual findings. The sponsor offered no explanation for the selection of the dose, 4 mg/kg.

4. NDA 21-481 Reference 2003, Volume 4. IND [redacted] Serial 085.
   Pharmacokinetic analyses from "Pharmacokinetics of T-20 in plasma and lymph following intravenous administration of a single dose in rats." [redacted] Study 6077T-20#2/ 6077T-20LY. Final Report, August 9, 2000.

This non-GLP study was designed to determine the plasma pharmacokinetics and lymphatic penetration of T-20 in albino Sprague-Dawley rats following administration of a single dose of T-20 by intravenous injection. The animal portion of the study was conducted in September 1995. Plasma and lymph samples and test solution aliquots were sent to Trimeris, Inc. for analysis.

Five adult male albino Sprague-Dawley Crl:CD®(SD)BR rats were obtained from [redacted] for this study. Trimeris, Inc. prepared the solution of T-20 in [redacted] saline for [redacted]. All five rats were administered 4 mg/kg intravenously in a dose volume of 0.8 mL/kg. Blood was collected at predose, 5, 15, and 30 minutes, 1, 2, 4, 6, and 8 hours post dosing from an [redacted] The "Statement of Work" states the volume of blood removed will be replaced with lightly heparinized plasma, although it does not state what plasma will be used for this replacement. Plasma was isolated by centrifugation and was flash frozen. Two days later, the rats were dosed again and lymph was collected at predosing and 1 hour intervals for up to 6 hours post dosing from an [redacted] Lymph was flash frozen. Trimeris measured T-20 in samples using an [redacted] with a [redacted]

Results:
There were no clinical data provided in this report. There were also no data verifying the T-20 dosing solution concentrations.

Trimeris states that T-20 exhibited biphasic pharmacokinetics following intravenous administration and that these results are comparable to those in the previous study [redacted] 6077#1 T-20).

T-20 in plasma:

Parameter	Present Study		Study 6077#1 T-20	
	Mean	SD	Mean	SD
T_{max} (hours)*	0.083	0.0	0.083	0.0
C_{max} (µg/mL)	63.4	4.9	40.3	4.3
$t_{1/2,\ terminal}$ (hours)	2.15	0.17	2.42	0.81
$AUC_{(0-\infty)}$ (µg-h/mL)	65.0	3.3	50.6	5.2

* Represents time of first blood collection.

The cannula of Rat No. 2 stopped flowing after the 2-hour collection and Rat No. 5 died before the end of the 4-5 hour interval. Therefore, the lymph calculations are based on data from three rats. Trimeris states that T-20 rapidly penetrated into the lymphatic system and equilibrated with the plasma reservoir of T-20 within approximately 1 hour after administration, and remained in equilibrium with plasma concentrations throughout the remainder of the study.

T-20 in lymph:

Parameter	Mean	SD
T_{max} (hours)*	0.5	0.0
C_{max} (µg/mL)	41.0	15.0
$t_{1/2, terminal}$ (hours)	1.4	0.3
$AUC_{(0-8h)}$ (µg-h/mL)	79.4	12.8
$AUC_{(0-\infty)}$ (µg-h/mL)	84.4	13.8

* Represents the midpoint of the first lymph collection interval (0 – 1 hour).

Reviewer comments:
The sponsor offered no explanation for the selection of the dose, 4 mg/kg.

Presumably, equilibrium of T-20 in lymph and plasma is deduced from the concentrations of T-20 in each fluid at similar time intervals in the separate experiments. The sponsor presents no calculations or graphs that directly combine or compare the data. By the concentration-time graphs (T-20 in lymph and plasma), it appears that equilibrium is not achieved at 1 hour as the sponsor states; concentrations of T-20 are considerably higher in lymph than in plasma. At two hours, lymph T-20 concentrations are still higher than plasma concentrations. Perhaps the equilibrium to which the sponsor refers is a broad, general "ballpark" agreement, vis-à-vis, the calculated pharmacokinetic parameters. However, it is not clear from the data that equilibrium is ever actually achieved.

5. NDA 21-481 Reference 2004, Volume 4. IND ——— Serial 284.
Ro 29-9800 (T20): A single dose subcutaneous pharmacokinetic study of two formulations in fed male rats. Roche Study 07399. Final Report RR 1004781, January 9, 2002.

This non-GLP study was conducted during May 2000 to compare the pharmacokinetics and microscopic injection site changes from two formulations of T-20. The sponsor is seeking a formulation with improved kinetic and toxicologic properties to reduce dosing frequency. The maximum subcutaneous injection volume for rats and the maximum achievable solution concentration of T-20 were selected for this study.

Nine male rats (——— HsdBrl ——— from ——— weight range 144 – 212 g) per group were administered a single subcutaneous injection of vehicle (1 mL) or T-20 at a dose of 50 mg/rat (1 mL). Groups A, B, C, and D were carbonate vehicle control, T-20 carbonate solution, suspension vehicle control, and T-20 suspension, respectively. The T-20 carbonate vehicle formulation is the current (March 2002) clinical formulation of T-20. The vehicle is 5.3 mg/mL sodium carbonate and 25 mg/mL mannitol in water, with pH adjusted to 9.0. The (experimental) suspension vehicle contains 10 mg/mL ——— and 1 mg/mL ——— in water.

Blood samples were collected from 3 rats/timepoint at 0.5, 1, 2, 4, 6, 8, and 24 hours post dosing. Plasma concentrations of Ro 29-9800 (T-20) and Ro 50-6343 (a T-20 metabolite) were determined by ——— Necropsies of 3 rats/group were conducted on Days 1, 3 and 6 post dosing. Skin tissue samples from the injection sites were prepared for histological examination.

Results:
The pharmacokinetics parameters for Ro 29-9800 and Ro 50-6343 are as follows:

Parameter	Units	Carbonate solution	Suspension
Ro 29-9800 (T-20)			
$AUC_{0\rightarrow 24\ h}$	µg-h/mL	36.2	54.3
AUC/dose	µg-h/mL/dose	0.7	1.1
C_{max}	µg/mL	8.1	3.5
t_{max}	Hours	2	8
Ro 50-6343			
$AUC_{0\rightarrow 24\ h}$	µg-h/mL	49.9	53.3
AUC/dose	µg-h/mL/dose	1.0	1.1
C_{max}	µg/mL	4.4	3.4
t_{max}	Hours	2	6

Subcutaneous administration of T-20 in the suspension formulation provided a greater exposure ($AUC_{0\rightarrow 24\ h}$) to T-20 than that provided by the current carbonate formulation. However, maximum plasma concentrations (C_{max}) of T-20 were approximately 2-fold lower and occurred 6 hours later with the suspension compared with the carbonate solution. These results suggest that the suspension improved drug exposure by providing a slower and more persistent absorption from the injection site. Both systemic exposures and peak concentrations of Ro 50-6343 were the same for both formulations.

Macroscopic injection site changes (subcutaneous edema, hemorrhage, nodules or accumulation of inoculum) were identified more often for the suspension groups (Groups C and D, 5/9, 8/9) than for the solution groups (Groups A and B, 0/9, 3/9).

Microscopic evaluation of injection sites treated with carbonate vehicle (Group A) revealed no effects attributed to the vehicle. Subcutaneous edema and infiltration of inflammatory cells were observed on Days 1 and 3 in the tissues of one or two animals where the T-20 carbonate solution (Group B) or the suspension vehicle (Group C) were administered, and on Days 1, 3 and 6 in the tissues of nearly all the animals where the T-20 suspension (Group D) was administered. Fibroplasia, ulcerative dermatitis or epidermal necrosis were observed in animals treated with either the T-20 suspension or suspension vehicle more than in the T-20 carbonate solution group. Subcutaneous accumulation of inoculum was observed in almost all animals treated with T-20 suspension. Microscopic changes in the subcutaneous skeletal muscle (coagulation necrosis, degeneration, atrophy) were observed in individual animals from Groups B, C, and D. Other changes (intramuscular edema, microvascular hemorrhage, vascular necrosis, epidermal necrosis, axonal degeneration with secondary demyelination) could not be clearly attributed to treatments. These changes suggest a greater potential for the T-20 suspension formulation to produce pathological changes compared with the current T-20 carbonate solution. Further, some of the changes were attributed to the suspension vehicle.

6. NDA 21-481 Reference 2005, Volume 4. IND —— Serial 284.
Ro 29-9800 (T-20 Fusion Inhibitor): A single dose subcutaneous or intravenous pharmacokinetic study in fed male rats. Roche Study 07431, Final Report RR 1006412, January 9, 2002.

This non-GLP study was conducted during August 2000 to assess whether the formation of the metabolite identified in the previous rat study (Roche Study 07399) is dependent on the route of administration. The metabolite (designated Ro 50-6343) is an acid hydrolysis product of T-20 (Ro 29-9800) resulting from deamidation of the C-terminal phenylalanine residue.

Twelve male rats (——— HsdBrl ——— from ——— VA; weight range 114 – 136 g) per group were administered T-20 ——— solution either subcutaneously or intravenously. Initially, both groups were to receive 50 mg of T-20 (approximately 400 mg/kg). But due to the deaths of the first two rats dosed intravenously, the intravenous dose was reduced to 4 mg/kg.

Blood samples were collected from 3 rats/timepoint at 10 minutes and 30 minutes and 1, 2, 4, 6, 8, and 24 hours post dosing from the rats in the intravenous group, and 0.5, 1, 2, 4, 6, 8, and 24 hours post dosing in the subcutaneous injection group. Plasma concentrations of Ro 29-9800 (T-20) and Ro 50-6343 were determined by ———.

Results:
The pharmacokinetics parameters for Ro 29-9800 and Ro 50-6343 are as follows:

Parameter	Units	Ro 29-9800 (T-20)	Ro 50-6343
Intravenous Dose			
$AUC_{0\rightarrow 24\,h}$	µg-h/mL	41	118
AUC/dose	(µg-h/mL/dose)/ (mg/kg)	10	30
C_{10min}	µg/mL	39	18
Cl	mL/h/kg	97	NA
$t_{1/2}$	hours	2	NA
V_{ss}	mL/kg	123	NA
Subcutaneous Dose			
$AUC_{0\rightarrow 24\,h}$	µg-h/mL	96	159
AUC/dose	µg-h/mL/dose	0.25	0.41
C_{max}	µg/mL	19.4	15.3
t_{max}	hours	1	4
F	%	2.4	NA

After subcutaneous injection of T-20, plasma concentrations reached maximum levels at 1 hour post dosing. The apparent bioavailability of T-20 after subcutaneous injection was 2.4%. The observed AUC of T-20 following subcutaneous treatment in this study is about 3-fold greater than that observed in the previous study, although the doses (mg/kg) were similar. The sponsor suggested that this might "reflect the early developmental phase of the sample processing techniques and bioanalytical method."

The metabolite Ro 50-6343 was detected in plasma following both routes of administration. The exposure (AUC) ratio of metabolite to parent drug was higher after intravenous injection than after subcutaneous injection (2.9 and 1.7, respectively), suggesting that the formation of the metabolite Ro 50-6343 is independent of the route of administration and does not occur solely in the subcutaneous space.

Single Dose PK – Non Rodent

7. NDA 21-481 Reference 2006, Volume 5. IND [redacted] Serial 000.
A pharmacokinetic study of T-20 peptide in cynomolgus monkeys. [redacted]
[redacted]. Study 20912. Final Report, October 31, 1995.

[redacted] conducted this non-GLP study for the sponsor during April and May 1995 to examine the pharmacokinetic profile of a single subcutaneous or intramuscular dose or multiple intravenous doses of T-20 in cynomolgus monkeys.

One male and one female cynomolgus monkey were administered T-20 intravenously (0.5 and 5.0 mg/kg), intramuscularly (4.1 mg/kg), and subcutaneously (6.7 mg/kg), with a minimum of 96 hours washout between doses. Blood samples were taken before dosing and up to 8 times (up to 24 hours) post dosing. Body weights were recorded on five days throughout the study; clinical observations were recorded daily. T-20 was measured in plasma samples using a [redacted] that includes a [redacted]

There were no deaths or abnormal clinical signs (including no rashes) reported over the two-week study period after administration of any of the doses. The male monkey gained 0.1 kg and the female monkey lost 0.2 kg. The female showed decreased activity during 13 of 20 days.

Plasma concentrations were similar in both the male and female monkey. IV AUC values were about 8.5 µg-h/mL at 0.5 mg/kg and 115 µg-h/mL at 5.0 mg/kg. The IV dose half-life was approximately 3 hours for both doses and peak plasma concentrations were recorded at about 3 hours for both doses.

Results for IM and SC dosing were similar to IV dosing, although with slightly longer half-lives. Bioavailability with SC dosing was greater relative to the IV AUC values (101% male, and 133% female), suggesting variance in the dosing solution concentrations or with the [redacted] assay precision. With IM dosing, bioavailabilty was 67.6% and 131% of the IV dose in the male and female, respectively. BDL suggested these differences were due to differences in absorption or elimination between the sexes or normal variation reflecting the use of only two animals.

8. NDA 21-481 Reference 2007, Volume 5. IND [redacted] Serial 000.
A pharmacokinetic study of T-20 peptide in three male cynomolgus monkeys.
[redacted] Study 31501. Final Report, May 1, 1996.

[redacted] conducted this non-GLP study for the sponsor during May and June 1995 to examine the pharmacokinetic profile of a single intravenous or intramuscular dose or multiple subcutaneous doses of T-20 in cynomolgus monkeys. This non-GLP study was conducted similarly to the previous study except three male monkeys were administered one dose IV (0.4 mg/kg), one dose IM (0.4 mg/kg), and three doses SC (0.2, 0.4, and 0.8 mg/kg) over a two-week period.

There were no deaths or abnormal clinical signs reported, including no rashes after administration of any of the doses. Fluctuations in body weights of 0.2 kg between measurements were common (one monkey gained 0.4 kg between days 1 and 3).

Plasma AUC increased linearly with the three SC doses. Peak plasma concentrations were recorded at approximately 2 hours after SC dosing and 1 hour after IM dosing. Plasma elimination half-lives were similar for all routes of administration (range 2.6 to 3.8 hours). Relative bioavailability of drug by the IM route was 81%. By the SC route, bioavailability values were 94%, 72% and 54% for the 0.2, 0.4, and 0.8 mg/kg doses, respectively.

9. NDA 21-481 Reference 2008, Volume 5. IND ——— Serial 047, 333.
A pharmacokinetic study of T-20 peptide in male and female cynomolgus monkeys. ——— Study 98-3646. Final Report, November 18, 1999, amended May 3, 2002. Trimeris report document EDS102.R01-00, March 11, 2002.

——— conducted this non-GLP study for the sponsor during March 1998 to examine the pharmacokinetics of T-20. Intravenous and subcutaneous injections of T-20 were administered to two male and two female cynomolgus monkeys at a dose of 0.8 mg/kg. The study was also designed to examine the rate of rise to steady state concentration and the steady state plasma concentration following a bolus loading dose of 0.8 mg/kg and a continuous subcutaneous infusion of T-20 over 24 hours at a dose level of 4.6 mg/kg/day. Finally, the study included changing the site of attachment of the device for continuous infusion to examine its effect on steady state plasma concentration.

——— amended the original study report to note that Trimeris did not analyze the dosing solutions as specified in the protocol. Trimeris conducted the analyses of plasma samples for this study, though not under GLP conditions.

The cynomolgus monkeys for this study came from ———

Study Design:
The same two male monkeys and two female monkeys were used for all five experiments. The study experiments were as follows:

Day	Route	Dose mg/kg	Conc. mg/mL	Vol. mL/kg	Rate mL/hour	Sampling times
1	IV bolus	0.8	0.8	1.0	-	Predose, <0.05, 0.5, 1, 2, 4, 6, 8, 12 h
4	SC bolus	0.8	1.6	0.5	-	Predose, 0.5, 1, 2, 4, 6, 8, 12 h
8	SC bolus + CSI (Site 1)	0.8 + 4.6/d infusion	0.8 loading	1.0 loading	0.084	Predose, 0.5, 1, 2, 4, 6, 8, 12 h
9	CSI (Site 2)	4.6/d	Adj.	-	0.084	Pre-Site change, 0.5, 1, 2, 8 h
9	CSI (Site 3)	4.6/d	Adj.	-	0.084	3 h

SC = subcutaneous; CSI = continuous subcutaneous infusion.
Adj. = concentration adjusted for each animal (mg/kg/d) for a constant infusion rate of 0.084 mL/h.

Animals were observed twice daily in their cages for mortality and signs of severe toxic or pharmacologic effects. Animals were removed from their cages and examined prior to initiating the study and 1 to 2 hours after test material administration. Examinations included general conditions, skin, fur, eyes, nose, abdomen, external genitalia, respiration, the cardiovascular system, and central and autonomic nervous system.

Animals were weighed prior to each experiment. Pretest blood was collected for hematology and clinical chemistry analysis. All pharmacokinetics blood samples were sent to the sponsor for analysis.

Results:
The experiments were apparently fraught with technical difficulties. During the subcutaneous infusion phase, interruptions in test material delivery were observed which involved displacement of the catheter and kinks in the tubing. Subsequently the infusion catheters were moved to a second site to partially compensate for these difficulties and infusion was continued for an additional 8 hours. Efforts were made to handle the monkeys less often, but these were only partially successful. The actual volumes of test material administered at Site 1 were 12% to 56% lower than those that were intended. Similar difficulties ensued at infusion Site 2 and the actual volumes delivered were approximately –4% to +8% of those intended.

After the discontinuation of subcutaneous infusion at Site 3 on Day 10, a firm mass was observed at the infusion site of one female and two males. The masses ranged in diameter from approximately 2 to 3 cm and a white or yellow exudate was present in two of the masses. Similar masses were not seen at Sites 1 and 2. The sponsor concluded that many irregularities and interruptions in dosing by continuous subcutaneous infusion made it doubtful that this method of delivery for T-20 would be practical.

There were no abnormal changes in body weights during this experiment. None of the animals' body weights varied more than 0.2 kg during the study.

Hematology and clinical chemistry values prior to the study were normal. Blood collection times varied from –2 to +7 minutes at a few timepoints. (Hematology and clinical chemistry was not performed in this study following dosing.)

Plasma pharmacokinetic parameters of T-20 in cynomolgus monkeys after a single 0.8 mg/kg intravenous or a single subcutaneous bolus dose.

Parameter	Intravenous bolus dose (0.8 mg/kg)	Subcutaneous bolus dose (0.8 mg/kg)
Cmax (µg/mL)	22.1 ± 1.7	3.9 ± 1.0
t_{max} (h)		1.8 ± 0.5
$AUC_{(0-12h)}$ (µg-h/mL)	34.8 ± 3.5	20.3 ± 3.6
$AUMC_{(0-12h)}$ (µg-h^2/mL)	93.0 ± 11.0	82.4 ± 15.3
t ½ (h)	3.0 ± 0.2	3.1 ± 0.3
Range (h)	4 - 12	6 – 12
$AUC_{(0-\infty)}$ (µg-h/mL)	36.6 ± 3.8	22.0 ± 3.8
$AUMC_{(0-\infty)}$ (µg-h^2/mL)	122.0 ± 18.8	111.0 ± 16.7
MRT (h)	3.3 ± 0.3	5.1 ± 0.2
CL/F (L/h)	0.06 ± 0.01	0.10 ± 0.02

Vss/F (L)	0.56 ± 0.08	
Vz/F (L)	0.72 ± 0.12	1.3 ± 0.2
Vc	0.10 ± 0.01	
%F		59.9 ± 6.3

N=4 for all calculations. AUMC = area under the first moment curve. MRT = mean residence time = $(AUMC_{(0-\infty)} / AUC_{(0-\infty)})$x 100%. Vss = steady state volume. Vc = central compartment = $Dose_{i.v.} / C(0)$, where C(0) is plasma concentration at t=0 h.

Individual plasma pharmacokinetic profiles were consistent among the 4 monkeys after a single intravenous bolus dose of T-20 (0.8 mg/kg). Plasma profiles were ——— with a short rapid decline lasting up to approximately 1 hour after dosing, followed by a longer terminal phase. Following a subcutaneous bolus dose, the mean half-life was similar to that observed after the intravenous dose (about 3 hours). Mean systemic bioavailability (%F) was approximately 60% for the 4 monkeys.

After a subcutaneous loading dose (0.8 mg/kg) followed by continuous subcutaneous infusion (CSI) at 4.6 mg/kg/day, mean peak plasma concentrations of approximately 4.7 µg/mL were observed at 2-4 hours after initial dosing. After 6 hours, mean steady-state plasma concentrations were below the expected target of ——— µg/mL. Without a loading dose, maximal concentrations would not have been achieved until 12 hours after the start of infusion. As the effect of the bolus dose diminished, the targeted plasma levels were not sustained.

10. NDA 21-481 Reference 2009, Volume 5. IND ——— Serial 079.
Pharmacokinetic analyses from "Bioequivalence testing of T-20 formulations following subcutaneous administration to cynomolgus monkeys." ———
EHAW-114. Final Report, June 6, 2000.

——— conducted this non-GLP study for Trimeris Inc. during December 1999 to assess the pharmacokinetics of two formulations of T-20 administered subcutaneously twice a day for four days to male cynomolgus monkeys.

Formulation 1 was a carbonate buffer formulation and formulation 2 was a Tris buffer formulation. The dosing schedule was as follows:

Session number	Group number	No. of animals	Treatment Administration			
			Formulation	Dose (mg/kg)	Dose Volume (mL/kg)	Dosing regimen
I	1	3	1 (carbonate buffer)	0.5	0.1	Days 1-4 q12h
	2	3		5.0	0.1	
II	1	3	2 (Tris buffer)	0.5	0.1	Days 15-18 q12h
	2	3		5.0	0.05	

The same six monkeys were used for both experiments ("sessions"). Blood was collected at predose, 0.25, 0.5, 1, 2, 4, 8, and 12 hours post dosing on Study Days 1, 4, 15, and 18. An additional blood sample was collected at 6 hours post dosing on Study Days 15 and 18. (However, the 6-hour blood collection on Day 18 was cancelled for Group 2 animals during the experiment.) Plasma was isolated by centrifugation and flash frozen for shipment to the sponsor.

Trimeris provided T-20 to ——— as a lyophilized powder. ——— provided dosing solution aliquots to Trimeris for analyses. Trimeris analyzed T-20 in plasma using an ——— and analyzed T-20 in dosing solutions using a ——— method ———

Results:
Dosing solutions were within 87.3% and 100.3% of nominal values. Animal weights were not provided.

C_{min}, C_{max}, and $AUC_{(0\text{-}12h)}$ varied in direct proportion to the dose levels for both formulations at all doses and times. Also, the pharmacokinetic curves and parameters determined for formulation 2 (Days 15 and 18) were comparable to those for formulation 1 (Days 1 and 4, respectively).

However, the Session 2 (formulation 2), Group 2 animals exhibited variable plasma concentrations over time on Study Day 18. This was the only group with a dose volume of 0.05 mL/kg and dose concentration of 100 mg/mL. Trimeris suggested that the concentration variability might be because these doses were less accurate and less precise than those delivered at 0.1 mL/kg. However, the plasma concentrations of Group 2 animals do not exhibit much variation on Day 15. Trimeris notes that the 6-hour time measurements were not taken for Group 2 animals on Day 18 and then states that it suspected "that the 6 hour samples were incorrectly labelled with respect to animal identity."

Group mean pharmacokinetic parameters, Study Day 1, Session I, Formulation 1:

Parameter	Dose Group	
	1 (0.5 mg/kg)	2 (5 mg/kg)
$t_{1/2,\ terminal}$ (hours)	3.25 ± 0.04	2.96 ± 0.52
t_{max} (hours)	2.00 ± 0.00	2.67 ± 1.15
C_{max} (µg/mL)	2.05 ± 0.34	15.8 ± 3.1
C_{min} (µg/mL)	0.275 ± 0.050	2.15 ± 0.42
$AUC_{(0\text{-}12h)}$ (µg-h/mL)	11.95 ± 1.93	100.7 ± 13.3

Group mean pharmacokinetic parameters, Study Day 4, Session I, Formulation 1:

Parameter	Dose Group	
	1 (0.5 mg/kg)	2 (5 mg/kg)
$t_{1/2,\ terminal}$ (hours)	3.15 ± 0.29	2.56 ± 0.27
t_{max} (hours)	1.67 ± 0.58	3.33 ± 1.15
C_{max} (µg/mL)	2.33 ± 0.43	20.3 ± 0.9
C_{min} (µg/mL)	0.341 ± 0.137	2.23 ± 0.50
$AUC_{(0\text{-}12h)}$ (µg-h/mL)	14.94 ± 3.16	127.5 ± 9.8

Group mean pharmacokinetic parameters, Study Day 15, Session II, Formulation 2:

Parameter	Dose Group	
	1 (0.5 mg/kg)	2 (5 mg/kg)
$t_{1/2,\ terminal}$ (hours)	3.32 ± 0.70	2.77 ± 0.29
t_{max} (hours)	2.67 ± 1.15	4.00 ± 0.00
C_{max} (µg/mL)	1.62 ± 0.19	14.5 ± 0.9
C_{min} (µg/mL)	0.294 ± 0.086	1.99 ± 0.31
$AUC_{(0\text{-}12h)}$ (µg-h/mL)	11.07 ± 1.55	89.43 ± 3.29

Group mean pharmacokinetic parameters, Study Day 18, Session II, Formulation 2:

Parameter	Dose Group	
	1 (0.5 mg/kg)	2 (5 mg/kg)
$t_{1/2, terminal}$ (hours)	3.06 ± 0.26	4.28 ± 1.31
t_{max} (hours)	2.00 ± 0.00	2.67 ± 1.15
C_{max} (µg/mL)	1.58 ± 0.01	13.98 ± 5.58
C_{min} (µg/mL)	0.260 ± 0.044	3.35 ± 2.28
$AUC_{(0-12h)}$ (µg-h/mL)	10.62 ± 0.57	102.5 ± 38.0

Generally, the concentration curves are similarly shaped in all experimental dose groups. With the data presented, it is not possible to determine the cause of the relatively large variation in plasma concentrations for the formulation 2, high dose group on Day 18. The graph of those data for the three animals does not particularly support Trimeris's contention that doses were mislabeled, because one animal's plasma concentrations were considerably lower than the other two for all data points from 2 to 12 hours, not just at the 6-hour timepoint. Thus there appears to be a real difference in distribution, metabolism, or elimination that is occurring with the Tris buffer formulation of T-20 at this route and dose compared with the carbonate buffer formulation, although the significance of that difference is indeterminate.

11. NDA 21-481 Reference 2010, Volume 5. IND ——— Serial 079.
Pharmacokinetic analyses from "Bioequivalence testing of T-20 formulations following subcutaneous administration to cynomolgus monkeys." ———. Study EHAW-116. Final Report, July 31, 2000.

———. conducted this non-GLP study for Trimeris Inc. during January and February 2000 to assess the pharmacokinetics of six different formulations of T-20 administered as single or repeated subcutaneous injections to male cynomolgus monkeys. This report includes the pharmacokinetic analysis of formulations 1, 2, and 3 only.

T-20 formulation 1 (carbonate buffer, 50 mg/vial) was given as a divided dose (two sites) to each of three monkeys, one time. T-20 formulation 2 (Tris buffer, 100 mg/vial) and formulation 3 (carbonate buffer, 100 mg/vial) were each given to 3 monkeys twice a day for four days at either 0.5 or 5.0 mg/mL. All doses were delivered at a dose volume of 0.1 mL/kg. The dosing schedule was as follows:

Session number	Group number	No. of animals	Treatment Administration			
			Formulation	Dose (mg/kg)	Dose Volume (mL/kg)	Dosing regimen
I	1	3	2 (Tris buffer, 100 mg/vial)	0.5	0.050	Days 1-4 q12h
	2	3		5.0	0.050	
	3	3	3 (carbonate buffer, 100 mg/vial)	0.5	0.050	
	4	3		5.0	0.050	
II	1	3	1 (carbonate buffer, 50 mg/vial)	5.0	0.050 (2 sites)	Day 9, once

Session I, Group 1 animals were used on Session II after a four-day washout period. Blood was collected at predose, 0.25, 0.5, 1, 2, 4, 6, 8, and 12 hours post dosing on Study Days 1, 4, and 9. Plasma was isolated by centrifugation and flash frozen for shipment to the sponsor.

Trimeris provided T-20 to ——— as a lyophilized powder. ——— provided dosing solution aliquots to Trimeris for analysis. Trimeris analyzed T-20 in plasma using an ——— and analyzed T-20 in dosing solutions using a ———method that ———

Results:
Dosing solutions were within 76% and 102% of the nominal values. Animal weights were not provided.

On Day 1 of Session I experiments (formulations 2 and 3), C_{min}, C_{max}, and $AUC_{(0\text{-}12h)}$ varied in direct proportion to the dose levels. Trimeris states that formulation 2 pharmacokinetic parameters are consistent with those observed in Study EHAW-114 and formulation 3 pharmacokinetic parameters are "similar to those observed with the 50 mg ——— formulation in earlier studies in cynomolgus monkeys."

Group mean pharmacokinetic parameters, Session I, formulation 2 (100 mg Tris):

Parameter	Study Day 1 Dose Group		Study Day 4 Dose Group	
	1 (0.5 mg/kg)	2 (5 mg/kg)	1 (0.5 mg/kg)	2 (5 mg/kg)
$t_{1/2,\ terminal}$ (hours)	4.12 ± 0.72	3.73 ± 0.22	3.81± 0.60	4.82 ± 0.84
t_{max} (hours)	2.67 ± 1.15	5.33 ± 1.15	3.33 ± 1.15	2.00 ± 1.73
C_{max} (µg/mL)	1.17 ± 0.25	10.7 ± 1.8	2.28 ± 0.61	7.56 ± 4.54
C_{min} (µg/mL)	0.220 ± 0.006	0.637 ± 0.230	0.437 ± 0.048	1.56 ± 0.61
$AUC_{(0\text{-}12h)}$ (µg-h/mL)	8.95 ± 0.85	74.6 ± 14.9	16.2 ± 2.67	53.9 ± 34.9

On Day 4 of Session I, formulation 2 (Tris buffer) animals in the low T-20 dose (0.5 mg/kg) exhibited similar plasma concentrations and kinetic profiles to one another, similar to those on Day 1, and similar to those observed on Study EHAW-114. The formulation 2 high dose (5.0 mg/kg) animals, however, exhibited plasma concentrations that are more variable from animal to animal and significantly lower on Day 4 than on Day 1. In the high dose group, Day 4 plasma concentrations are predictive of the dose level in only one of three monkeys. This variability in the high dose Tris formulation on Day 4 was seen in Study EHAW-114, but in that study the group mean pharmacokinetics were similar to those seen following the Day 1 dose, and they are not similar in this study. Trimeris states that one animal in the high dose group received a dose on Day 4 that was approximately 68% of the target dose and the animal displayed plasma concentrations that were lower than expected. However, this discrepancy does not fully account for the variability observed because two animals exhibited similar low plasma concentrations. Trimeris confirms that the stability of the Tris formulation is not a concern and that the dosing volume does not appear to be in error. Trimeris suggests that the administration of drug (e.g., inconsistent needle insertion or injection at a non-uniform depth) "should not be excluded as a plausible explanation for the uncharacteristic pharmacokinetics observed for group 2 animals on Study Day 4."

Group mean pharmacokinetic parameters, Session I, formulation 3 (100 mg Carbonate):

Parameter	Study Day 1 Dose Group		Study Day 4 Dose Group	
	3 (0.5 mg/kg)	4 (5 mg/kg)	3 (0.5 mg/kg)	4 (5 mg/kg)
$t_{1/2,\ terminal}$ (hours)	2.83 ± 0.18	3.42 ± 0.18	2.99 ± 0.24	3.23 ± 0.44
t_{max} (hours)	2.00 ± 0.00	4.00 ± 0.00	2.00 ± 0.00	4.00 ± 0.00
C_{max} (µg/mL)	2.00 ± 0.60	17.1 ± 3.8	2.03 ± 0.74	16.6 ± 4.4
C_{min} (µg/mL)	0.211 ± 0.106	1.17 ± 0.42	0.240 ± 0.043	2.47 ± 0.92
$AUC_{(0\text{-}12h)}$ (µg-h/mL)	11.5 ± 2.8	115 ± 22	12.5 ± 4.1	110 ± 20

Study Day 4, formulation 3 pharmacokinetic parameters and curve profiles for both dose groups are comparable to those seen on Study Day 1. Both groups show a dose response on both days and these results are similar to those observed with the 50 mg formulation in Study EHAW-114.

Session II, Study Day 9 results are similar to the Session I, formulation 3 results and the Study EHAW-114 results.

Group mean pharmacokinetic parameters, Session II, formulation 1 (50 mg Carbonate):

Parameter	Study Day 9 Dose Group 1 (5 mg/kg)
$t_{1/2,\ terminal}$ (hours)	3.35 ± 0.69
t_{max} (hours)	4.00 ± 0.00
C_{max} (µg/mL)	20.5 ± 3.9
C_{min} (µg/mL)	2.02 ± 0.65
$AUC_{(0\text{-}12h)}$ (µg-h/mL)	133 ± 20

Trimeris concludes that the T-20 carbonate formulations display reproducible dose response pharmacokinetics and are consistent with earlier results. The T-20 Tris buffer formulation pharmacokinetics are similar to the carbonate formulation and exhibit dose dependence for both dose levels on Day 1 and for the low dose (0.5 mg/kg) on Day 4. However, the Tris formulation high dose (5.0 mg/kg) Day 4 plasma concentrations are lower than those on Day 1 (exhibit much faster extinction than on Day 1) and exhibit higher variability among the three monkeys than any of the other plasma time curves in this study. These Day 4 results with the Tris formulation, 5 mg/kg, are similar to those seen in Study EHAW-114. Trimeris suggests these observations may be due to dosing artifacts.

Trimeris concludes that the 100 mg Tris and 100 mg carbonate formulations are equivalent and these results warrant further evaluation of the two T-20 formulations in clinical studies without modification of the dose. This reviewer disagrees that they are equivalent. The contention that the T-20 plasma concentration variances are due to dosing artifacts is not supported because the effects are seen in at least 4 of 6 monkeys across two studies with one formulation (Tris) at one dose and not in any other group in either study (21 other monkeys). Until further information is provided, the Division considers that the pharmacokinetics of the T-20 Tris buffer formulation are not equivalent to those of the carbonate buffer formulation.

In early 2000, Trimeris began a 48-week clinical study (T20-208) titled, "Tolerance and pharmacokinetics of carbonate and Tris buffer high strength (100 mg/mL) formulations of T-20 compared to the current carbonate formulation (50 mg/mL), each at doses of 100 mg bid and 75 mg bid in treatment experienced HIV infected patients." Thirty-six patients total were to be

enrolled on two 100 mg/mL carbonate buffer formulation cohorts and 24 patients total were to be enrolled on two 100 mg/mL Tris buffer formulation cohorts. In February 2001, Trimeris informed the Division that the study directors had decided to move into the Phase III portion of the study. They extended the study duration an additional 48 weeks and switched all patients (on or before the end of the initial 48 weeks) to the 100 mg/mL carbonate buffer formulation. Only one of the two Tris buffer cohorts had been enrolled (12 patients) and administration of the Tris formulation was being discontinued. In their justification, the study directors reference positive results from another clinical study (T20-206) of the T-20 100 mg/mL carbonate buffer formulation but they do not mention the Tris buffer formulation. (IND —— Series 126, Letter Date February 12, 2001.)

Red Cell Partitioning and Protein Binding

12. NDA 21-481 Reference 2200, Volume 6. IND —— Serial 084.
Plasma protein binding and blood distribution studies with ^{3}H-T-20. ——
——/03. Final Report, August 22, 2000.

—— conducted this non-GLP study during March 2000 for Trimeris, Inc.

Human plasma and ^{3}H-T-20 were mixed (100, 50, 10, 1, 0.5, 0.05 µg ^{3}H-T-20/g plasma) and the extent of binding of ^{3}H-T-20 to plasma protein was determined by ——
——; followed by —— The extent of protein binding of ^{3}H-T-20 to human serum albumin (0.58 mM) and α-1 acid glycoprotein (0.016 mM) was determined at concentrations of 10 and 100 µg ^{3}H-T-20/g by the same procedure.

The potential for displacement of protein-bound T-20 by co-administered compounds was investigated by adding saquinavir, nelfinavir, efavirenz, and —— at concentrations of 10 µg/g to human plasma containing 10 µg/g of ^{3}H-T-20. Samples (n = 4) of each solution were centrifuged and radioactivity levels in the ultrafiltrate were compared with controls (n = 4) that contained only ^{3}H-T-20.

Whole blood from two human volunteers was collected and aliquots were incubated with 1, 10, or 100 µg ^{3}H-T-20/g for 30 minutes at 37 °C. Radioactivity of aliquots of the whole blood and centrifuged plasma were analyzed and compared.

The centrifugation system was initially tested for suitability using —— plasma to determine the extent of nonspecific binding of T-20 to the filter unit and membrane.

Results:
—— states that the recovery of radioactivity from the —— during the system suitability testing was about —— and that the data were consistent (replicate samples, n = 5). Those data were not provided. The amounts of T-20 bound to plasma protein, human serum albumin, α-1 acid glycoprotein and drugs, and the amounts of T-20 associated with whole blood cells are presented below:

Amount of T-20 that is protein bound.

	Nominal T-20 concentration (µg/g)	Actual T-20 concentration (µg/g)	% Bound ± SD
Plasma			99.1 ± 0.0
Plasma			98.3 ± 0.1
Plasma			97.5 ± 1.8
Plasma			98.0 ± 1.5
Plasma			97.3 ± 3.9
Plasma			ND
Human serum albumin			94.8 ± 0.1
Human serum albumin			95.5 ± 0.7
α-1 acid glycoprotein			36.6 ± 1.4
α-1 acid glycoprotein			59.2 ± 0.5

ND – Not determined, radioactivity below the limits of detection.

Effect of four drugs on the plasma protein binding of T-20

Drug (Nominal concentration = 10 µg/g)	T-20 plasma concentration (µg/g)	% Bound to plasma protein ± SD
None		98.2 ± 0.4
Saquinavir		97.9 ± 0.7
Nelfinavir		98.0 ± 0.3
Efavirenz		98.6 ± 0.3
Nevirapine		98.5 ± 0.4

Concentrations of T-20 in blood cells and plasma following incubation of ^{3}H-T-20 at 3 concentrations in whole blood at approximately 37 °C for 30 minutes.

Donor	Nominal T-20 Concentration of T-20 (µg/g)	Replicate number	T-20 Concentration (µg/g) in Blood Cells	T-20 Concentration (µg/g) in Whole Blood	% T-20 in Blood Associated with Blood Cells
A*					
B*					

* The hematocrit values of donors A and B were 44.4% and 47.8%, respectively.

The extent of T-20 binding to plasma protein was independent of T-20 concentration (97 – 99% bound) across a 2000-fold concentration [redacted] µg T-20/g plasma). Similar results were obtained with [redacted] solution (T-20 approximately 95% bound). Binding to α-1 acid glycoprotein was lower (37% bound at 100 µg/g and 60 % bound at 10 µg/g). Coadministered drugs (saquinavir, nelfinavir, efavirenz, and nevirapine) had no obvious effect on binding of T-20 to plasma protein.

[redacted] states that there was no "unusual" binding of T-20 in whole blood "other than that expected for general distribution within the blood components."

[redacted] concludes that the main plasma protein that is binding T-20 in vivo is albumin. This study supports that conclusion.

13. NDA 21-481 Reference 2201, Volume 6. IND [redacted] Serial 286.
Ro 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. [redacted] Study D01034. Roche Final Report, RR 1007511, February 15, 2002.

[redacted] conducted this non-GLP study for the sponsor during 2001 to compare the in vitro binding of T-20 to human plasma proteins from normal healthy volunteers and from persons with HIV infection. The potential plasma protein displacement of T-20 by concomitant drugs (warfarin, midazolam, [redacted] amprenavir, lopinavir, and efavirenz) and the potential plasma protein displacement of the same concomitant drugs by T-20 were also studied in plasma from normal healthy volunteers. Both studies utilized a [redacted] instrument to measure time to equilibrium. Protein binding was determined by counting radio-labeled T-20 or radio-labeled other-drug on each side (buffer and plasma sides) of the equilibrium membrane. Samples were analyzed in triplicate.

Results:
Time to equilibrium (<10% change between successive measurements) was 20 hours for Ro 29-9800 (T-20) and 4 hours for the other drugs. Ro 29-9800 (T-20) at a concentration of 2 µg/mL in plasma was found to be stable for up to 24 hours at 37°C.

Protein binding of ^{3}H-Ro 29-9800 (T-20) in healthy and HIV+ human plasma

Drug	Concentration	Donor side	% Bound	% Free
^{3}H-T-20	[redacted]	Healthy plasma	95.3	4.7
^{3}H-T-20		HIV plasma	92.5	7.5
^{3}H-T-20		HIV plasma	92.0	8.0
^{3}H-T-20		HIV plasma	92.1	7.9

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Displacement of co-administered drugs on ^{3}H-Ro 29-9800 (T-20) protein binding.

T-20 concentration	Test drug	Concentration	% Bound	% Free
	None		95.3	4.7
	Warfarin		95.1	4.9
	Warfarin		95.1	4.9
	Midazolam		95.1	4.9
	Midazolam		94.9	5.1
	Itraconazole		94.3	5.7
	Itraconazole		94.1	5.9
	Lopinavir		95.0	5.0
	Lopinavir		94.8	5.2
	Amprenavir		94.0	6.0
	Amprenavir		93.2	6.8

Note: Lopinavir was used in combination with ritonavir (i.e., Kaletra) because a commercial source for lopinavir alone was not located.

Displacement of Ro 29-9800 (T-20) on protein binding of co-administered drugs.

Test drug	Concentration	T-20 concentration	% Bound	% Free
^{3}H-Warfarin			98.7	1.3
^{3}H-Warfarin			98.8	1.2
^{3}H-Warfarin			98.5	1.5
^{3}H-Warfarin			98.8	1.2
^{3}H-Midazolam			88.5	11.5
^{3}H-Midazolam			88.8	11.2
^{3}H-Midazolam			89.8	10.2
^{3}H-Midazolam			89.5	10.5
^{3}H-Amprenavir			91.3	8.7
^{3}H-Amprenavir			91.3	8.7
^{3}H-Amprenavir			91.7	8.3
^{3}H-Amprenavir			91.0	9.0
14C-Efavirenz			99.2	0.8
14C-Efavirenz			99.2	0.8
14C-Efavirenz			99.2	0.8
14C-Efavirenz			99.2	0.8

Plasma protein from healthy volunteers bound 3% to 4% more T-20 than plasma protein from HIV-infected persons. The largest effect on T-20 binding to healthy donor plasma protein when tested with five other drugs was a decrease of less than 3% (with 16 µg/mL of amprenavir). The largest effect of T-20 on the binding of four other drugs to plasma protein was an increase in binding of midazolam (5 µg/mL) by less than 2% (with 5 µg/mL of T-20). None of the changes were statistically significant.

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Metabolic Studies In Vivo

14. NDA 21-481 Reference 2202, Volume 6. IND [redacted] Serial 080.
An in vivo study of enzyme induction by T-20. [redacted] Study Rt99-TRI1, [redacted] Final Report, March 24, 2000. Trimeris, Inc. signed April 6, 2000.

[redacted] conducted this study for Trimeris, Inc. to determine whether T-20 induces hepatic metabolic enzymes in rats following administration by subcutaneous injection twice daily for 12 days. The Sprague-Dawley Crl:CD®(SD)BR male rats used in this study were purchased from [redacted] All animals were approximately 52 days old at the time of dosing and weighed 237-249 g. The sponsor provided the T-20 and control solutions to [redacted]

The in-life portion of this study was conducted under FDA Good Laboratory Practices regulations (21 CFR Part 58). Portions of the study not conducted under GLPs were the excision of livers, preparation of microsomes, and assays of enzymatic activity. Also, formulation of T-20 was by the sponsor was not conducted under GLPs. There is a signed quality assurance statement filed with the [redacted] s study report.

Four rats were administered T-20 subcutaneous injections at doses of approximately 15 mg/kg/dose (range 11.1-17.8 mg/kg, mean 14.4 ± 0.9 mg/kg) for 12 days, twice a day. Four more rats were administered vehicle control ([redacted] on the same schedule. Four more rats were administered [redacted] for 4 days, once a day, as a positive control. Subcutaneous dosing was approximately 0.4 mL/kg.

On the day following the final doses, the rats were weighed and killed. Their livers were immediately removed, weighed, placed in ice-cold [redacted] buffer (pH 7.4) containing 0.15 M KCl. The buffer rinse was used to remove excess blood. The livers were then homogenized and centrifuged. Supernatant was removed and centrifuged to pellet the microsomes. The microsomes were removed, resuspended, centrifuged again, and resuspended at a concentration of 17-28 mg protein/mL for analysis.

The assays were as follows:

[redacted]

Results:
There were no statistically significant differences between mean values of liver weights, liver weights as percent of body weights, cytochrome P450, cytochrome b_5, CYP1A2 activity, CYP3A1/2 activity, or CYP2B1/2 activity in the T-20-dosed rats compared with the control vehicle-dosed rats. The phenobarbital-dosed rats had mean values of all parameters except cytochrome b_5 that were statistically significantly higher than the controls. [redacted] indicated that the positive control results demonstrated that the rats were not refractory to P450 enzyme induction.

Trimeris indicated to [redacted] that dosing the rats with approximately 15 mg/kg T-20 twice a day for 12 days results in C_{max} values that are approximately 1.4 µM—the same approximate concentration found in humans receiving the highest clinical dose (100 mg, twice a day).

states that the results of this study suggest there is no potential for T-20 to induce oxidative metabolism in mammals following chronic administration and that this finding might represent an advantage over protease inhibitor drugs which have a marked effect on P450 enzymes.

Metabolic Studies In Vitro

15. NDA 21-481 Reference 2203, Volume 6. IND Serial 080.
Assessment of the inhibition of the activities of human hepatic microsomal cytochrome P450 isozymes by T-20 in vitro. Report 64C-07482-006, April 11, 2000, memorandum of typographical error, August 3, 2002. Trimeris, Inc. signed the original report on April 6, 2000.

conducted this study for Trimeris, Inc. For this study, a pooled sample of human liver microsomes was obtained from the . Aliquots of the microsomes were incubated with T-20 at concentrations of 10 µM or 100 µM. Additional reagents varied with individual assays. Control preparations contained (T-20 product vehicle). All preparations were conducted in triplicate. , provided the T-20 solutions to Vehicle was supplied by the sponsor.

Assays were as follows:

1. [redacted]
2.
3.
4.
5.
6.
7.
8.
9.
10.

Results:
At the µM T-20 concentration the mean activity of CYP2C19 was statistically significantly lower (-21%) than the mean control activity, and at the 10 µM T-20 concentration the mean activity of CYP2D6 was statistically significantly higher (+59%) than the mean control activity. All the other (18 of 20) mean T-20 assay activities were similar to control means. The increase in mean CYP2D6 activity compared with controls was not observed at the higher T-20 concentration.

states that the data indicate there is little potential for T-20 to inhibit the cytochrome P450-mediated metabolism of drugs with which it is coadministered. notes that human subjects receiving the highest dose of T-20 (100 mg/kg twice daily) achieve a plasma T-20 C_{max} of approximately 1 µM or less, which is 10- and 100-times lower than the T-20 concentrations employed in this study.

16. NDA 21-481 Reference 2204, Volume 6. IND [redacted] Serial 144.
Ro 29-9800: Identification of a metabolite of T-20 found in rat plasma and (rat and human) liver microsomal incubations. Roche Final Report RR 1003497, January 30, 2001.

An unknown [redacted] peak (LC retention time approximately 3.8 minutes) was observed in rat plasma samples from rats administered T-20, and a similar peak was observed after in vitro incubation of T-20 with rat or human liver microsomes. The peak area profile followed the pharmacokinetic (concentration vs. time) curve for T-20. Roche hypothesized that the peak resulted from deamination of the C-terminus of T-20.

Roche chemists synthesized the proposed metabolite (designated Ro 50-6343) and studied it in controlled experiments with rat and human liver microsomes using two different types of [redacted] Also, treated and control rat plasma from a previous study (Roche Study 07431) was [redacted] with Ro 29-9800 and Ro 50-6343 and analyzed with [redacted]

The MS fragmentation pattern of Ro 50-6343 matched the pattern of the metabolite and the [redacted] rat plasma analyses resulted in a single superimposed peak for the microsomal metabolite and [redacted] Ro 50-6343, thus confirming the identity of the metabolite as Ro 50-6343. Ro 50-6343 is an amide hydrolysis product of the C-terminus phenylalanine of T-20.

17. NDA 21-481 Reference 2205, Volume 6. IND [redacted] Serial 286.
Ro 29-9800: In vitro metabolism of [^{3}H] T-20 by rat, monkey, and human hepatocytes. [redacted] Study 6131-321, Final Report September 4, 2001. Roche Final Report RR 1006292, February 13, 2002.

[redacted] conducted this non-GLP study for the sponsor during 2000 and 2001.

Primary rat and monkey hepatocytes ([redacted] and primary human hepatocytes from three donors ([redacted] were incubated at 10^6 hepatocytes/mL with 1 or 10 µM ^{3}H-T-20 for 0, 30, 60, 120, and 240 minutes to evaluate metabolism. T-20 and metabolites were analyzed using HPLC and [redacted]

Results:
The apparent rates of metabolism as measured by the disappearance of T-20 are presented in the following table:

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Species	Initial T-20 concentration (μM)	Disappearance of T-20 (pmol/L x 10^6 hepatocytes/min)
Rat	1	27
	10	124
Monkey	1	11
	10	25
Human 1	1	18
	10	111
Human 2	1	23
	10	143
Human 3	1	19
	10	113

In the rat hepatocytes, T-20 (1 μM) was essentially completely metabolized at 30 minutes. In the monkey and human hepatocytes, T-20 at 1 μM was nearly completely metabolized at 4 hours.

At 10 μM, T-20 was essentially all metabolized by the rat hepatocytes at 4 hours, but not all the T-20 was metabolized by the human or monkey hepatocytes at 4 hours. The hepatocellular (culture) rate of T-20 metabolism in the monkey was relatively slower than that in humans. In humans, the rates of metabolism were initially rapid, then decreased after 30 minutes (both T-20 concentrations) and then were nearly linear to 120 minutes

———— from 60-minute incubation samples revealed one primary, highly polar metabolite (M-1) in the rat and monkey cultures. The same metabolite was predominant (— to — at 240 minutes) in humans but additional metabolites, including a band eluting at about 33 minutes (peak M-3, less polar than T-20), were noted in the human samples. ——— could not rule out the presence of M-3 metabolite(s) in the rat and monkey ———— due to differences in the sensitivity between the human and animal assays. M-3 is apparently converted to M-1 over time. ——— compared the M-3 band with a ———— of Ro 50-6343 and concluded that Ro 50-6343 was the metabolite producing the M-3 band. However, ——— did not attempt to identify the structures of the metabolites. Another, smaller, polar peak (M-2) was seen in the human———— and is barely present in the monkey ————

Note: Two of the human hepatocyte donors were significant drinkers, all three were significant tobacco smokers, and all three took medications regularly. Neither ——— nor the sponsor addressed the significance of the human donor histories.

Distribution

18. NDA 21-481 Reference 2300, Volume 7. IND——— Serial 084.
Preliminary metabolism, distribution and pharmaceutical study of ^{3}H-T-20 following intravenous administration to Sprague-Dawley rats. ———— Study —/01. Final Report, June 27, 2000.

———— conducted this study during January and February of 2000. This study was not conducted under FDA Good Laboratory Practices (21CFR Part 58), although it was conducted under the UK Good Laboratory Practice Regulations 1999,

OECD Principles of Good Laboratory Practice (Paris, 1998), and the EC Commission Directive 1999/11/EC of March 1999.

Twelve albino male Sprague-Dawley Crl:CD®BR rats were used for this study. Each rat was administered a single bolus intravenous (target) dose of 4 mg/kg ^{3}H-T-20 via the dorsal vein of the penis. The radioactivity dosed to each animal was determined by weight difference of the syringe before and after administration.

Five of the rats were bled (approximately 300 µL) from the tip of the tail at post-dose times of 5, 15, 30 minutes and 1, 2, 4, 8, and 24 hours. Blood was centrifuged and plasma was analyzed by [redacted] Residual plasma was retained for possible HPLC analysis. At 24 hours, the animals were sacrificed and the carcasses were retained for possible future analysis.

Three additional rats were exsanguinated via the dorsal aorta by cardiac puncture at 2, 4, or 8 hours post dosing. Plasma samples from these animals were analyzed by [redacted] and HPLC.

Four additional rats were dosed and the urine and feces were collected for analysis of radioactivity. These rats were housed in glass "metabolism" cages that could be carefully rinsed. Collection times for urine were pre-dose, 0 – 6 hours, 6- 24 hours, and 24-48 hours. Collection times for feces were predose, 0 – 24 hours and 24 – 48 hours. Liver, lungs, kidneys, skeletal muscle and penis from two of these rats were obtained for radioactivity analysis at 48 hours and plasma and carcasses were retained from all animals for possible future analysis.

Results:
Mean ^{3}H-T-20 administered (n = 12) was 4.284 ± 0.050 mg/kg, and 13.70 ± 0.16 MBq/kg.

For the first 5 rats, C_{max} was 49 ± 6 µg/g and T_{max} was the first collection time (0.083 hours). $T_{1/2}$ using the 4, 8, and 24-hour data (n = 5) was 10.9 ± 0.9 hours; or, along with the next three rats and using the 2, 4, and 8-hour data (n = 8) $T_{1/2}$ was 4.3 ± 0.3 hours.

A corrected $T_{1/2}$ was calculated by scaling the $T_{1/2}$ obtained from [redacted] by a percent of integrated area attributed to the parent compound, determined by HPLC in the second group of three rats. This correction is appropriate because the HPLC traces show a very significant second peak whose height exceeds the parent peak at the 4 hours measurement. At 8 hours the second peak is greater than 3 times the height of the parent peak. The corrected plasma concentrations (three data times) are more similar to the values obtained in previous rat studies by [redacted] (and provided to [redacted] by Trimeris) than the plasma concentrations obtained by [redacted] uncorrected.

In the last four rats, the mean recovery of radioactivity, expressed as a percent of the dose administered is as follows:

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Radioactivity in Rat Tissues as Percent of Dose Administered.

Tissue	Collection Time	Percent of Dose Administered
Urine	Predose	
	0 – 6 h	
	6 – 24 h	
	24 – 48 h	
	Total	
Feces	Predose	
	0 – 24 h	
	24 – 48 h	
	Total	
Cage wash (water)	48 h	
Cage wash (methanol)	48 h	
Total excreted	48 h	
Liver	48 h	
Lung	48 h	
Kidney	48 h	
Penis*	48 h	
Muscle**	48 h	
Total	48 h	

*Penis is the injection site. **Muscle values were calculated on the assumption that skeletal muscle accounts for 45.5% of body weight. Limits of detection not specified.

Reviewer comments:
When the $T_{1/2}$ of T-20 obtained from the ——— is corrected by subtracting the radioactivity attributed to the metabolite, the result is consistent to the $T_{1/2}$ obtained by——— ———states that this agreement suggests that the T-20 metabolite was not detected by the ——— Then ——— states that since the ——— is considered to be specific for pharmacologically active T-20, the T-20 metabolite is presumed to be pharmacologically inactive. This presumption may or may not be correct and neither the sponsor nor the ——has provided data to confirm it.

Recovery of radioactivity from urine, feces and cage wash (n = 4) at 48 hours was approximately ——. When liver, lung, kidney, penis (injection site), and muscle (calculated assuming skeletal muscle accounts for 45.5% of body weight) are included (n = 2), recovery goes up to —— The discrepancy from —— recovery includes radioactivity in organ tissues not assayed and expired air (not collected).

19. NDA 21-481 Reference 2301, Volume 7. IND —— Serial 084, 286.
Metabolism and distribution study of ^{3}H-T-20 following intravenous administration to Sprague-Dawley rats:——— Study —/02. Final Report, June 29, 2000. Amendment 1, March 1, 2001.

——— conducted this study during March of 2000. This study was not conducted under FDA Good Laboratory Practices (21CFR Part 58), although it was conducted under the UK Good Laboratory Practice Regulations 1999, OECD Principles of

Good Laboratory Practice (Paris, 1998), and the EC Commission Directive 1999/11/EC of March 1999. The original final report was amended to effect the following changes: (1) change in study title, (2) clarification of how many animals' excreta were collected, (3) correction of the reason for using the intravenous route of administration on this study, and (4) indication that retained animal carcasses were analyzed and data were presented in another study report (——/04).

Four male and four female albino Sprague-Dawley Crl:CD®BR rats were used for this study. Each rat was administered a single bolus intravenous (target) dose of 4 mg/kg ^{3}H-T-20 via a lateral tail vein. The radioactivity dosed to each animal was determined by weight difference of the syringe before and after administration. The rats were then immediately returned to their cages (metabolic, glass) for the duration of the study.

Urine was collected in a container cooled in solid carbon dioxide according to the following schedule: predose, 0 – 6, 6 – 24, 24 – 48, 48 – 72, 72 – 96, 96 – 120, 120 – 144, 144 – 168 hours.

Feces and expired air (trapped by duplicate serial water traps) were collected from 2 males and 2 female rats only, according to the following schedule: predose, 0 – 24, 24 – 48, 48 – 72, 72 – 96, 96 – 120, 120 – 144, 144 – 168 hours.

At 168 hours all rats were killed by overdose of anaesthetic. The cages were rinsed with water and methanol and the rinse solutions were collected. All samples were analyzed for ——— ——————— Next, the urine samples up through 48 hours (predose, 0 – 6, 6 – 24, 24 – 48) were pooled by sex and profiled by HPLC (8 samples total). Carcasses were retained from all animals for possible future analysis.

Results:
The mean concentration of T-20 administered to male and females rats was 3.86 mg/kg and 4.02 mg/kg, respectively. The radioactivity of samples expressed as a percent of the dose administered, is presented below:

Radioactivity Recovered from Rats Administered ^{3}H-T-20 as a Single Intravenous Dose, Expressed as a Percent of Dose Administered

Animal	Urine	Feces	Expired Air	Cagewash Water	Cagewash Methanol	Total
Male 1	11.1	6.7	9.2	0.6	0.03	27.6
Male 2	10.4	9.1	9.0	0.8	0.03	29.3
Male 3	10.4	9.9	NA	1.7	0.02	22.0
Male 4	7.7	10.6	NA	0.7	0.03	19.0
Mean Males	9.9 ± 1.5	9.1 ± 1.7	9.1 ± 0.1	0.9 ± 0.5	0.03 ± 0.01	NA
Female 1	23.9	11.5	10.4	0.6	0.02	46.5
Female 2	15.4	11.2	12.0	0.9	0.02	39.4
Female 3	13.2	10.3	NA	0.7	0.02	24.2
Female 4	17.1	12.2	NA	0.6	0.19	30.0
Mean Females	17.4 ± 4.6	11.3 ± 0.8	11.2 ± 1.1	0.7 ± 0.1	0.06 ± 0.09	NA

The rate of excretion of radioactivity by urine, feces, and expired air was fastest during the first 24 or 48 hours and was fairly constant thereafter. Females excreted radioactivity by all routes at a higher rate than the males and the difference was most pronounced in the urine samples.

At the end of one week the total recovery of radioactivity from animals from which expired air was collected was ——for the males and —— for the females. The low total radioactivity excreted indicates that T-20 metabolites are incorporated into intermediate metabolic pathways.

Two peaks showed up in the HPLC———— of pooled urine samples. Peak maximums were significantly greater for the males, but the relative positions and shapes of the peaks for both sexes at each collection time were similar. Over the time intervals 0 – 6 hours to 6 – 24 hours, to 24 – 48 hours, the second peak (more polar compound) gained in height and the first peak diminished in height.

———— states that the HPLC ———— indicate that ^{3}H-T-20 is "essentially completely metabolized to at least two radioactive compounds which are excreted in urine." It also states that the presence of trace amounts of ^{3}H-T-20 in ———— due to carryover of material cannot be ruled out due to the highly adsorptive nature of the compound.

20. NDA 21-481 Reference 2302, Volume 7. IND —— Serial 286.
Mass balance study of ^{3}H-T-20 following intravenous administration to Sprague-Dawley rats. —— Study——/04. Final Report, March 1, 2001.

This ———— report contains an analysis of the radioactivity in the carcasses of the eight rats (4/sex) from study ——/02. In that study, Sprague-Dawley Crl:CD®BR rats were administered a single bolus intravenous (target) dose of 4 mg/kg ^{3}H-T-20, and urine, feces, and expired air were collected from them for 7 days (except expired air was collected from 2 animals/sex, only). All excreta samples were analyzed by ———— and pooled urine samples were additionally analyzed by HPLC. Following the issue of the final report of study TRS/02, the sponsor requested that———— analyze the radioactivity of the animal carcasses. The animal carcasses were digested in whole (no attempt was made to divide or section them).

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Results:
Radioactivity Recovered from Rats Administered ^{3}H-T-20 as a Single Intravenous Dose, Expressed as a Percent of Dose Administered

Animal	Urine	Feces	Expired Air	Cagewash Water	Cagewash Methanol	Carcass	Total
Male 1	11.1	6.7	9.2	0.6	0.03	72.8	100.4
Male 2	10.4	9.1	9.0	0.8	0.03	70.4	99.7
Male 3	10.4	9.9	-	1.7	0.02	60.9	-
Male 4	7.7	10.6	-	0.7	0.03	70.3	-
Mean Males	9.9 ± 1.5	9.1 ± 1.7	9.1 ± 0.1	0.9 ± 0.5	0.03 ± 0.01	68.6 ± 5.3	NA
Female 1	23.9	11.5	10.4	0.6	0.02	52.3	98.8
Female 2	15.4	11.2	12.0	0.9	0.02	58.1	97.5
Female 3	13.2	10.3	-	0.7	0.02	63.7	-
Female 4	17.1	12.2	-	0.6	0.19	57.9	-
Mean Females	17.4 ± 4.6	11.3 ± 0.8	11.2 ± 1.1	0.7 ± 0.1	0.06 ± 0.09	58.0 ± 4.7	NA

The mean recovery of radioactivity associated with the carcasses (n = 4/sex) was [redacted] for the males and [redacted] for the females. The total mass balance (above) could only be determined for n = 2 animals/sex because the expired air fraction was collected from only 2 animals/sex.

These results indicate that a large amount of the radioactivity remained in the carcasses at the time of the last sample collection. [redacted] indicated that there was an insufficient number of animals to determine whether the difference in radioactivity in carcasses by sex was significant.

21. NDA 21-481 Reference 2303, Volume 7. IND [redacted] Serial 211.
Ro29-9800/000 (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. Roche Study 07461. Final Report RR 1004433, September 4, 2001.

Hoffmann-La Roche Inc. conducted this non-GLP rat study during October 2000 to explore a method for monitoring T-20 levels in lymph nodes. Eighteen male rats were administered a single dose of T-20 (25 mg/kg, 2 mL/kg) subcutaneously in the nape of the neck. Six rats received injections of sterile water. T-20 treated rats were sacrificed at 0.5, 1 and 6 hours post dosing (6 each). Control animals were sacrificed at 6 hours post dosing. Blood samples and lymph nodes (mandibular, axillary, and brachial) were collected at necropsy. Half the lymph nodes collected were assayed for Ro 29-9800 (T-20) and its metabolite Ro 50-6343 by [redacted] The remaining lymph nodes and skin samples were assayed for T-20-specific antibodies by [redacted]

Results:
There were no unscheduled deaths or treatment-related clinical signs following administration of T-20, and no treatment-related findings at necropsy.

Following subcutaneous injection of T-20 at 25 mg/kg, T-20 could be detected in plasma by 0.5 hour post dosing ([redacted]). T-20 (Ro 29-9800 peaked at 1 hour post dosing ([redacted]) and decreased at 6 hours post dosing ([redacted]). Concentrations of the T-20 metabolite Ro 50-

6343 were detectable at 0.5 hour (———— and continued to increase at 1 hour (————) and 6 hours (————→post dosing. In the lymph nodes, Ro 29-9800 and Ro 50-6343 could not be detected by the ———— method.

Using ————————————————————————————, Ro 29-9800 was observed in the dermis and subcutaneous injection site tissue at 0.5 hour post dosing. The intensity of staining decreased at 1 hour post dosing and disappeared completely by 6 hours post dosing. Sections of lymph nodes from most of the animals at 0.5 and 1 hour post injection resulted in minimal to intense staining for Ro 29-9800 in the subscapular sinus, paracortical area, medullary sinusoids and lumen of the vasculature. Except for minimal to mild staining in a few lymph nodes, Ro 29-9800 was cleared from most of the lymph nodes at 6 hours post dosing.

The sponsor suggested that factors explaining why Ro 29-9800 and Ro 50-6343 were not detectable in lymph nodes by ———— include inefficient extraction from the tissue, nonspecific binding, or retention of peptide to the glassware. The sponsor concluded that the ————————————————method may be applicable to human tissues.

22. NDA 21-481 Reference 2305, Volume 7. IND ——— Serial 297.
Ro 29-9800: Mass balance study of ^{3}H Ro 29-9800 (^{3}H T20) following subcutaneous administration to male Long Evans rats.———————————— Study ——/01. ————Final Report, April 26, 2001. Roche Study D01021. Roche Final Report, RR 1004809, June 12, 2001.

Roche previously conducted a mass balance study in which all of the radioactivity could not be accounted for and in which expired air was not collected. Therefore, Roche contracted with ————, which had the capability to collect expired air, to conduct the present study.

————————————, conducted this non-GLP study during March and April 2001 to investigate the total mass balance distribution and excretion of Ro 29-9800, including expired air. A single subcutaneous dose of ^{3}H-Ro 29-9800 was administered (nominally 1 mL/250 g rat) to 4 unfasted male Long Evans rats. The rats were approximately 9 weeks of age at the time of dosing and weighed from 214 to 240 grams. The mean dose administered was 202 mg ^{3}H-Ro 29-9800/ kg, and 625 µCi ^{3}H- Ro 29-9800/ kg. Urine, feces, and expired air were collected in 24-hour increments for one week. (Urine was also collected initially at 0 to 6 hours and 6 to 24 hours.)

Results:
Excretion of radioactivity as percent of dose administered over 168 hours was as follows:

Urine	Feces	Expired air	Cage wash water	Cage wash methanol	Carcass	Total
6.5 ± 2.6	14.3 ± 1.6	12.0 ± 0.3	2.0 ± 0.4	0.02 ± 0.01	57.2 ± 1.2	92.2 ± 1.4

Rates of excretion in urine, feces, and expired air were relatively constant (linear) after the first 24 hours. Mean excretion of radioactivity in urine was 1.8% of dose administered during the first 24 hours and then decreased slowly to approximately 0.6% per day at Day 7. Mean excretion of radioactivity in feces was 4.3% of dose administered during the first 24 hours and then deceased slowly to 1.1% per day at Day 7. Mean excretion of radioactivity in expired air was 1% of

administered air at the end of 24 hours and increased to >2% per day at Day 3, then decreased slowly to 1.6% per day at Day 7.

HPLC [redacted] of rat urine showed two polar peaks at approximate retention times 5 and 7 minutes. The more polar of the two increased while the less polar peak decreased over the sample sequence 0-6 hours, 6-24 hours, and 24-48 hours. There was no evidence of parent Ro 29-9800 in the urine samples for the time increments analyzed. Analysis of pooled urine samples from 6-24 hours before and after lyophilization showed that the more polar peak decreased following lyophilization and the second peak remained constant. [redacted] stated this indicates that the more polar peak represents two or more compounds, some of which are associated with tritiated water, and some that are not. In the 6-24 hour urine sample, the radioactivity associated with water was 25% and in the 72-96 hour urine sample (Day 4) the radioactivity associated with water was 69% by this method.

Reviewer comments:
In Study D00111 (NDA 21-481 Reference 2306, follows this review), the total 7-day proportions of radioactivity excreted in urine and feces were similar to these results, though the proportion of radioactivity present in the carcass was less (45% compared with 57% in this study). In that study 34% of administered radioactivity was not accounted for. In this study expired air accounted for 12% of administered dose at the end of 7 days and 8% was unaccounted for. Thus the difference in the results between the studies is nearly all in the carcass. Perhaps radioactivity was lost in the previous study during the process of removing organs for individual radioactivity counting. In this study, organs were not removed from the carcass.

The [redacted] in Study D00111 showed that the parent drug, Ro 29-9800, was still detectable at 4 hours but undetectable at 8 hours. This study shows the parent compound is undetectable in urine from the 0- 6 hours interval.

23. NDA 21-481 Reference 2306, Volume 8. IND [redacted] Serial 286.
Ro 29-9800: Mass balance and [redacted] in rat following subcutaneous administration of ^{3}H Ro 29-9800. Roche Study D00111. Final Report RR 1005251, February 14, 2002.

The sponsor conducted this non-GLP study (dates not presented) to determine the mass balance, routes of excretion, and tissue distribution following a single subcutaneous dose (0.5 mL) of ^{3}H-Ro 29-9800 to 12 unfasted male Long Evans Crl:(LE)BR rats. The rats were approximately 9 weeks of age at the time of dosing and weighed from 191 to 225 grams. The mean dose administered was 222 mg ^{3}H-Ro 29-9800/ kg, and 577 µCi ^{3}H- Ro 29-9800/ kg.

Nine rats were sacrificed at 30 minutes, 2, 4, 8, or 24 hours post dosing and their bodies were immediately frozen for sagittal sectioning (20 µm). (The sponsor does not report how many rats were sacrificed at each timepoint.); [redacted] were placed against selected sections for 7 to 10 days of exposure to generate [redacted] which were analyzed by an [redacted]

From 3 additional rats, urine, feces, whole blood, and sera were collected at 24-hour intervals for one week following dosing. At 168 hours post dosing, the animals were killed and the large

intestine, small intestine, stomach, liver, kidneys, lungs, heart, brain, adrenals, thymus, testes, spleen, pancreas, and bone were removed, weighed, homogenized, and frozen. The urine, feces, whole blood and sera, cage wash, and remaining carcasses were also retained for [redacted].

HPLC [redacted] were also generated from sera samples.

A control urine sample from a male rat (not on the previous experiments?) was fortified with ^{3}H-Ro 29-9800 (from the same batch administered to rats in these two experiments) to investigate the possibility that $^{3}H_2O$ was forming from ^{3}H-Ro 29-9800 by tritium exchange with water. The fortified urine was vortexed and kept at room temperature. Two samples were collected at 0, 3, and 22 hours; one sample was counted at the collection time and the other was dried overnight to evaporate the volatile portion, and then counted.

Results:
In the mass balance study (n = 3), total excretion of radioactivity at the end of 7 days by feces and urine was 6% and 15% (mean values), respectively. Values were highest in the first 24-hour interval and tapered off in subsequent 24-hour intervals. In animal tissues at the end of 7 days, the percent of administered radioactivity was highest in the carcass (38%, without 14 organs that were removed), followed in magnitude by the liver (3%), small intestine (1%), kidneys (0.8%), and large intestine (0.6%). Total body tissue radioactivity at the end of 7 days was 45% of the administered dose. The mass balance study did not account for 34% of the administered dose, which was presumed to be lost in expired air and water (not collected). The sponsor did not present radioactivity per weight of tissue sample collected in this experiment.

Radioactivity was detectable in both blood and sera at the end of 1 week. The ratio of radioactivity in blood to sera was 0.7 at 2 hours and 2.6 at the end of 1 week. Cmax in sera was approximately 225 µg-equivalents of T-20 per mL at (perhaps) 12 hours and Cmax in whole blood was approximately 175 µg-equivalents of T-20 per mL at 48 hours. (The sponsor presented these data graphically only. It appears that blood samples were not collected at the time intervals described in the report narrative.)

The [redacted] showed that 80% of the total radioactivity in the samples at 0.5 hour was at the retention time for the parent compound (^{3}H-Ro 29-9800, RRT about 17 minutes). At 2, 4, and 8 hours it was 40%, 6.5%, and non-detectable, respectively. With the decrease of radioactivity associated with the ^{3}H-Ro 29-9800 peak, additional peaks emerged at 1.6 minutes and 10 minutes (relative retention times).

Urine radioactivity was measured both before and after drying. The volatile portion of urine contained 36% of the radioactivity in the first 24-hour sample and 93% of the total over the last 72 hours of the week. The sponsor stated that this indicates that the composition of the radioactivity in urine is changing over time and that an increasing portion is being excreted as water. Subsequently, the sponsor conducted the "spiked" control urine sample experiment.

After incubation with ^{3}H-Ro 29-9800 in control rat urine for 0, 3, and 22 hours, there was no difference in the radioactivity values determined for total ^{3}H and non-volatile ^{3}H at any of those times. This result indicates that no exchange of tritium with water occurred in vitro over 22 hours