

and that any $^3\text{H}_2\text{O}$ (or other volatile tritiated compounds) generated in the study rat experiments was generated by in vivo metabolism.

From the whole body _____ the concentration of radiation in 24 tissues plus the contents of the stomach, large intestine, and small intestine were determined at 0.5, 2, 4, 8, and 24 hours. At 0.5 hour, the largest concentration of radioactivity was at the site of injection (35,376 μg -equivalents of T-20 per gram of tissue), followed by the brown fat (33 μg -equivalents/g), eye (26 μg -equivalents/g), stomach (23 μg -equivalents/g), and kidney (18 μg -equivalents/g). The radioactivity at the injection site climbed to 44,985 μg -equivalents/g at 2 hours and tapered off to non-detectable at 24 hours. The radioactivity concentrations peaked in most of the other tissues at the 4-hour or 8-hour timepoints. Tissues having their highest values at 24 hours were kidney, lungs, thymus, testes, heart, and muscle (357, 161, 125, 77, 72, and 52 μg -equivalents/g, respectively). However: (1) radioactivity concentrations fluctuated over time in individual tissues, (2) raw data were not presented, (3) the uncertainty and the n of each estimate was not presented, and (4) there are no measurements beyond 24 hours.

Reviewer comments:

This was an exceptionally difficult report to review for the following reasons: Not all the data from the study were included in the report. The narrative alternated between portions of the two main experiments. Study design description was written into the results section and results were presented in the study design sections. It was not possible to track the fate of each animal. It is not entirely clear which animals were bled for the _____. It is not clear how many animals were sacrificed for each of the _____ or how many _____ were generated from each animal, or how the _____ data table was generated from the _____.

The most interesting result of this study is that the _____ ^3H -Ro 29-9800 peak was gone by the end of 8 hours. Therefore, by the time the radioactivity is maximized in serum (12 hours) or whole blood (48 hours), virtually all of the parent compound has been metabolized to something else. Pharmacokinetic and in vitro metabolism studies indicate that humans metabolize Ro 29-9800 more slowly than rats.

It would have been interesting if the sponsor had compared the _____ generated in this study with those generated with the _____ system in other rat studies.

It would have been interesting to see how the radioactivity per gram of tissue determined in the mass balance experiment compares with the same quantities calculated from the _____.

24. NDA 21-481 Reference 2307, Volume 8. IND _____ Serial 319.

Ro 29-9800: Milk secretion and placental transfer studies with ^3H -Ro 29-9800 (^3H -T-20) following subcutaneous administration to female Wistar Hanover rats. _____ Study _____ 02. _____ Final Report, March 6, 2002. Roche Study D01022. Roche Final Report RR 1007715, April 23, 2002.

_____ conducted this non-GLP study for the sponsor beginning in May 2001 to investigate lacteal and placental transfer of ^3H -Ro 29-9800 following a

single subcutaneous dose. _____ rats _____, were used for this study. In both the milk secretion study and placental transfer study, the target dose was 200 mg/kg and 600 μ Ci/kg.

In the milk secretion study (n = 24), ^3H -Ro 29-9800 was administered by subcutaneous injection at approximately Day 14 post-parturition and 3 rats per time period were sacrificed and blood and milk samples were collected from 3 rats per time period at 0.5, 1, 2, 4, 6, 8, 24, and 48 hours post dosing. _____ was then used to measure radioactivity in blood, plasma, milk, and carcasses, and HPLC was used to measure the concentration of Ro 29-9800 in plasma and milk.

In the placental transfer study (n = 24), ^3H -Ro 29-9800 was administered by subcutaneous injection to the dams on approximately Day 18 of gestation and 3 rats per time period were sacrificed at times 0.5, 1, 2, 4, 6, 8, 24, and 48 hours post dosing. The following tissues of the dams were collected: amniotic fluid, brain, heart, kidneys, liver, lungs, mammary glands, ovaries, placenta, and uterus. In addition, 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 Ro 29-9800 in amniotic fluid and placental homogenate.

Results:

The doses of ^3H -Ro 29-9800 that were administered to the rats in the milk secretion study and in the placental transfer study were 197 mg/kg and 196 mg/kg, respectively, and 628 μ Ci/kg and 566 μ Ci/kg, respectively.

Mean concentrations of radioactivity in the milk secretion study were as follows:

Time (hours)	Blood ($\mu\text{g equiv/g}$)	Plasma ($\mu\text{g equiv/g}$)	Milk ($\mu\text{g equiv/g}$)	Carcasses ($\mu\text{g equiv/g}$)
0.5	13.8 \pm 4.9	15.5 \pm 5.9	1.1 \pm 0.6	95.3 \pm 0.3
1	26.6 \pm 5.6	28.0 \pm 5.9	4.9 \pm 3.0	96.5 \pm 0.3
2	58.8 \pm 8.1	41.5 \pm 9.4	46.9 \pm 20.1	94.1 \pm 1.9
4	99.1 \pm 4.7	54.5 \pm 5.6	130.3 \pm 22.7	92.3 \pm 1.2
6	85.8 \pm 13.1	48.4 \pm 1.1	135.4 \pm 29.0	86.8 \pm 2.8
8	93.9 \pm 25.9	50.5 \pm 5.4	141.3 \pm 59.8	81.3 \pm 2.6
24	81.4 \pm 9.6	26.8 \pm 4.1	33.7 \pm 8.6	61.1 \pm 2.7
48	82.3 \pm 11.0	43.7 \pm 9.7	37.1 \pm 5.9	52.5 \pm 2.6

Mean concentrations of radioactivity in the placental transfer study were as follows:

Time (hours)	Blood ($\mu\text{g equiv/g}$)	Plasma ($\mu\text{g equiv/g}$)	Amniotic fluid ($\mu\text{g equiv/g}$)	Dam carcasses ($\mu\text{g equiv/g}$)	Fetuses ($\mu\text{g equiv/g}$)
0.5	10.8 \pm 1.3	12.2 \pm 1.6	0.9	263.1	2.1
1	33.4 \pm 2.6	29.2 \pm 1.7	3.2	239.5	14.4
2	110.3 \pm 7.0	54.7 \pm 7.1	16.5	220.4	72.6
4	174.7 \pm 16.2	68.7 \pm 14.6	23.9	219.4	114.6
6	222.6 \pm 24.3	54.2 \pm 1.3	31.2	211.1	142.4
8	232.1 \pm 11.8	56.0 \pm 9.8	30.9	190.8	146.1
24	150.7 \pm 7.8	46.6 \pm 6.8	44.0	183.3	189.4
48	135.0 \pm 2.6	44.2 \pm 6.0	42.4	186.1	166.8

Mean concentrations of radioactivity of dams' organs ($\mu\text{g equiv/g-wet tissue}$) in the placental transfer study were as follows:

Time (hours)	Brain	Heart	Kidneys	Liver	Lung	Mammary gland	Ovaries	Placenta	uterus
0.5	1.3	3.8	16.6	11.6	4.7	2.5	5.3	4.0	3.6
1	8.1	19.6	65.8	82.6	21.0	12.1	32.3	20.9	20.4
2	34.0	67.2	172.7	251.2	77.2	46.1	143.8	99.7	78.2
4	46.1	95.2	226.7	290.6	107.3	78.5	196.8	161.3	111.7
6	56.3	115.8	263.8	325.1	133.9	96.3	240.2	201.1	151.4
8	58.6	108.6	265.2	316.9	137.4	97.7	247.8	207.8	158.4
24	65.5	117.5	225.8	266.0	125.8	101.1	185.7	239.0	172.8
48	66.4	113.9	212.9	259.5	114.4	65.4	167.0	223.9	146.0

In the milk transfer study, following a single subcutaneous injection of $^3\text{H-Ro 29-9800}$, radioactivity was initially highest in the carcass (compared with three other fluids), and began to diminish from the carcass after 4 hours. The radioactivity levels were highest in whole blood, plasma, and milk in the samples collected at 4, 6, and 8 hours, but it was much higher in milk (by at least 30%) during this period than in either whole blood or plasma. At 24 and 48 hours, the radioactivity levels were diminished in all four tissues/fluids, but were more reduced in plasma and milk than in whole blood or in the carcasses.

In the placental transfer study, following a single subcutaneous injection of $^3\text{H-Ro 29-9800}$, radioactivity was initially highest in the carcass, and began to diminish from the carcass after 4 hours. Radioactivity in most other organs and tissues increased rapidly with the 2-hour samples and reached their maximum values in the samples collected at 8 hours or 24 hours. The amniotic fluid, the brain, and the plasma had the lowest levels of radioactivity on a per-weight basis throughout the study. 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 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) greater than 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 Ro 29-9800 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 Ro 29-9800. These results are consistent with the rapid metabolism of Ro 29-9800 and the subsequent widespread distribution of amino acids throughout the body. These results are consistent with earlier mass distribution studies of Ro 29-9800 except that in Roche Study D00111 (where approximately the same dose was administered) Ro 29-9800 was detected in sera samples collected up to 4 hours. In this report, the _____ stated that the extraction or analysis methods may have been "inefficient" or "suboptimal," and therefore the absence of Ro 29-9800 in the chromatograms is not conclusive evidence that parent compound was not present in the samples.

Reviewer comments:

Radioactivity levels in the fetuses and milk do not conclusively settle whether the rat fetuses were exposed to Ro 29-9800 in utero or whether the nursing pups were exposed to Ro 29-9800 in milk. The _____ and the sponsor concluded that if exposures to fetuses and nursing pups

occurred that they were probably low or negligible. However, the study results do not justify this conclusion.

TOXICOLOGY STUDY REPORTS

Single Dose

25. NDA 21-481 Reference 3000, Volume 9. IND _____ Serial 000.

A single dose toxicity study of T-20 in the albino rat. _____ Study 680.
Final Report, May 10, 1996.

_____ conducted this GLP study during February 1996 for Trimeris, Inc.

T-20 was administered intravenously to 5 male and 5 female CDF (Fischer) rats per group at 10, 20, 50, and 100 mg/kg. Planned intravenous doses of 200 mg/kg were not administered due to deaths at 100 mg/kg. Also, 3 male and 3 female rats were administered T-20 intraperitoneally at 100 mg/kg. Two control groups (5 rats/sex/group) received either _____ saline (20.0 mL/kg, twice the high dose volume) or nothing. The rats were approximately 4 weeks old at the time of dosing. Clinical observations were made before and after dosing, and one hour after dosing. Body weights were recorded at randomization, before dosing and before necropsy (fasted weight). Animals were euthanized and necropsied (except those dosed intraperitoneally) at approximately 24 hours post dosing. Selected body organs were weighed. Lungs from each group (and from animals that died) were preserved for histological examination.

All animals, except one male, that were treated intravenously with T-20 at 100 mg/mL died after dosing. Clinical signs observed for animals that died included foamy pink discharge from the mouth and/or nose, rapid respiration, ataxia, languid behavior, convulsions, labored breathing, dyspnea, and/or clear nasal discharge. The surviving animal showed languid behavior and rapid respiration after dosing, but appeared normal one hour later. 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. All animals treated intraperitoneally at 100 mg/kg survived.

At necropsy, all animals that died had red, darkened lungs. Red lesions on the lung were also observed in one female treated with T-20 at 20 mg/kg and one male and two females treated at 50 mg/kg. Other animals appeared normal, including the one surviving male from the high intravenous dose group. The pathologist stated that the incidence of macroscopic pulmonary lesions was suggestive of a test-article-related effect as well as a dose-effect, because 1 of 10, 3 of 10, and 9 of 10 animals in the 20, 50, and 100 mg/kg groups, respectively, had dark red lesions involving all lobes of the lungs.

Absolute and relative weights of liver, spleen, and thymus, from animals from the 10, 20, and 50 mg/kg groups were normal. Organ weights were not recorded for the 100 mg/kg groups.

Microscopically, lungs from animals receiving T-20 in the 20, 50, and 100 mg/kg i.v. groups exhibited non-specific congestion in a dose-related fashion similar to lesions seen

macroscopically. Congestion tended to be multi-focal to diffuse and included over 50% of alveolar tissue. Hemorrhage tended to occur in focal to multi-focal distribution and edema was seen mainly perivascularly around large pulmonary vessels. The pathologist stated that he could not suggest a mechanism to explain these observations.

The authors considered the cause of animal deaths at 100 mg/kg. They ruled out receipt of a large volume of solution because control animals received a larger volume; they ruled out rate of delivery because the intraperitoneal group received the same dose and did not exhibit toxic effects. They concluded that the i.v. 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.

The pathologist concluded that the red lesions in the lungs of animals treated with T-20 at 20 and 50 mg/kg were similar to those seen in animals that died after a single i.v. dose of 100 mg/kg, and they were related dose-dependently to the test article.

The study authors concluded that administration of T-20 at 10 and 20 mg/kg did not cause acute toxicity, though it did produce pathological effects at doses of 50 mg/kg and higher.

Reviewer comment:

It is not clear why the sponsor concluded that there was no acute toxicity at 20 mg/kg when the pathologist noted that adverse findings in one rat at that dose were the same as those that were considered drug-related at higher doses.

26. NDA 21-481 Reference 3001, Volume 9. IND _____ Serial 000.

Single-dose toxicity study of a test article (T-20) administered intravenously to cynomolgus monkeys. _____ Study 3-B44. Final Report, June 11, 1996.

_____ conducted this GLP study during March and April 1996 for Trimeris, Inc. to determine the acute toxicity associated with a single intravenous dose of T-20 in cynomolgus monkeys. The cynomolgus monkeys (*Macaca fascicularis*) came from _____

Under ketamine HCl sedation, five female cynomolgus monkeys were given 0, 5, 10, 25, or 50 mg/kg T-20 intravenously via a cephalic vein. Animals in the first four dose groups were administered the drug in dose volumes of 2.5 mL/kg (7.25 mL or 7.75 mL each); the high dose animal received 5 mL/kg (14.0 mL). The sponsor provided T-20 to the study director in frozen solutions of (nominally) 10 mg/mL, but there were no certificates of analysis provided to verify the potency of the solutions. The study personnel calculated all doses based on the nominal concentration. Animals in the first four dose groups were administered the drug consecutively. The animal receiving 25 mg/kg of T-20 was observed for abnormal clinical observations (specifically nasal hemorrhaging) for approximately 15 to 30 minutes before administering T-20 to the 50 mg/kg dose animal. The 50 mg/kg animal was also observed for 15 to 30 minutes post dosing. All animals were observed clinically at 1, 2, 4, and 24-hours post dosing and sacrificed at 24 hours post dosing. Animals were examined for gross lesions at necropsy and the liver, spleen, and thymus were weighed.

T-20 was well tolerated by all except the high-dose (50 mg/kg) monkey. This animal recovered more slowly than expected from ketamine and had pale mucous membranes which resolved between 6 and 24 hours. There were no other abnormal clinical observations in this animal or in any of the other animals, including rashes on the lower back.

At necropsy, the monkeys weighed between 2.6 kg (the control) to 3.0 kg (the 5 mg/kg animal). Liver weights in all T-20 animals were increased relative to the control (range +19% to +39%). Relative liver weights (as a percentage of body weights) were increased compared with the control (+27% to +44%). Absolute and relative spleen weights were also increased in all T-20 animals (+68% to +122%) compared with the control. The sponsor reported that none of the differences in body weights or liver or spleen weights were significant although no statistical analysis was performed.

At necropsy, there were no obvious patterns or consistent occurrences of abnormalities among the animals. Lung foci were noted in the 0 and 25 mg/kg animals, but not in the others. Spleen foci and/or discoloration were noted in the 10 and 50 mg/kg animals, but not in the others. Other observations (liver foci, large intestine nodules, kidney discoloration, administration site focus) occurred in different animals. The high dose (50 mg/kg) monkey exhibited spleen and kidney discoloration and discoloration (red) at the administration site that was 6 inches in diameter. The sponsor stated there were no gross observations at necropsy attributable to effects of T-20. This study did not include histological examinations.

(A sixth study animal was to have received 100 mg/kg, but was removed from the study on the day of dosing without explanation. The study report merely states that the animal was removed at the request of the sponsor. The sponsor signed the amendment to the protocol three days after the study was completed.)

The sponsor concluded that intravenous T-20 was well tolerated up to 25 mg/kg in cynomolgus monkeys and that toxicity in the monkey that received 50 mg/kg was limited to the clinical signs at the time of dosing.

Reviewer comment:

Evidence that findings occurring at 25 mg/kg and below are drug-related is equivocal.

Repeat Dose - Rat

27. NDA 21-481 Reference 3100, Volume 9. IND _____ Serial 000, 168.

A 28-day repeat dose toxicity study of T-20 in the albino rat followed by a 2-week recovery period. _____ Study 682. August 2, 1996.

_____ performed this GLP study during March and April 1996 for Trimeris, Inc.

Fifteen male and 15 female CDF (Fischer F-344/CrlBR) rats (from _____) per group received intravenous injections of T-20 in doses of 0, 1.0, 3.0, and 10.0 mg/kg/day for 28 or 29 days. Control animals received the same volume injection as the highest dose group.

_____ received three drug vials of liquid T-20 that were assayed at _____, and _____ of nominal value (10 mg/mL) _____ assigned the vial that assayed the lowest to the 1.0 and 3.0 mg/kg dose groups and the other two lots to the 10 mg/kg dose groups. The researchers indicated they adjusted the dose volumes so that the animals received the doses of drug specified by the protocol, and they sent samples of the solutions prepared for dosing the animals to the sponsor for analysis.

Two male rats received from _____ died during the acclimation period (21 days) before the study began. The rats used on the study were all considered healthy.

Observations for mortality and gross reactions to treatment were performed twice daily. Clinical observations were performed after dose administration and weekly at the time of body weighing. Body weights were recorded before initial dosing, weekly thereafter, and before necropsy (fasted weight). Other metrics include food consumption, eye exams, hematology and blood chemistry (before scheduled necropsies). Five animals per each sex and group were put on 2-week recovery before necropsy.

All animals survived until scheduled sacrifice. Adverse signs recorded during the study include occasional chromodacryorrhea (blood-stained tears, 3 control animals and 1 animal each in the low and intermediate dose groups), alopecia around the eye of one control animal, and mild nose swelling of another control animal. Four animals (two controls, one each in the higher two dose groups) were found to have ophthalmoscopic abnormalities at the Week 4 examination. A corneal dystrophy was noted in one control animal at Week 6 (recovery). No observable differences in group mean body weights or food consumption were noted during the study.

High dose (10 mg/kg/day) male rats, but not females, had a statistically significant decrease (-12%) in total leukocyte count at Day 28 or 29 sacrifice. After recovery, all T-20 male groups, but not females, had statistically significant increase in erythrocyte count (+17%), hematocrit (+17%), and hemoglobin (+9%), and decreases in mean corpuscular hemoglobin (-8%) and mean corpuscular hemoglobin concentration (-7%). Statistically significant increases in sodium levels were noted in females in the 3 and 10 mg/kg dose groups (+1% to +2%) at terminal sacrifice, and in males in the 3 and 10 mg/kg dose groups and females in the 3 mg/kg/day dose group after recovery (group mean control values compared with controls). Albumin was increased (+4 to +6%) in males and females after recovery in the 10 mg/kg dose groups and females in the 1.0 mg/kg dose group.

High dose male and female mean absolute and relative thyroid/parathyroid weights were higher (+38% to +45%) than the means of respective control animals. High dose relative male spleen weights were higher (+7%) than control animals. After recovery, all values were similar to controls.

Microgranulomas occurred more frequently in the lungs of animals in the high dose (10 mg/kg/day) groups (male and female) than in the control groups: 7/10 (high dose males), 2/10 (control males), 6/10 (high dose females), 1/10 (control females). (Tissues in the two low-dose T-20 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. However, the pathology report does not contain data on foreign material in microgranulomas, and it cannot be determined how often or

in what distribution among study groups this occurred. "Minimal focal inflammation" was also found in the lungs of high dose animals (5/10 males, 1/10 females) and controls (2/10, 2/10). The pathologist indicated that the focal inflammation lesions in males were possibly related to T-20 administration, but he discounted their significance because they occurred in controls and were of negligible significance histopathologically.

— did not attribute any effects to T-20. The study director concluded that administration of T-20 at doses up to 10 mg/kg/day for 28 days was not associated with macroscopic or microscopic lesions, and changes observed in some hematological and blood chemistry parameters were not considered biologically meaningful.

Reviewer comments:

The pathologist who reviewed tissues for this study (— 682) was not the same pathologist who reviewed tissues in the previous (acute dose) rat study (— 680). The acute dose study indicated that lung lesions in rats might be a consequence of T-20 administered intravenously and were dose-related. Therefore, it is not prudent to discount the lung lesions observed in this study, because they occurred in the acute study and they occurred more frequently in this study in the high dose groups than in the controls.

Lung lesions should be considered a consequence of repeat intravenous dosing of T-20 in rats at doses at least as low as 10 mg/kg/day. However, because histopathologic examinations of tissues were not performed for the lower dosed animals, a NOAEL cannot be determined from this study.

28. NDA 21-481 Reference 3101, Volume 10. IND — Serial 001.

A maximum tolerated dose (MTD) determination and a 7-day intravenous toxicity study of — batches of T-20 in Fischer-344 rats. — Study 793. Final Report November 6, 1996.

— conducted this GLP study during July and August 1996 for Trimeris, Inc. to compare the toxicities of — different preparations of T-20. The — preparations are designated — 209601A and 3U1. The two study parts are a single, intravenous injection maximum tolerated dose (MTD) experiment in rats and a repeat-dose, intravenous injection study in rats.

The Fischer-344 rats used in this study came from —

For the maximum tolerated dose (MTD) study, 2 Fischer-344 rats/sex/group received one or the other preparation of T-20 intravenously at doses of 50, 75, 100, 110, or 125 mg/kg. Animals were observed twice daily, at least 6 hours apart for mortality and signs of reaction to the treatment. Clinical observations were performed at dosing and on days 1 through 14, except that the animals that received 50 mg/kg were watched for 9 days and then dosed at 125 mg/kg and then watched for 14 days (i.e., the 50 mg/kg rats were dosed a second time in the study). Animal weights were collected at randomization and on Day 1 prior to dosing, Days 8 and 14, and prior to necropsy (fasted weight) on Day 15.

For the 7-day repeat-dose toxicity study, 5 Fischer-344 rats/sex/group received either preparation of T-20 at dose levels of 2, 7, or 15 mg/kg/day or placebo. The same observation schedule was

followed as with the MTD study through Day 7, at which time animals were necropsied. In addition, — researchers collected blood before the necropsies for hematologic and clinical chemistry examinations, and they collected tissues of the placebo and high dose animals for histopathologic examinations.

Trimeris, Inc. provided — lots of each preparation, and control solutions (— saline), to — for testing and specified which lot was to be used for which animal group. Single lots of each preparation were used for all dose groups in the MTD study and separate lots of each preparation were used for each dose group in the repeat-dose study.

Results:

MTD STUDY:

One male rat in the high dose group (125 mg/kg) of T-20 3U1 and both male rats in the high dose group of T-20 —209601A were found dead on the day of dosing. Also, the two male rats in the next-to-highest dose (110 mg/kg) of T-20 —209601A were found dead on the day of dosing or 2 days post dosing. All other rats lived until necropsy.

The male rat that survived in the 125 mg/kg T-20 3U1 group exhibited languid behavior through at least 6 hours post dosing. It was also prostrate, with paralyzed limbs, and exhibited rapid respiration through the first hour post dosing, and exhibited ataxia immediately after dosing. Both the female rats in the 125 mg/kg 3U1 group exhibited languid behavior and ataxia, but the two females in the 125 mg/kg —209601A group did not exhibit any clinical signs.

Both males and one female treated with 110 mg/kg T-20 3U1 exhibited ataxia and languid behavior during the hour after dosing, then were normal through Day 14. One male in the 110 mg/kg —209601A group became prostrate after dosing then died approximately 5 hours later. The other male in this group exhibited ataxia, languid behavior, prostration, and then died on Day 2. The treated females (110 mg/kg —209601A) were languid after dosing and at 1 hour.

Clinical signs after administration of T-20 3U1 at 125 mg/kg were ataxia, languid behavior and rapid respiration. One male also exhibited prostration and limb paralysis on the day after dosing. One male treated with T-20 —209601A at 125 mg/kg was ataxic and exhibited rapid respiration. Both males died within 30 minutes of dosing. Both females at this dose showed no adverse clinical signs. The only clinical sign recorded for animals from the lower dose groups was discoloration of the tail in one female (50 mg/kg T-20 —209601A).

Rats exhibited dose-related decreases in weight gains with — of the T-20 preparations. Differences in weight changes between the — preparations were unremarkable.

— noted that mortality occurred at 125 mg/kg for the T-20 3U1 preparation and 110 mg/kg for the T-20 —209601A preparation. Therefore, the MTD for T-20 3U1 is 110 mg/kg and the MTD for T-20 —209601A is 100 mg/kg.

Reviewer comment:

Nominally, there were more deaths, and deaths at a lower dose, with the —209601A preparation compared to the 3U1 preparation. However, the rats dosed with the 3U1 preparation exhibited

more adverse clinical signs than the rats that survived dosing with the —209601A preparation. With the small number of animals on this study, it isn't clear than there are any biologically significant differences between the two preparations.

SEVEN-DAY REPEAT DOSE STUDY:

All animals survived to Day 8 necropsy on the repeat-dose study. The only adverse clinical signs noted were discoloration or bruising of the rat tails. There were no notable differences in weight gains between the different dose groups and placebo groups or between groups dosed with the two T-20 preparations.

Hematologic changes compared to placebo were noted with both sexes and both preparations. They include slight depression of red blood cell counts and hematocrit (-2% to -15%) that appeared dose-related with some groups (e.g., males dosed with T-20 3U1). Mean corpuscular volume was slightly elevated (as high as +4%) compared with controls at all doses of T-20, — preparations, and for both sexes.

Serum chloride was elevated (+1% to +4%) for all males with — preparations compared with controls (statistically significant for all 3U1 male dose groups and the high-dose —209601A males). Nominally, both groups' means followed a dose-dependent increase, but because the changes are small, the trend may be an artifact. Aspartate aminotransferase (AST) was elevated (statistically significant, +11%,) in males in the high-dose group dosed with T-20 3U1.

There were no important gross necropsy observations in the repeat-dose study. Microscopic evaluation of tissues included only the placebo and high-dose groups. The only microscopic findings among the high-dose (15 mg/kg/day) animals were one female (—209601A) with minimal unilateral mineralization of the kidney cortex, and one male (3U1) with minimal increased extramedullary hematopoiesis of the spleen. The same observations were noted in the control animals (one each) plus one control rat had a foreign body and chronic inflammation in the lung. The pathologist reported that the latter was consistent with a hair shaft introduced at dosing. — concluded that no gross necropsy or histopathologic findings were related to drug treatment.

— concluded, based on statistically significant differences in some hematologic values and placebos, that the no-effect level for T-20 3U1 was 2 mg/kg/day and the no-effect level for T-20 —209601A was not established under the conditions of the study.

Reviewer comment:

The hematologic changes, and perhaps the small blood chemistry changes appear to be drug-related. However, I would not place much credence in distinguishing between the two drug preparations by the places in the data where statistical significance was demonstrated. This study and the changes measured are too small for that inference. I do not believe that this study demonstrated any biologically significant differences between the two drug preparations.

29. NDA 21-481 Reference 3102, Volumes 11-14. IND — Serial 018, 026, 035, 047, and 202.

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. Final Report, December 13, 1999, amended October 2, 2001.

_____ conducted this GLP study for Trimeris, Inc. to assess the toxicity, pharmacokinetics and potential antibody induction of T-20 when administered at three dose levels twice per day via subcutaneous injection to Sprague-Dawley _____ rats. The protocol for this animal study was signed on April 9, 1998, and the first animal was dosed on April 22, 1998. Dosing was completed on October 21, 1998, and the recovery sacrifice occurred on November 18, 1998.

Three dose levels of T-20 (plus placebo controls) were evaluated during the first 28 days of the study (0, 2.76, 10.35, and 34.50 mg/kg/day) and another three dose levels (plus placebo controls) were evaluated from Day 29 to Day 183 (0, 2.4, 9.0 and 30 mg/kg/day). The reason that the dosing changed was that the dosing solutions originally provided to _____ by the sponsor were incorrectly labeled. The vials contained 57.5 mg of drug substance instead of the nominal 50 mg. The dose levels and concentrations were reduced to the nominal levels of drug substance beginning on Day 29. All animals were dosed twice a day (i.e., at half the daily doses, 12 hours apart). There were 25 rats/sex/group in Groups II and III, and 30 rats/sex/group in Groups I and IV. After six months of treatment, control and high-dose animals (5/sex/group) were held for a one-month treatment-free recovery period. Ten animals/sex/group were sacrificed at Day 29, 15/sex/group were sacrificed at 6 months (days 184 and 185), and 5/sex/group (Groups I and IV) were sacrificed at 7 months (on Day 211). Control animals (Group I) received placebo at the same dose volume (1 mL/kg) as administered to the treated animals.

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 twice predosing and weekly during the study for general conditions, and eyes, nose, throat, oral cavity, abdomen and external genitalia, skin and fur, respiration, and palpation for tissue masses. Ophthalmoscopic examinations were conducted predosing, on Day 8 and at necropsy (Day 183 or Day 210). Animals were weighed two times before dosing and weekly throughout the study and at necropsy. Food consumption was measured weekly.

Blood was collected for hematology and blood chemistry analyses at the end of Months 1, 3, 6, and 7. Blood for toxicokinetics evaluations was collected at Days 1, 7, 36, and at the end of each month. Blood for serum antibody measurements was collected at predosing, and at the end of Week 1, Months 1, 2, 3, 4, 5, 6, and 7. Blood samples collected for toxicokinetics and antibody testing were sent to Trimeris for analysis.

Macroscopic examinations were performed and selected tissue weights were collected at necropsy. Thirty-six tissues were collected from all animals. Slides were made from all tissues for Groups I and IV animals and from only injection sites, kidneys, liver, lungs, and spleen for Group II and III animals. Histopathologic slides were examined at _____

Results:

Results of toxicokinetics analyses are included under Reference 3115 and results of antibody analyses are included under Reference 3114 of this NDA.

There were no mortalities. — did not attribute any adverse physical observations to T-20, including adverse ophthalmological findings, body weight changes, or food consumption. Food consumption was statistically significantly increased in males compared with controls, but was considered in the normal range.

Reviewer comment:

Mean body weights of all animal groups increased over the duration of the study but T-20-treated male rats gained less weight than control animals and T-20-treated female rats gained more weight than control animals. In contrast to body weight gains, food consumption in all animals dropped from greater than 90 mg/kg/day in males and 95 mg/kg/day in females at the start of dosing, to below 50 mg/kg/day (males) or 65 mg/kg/day (females) at the end of 6 months. The males in the high-dose group (that had the greatest body weight depression compared with controls) generally had the highest food consumption. Females in the high-dose group (that had higher body weight increases compared with controls) also maintained the highest food consumption. Perhaps T-20 is a minor appetite stimulant.

There was also a higher incidence of maloccluded or broken/missing incisors among the high-dose animals and all T-20 animals compared with controls. The following table presents the sum of counts per week of abnormal oral findings by dose group over the full 33 weeks of the study. The data suggest that the drug may affect the way the animals eat.

Total counts mg/kg/day	Incisors maloccluded		Incisors broken / missing		Oral sores	
	Males	Females	Males	Females	Males	Females
0	1	9	6	11	0	0
2.4	26	9	25	5	1	0
9.0	24	0	22	0	0	0
30.0	53	16	59	25	0	5

Mean hematologic values of T-20 dosed rats were mostly similar to controls. Mean absolute neutrophil counts were increased (+39%) compared with controls in low dose females at 1 month, and in low-dose (+61%) and high-dose (+64%) males at 3 months. Mean absolute lymphocyte counts were decreased (-18%) in the middle-dose males compared with controls at six months. These deviations do not suggest a trend or necessarily a drug effect.

Mean clinical chemistry values of T-20 treated rats were mostly similar to control rats. In females, mean aspartate aminotransferase (AST) and alanine aminotransferase (ALT) values were sometimes different from controls. At 3 months, mean AST values were increased in the low-dose group (+17%) and high-dose group (+21%) from controls, and mean ALT values in the low-dose group were increased (+12.5%) from controls. At six months (termination), mean AST values were increased in the low dose (+16%) and high dose (+27%) groups compared with controls, and mean ALT values were increased in the low dose (+25%) and high dose (+25%) groups. After recovery, female mean AST values were decreased (-16%) and mean ALT values were decreased (-24%) compared with controls.

Reviewer comment:

— did not indicate that any of these blood chemistry differences were statistically significant. The magnitude of the differences between T-20 dosed females and controls was greater than

10% in the low and high dose groups only (not the middle-dosed females), and therefore these changes were not dose-related. AST elevation was seen previously in male rats in the T-20 preparation bridging study (intravenous injections, 15 mg/kg daily for 7 days, mean AST +11% compared with controls, n = 5/group). From these data, it is not clear whether differences in AST and ALT values are drug related.

Mean organ weights of T-20 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 change. However, there were only five animals in each group and the rat adrenal weights are small (<0.1 g). At 28-day sacrifice and terminal sacrifice, there were no dose-related trends (changes in organ weights) that occurred in both absolute and relative organ weights.

At the 28-day sacrifice, there were higher incidences of discoloration at injection sites in animals treated with T-20 than with controls (8/30 T-20 males, 2/10 male controls; 16/30 T-20 females, 4/10 female controls). The incidences did not show exact correlation with dose. — indicated it was uncertain whether the discoloration was due to a toxic effect of the compound or simply related to the trauma of the injection procedure. — did not consider any other macroscopic findings were related to treatment with T-20. Other gross findings at 28 days did not affect more than one animal per group.

At the 28-day sacrifice, histological findings were consistent with the macroscopic findings. Incidence of injection site abnormalities outnumbered other findings. These included subdermal hemorrhages, subdermal chronic inflammation/fibrosis, subdermal edema, and degeneration of the cutaneous muscle. — indicated that the injection site effects were likely due to trauma but that a toxic or irritant effect could not be ruled out. — did not attribute any microscopic findings "confidently" to toxicity of T-20.

One interesting finding that is unlikely drug-related is that all the male dose groups (including controls) had animals with slight kidney cortical tubule dilation. None of the females showed this, but the female groups all included animals with kidney corticomedullary mineralization and none of the male groups did.

At the 6-month sacrifice, the macroscopic and microscopic results were similar to the 28-day sacrifice. The incidence of (macroscopic) discoloration at injection sites was as follows: 18/45 T-20 males, 1/15 control males; 12/45 T-20 females, 4/15 control females. Histopathologically, injection site subcutaneous edema was seen in 39 of 60 males and 22 of 60 females. Kidney corticomedullary mineralization was still elevated in females (21 of 60 females) compared to males (2 of 60). Perivascular/peribronchiolar chronic inflammatory cells in the lungs was prominent in the males (31 of 60) but not the females (8 of 60). Hemosiderosis in the spleen was prominent in females (25 of 60) but not in males (0 of 60). Again, these findings were unlikely drug-related because the incidence in controls was similar to that of T-20 dosed animals. Changes in the optic nerves (axonal degeneration, hemorrhage, or vacuolation) were seen in 5 males and 5 females in the high dose groups, and 7 female control animals. — attributed these changes to the blood sampling procedure and not to the T-20 treatment.

At the end of the 4-week recovery period, none of the males had any gross observations or histopathologic findings. Red areas or foci in the lungs were found in 3 of 5 females in the high-dose group and in 1 control female.

Two neoplasms were seen: a mammary gland carcinoma in a female and a pituitary adenoma in a male. Both were in control animals.

— concluded that subcutaneous injection of T-20 to rats at doses of 1.2, 4.5, and 15 mg/kg twice daily (2.4, 9.0, and 30.0 mg/kg/day) for six months did not produce any adverse effects, and that the no observed effect level (NOEL) (actually, the no observed adverse effect level, NOAEL) under the conditions of this study was 30.0 mg/kg/day.

Reviewer comments:

When this study was first reviewed this reviewer concluded that no adverse events in this study stand out as clearly drug-related but that frequent adverse findings at the injection sites were possibly related to T-20. In subsequent animal and human studies, injection site reactions were identified as primary adverse drug effects.

The NOAEL in this rat study for adverse systemic effects (30 mg/kg/day) is approximately a human equivalent dose (HED) of 4.8 mg/kg/day, on a body surface area basis. The safety margin of the clinical dose (180 mg/day) would be approximately 1.6 for a 60 kg human. However, this calculation is not appropriate when considering hypersensitivity reactions because they are not dose-dependent. For local adverse reactions (e.g., injection site reactions) the rat NOAEL (30 mg/kg/day) is approximately 10 times the human dose on a mg/kg basis.

30. NDA 21-481 Reference 3103, Volume 15. IND—Serial 284.

Ro 29-9800/000 (T-20 Fusion Inhibitor): A seven-day subcutaneously (b.i.d.) toxicity, local tolerance and toxicokinetic study in male rats. Roche Study 07496. Final Report RR 1005285, February 27, 2002.

Roche conducted this non-GLP study during January 2001 to determine the maximum dose of T-20 to be administered in subsequent long-term repeat-dose toxicology studies in rats. — analyzed the plasma samples for this study using an — assay.

Five groups of 9 male rats (HsdBrH—) were administered subcutaneous doses of 0, 31.25, 62.5, 125, or 250 mg/kg/day twice daily (0, 15.625, 31.25, 62.5, or 125 mg/kg/dose) for seven days. The lowest T-20 dose (31.25 mg/kg) was chosen to approximate the highest dose administered in Trimeris — study 98-2579 (34.5 mg/kg). Trimeris, Inc. considered that dose to be a NOAEL. The highest dose in this study was chosen to approximate the maximum subcutaneous injection volume for rats and the maximum achievable concentration of T-20.

Rats were approximately 6 weeks old and the mean body weight was 190.4 grams at the start of the study.

Clinical observations were made daily beginning on Day -4, and were recorded on Days 0, 7, and before and after dosing on other days when treatment-related observations were made. Body weights were recorded on Days -4, 0, and 7. Food consumption was recorded on Day 7 only. Baseline food consumption was lost to technical error. Blood samples for toxicokinetic evaluation of Ro 29-9800 (T-20) and Ro 50-6343 (metabolite) were collected on Days 0 and 6 prior to dosing and at 0.5, 1, 2, 4, 6, and 12 hours post dosing. Each animal was bled two or three times on each day. Hematology analyses were performed on the same blood samples that were collected for toxicokinetics on Day 6 at 0, 0.5, and 1 hour post dosing and on samples collected at necropsy (after an overnight fast). Blood for clinical chemistry analyses was collected at necropsy. Gross examination of tissues was conducted at necropsy on Day 8 and approximately 40 tissues from each animal were prepared for histopathologic examination. Microscopic examination was conducted of all injection site tissues, as well as any gross lesions or masses from all animals in all groups.

Results:

There were no deaths on the study and no treatment-related clinical observations. Mean body weights were not reduced during the study. Mean body weight gain was reduced (-16%), but not statistically significantly, in groups receiving T-20 at 125 and 250 mg/kg/day.

Mean red cell distribution width (RDW) was statistically significantly decreased (-10%) in rats dosed at 125 and 250 mg/kg/day. The sponsor indicated that this change was due to elevated control values compared with historical controls. Statistically significant decreases in total protein, albumin, calcium, phosphorus and triglyceride levels (-3%, -7%, -4%, -9%, and -40%, respectively) were observed in rats receiving 250 mg/kg/day. The sponsor considered that the values of all of these chemistry values were within historical limits and not toxicologically relevant.

Treatment-related macroscopic findings (reddish discoloration) were observed in the injection site tissues in all groups receiving T-20.

Microscopic changes at the injection sites included serocellular crusts (scabs) on the epidermal surfaces, occasionally with acute inflammatory cells (neutrophils) and erosion of the epidermis. Adjacent epidermis was often characterized by epithelial hyperplasia. Slight to moderate hemorrhage and edema (with macrophages) was seen in many of the treated animals. Subcutaneous skeletal muscle consistently presented with minimal to marked chronic inflammation, degeneration, and regeneration. Incidence and severity of inflammation, epidermal erosion, subcutaneous hemorrhage, subcutis inflammation, and inflammatory degeneration in subcutaneous skeletal muscle increased with dose. Epidermal scabs and epithelial hyperplasia (acanthosis) occurred in all dose groups, including controls, but occurred more frequently (11/36 injection sites) in the high dose group. Edema occurred in all dose groups without dose proportion.

A well-defined granuloma with necrotic center, neutrophils, hemorrhage, macrophages and fibroblasts was present in the subcutis of one animal given 250 mg/kg/day. Giant cells containing non-birefringent foreign material in the cytosol were present around a large blood vessel in the tissue of another high-dose animal.

Toxicokinetic parameters were as follows:

Dose mg/kg/day	AUC _{0→12h} μg-h/mL	AUC/dose (μg-h/mL)/ (mg/kg)	C _{max} μg/mL	T _{max} hours
Ro 29-9800 (T-20)				
Day 0				
31.25	18	1.2	5.5	2
62.5	19	0.59	5.3	1
125	25	0.40	6.2	2
250	19	0.15	5.6	0.5
Day 6				
31.25	38	2.5	9.7	2
62.5	59	1.9	15	1
125	77	1.2	25	0.5
250	142	1.1	24	4
Ro 50-6343				
Day 0				
31.25	22	1.4	2.8	2
62.5	22	0.69	2.8	2
125	26	0.41	3.2	4
250	21	0.17	3.3	2
Day 6				
31.25	41	2.6	5.4	4
62.5	58	1.9	7.3	6
125	55	0.87	6.6	2
250	95	0.76	13	4

There was no appreciable increase in AUC_{0→12h} or C_{max} with increasing T-20 dose, on Day 0 for either T-20 or the metabolite Ro 50-6343. Less than dose-proportional increases in AUC_{0→12h} and C_{max} were observed at all doses on Day 6, although saturation of absorption was not observed. Exposures were 2- to 7-fold higher on Day 6 compared with Day 0. The sponsor suggests that this may be due to increased absorption of T-20 on Day 6 compared with Day 0 since the elimination slope of T-20 is unchanged.

The sponsor concluded that T-20 at repeat doses up to 250 mg/kg/day for one week was well tolerated in male rats.

31. NDA 21-481 Reference 3111, Volume 15. IND ——— Serial 284.

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. Roche Study 07595. Final Report, RR 1005341, February 13, 2002.

Roche conducted this non-GLP study during May, June and July 2001 to determine the plasma kinetic profile of Ro 29-9800 (T-20) and Ro 50-6343 in female rats after a single subcutaneous dose of 60, 125, or 250 mg/kg. Twelve rats (from _____) were assigned to each group. Bleeding times were 0, 0.5, 1, 2, 4, 6, and 12 hours post dosing. Clinical observations were recorded prior to dosing and approximately 30-60 minutes after dosing. Body weights were recorded on the day of dosing to determine dose volume. _____ analyzed the plasma samples for this study using an _____ assay.

Results:

There were no deaths and no adverse clinical signs recorded during this study.

Toxicokinetic parameters were as follows:

Dose mg/kg	AUC _{0-12h} µg-h/mL	AUC/dose (µg-h/mL)/ (mg/kg)	C _{max} µg/mL	T _{max} hours
Ro 29-9800 (T-20)				
60	59	0.98	16	1
125	93	0.74	15	2
250	120	0.48	26	0.5
Ro 50-6343				
60	23	0.38	3	4
125	32	0.26	4	6
250	52	0.21	8	6

There was a less-than-proportional increase in exposure to both T-20 and Ro 50-6343 from increased doses of T-20. Exposure values for T-20 at 125 mg/kg and 250 mg/kg were about 75% and 45%, respectively, of the exposures calculated from the ratio of doses. Exposure values for Ro 50-6343 at 125 mg/kg and 250 mg/kg were approximately 65% and 58%, respectively, of the calculated exposures from increased doses.

Exposure ratios (Ro 50-6343:Ro 29-9800) were approximately 40% and 23% for AUC and C_{max} values, respectively.

The sponsor noted that T-20 absorption did not appear to be saturated in this study since the AUC curves did not plateau at the C_{max} values in the upper two dose groups. Peak plasma concentrations were similar in the two higher dose groups, but one animal had an unusually low plasma T-20 concentration and unusually high plasma Ro 50-6343 concentration at the 1-hour timepoint, which suggests higher metabolism in this rat.

Reviewer comment:

In Roche Study 07431, the sponsor attributes the variation in toxicokinetic parameters to possible variation in methodology. In this study, plasma drug variability was attributed to the metabolism of the rats. Since the earliest T-20 rat studies (1995-1998 Serial nos. 000, 047) rat plasma concentrations of T-20 have included high variability. The initial analytical method was ~~_____~~. See, for example, Trimeris ~~_____~~ study 98-2579 (Serial 047). The monkey model does not seem to have demonstrated as high exposure variability as the rat model.

32. NDA 21-481 Reference 3112, Volumes 16-18. IND ~~_____~~ Serial 319.

Ro 29-9800/000 (T-20, Fusion Inhibitor): A four-week b.i.d. subcutaneous (injection) toxicity study in rats with T-20 and impurities. Roche Study 07678. Final Report RR 1006069, April 22, 2002.

Roche conducted this GLP study during October and November 2001 to qualify a batch of T-20 that had impurities that had not been present in drug batches used in previous nonclinical or clinical studies. That batch was designated the "Qualification Batch" and in this study it was

compared with a "Comparator Batch" that had been previously used in nonclinical and clinical studies.

Doses of 10 or 30 mg/kg/day of the Qualification Batch or 30 mg/kg/day of the Comparator Batch were administered subcutaneously for 4 weeks to 12 rats/sex/group in the toxicity study and 10 rats/sex/group in the toxicokinetic study. Both the toxicity and toxicokinetic portions of the study had a vehicle control group. All solutions were administered b.i.d. (half the daily dose and approximately 12 hours apart) in a volume of 1 mL/kg/injection.

Qualification Batch: Roche Colorado Corporation, drug substance lot no. B00101R001.
Comparator Batch: _____, T20 for Injection, lot no. _____ 006 (Trimeris lot no. ADP0415). The vehicle control was _____. The rats were Crl:CD[®](SD)IGS BR rats from _____ and were about six weeks old and weighed an average of 189 grams (males) and 156 grams (females) at the beginning of dosing.

Plasma samples (at least 0.3 mL) were collected from 3 rats/sex/time point in the toxicokinetic study on nominal Day 0 (first day of treatment) and Day 28. Animals were bled prior to morning dose and at 30 minutes, and 1, 2, 4, 6, and 12 hours after dosing (and prior to the second dose). Samples were prepared and sent to _____ for analysis of Ro 29-9800 (T-20) and the metabolite Ro 50-6343 by a validated _____ method. The lower limit of quantitation for both compounds was _____.

At necropsy, serum samples from toxicology rats were collected for possible antibody determination. The protocol was amended to state that the antibody analyses were not done but the sponsor might do them. From all toxicity animals, 6 organs per animal were weighed and approximately 40 tissues per animal were collected for histopathologic examination.

Results:

All dosing solutions were within the acceptable range (90% to 110%) of nominal values at the beginning of treatment. At the end of treatment the Qualification Batch solutions were still within range, but the Comparator Batch solution assayed at 115.3% of the nominal concentration (____ mg/mL solution). One month later, the Comparator solution was at 110% of nominal value. The sponsor explained in a correspondence with the Division (IND _____ Serial 387) that the Comparator solution assayed at the end of four and eight weeks was from a different reconstituted vial than the dosing solution used at the beginning of the study. It assayed at _____ mg/mL and _____ mg/mL at the time it was submitted (end of study Week 4) and one month later, respectively. The decrease in Ro 29-9800 concentration at study Week 8 was attributed to degradation due to nonstandard storage conditions (5°C).

Mean C_{max} values for Ro 29-9800 (T-20) were higher by about 32% in the rats that received Qualification Batch solution at 15 mg/mL compared with those that received Comparator Batch solution at the same concentration, except that females on Day 28 that received Comparative Batch solution had a lower mean C_{max} value by about 32% compared with those females that received Qualification Batch solution. All rats that received Qualification Batch solution at 5 mg/mL had lower AUC values than those rats that received 15 mg/mL solutions. But on a per dose basis, the rats that received the smaller concentration had higher AUC values (i.e., exposure was less than dose-proportional). AUC values of Ro 29-9800 were 1.4 to 2.5 times greater on

Day 28 compared with Day 0. Males had 2% to 55% higher AUC values for a given dose than females.

C_{max} values for the metabolite Ro 50-6343 were also higher in those rats that received Qualification Batch solution at 15 mg/mL compared with those that received Comparator Batch solution at 15 mg/mL. The differences ranged from 3% to 57% over the Comparator Batch C_{max} values. AUC values were similar between the two batches at the higher concentration, were lower with the lower concentration of Qualification Batch solution, but higher on an AUC per dose basis with 5 mg/mL (Qualification Batch solution) compared with the — batch solutions at 15 mg/mL.

T_{max} values ranged from 0.5 hour to 2 hours for Ro 29-9800 and 1 to 6 hours for Ro 50-6343.

Three vehicle control samples tested positive for Ro 29-9800. Two of those (7 and 9 ng/mL) were close to the limit of detection (—). The third sample (76 ng/mL) was a 12-hour time sample from a male rat on Day 28. The sponsor stated that there was no record indicating that misdosing had occurred and no detection of Ro 29-9800 in a sample from this animal drawn two hours earlier.

In the toxicity study, there were no mortalities or early sacrifices and no treatment-related effects on body weight, food consumption, neurological findings, ophthalmological findings, hematology, or organ weights.

All the treated males had increases (+26% to +30%) in mean cholesterol levels compared with controls and the Qualification Batch-treated males had an increase in mean triglyceride levels (+29% and +45%, 10 and 30 mg/kg/day groups, respectively) compared with controls. The sponsor did not consider that these changes were treatment-related because (the sponsor stated) they were within the normal historical reference ranges and lacked dose response relationships. However, the sponsor did not provide the reference ranges and triglyceride changes do show an increase with dose.

Clinical findings were restricted to injection sites (at the nape of the neck) and included swelling and/or hardened skin in the two high dose groups, and scabs in some of the animals in the high dose Qualification Batch group. Gross findings at necropsy among animals treated with 30 mg/kg (either the Qualification or Comparator Batch solution) included discoloration (reddish) (19/24 and 19/24 animals) and firmness at the injection sites (20/24 and 20/24 animals). Histopathologic findings at the 30 mg/kg/day dose level included accumulation of eosinophilic-hyaline material, chronic and granulomatous inflammation, necrosis, and fibrosis in the subcutaneous areas at the injection sites. These findings were similar in incidence and severity in animals receiving the Qualification Batch and the Comparator Batch drug and therefore the sponsor did not review the injection site tissues of lower dose Qualification Batch rats.

The sponsor concluded that repeated twice daily subcutaneous injections of two different batches of Ro 29-9800 to rats for four weeks at doses up to 30 mg/kg/day produced generally comparable toxicokinetic profiles and no adverse systemic toxicities. Treatment-related toxicities were limited to inject site reactions and were similar to those observed in previous studies of the drug. The sponsor concluded that the presence of — impurities in the Qualification Batch did not affect the toxicokinetic or toxicologic profile of Ro 29-9800.

Reviewer comments:

The sponsor's conclusions are generally supported by the data. While the increase in triglyceride levels in males receiving the Qualification Batch drug (compared with controls) cannot be ruled out as a drug-related and batch-specific effect, the magnitude of the change is moderate and the biological significance of the observation is not known.

Anomalies in dose solution analyses and rat plasma drug concentration analyses suggest that the analytical methods for Ro 29-9800 and Ro 50-6343 are prone to errors.

33. NDA 21-481 Reference 3113, Volume 18. IND _____ Serial 339.

A toxicokinetics report on a 28-day toxicity study of T-20 peptide administered subcutaneously via continuous infusion in the rat. _____ Study 98-3645. Trimeris document EDS105.R01-00/01, March 11, 2002, amended April 30, 2002.

This toxicokinetics study was part of a larger toxicology study, which the sponsor did not submit. _____ conducted the toxicology study and collected and shipped plasma samples to Trimeris, Inc. for analysis. The toxicokinetics portion of the study was not conducted under GLP conditions.

Four groups of 10 rats (5 male and 5 female, albino, outbred, VAG/Plus[®], CrI:CD[®] (SD)IGS BR, _____) were administered 0, 1, 4, or 13 mg/kg/day of T-20 by continuous subcutaneous infusion (0.02 mL/h). Blood samples for toxicokinetic analysis were collected from 2 or 3 rats/sex/group on Day 1 at pre-dosing, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 3.5, 4.5 and 5.5 hours post dosing and on Days 7 and 14 (single samples, collection times post dosing not given).

The study protocol called for dosing for 28 days, bleeding a different number of rats than were actually bled, and for blood samples to be collected on Days 1, 7 and 28 rather than Days 1, 7, and 14. However, the toxicology study was terminated on Day 11 after the study administrators determined that the intended doses could not be assured. There were problems with the dosing procedures including precipitate formation on the exterior surface of the injection cap, occlusions in the infusion catheter and lumps at the tip of the catheter. Blood samples collected according to protocol for serum antibody assessment were not analyzed.

Plasma samples collected on Days 1 and 7 were analyzed by a _____ labeled _____ method (lower limit of quantitation approximately _____). Plasma samples collected on Day 14 (controls and low dose group only) were analyzed using a T-20 _____ method (lower limit of quantitation _____).

Results:

Most, but not all of the plasma samples collected on Day 1 had detectable levels of T-20. Day 1 T-20 plasma concentrations were erratic (moved up and down repeatedly) over time (post dosing) in all dose groups. Data were presented for one rat (low dose group) bled on Day 1, whose sex was not known.

Day 1 calculated pharmacokinetics parameters (each dose group n = 2 or 3).

Dose (mg/kg/day)	C _{max} (µg/mL)	C _{ave} ^a (µg/mL)	t _{max} (h)	AUC _(0-5.5h) (µg-h/mL)	AUC _(0-24h) (µg-h/mL)
1	0.95	0.53	2	2.9	10.2
4	5.6	2.4	1.5	13.3	42.5
13	13.8	28.5	3	28.5	107.6

^a C_{ave} is the average T-20 plasma concentration over the interval 0 to 5.5 hours.

Observed C_{max} and AUC_{0-24h} values on Day 1 increased approximately proportionally to administered dose among the lower two dose groups, but increased less than dose proportionally for the high dose group.

On Day 7, one rat in the low dose group had a detectable plasma T-20 concentration (— µg/mL). All the other rats bled on Day 7 and all the rats bled on Day 14 had no detectable plasma levels of T-20.

Reviewer comment:

This study report does not specify when the study was conducted. It appears that this study was an early study that Trimeris conducted when it was exploring different T-20 routes of administration. This study is one of several that showed that continuous subcutaneous infusion was not a dependable route of administration.

34. NDA 21-481 Reference 3114, Volume 159. IND — Serial 381.

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). Trimeris document, EDS107.R01-00, June 3, 2002.

This report contains the results of serum antibody analyses from samples collected from the 6-month repeat-dose T-20 rat study that — conducted for Trimeris, Inc. in 1998 (— Study 98-2579, Reference 3102 of this NDA). The Trimeris Exploratory Development group completed the antibody analyses in 2002 under GLP conditions. These are the only anti-T-20 serum antibody analyses of samples from rats submitted to this NDA.

Four groups of 5 rats/sex/group were treated twice daily with T-20 via subcutaneous injection. Doses in the first 28 days were 0, 1.38, 5.175, and 17.25 mg/kg/dose. Doses from Day 29 to the end of the study were 0, 1.2, 4.5, and 15 mg/kg/dose. Samples from the four dose groups were collected at predose, week 1, and months 1 through 7. Trimeris received a total of 340 serum samples from — Antibody titers were measured using an indirect — method. The endpoint titer corresponds to the highest dilution at which the mean signal to noise ratio is 3:1 and the minimum measurable titer was 1:100.

There were no measurable anti-T-20 antibody titers for any rats from Groups I or II (placebo and low T-20 dose groups) or from any pre-test samples. Among the 80 Group III samples and 90 Group IV samples, 2 and 6 serum samples, respectively, had positive titers. All titers, except one, were 1:100. The exception—from a Group IV male at month 2—was 1:400. The sponsor

concluded that rats develop little or no measurable T-20 reactive antibody response when chronically treated with T-20 under the conditions of the study.

Trimeris also retested 10 serum samples from the 9-month cynomolgus monkey study (conducted in 1998-1999) to evaluate the stability of the antibody titers under storage conditions. Six of the 10 samples were originally assayed in 1999 and the remaining 4 in 2001. Of the 10 samples, none showed a change in the endpoint titer from the original assay. These results (not provided) suggest that the frozen anti-T-20 serum antibodies remain stable over time.

35. NDA 21-481 Reference 3115, Volume 193. IND ~~Serial~~ Serial 018, 047, and 202.

Toxicokinetic analyses 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."

Trimeris Inc. final report, November 23, 1999.

~~Study~~ Study conducted the in-life portion of this GLP study (~~98-2579~~, Reference 3102 of this NDA) for the sponsor. Trimeris, Inc. conducted the toxicokinetic analyses.

Blood was collected from 5 animals/sex/group at 1.5 hours post dose (expected C_{max}) on study Day 1 and at the end of each month, and from 3 animals/sex/group on study days 7 and 36 at times 0.5, 1, 2, 4, 6, and 8 hours. Blood collection was rotated among the animals in each group throughout the study. On days 7 and 36, a maximum of 3 blood samples were collected from any one animal.

T-20 concentrations were determined by a ~~method~~ method to verify doses. Mean concentrations of the three dose solutions throughout the study were 95.3%, 99.7%, and 96.3% of nominal values (n=7 for each dose). The greatest deviation from theoretical dose was that the low dose group was 81.7% of nominal dose 1.2 mg/mL at 5-months.

Reviewer comments:

Table 1 showing the analyses of T-20 solutions makes no reference to the change in dosing from the first 28 days of the study (0, 2.76, 10.35, and 34.50 mg/kg/day) to the final 5 months of the study (0, 2.4, 9.0 and 30 mg/kg/day). All analytical data are compared with theoretical doses from the latter series. Trimeris indicated that the change in dosing regimen made no difference to the goal of attaining $\mu\text{g-h/mL}$ T-20 plasma concentrations in the high dose group. Because the Table 1 calculated "% of Theoretical Concentrations" values for days 1-28 are apparently incorrectly based on the post-Day 28 solution concentrations, the first three sets of calculated percentages are higher in the table than they should be. This discrepancy should not affect other results.

The plasma concentration-time curves at days 7 and 36 are parallel and similar. However, the error bars are large, which the sponsor attributes to the sparse data and rotating sampling technique. The sponsor also mentions that the standard curve for the analyses on Day 36 did not extend to the normal lower limit of quantitation but that the data were acceptable (though not fully valid) based on other criteria.

Trimeris refers to the single monthly measurements at 1.5 hours post dosing as “C_{max} values,” but this is not necessarily correct. They are replicate interval (1.5 hours) concentration values. On Days 7 and 36 multiple blood samples were collected and values of t_{max} varied between 1.4 and 2.1 hours. Group mean replicate interval values were relatively constant throughout the 6 months. (Month 3 data were discarded because of “unacceptable assay performance.”) Trimeris stated that the relative consistency of the “C_{max} values” indicates that the target levels were maintained. However, since they are not necessarily C_{max} values, the conclusion is not valid, although the AUC variance is apparently not large.

Values of AUC_(0→12) for the 15 mg/kg/dose group calculated from multiple time measurements on days 7 and 36 were within 89% and 98% of the target exposure level of — μg-h/mL. Trimeris concluded that the highest dose in the current study exceeded the maximum AUC observed in the clinic by a factor of 1.1, but it is not clear which clinical data they are comparing these results to.

	Dose level (mg/kg/dose) (Study day)					
	1.2 (7)	4.5 (7)	15.0 (7)	1.2 (36)	4.5 (36)	15.0 (36)
t _{1/2} , elim. (h)	2.3	2.4	2.2	2.1	1.5	1.3
C _{max} (μg/mL)	2.55	4.45	8.42	1.84	3.82	8.27
t _{max} (h)	1.6	1.4	1.6	1.6	2.1	2.1
AUC _(0→8h) (μg-h/mL)	10.5	18.0	33.1	7.43	18.8	37.2
AUC _(0→12h) (μg-h/mL)	11.5	19.9	35.7	8.00	20.1	39.1

In conclusion, this toxicokinetics study produced consistent and apparently reliable data.

Repeat Dose – Monkey

36. NDA 21-481 Reference 3106, Volume 19. IND ——— Serial 000, 003, 247.

Twenty-eight day repeated dose toxicity study of T-20 peptide administered intravenously to cynomolgus monkeys. ——— Study 3-B45. Final Report, December 13, 1996, amended March 27, 2002.

————— performed this GLP study during June and July 1996 for Trimeris, Inc. The cynomolgus monkeys (*Macaca fascicularis*) came from ———

This study consisted of three T-20 dose groups plus placebo: 0, 0.2, 0.6, and 2.0 mg/kg/dose, administered intravenously via the saphenous or cephalic vein twice daily for 28 days (i.e., total daily doses of 0, 0.4, 1.2, and 4.0 mg/kg/day). Each dose group consisted of four monkeys of each sex (32 monkeys total).

Cage-side observations for mortality and morbidity were performed twice daily and clinical observations were performed daily. Body weights were recorded at Day -1 and weekly thereafter. Food consumption was recorded daily. Ophthalmic examinations and electrocardiograms were conducted prior to treatment and one or two days prior to necropsy. Blood was collected for hematology, clinical chemistry, and pharmacokinetics and antibody

analyses. Forty or more organs were examined at necropsy; eight organs were weighed at necropsy, and tissues in the control and high-dose groups were examined microscopically (histology).

Results:

There were no deaths during the study. The primary clinical observation was bruising at the sites of dosing and blood collection. There were no important changes noted in body weights or food consumption during the study. There were no ophthalmological findings attributed to T-20 and no abnormal electrocardiograms at pre-study or at the end-of-study examinations. Fluctuations in red blood cell counts and white blood cell counts were all within the range of control values during the study and there were no significant differences in any hematological parameters between treated and control animals.

Chloride levels were elevated in mid-dose males on Day 1 (+6%), and in all three dose groups on Day 15 (to +5%) compared with controls. At the end of the study there were no differences in chloride levels between the T-20 groups and controls.

Glucose values in high dose males were elevated on Day 15 (+35%) and reduced in mid-dosed males on Day 29 (-26%) compared with controls, but the values were within the range (69 to 101 mg/dL) exhibited by controls during the study.

Low-dose females showed decreases in calcium (-7%) and triglycerides (-61%) on Day 15 compared with controls. High dose males and females showed a decrease in blood urea nitrogen (BUN) on Day 29 compared with controls (-21% and -38%, respectively).

Gross necropsy observations included red or purple foci at the sites of dose administration, spleen lymphoid hyperplasia, foci in spleen, liver, lungs, and nodules in cecum, discolored lymph nodes, and small testes. The researchers attributed the foci and nodules to parasites. Malarial parasites were noted in red blood cells in 1 male and 5 females on Day 29. The researchers attributed small testes to sexual immaturity of young monkeys. None of the observations occurred in all four animals in a group and most of the more important observations (spleen lymphoid hyperplasia, foci in the lungs and liver) that occurred in the high dose group also occurred in the controls.

Histologically, there were more animals with lung foreign body emboli in the T-20 dosed animals than in the controls (8/12 T-20 males, 1/4 control males, 5/12 T-20 females, 0/4 control females). However, lung emboli occurred in controls and there was no clear dose-related response in the T-20 dosed animals.

The frequency of nuclear inclusions and erythroid depletion in bone marrow was greater in the T-20 animals than in the controls. (See table below.)

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Histopathologic Incidence, Bone Marrow, Day 28 Necropsy

	0 mg/kg/d		0.4 mg/kg/d		1.2 mg/kg/d		4.0 mg/kg/d	
	Males N = 4	Females N = 4	Males N = 4	Females N = 4	Males N = 4	Females N = 4	Males N = 4	Females N = 4
Bone marrow								
Nuclear inclusions	1	1	4	1	4	3	4	2
Erythroid depletion	1	0	3	0	3	2	3	1
Increased blasts	0	0	0	0	1	0	0	0

The severity of spleen hyperplasia also appears to be increased in the T-20-dosed animals. The pathologist noted splenic hyperplasia in 7 of 8 animals in the control group and in the first two T-20 groups, and 8 of 8 animals in the high dose group. But the grading increased from 1 animal with minimal splenic hypertrophy, 3 mild, and 3 moderate in the control group, to 1 mild, 4 moderate, and 2 marked in the middle dose group, and 1 minimal, 1 mild, 5 moderate, and 1 marked in the high dose group. Similarly, the spleen weights and the spleen weights as a fraction of body weights at necropsy increased in the T-20 dosed groups.

T-20 dose	0 mg/kg/d	0.4 mg/kg/d	1.1 mg/kg/d	4.0 mg/kg/d
Mean spleen weight, N=8	10.4 g	7.9 g	13.1	21.7
Mean spleen weight/body weight ratio	0.33	0.26	0.42	0.77

states there were no statistically significant differences in organ weights between T-20-dosed animals and controls. One female in the high-dose group had a large spleen: 89.3 grams compared with the female control mean spleen weight of 7.29 grams and a range among other female spleens in the same (high) dose group of 5 to 17 grams. Hence, according to the sponsor, there was no drug effect since the other female spleen weights in the high-dose group were normal.

Reviewer comment:

The one large (female) spleen is 3.3% of its body weight, and almost 5 times larger than the next largest animal spleen as a percent of body weight. Histologically, this spleen was graded a "3", or moderate hyperplasia (not the highest grade).

Blood was drawn from two monkeys of each sex per dose group on days 1 and 7 for pharmacokinetics measurements. Blood collection was made at predosing, immediately after dosing, and at 0.25, 0.5, 1, 2, 3, 6, and 12 hours post dosing (at the first dosing of the day, only). Because the T-20 concentrations in males and females were similar within each dose level, the means were calculated from all four animals per dose group, except one value (0.2 mg/kg female, Day 7) was low and was not included in the mean.

Mean plasma C_{max} levels and $AUC_{(0-\infty)}$ values increased approximately linearly over the range of dosing on both days 1 and 7. Mean terminal plasma elimination half-life ($t_{1/2}$) was approximately 3 hours regardless of dose level and day of study.

T-20 dose mg/kg	C _{max} µg/mL		AUC _(0-∞) µg-h/mL		t _{1/2} hours	
	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7
0.2	4.79 ± 0.58	3.78 ± 0.49	6.8 ± 0.91	5.7 ± 0.80	3.1 ± 0.16	3.1 ± 0.35
0.6	14.63 ± 1.07	11.88 ± 0.84	20.5 ± 4.51	17.7 ± 3.72	3.2 ± 0.26	2.8 ± 0.21
2.0	45.91 ± 9.12	41.17 ± 3.68	51.6 ± 10.85	48.4 ± 10.40	2.8 ± 0.05	2.7 ± 0.26

Blood was collected from one male and female from each dose group for antibody testing. Animals were selected that were not a part of the pharmacokinetics study. Blood was collected prior to the first treatment and weekly thereafter. Antibodies to T-20 were detected in animals in the 2.0 mg/kg/dose group only, and the titers increased from Day 7 (1:100) to Day 28 (1:8000 in the male and 1:900 in the female).

_____ attributed no changes in behavior, general physiology, body weights, ophthalmological and EKG measurements, hematology, clinical chemistry, organ weights, or gross and microscopic pathology to T-20. _____ concluded twice daily dosing of cynomolgus monkeys with T-20 for 28 days was well tolerated at all dose levels, and the no observable effect level (NOEL) was 4 mg/kg/day (2.0 mg/kg/dose).

Reviewer comments:

It is unclear whether effects noted in the lung, spleen, and bone marrow are drug-related or not. The researchers considered that all of the histologic and organ weight findings were incidental and not drug-related. But the differences in the frequencies of occurrence of lung foreign body emboli, bone marrow nuclear inclusions, and bone marrow erythroid depletion between T-20 treated animals and controls, the difference in severity of splenic hypertrophy between the T-20 treated animals and the controls, and the exceptionally large spleen in one female, are notable and suggest an immune response. If there are dose-response effects, they are not obvious from these data. If the effects are drug-related, this study cannot address whether or not they are reversible because there was no recovery period included in its design.

The _____ claimed that the high dose in this study (4 mg/kg/day) is a NOEL. This is doubtful. However, subsequent to this study, Trimeris switched to a subcutaneous route of drug administration, which has shown to have a much lower risk for adverse systemic effects. Subsequent clinical and animal studies support the likelihood that adverse effects at the injection sites and antibody production are related to the administration of T-20.

37. NDA 21-481 Reference 3107, Volume 20. IND _____ Serial 024, 025, 047, 333.

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. _____

_____ Study 98-3373. Final Report, November 18, 1999, amended May 9, 2002.

_____ conducted this GLP study during July and August 1998 to compare pharmacokinetics, antibody formation, and potential toxicity to two different formulations of T-20 (Route 1 and Route 2) when administered to cynomolgus monkeys via twice daily subcutaneous injections. _____ sent blood for toxicokinetics and antibody measurements to Trimeris, Inc. for analysis and reporting. Trimeris did not conduct

analyses under GLP conditions. The cynomolgus monkeys (*Macaca fascicularis*) used in this study were from _____

In this study, "Route 1" and "Route 2" are two manufacturing procedures. Trimeris, Inc. introduced the Route 2 method to the FDA in a letter (IND _____ Serial 019) dated June 9, 1998, and informed the FDA in another letter dated November 19, 1998 that it would be using Route 2-manufactured product in its clinical studies.

T-20 from one of two formulations ("Route 1" and "Route 2") was administered to cynomolgus monkeys (2/sex/group) twice daily via subcutaneous injections at dose levels of 0, 10 and 20 mg/kg/day for a period of 28 days (20 monkeys total). All animals received injections at a dose volume of 1 mL/kg/injection.

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 twice predosing and weekly during the study for general conditions, eyes, nose, external genitalia, skin and fur, respiration, cardiovascular system, and the central and autonomic nervous system. Ophthalmoscopic examinations were conducted predosing and on Day 27. Animals were weighed two times before dosing and weekly during the study and at necropsy (fasted). Food consumption was visually estimated and scored (0 to 5) daily for one week before dosing and daily throughout the study for each animal. Blood was collected from animals for hematology, clinical laboratory examinations, toxicokinetic evaluations, and serum antibody analysis.

Macroscopic examinations were conducted immediately postmortem. Seven selected organs were weighed from all animals. Microscopic (histopathologic) slides were prepared and examined for 37 tissues from animals in Groups I, III, and V (the placebo and high-dose groups of each formulation).

Results:

There were no mortalities. Apart from injection site effects, _____ attributed no adverse affects to the administration of T-20.

Discoloration at the injection sites was seen macroscopically in all 20 animals (including controls). Thickening of the skin at one of the dose sites was seen in two males and one female from Group III (the high dose, 20 mg/kg/day, Route 1 group).

Microscopically, increases in tissue damage, hemorrhage, and inflammatory lesions were noted at the subcutaneous injection sites of both groups of moneys administered 20 mg/kg/day when compared with the control animals. Eight of nine examples of the most serious ("moderately severe" or "severe") findings of inflammation or hemorrhage were noted in Group III animals (males and females). There were seven instances of necrosis at injection sites and all of them were observed in Group III animals.

One female (Group V, high dose, Route 2) was slightly emaciated at necropsy and had lost hair on both hindlimbs.

There were no obvious T-20 effects seen in hematology and clinical chemistry values. Both males in Group II (low dose, Route 1) had elevated AST values at necropsy compared with their

predose values (mean of +245%) and compared with controls (+474%). But these effects were not seen in Group III (high dose, Route 1) monkeys.

One male (Group IV, low dose, Route 2) had large adrenals (155% of placebo average) and a large spleen (125% of placebo average). The largest thymus weight was a factor of 8.6 times larger than the smallest thymus weight but both of them were Group V males. The largest testes/epididymides weights recorded were a factor of 13 greater than the smallest (a control animal).

Reviewer comment:

— attributes the wide variation in the testes weights (in this study and in previous studies) to the variable sexual maturity of young males. That may be true, but it begs the question: What can be discerned from weighing the testes of variably sexually mature male monkeys?

— concluded that the inflammatory lesions in high-dose Route 1 animals were more severe compared with high-dose Route 2 animals, based on the presence of focal or multifocal necrosis in Route 1 animals only. Secondly, the no observable effect level (NOEL) for systemic toxicity under the conditions of this study was 20 mg/kg/day.

Reviewer comments:

The Route 1 formulation appears to be significantly more toxic than the Route 2 formulation for the reasons that — presents. However, a NOEL (NOAEL) of 20 mg/kg/day for systemic toxicity "under the conditions of this study" is a very limited NOEL because of the small number of animals per group (two per sex), and the small number of observations and measurements.

We know from the 9-month monkey study (— 98-3371) that subcutaneous administration of 20 mg/kg/day of T-20 is not a NOAEL in cynomolgus monkeys. It is noted that the present study ended before both the 6-month rat study (— 98-2579) and the 9-month monkey study were completed. In the 9-month monkey study, elevated eosinophils and antibody production indicated an immune response to T-20 at 10 mg/kg/day. In this study, elevated eosinophils were present but they were not considered significant because there were too few data to derive significance from them. Also, the 9-month monkey study showed antibody production by Day 29, but — would not have had that information at this study's completion and — did not do the antibody analysis for this study.

Blood was collected for pharmacokinetics analyses from all animals on study days 1 and 28 at 0.5, 1, 2, 3, 6, and 8 hours post dosing. Dosing solution concentrations were confirmed by spectrophotometric methods and T-20 concentrations in serum were measured using — and —

Results:

Dosing concentrations of the T-20 solutions were between 89% and 109% of the nominal values. All of the high dose solutions (10 mg/mL) were greater than 100% of nominal value, and three of four low dose solutions (5 mg/mL) were below the nominal value.

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Pharmacokinetics parameters on Day 1 were as follows:

	T-20 Synthesis Route / Dose level			
	1 / 5 mg/kg	1 / 10 mg/kg	2 / 5 mg/kg	2 / 10 mg/kg
T_{max} (hours)	1.8 ± 0.5	2.0 ± 0.0	2.3 ± 1.0	2.0 ± 0.8
C_{max} (µg/mL)	16.6 ± 3.5	33.3 ± 3.4	14.0 ± 1.8	31.6 ± 6.8
$AUC_{(0-8h)}$ (µg-h/mL)	79.4 ± 14.7	172.8 ± 23.2	71.5 ± 7.7	147.1 ± 50.0
$AUC_{(0-∞)}$ (µg-h/mL)	95.1 ± 13.1	215.4 ± 46.9	106.0 ± 21.3	174.1 ± 69.4

Pharmacokinetics parameters on Day 28 were as follows:

	T-20 Synthesis Route / Dose level			
	1 / 5 mg/kg	1 / 10 mg/kg	2 / 5 mg/kg	2 / 10 mg/kg
T_{max} (hours)	1.6 ± 0.8	2.0 ± 0	1.8 ± 1.0	2.0 ± 0.0
C_{max} (µg/mL)	21.6 ± 7.3	45.3 ± 14.3	18.0 ± 2.2	33.2 ± 6.2
$AUC_{(0-8h)}$ (µg-h/mL)	104.0 ± 28.5	217.8 ± 75.7	100.0 ± 32.0	168.0 ± 48.3

Percentage increase in AUC values from Day 1 to Day 28:

	T-20 Synthesis Route / Dose level			
	1 / 5 mg/kg	1 / 10 mg/kg	2 / 5 mg/kg	2 / 10 mg/kg
$AUC_{(0-8h)}$ (µg-h/mL)	30%	25%	38%	6.8%
$AUC_{(0-∞)}$ (µg-h/mL)	39%	25%	48%	44%

In the T-20 concentration vs. time plots there appears to be slightly more variation among the Route 2 animals compared with the Route 1 animals. In the composite data plot, the curves (T-20 concentration vs. time) are generally alike. Variations at 8 hours in the Route 2 plots are due in part to missing data points at 8 hours from two different animals—one on Day 1 in the low dose group and another on Day 28 in the high dose group. These discrepancies effectively raise slightly the mean AUC values of Route 2 low dose at Day 1 and Route 2 high dose at Day 28.

Exposures (C_{max} and $AUC_{(0-8h)}$) were slightly higher with Route 1 drug than with Route 2 drug. The differences between Route 1 and Route 2 $AUC_{(0-8h)}$ values were greater on Day 28 than on Day 1. The mean rate of systemic absorption was apparently slightly greater for Route 1 drug compared with Route 2 drug, particularly on Day 28 and at the higher dose. The sponsor indicated that these differences do not suggest notable differences in the in vivo pharmacokinetic behavior of the Route 1 and Route 2 material. Mean t_{max} values were consistently at approximately 2 hours; mean $t_{1/2}$ values were within the range of 2 to 5 hours.

Trimeris, Inc. concluded that the mean plasma concentrations at each time point for all animals revealed a clear positive dose response for both Route 1 and Route 2 T-20 formulations on both Day 1 and Day 28. C_{max} and AUC values increased proportionately with dose on both study days. AUC values were generally greater by 10%-40% on Day 28 than on Day 1, suggesting that there was not a marked increase in systemic T-20 exposure after repeated dosing. However, the

relationship did not hold for $AUC_{(0,\infty)}$ values for low dose animals on Day 1 probably because of missing in Route 2 animals at 8 hours.

Trimeris used an indirect _____ to measure antibody levels. The method is a _____ that utilizes T-1249 and T-20 passively bound to the solid phase. The end point titer corresponds to the highest sample dilution at which the mean signal to noise ratio is 3:1; the minimum sample dilution is 1:100. The method determines antibody reactivity against both T-1249 and T-20.

There were no measurable end point titers to T-20 on study Day 1 before dosing at either T-20 concentration with either T-20 formulation or in the negative control assay. On study Day 28 pre-dosing, the negative control was still negative, but all monkeys administered T-20 (both formulations, both concentrations) had measurable T-20 titers. At the low T-20 dose, titers ranged from 1:400 to 1:3200. At the high dose, Route 1 drug produced higher titers (up to 1:12800) and higher average titers (1:7000 vs. 1:2625) compared with Route 2 drug. T-1249 titers followed the same pattern as T-20 titers but were lower by half or more for all Day 28 measurements. One animal had a minimal T-1249 titer (1:100) in the control arm at Day 28 and one animal had a T-1249 titer on study Day 1 pre-dosing. In summary, on study Day 28, the overall magnitude of anti-T-20 antibody response was similar for both formulations in the low dose group, but somewhat greater with Route 1 drug than Route 2 drug in the high dose group.

38. NDA 21-481 Reference 3109, Volumes 21-22. IND _____ Serial 029, 031, 037, 047, 333. A 9-month toxicity study of T-20 peptide administered twice per day via subcutaneous injection in the non-human primate. _____ Study 98-3371. Final Report, December 13, 1999, amended May 3, 2002.

_____ conducted this GLP study to assess the toxicity of T-20 when administered at two dose levels twice per day via subcutaneous injection to cynomolgus monkeys for a period of up to nine months. Portions of the study were conducted at _____. The protocol for this study was signed on August 5, 1998, and the first animal was dosed on August 12, 1998. Dosing was completed on May 11, 1999, and the recovery sacrifice occurred on June 8, 1999.

The study report was amended to note that Trimeris did not conduct the analyses of T-20 in dosing solutions or in toxicokinetics plasma samples in compliance with GLPs and did not conduct the analyses of serum antibodies in blood samples in compliance with GLPs. The report and review of toxicokinetic analyses and serum antibody analyses are included under Reference 3116 for this NDA.

The cynomolgus monkeys (*Macaca fascicularis*) used in this study were from _____

In this study, subcutaneous injections of two dose levels of T-20 (5 and 10 mg/kg/dose, 10 and 20 mg/kg/day) and placebo were administered twice a day for nine months. All animals received injections at a dose volume of 1 mL/kg, on one of four quadrants on a shaved area of the animal's back. The quadrant sites were rotated so each quadrant site received an injection every other day.

There were 8 animals/sex/group in the T-20 dose groups, and 6 animals/sex in the controls. After three months, necropsies were performed on 3 animals/sex/group of the T-20 dosed animals and 2 animals/sex/controls. At nine months, necropsies were performed on 4 animals/sex/group of the T-20 dosed animals and 3/sex/controls. At Month 10, the remaining animals (1 animal/sex/group) were sacrificed.

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 twice predosing and weekly during the study for general conditions, eyes, nose, abdomen and external genitalia, skin and fur, respiration, cardiovascular system, and the central and autonomic nervous system. Ophthalmoscopic examinations were conducted predosing (Day -6), and on Day 87 (Month 3) and at Day 267 (Month 9). Animals were acclimatized to electrocardiograms and ECGs were recorded predosing and approximately two hours after dosing at months 3 and 9. Animals were weighed three times before dosing and weekly throughout the study and at necropsy. Food consumption was visually estimated and scored (0 to 5) daily throughout the study for each animal. Blood was collected from animals for clinical laboratory examinations, toxicokinetic evaluations, and serum antibody analysis.

Macroscopic examinations were conducted immediately postmortem. Eight selected organs were weighed from all animals, and microscopic (histopathologic) slides were prepared and examined for 31 tissues from all animals. Samples of liver, kidney, lung, spleen, and heart were flash frozen in liquid nitrogen for possible future _____ assessment of complement fixation.

Results:

There were no mortalities. Swelling/edema at the dose site in several T-20 treated animals beginning during the fifth month of the study (Week 17) and continuing through the end of dosing was notable. Incidence was higher in males than in females, and the presentation of signs and symptoms varied from animal to animal. Incidence was similar in groups receiving either 10 or 20 mg/kg/day T-20. The total number of animals exhibiting swelling/edema at the dose site between weeks 17 and 39 were 4 of 5 males from each treated group, 3 of 5 females from the 10 mg/kg/day group and 5 of 5 females from the 20 mg/kg/day group. No edema was seen during the recovery period.

There were no abnormalities observed during the ophthalmoscopic examinations or in the electrocardiograms.

During the nine months of the study, T-20-treated males gained more weight than control males and T-20-treated females gained less weight than control females. By Week 39, both male T-20-treated groups' mean weight gain was 1.1 kg compared with 0.6 kg for male controls (+8% weight difference in treated vs. control monkeys). Mean female T-20 groups' weight gain were 0.3 kg (10 mg/kg/day group) or 0.2 kg (20 mg/kg/day group) compared with 0.8 kg for female controls (-19% weight difference compared with controls). When _____ compared the T-20-dosed animals' weight gains to historical controls (N= 21), there were no weight gain differences.

_____ stated 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, this comment apparently

resulted from a visual evaluation of the data tables since there are no numerical analyses or graphs of the food consumption data.

Reviewer comment:

These weight gain trends were opposite the results from the 6-month rat study (NDA 98-2579), i.e., the male rats gained less than controls and the female rats gained more than controls. However, the maximum weight differences between treated and control rats were approximately 5% whereas in monkeys it is closer to 20%. The comparison to historical controls suggests that the weight changes in monkeys were not biologically significant (and not drug related), however, historical control data was not provided. Supporting the researchers suggestion that the weight changes are not drug-related is the observation that the greatest divergence in weight gains in females occurs after Week 13, the three-month sacrifice. At that time, the two smallest monkeys were removed from the control pool and the second-largest monkeys (and one medium weight monkey) from each T-20 group were removed from the female T-20 groups. This effectively maximized the difference between the mean weights of the T-20 and control dose groups (or nearly so).

T-20 administration was associated with elevated numbers of eosinophils compared with control values in most ($\geq 70\%$) treated animals. The numbers of males and females whose values exceeded the control means did not differ by more than one animal in all months. The deviations from control means were most pronounced in low-dose (10 mg/kg/day) males. The maximum 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. (In Month 9, the control mean was exceptionally low, 0.63 % WBC, compared with the mean of control means, 2.98 % WBC.) One of the four animals held over to 10 months (recovery period) had an elevated eosinophil count at necropsy. This animal (a female) had approximately twice the eosinophil count as the control.

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.

These findings were consistent with microscopic tissue examination at dose sites (inflammatory infiltrate with significant eosinophilic polymorphonuclear component) and suggestive of a hypersensitivity (allergic) response to T-20.

_____ concluded that there were no effects of the administration of T-20 on the clinical chemistry values. There were a few treated animals that 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 histopathologic findings in the livers or spleens of the T-20 dosed animals. Also, the ALT values did not correlate well with the elevated eosinophil values.

Reviewer comment:

The considerable elevation of some ALT values from the norm suggests that some monkeys were not entirely healthy. They were pre-screened for Salmonella, Shigella, and tuberculosis and all animals were negative for those diseases. Blood for initial clinical chemistry measurements was collected on Day -8 (pre-dosing) of the study. There were no exclusion criteria in the protocol and the researchers did not elect to exclude any study animal. The elevated ALTs are bothersome because they cannot be explained.

There were no obvious effects of the administration of T-20 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. Non-statistical dose-related trends occurred in the following organ tissues. These may have no biological significance. The small number of animals at each necropsy and the small weights of some organs limit the interpretation of these data.

Percent differences in mean organ weights in high-dose group (20 mg/kg/day) compared with controls							
Necropsy	adrenal	heart	kidney	liver	spleen	testes	thymus
Males 3 months	-23%	-11%			-40%		-54%
Males 9 months	+19%	+15%	+16%	+17%		+25%	
Females 3 months	-15%	+18%					
Females 9 months		-32%	-16%				-56%

The most frequent macroscopic observation was discoloration at injection sites. At terminal sacrifice the injection sites of T-20 administration showed discoloration, thickening, and cysts. There was some discoloration at the sites of placebo control injection sites but the incidence and severity was much less than with the drug-treated injection sites. Discoloration and thickening correlated microscopically with subcutaneous hemorrhage, edema and fibrosis. At recovery sacrifice, discoloration was still apparent where T-20 had been injected, but not at placebo injection sites.

Microscopic changes in the spleen were attributed to T-20 dosing. Following three months dosing, the spleens of T-20 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.

Cortical lymphocyte depletion was observed in the thymuses of many T-20-treated animals following three months (1 of 6 males, 4 of 6 females) and nine months (6 of 8 males and 1 of 8 females) of dosing. One female control animal showed this effect at three months and one female control showed it at 9 months. No animal showed this at the 10-month recovery necropsy. This suggested the effect might represent a non-specific, stress-related response to the experimental

procedure. The incidence was higher in T-20 treated animals but there was not an obvious dose-related response.

Microscopic observations of injection sites following three months of T-20 dosing revealed varying degrees of subcutaneous hemorrhage, edema, and inflammatory infiltrate as well as degeneration of the fibers of the *panniculus carnosus* muscle. Edema and inflammatory infiltrate were increased at injection sites by the presence of T-20 relative to control animals but hemorrhage and muscle degeneration were of comparative severity in T-20 and control animals. Focal necrosis and fibrosis of the subcutaneous tissue were also observed in some T-20 animal injection sites, but not at the injection sites of control animals.

At the end of nine months of dosing, subcutaneous hemorrhage, edema and inflammatory infiltrate were present with a markedly greater incidence and severity at the sites of T-20 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 T-20 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 dosage groups.

Reviewer comment:

Apparently T-20 caused significant adverse effects at injection sites. The data also suggest that the placebo (mannitol and carbonate buffer) or the injection procedure caused significant adverse reactions.

Reviewer summary:

This study demonstrated adverse T-20 effects at injection sites, increased activity in the spleen, increased eosinophil (and total lymphocyte) counts, antibody titers to T-20, and a change in the absorption characteristics of the drug over the duration of the study. These effects are consistent with an immune (hypersensitivity) reaction. In a previous study (98-2397) T-20 produced delayed hypersensitivity in the guinea pig. In the 6-month rat study (98-2579), frequent and sometimes severe injection site pathologies were noted but were not definitely attributed to the drug.

Adverse reactions at injection sites in this study were also noted in control animals suggesting trauma from the injection procedure, but these effects were not as severe as those at T-20 injection sites.

Other observations (e.g., body and organ weight changes and high ALT values in some monkeys) cannot be conclusively attributed to the administration of the study drug from the data generated in this study, and their etiology remains unknown.

39. NDA 21-481 Reference 3110, Volumes 23-24. IND Serial 337.

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 6131-323 Final Report, May 9, 2002. Roche Study 07447. Final Report RR 1002995, May 13, 2002.

_____ conducted the in-life portion of this GLP study during September, October, and November 2000 to assess the safety and potential toxicity associated with administration of Ro 29-9801 (T-1249) by subcutaneous injection in 6 cynomolgus monkeys/sex/group for 28 days (Phase 1) followed by administration of Ro 29-9800 (T-20) by subcutaneous injection in 2 of the Phase 1 cynomolgus monkeys/sex/group for 28 days (Phase 2). Phase 1 monkeys received 0, 12.5, or 25 mg/kg/day of Ro 29-9801 in once-daily injections. Phase 2 monkeys received 0 or 50 mg/kg/day of Ro 29-9800 in twice daily injections (25 mg/kg/dose b.i.d.). At the end of Phase 1, 3 monkeys/sex/group were sacrificed and necropsied, and 1 monkey/sex/group was held on study for a four-week recovery period. At the beginning of Phase 2, the two control monkeys/sex were switched from Ro 29-9801 vehicle control to Ro 29-9800 vehicle control. The placebo for T-1249 was described only as a clear, colorless solution; the placebo for T-20 was described as a white powder.

The following observations or measurements were made of all monkeys during the study: clinical observations, food consumption, body weights, hematology, clinical chemistry, organ weights, histology, ophthalmoscopy, electrocardiology, toxicokinetics, and antibody analysis. In addition, blood was drawn for HIV-1 serology testing. The latter test was conducted on sera from placebo and T-1249-treated monkeys to see whether the presence of anti-T-1249 antibodies in primate sera would cause an HIV-1 ELISA/western blot procedure to test positive for HIV-1.

Trimeris, Inc., Durham, NC, conducted serum antibody assessments. _____, _____, conducted plasma test article determinations. Hoffmann-La Roche, Nutley, NJ conducted toxicokinetic analyses and _____ of T-20 injection site biopsies. _____, conducted HIV-1 serological testing. Monkeys used on the study were from _____ and were approximately 2 to 3 years of age and weighed from 1.7 to 2.6 kg (males) and 1.6 to 2.1 kg (females) at the initiation of dosing.

Blood samples for toxicokinetic analyses were drawn on Days 1 and 21 at six timepoints through 24 hours post dosing (Phase 1) and on Days 29 and 49 at seven timepoints through 24 hours post dosing (Phase 2). Blood samples for antibody analyses were drawn on Day -7 (pre-dosing), and prior to dosing on Days 14 and 28 (Phase 1), and Days 42 and 56 (Phase 1 Recovery). Blood samples for HIV-1 serology testing were drawn from 1 monkey/sex/group at baseline, Day 42 and Day 56.

Results:

Certificates of analysis were available for only _____ T-1249 lots used on the study (purity = _____) and no T-20 lots. Dosing solutions were analyzed for T-1249 on Days 1 and 27 (95.2% and 96% of nominal dose, respectively) and for T-20 on Days 29 and 56 (141.2% and 135.6% of nominal dose, respectively).

All monkeys survived to necropsy. There were no effects of T-1249 or T-20 evident in the body weight data, ophthalmic and electrocardiographic examinations, clinical pathology, or organ weights.

Administration of T-1249 or T-1249 followed by T-20 b.i.d. caused a necrotizing-granulomatous inflammatory response (observed microscopically) and swelling (observed macroscopically)