during Phase 2) at the injection sites. The necrotizing-granulomatous inflammatory response often contained giant cells, eosinophils, and perivascular mononuclear cell infiltration, and did not substantially resolve following the 28-day recovery period. During Phase 2, the T-20 injection sites became raised and firm within 3 or 4 days of dose initiation.

The [redacted] considered all other study findings to be unrelated to administration of T-1249 and T-20 or unimportant.

Monkeys given T-1249 had decreased food consumption (qualitative observations only) but they gained weight. Statistically significant changes were observed in a very few hematology parameters, leukocyte counts, and serum chemistry data, but they were small and not dose related.

Inflammation of the heart, lung, liver, kidneys and trachea was noted in histological examinations in numerous Phase 1 (T-1249) animals, across groups including control animals. Inflammation of the liver and kidney persisted in more than one animal after the 28-day recovery period. Inflammation of the lung, liver, kidney, and trachea persisted in animals that were dosed with T-20 (Phase 2), except that control animals did not show lung and trachea inflammation at the end of Phase 2. Because there were few animals in each group (range 1 to 3), the significance of these findings is not known.

The study report lists numerous minor protocol deviations. One high-dose male was given the incorrect dose on Days 30-34, although the dose administered was not indicated.

The [redacted] concluded that because the necrotizing-granulomatous inflammatory response at the T-1249 injection sites did not substantially resolve following the 28-day recovery period, it did not identify a no-observable-adverse-effect level (NOAEL).

Calculated toxicokinetic parameters are presented in the following tables:

Phase 1 (T-1249 administration).

Dose	Parameter	Units	Male	Female
Day 1				
12.5 mg/kg	$AUC_{0\text{-}24h}$	µg-h/mL	845 ± 97	859 ± 83
	AUC/Dose	µg-h/mL/mg/kg	67.6 ± 7.7	68.7 ± 6.6
	Cmax	µg/mL	72.8 ± 7.8	76.5 ± 12.0
	Tmax	h	4.5 ± 1.6	5.0 ± 1.6
25.0 mg/kg	$AUC_{0\text{-}24h}$	µg-h/mL	1550 ± 211	1450 ± 193
	AUC/Dose	µg-h/mL/mg/kg	62.0 ± 8.4	57.9 ± 7.8
	Cmax	µg/mL	122 ± 21	118 ± 15
	Tmax	h	5.5 ± 1.2	4.0 ± 1.6
Day 21				
12.5 mg/kg	$AUC_{0\text{-}24h}$	µg-h/mL	813 ± 398	711 ± 173
	AUC/Dose	µg-h/mL/mg/kg	65.0 ± 31.9	56.9 ± 13.8
	Cmax	µg/mL	65.4 ± 20.3	61.8 ± 14.7
	Tmax	h	4.0 ± 1.6	4.5 ± 1.6
25.0 mg/kg	$AUC_{0\text{-}24h}$	µg-h/mL	986 ± 190	871 ± 157
	AUC/Dose	µg-h/mL/mg/kg	39.4 ± 7.6	34.9 ± 6.3
	Cmax	µg/mL	90.2 ± 20.8	85.1 ± 10.0
	Tmax	h	4.0 ± 1.6	3.0 ± 0.0

Plasma concentrations of T-1249 peaked at approximately 3 to 6 hours post dosing, indicating a relatively slow absorption process. Approximately dose-proportional increases in $AUC_{0\text{-}24h}$ and Cmax were observed on study Day 1 and less than proportional increases were observed on Day 21. The decrease in exposure with time was more pronounced with the higher T-1249 dose. No obvious gender differences were observed in toxicokinetics of animals dosed in Phase 1.

Phase 2 (T-20 administration).

Dose	Parameter	Units	Male	Female
		Day 29		
50 mg/kg/day*	$AUC_{0\text{-}12h}$	µg-h/mL	455	759
	AUC/Dose	µg-h/mL/mg/kg	18.2	30.4
	Cmax	µg/mL	81.3	118
	Tmax	h	3.0	3.0
50 mg/kg/day**	$AUC_{0\text{-}12h}$	µg-h/mL	452	472
	AUC/Dose	µg-h/mL/mg/kg	18.1	18.9
	Cmax	µg/mL	77.7	68.5
	Tmax	h	2.3	3.0
		Day 49		
50 mg/kg/day*	$AUC_{0\text{-}12h}$	µg-h/mL	219	413
	AUC/Dose	µg-h/mL/mg/kg	8.78	16.6
	Cmax	µg/mL	47.3	60.7
	Tmax	h	1.5	1.5
50 mg/kg/day**	$AUC_{0\text{-}12h}$	µg-h/mL	301	435
	AUC/Dose	µg-h/mL/mg/kg	12.1	17.4
	Cmax	µg/mL	66.6	62.0
	Tmax	h	1.5	2.3

* after prior treatment with 12.5 mg/kg T-1249; ** after prior treatment with 25 mg/kg T-1249.

Plasma T-20 concentrations peaked at 1 to 3 hours post dosing. Exposures decreased between Days 29 and 49. Exposures were greater in females than in males, however, there were only two animals/sex/group. There was not any clear effect of prior dosing with T-1249 on the toxicokinetics of T-20.

Metabolite Ro 50-6343 plasma concentrations were 1.5 to 6 times lower than T-20 concentrations and peaked 1.5 to 6 hours post T-20 dosing. Ro 50-6343 $AUC_{0\text{-}12h}$ and Cmax values were similar to those of T-20 although they increased (instead of decreased) with time. There were no obvious gender differences in Ro 50-6343 concentrations.

Antibody titers reactive against T-1249 were similar in magnitude in animals dosed at 12.5 mg/kg and 25 mg/kg, and they persisted in animals following cessation of treatment (in both recovery animals and T-20-treated animals). Anti-T-1249 antibody titers ranged in magnitude from 1:100 to 1:204800. Most monkeys dosed with T-1249 had measurable anti-T-1249 antibodies in all sera samples analyzed after initiation of treatment, but one animal had no anti-T-1249 antibodies. One animal had anti-T-1249 antibodies prior to treatment.

Antibody titers to T-20 in Phase 2 animals were generally lower than titers to T-1249. Anti-T-20 antibody titers ranged in magnitude from 1:100 to 1:3200. Not all T-20 treated animals had anti-T-20 antibodies, and animals that had anti-T-20 antibodies often did not have them in all sera samples (at all time points). Many animals had measurable anti-T-20 antibodies during Phase 1

(prior to treatment with T-20), indicating their anti-T-1249 antibodies were cross-reactive to T-20. Most animals with anti-T-20 antibodies showed an increase in titer between Days 28 and 56 (after initiation of treatment with T-20). Some of these animals also showed an increase in T-1249 antibodies over the same interval, also indicating cross-reactivity.

In a separate non-GLP study, six monkey sera samples were tested to see whether the presence of anti-T-1249 and anti-T-20 antibodies would cause HIV-1 ELISA/western blot assays to produce positive results. The sponsor concluded that since the HIV-1 ELISA and western blot tests were negative for all six sera samples, anti-T-1249 antibodies and anti-T-20 antibodies do not react with those tests. However, only one of the sera samples tested had a measurable anti-T-1249 titer and it was a low titer (1:400) and none of the samples had measurable anti-T-20 titers. Therefore, the sponsor's conclusion is not supported by the study and the question that the study was meant to address remains unanswered.

In a separate non-GLP study, the antiviral potency of T-1249 and T-20 was tested in vitro with three sera samples (Days 28, 42, and 56) from two animals from this study that had relatively high anti-T-1249 antibody titers (maximum = 1:204800) and relatively low anti-T-20 antibody titers (maximum = 1:400). The sera antibodies from both animals reduced T-1249 binding with HIV-1 in vitro (reduced its potency), but did not reduce T-20 binding with HIV-1 in vitro.

40. NDA 21-481 Reference 3116, Volume 193. IND ——— Serial 029, 031, 037, 047, 333. Toxicokinetic analyses and serum antibody assessments from "A 9-month toxicity study of T-20 peptide administered twice per day via subcutaneous injection in the non-human primate. Trimeris final report, December 13, 1999, amended May 3, 2002.

——————— conducted the in-life portion of this 9-month monkey study (——— Study 98-3371, Reference 3109 of this NDA) for the sponsor and provided blood samples to Trimeris, Inc. for toxicokinetic analyses and anti-T-20 antibody analyses. This study was designed to assess the toxicity of T-20 when administered at two dose levels (5 mg/kg/dose and 10 mg/kg/dose) twice per day via subcutaneous injection to cynomolgus monkeys for a period of up to nine months. This was a GLP study; however, 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.

Blood for toxicokinetic analysis was collected from 3 animals/sex/group on Day 1 and months 3 and 9. Collection on Day 1 was at pre-dose, and post dose at 5, 15, and 30 minutes, 1, 2, 4, and 6 hours. The collection schedule at months 3 and 9 was pre-dose, and 0.5, 1, 2, 4, 6, 8, and 12 hours post-dose.

Results:
At Day 1 and Month 3, T-20 exhibited linear pharmacokinetics within the range of doses tested. A directly proportional increase in C_{max} and AUC were demonstrated with dose. At Month 9, C_{max} of the higher-dose group was 1.3-fold higher, on average, than C_{max} of the lower-dose group, and AUC was unchanged. Trimeris suggested that tissue damage and the production of anti-T-20 serum antibodies might have contributed to the rate of T-20 absorption and to the apparent loss in dose response relationship.

T-20 in plasma	C_{max} (µg/mL)		$AUC_{(0-6h)}$ (µg-h/mL)	
	5 mg/kg	10 mg/kg	5 mg/kg	10 mg/kg
Day 1	14.5 ± 4.79	29.2 ± 5.97	66.1 ± 19.1	130.6 ± 21.9
Month 3	11.4 ± 3.8	28.6 ± 8.5	58.9 ± 17.0	142.6 ± 39.8
Month 9	15.0 ± 8.6	20.0 ± 8.0	83.5 ± 50.2	98.7 ± 37.8

Blood was collected for serum antibody testing by indirect ——from 5 animals/sex/group on Day 1, at Week 1, and monthly. Animals that received only placebo showed no evidence of anti-T-20 antibody production, and no pre-dose sera from T-20 treated animals showed T-20 antibodies. All low-dose T-20 animals developed T-20 antibodies by Day 29. Titers from low-T-20 dosed animals at Month 9 ranged from 1:1,600 to 1:204,800. One animal exhibited sporadic low titers (maximum 1:1,600) and tested negative (<1:50) beyond Week 30. Eight of 10 high dose animals showed anti-T-20 antibodies by Day 29 (the first test day that any titers were measurable). At Month 9, titers were as high as 1:25,600—the highest titer achieved in the high-dose group. Several monkeys exhibited titers that went up and down, or up and down and up (etc.), over the duration of the study. Two high-dose animals showed no titers throughout the study. One animal showed its first titer at 9 months, and one animal had no titer but was only tested once. Correlations of toxicokinetics with tissue antibodies cannot be made because different animals and different blood collection times were used for each study.

——concluded that administration of T-20 for doses of 10 and 20 mg/kg/day for three and nine months produced a local immunogenic and hypersensitivity response.

Segment I Fertility

41. NDA Reference 3200, Volume 25. IND ——: Serial 047, 323.
Study of the effect of T-20 peptide on fertility and early embryonic development to implantation in the rat via subcutaneous administration. —— Study 99-4162. Final Report, November 18, 1999; amended, April 25, 2002.

——, conducted this study during April and May 1999 for Trimeris, Inc. to assess fertility and early embryonic development in rats following T-20 administration twice daily via subcutaneous injection. This was a GLP study except that the sponsor did not analyze the dose solutions for the concentration, stability, and homogeneity of the study drug as indicated in the study protocol. The concentrations of dosing solutions were assumed to be —— pure for the purposes of interpreting the study results.

The rats used on this study were Albino Rats Crl:CD®(SD) IGS BR from ——, conducted the sperm evaluations for ——

Dose levels to both male and female rats were 0, 2.4, 9.0, and 30 mg/kg/day administered as 0, 1.2, 4.5, and 15 mg/kg/dose twice a day by subcutaneous injection. Placebo was mannitol and carbonate buffer. Dose volumes were 1 mL/kg for all doses. Male rats (25/group) were treated for four weeks (pre-mating) and for two weeks post-mating and then necropsied. Female rats (25/group) were treated two weeks pre-mating, for a three-week mating period until evidence of

mating was observed, and daily through gestation day 7. Female rats were necropsied on gestation day 15.

All animals were observed twice a day for mortality and for toxicologic effects. Detailed physical observations were recorded before randomization and weekly through the study, at necropsy, and on gestation days 0, 3, 7, 11, and 15 for all mated females. Body weights and feed consumption were recorded twice pre-dosing, twice weekly, and on gestation days 0, 3, 7, 11, and 15 for mated females. Body weights were also recorded at necropsy. Estrus cycle evaluations were recorded daily for ten days prior to initiation of treatment and during the 21-day mating period or until evidence of mating occurred.

Macroscopic post-mortem evaluations of female rats included counting corpora lutea and the number and location of live, dead, and resorbed fetuses. Macroscopic post-mortem evaluation of males included weighing the testes and left epididymis and preserving them in Bouin's fixative. The right epididymis from 10 animals per group was processed for counting caudal sperm and assessing sperm morphology and motility. The remaining right epididymides were weighed and preserved in Bouin's fixative.

Results:
One high-dose male was found dead during the post-mating period of the study. —— stated the rat died accidentally. The gross examination report of that animal stated the "upper thoracic - upper sternum appears damaged." All other males and females lived until necropsy.

—— reported that there were no toxicologically relevant observations seen in any of the rats during the study and that all findings, such as alopecia, chromodacryorrhea, lacrimation, incisors broken/missing or maloccluded, swollen/red ears (in females), and decreased fecal volume, occurred sporadically and are common laboratory findings.

—— reported that sporadic statistically significant weight increases and decreases in male rats during mating and post-mating treatment periods were observed in the T-20 treatment groups but the overall weight gains were not affected by treatment. Female rat weights were comparable to control weights throughout the study.

There were increases in feed consumption by males during parts of the study —— was unclear as to why but considered it not toxicologically relevant.

Reviewer comment:
This effect on feed consumption was seen in the previous 6-month rat study (——98-2579, Reference 3102 in this NDA).

There was no effect of T-20 treatment on estrus cycling. All female rats had at least one estrus cycle during the 21-day period prior to mating. Mating index and pregnancy rates for T-20-treated female rats were comparable to controls.

There were no effects of T-20 treatment on cohabitation or mating. Male and female mating and fertility indices were all comparable to controls. The majority of all female rats in the control and T-20 treatment groups mated during the first 4 days of cohabitation.

There were no reported effects of T-20 identified during macroscopic post-mortem examinations.

There were no observed effects of T-20 treatment on mean or relative weights for the testes and epididymides.

There were no differences of T-20 treatment compared with controls on uterine implantation, corpora lutea, pre- and post-implantation loss, mean number of live fetuses per female rat, or early and late resorptions.

There were no apparent treatment-related effects in sperm/gram of cauda epididymis, sperm motility, or incidence of head/tale abnormalities.

[redacted] concluded that there were no adverse effects to either male or female rats administered T-20 subcutaneously up to 30 mg/kg/day, and that the no observed effect level of T-20 under the conditions of this study is 30 mg/kg/day.

Reviewer comments:
The high dose in this study should have been chosen to demonstrate unequivocal maternal toxicity, but it was not. In the 6-month rat study, [redacted] concluded that 30 mg/kg/day was a no observed effects level (NOEL) in the rat. It is noted that the laboratory (dosing and necropsy) portion of the 6-month rat study ended in November 1998, before this study began (March 1999).

In the protocol for this study, [redacted] states that dosing prior to mating "assumes that from repeat dose toxicity studies of at least one month duration, there have not been any adverse effects on weight of the reproductive tissues or any histopathologic changes to the reproductive organs. This pre-mating treatment regimen and dosing of mated females to Day 7 of gestation is consistent with the ICH 4.1.1 guidelines as per Section 3.1."

The ICH guidance recommends that the sponsor make use of data from repeat-dose toxicity studies of at least 1-month duration in the study design, especially for effects on spermatogenesis, in planning the administration period of the test article. The intent is that maximum dosing should occur which is absent adverse effects to the reproductive tissues. However, the guidance also recommends that the selection of dosages induce toxicity in the high-dose dams (ICH – S5A, Appendix A, #7).

On June 6, 2001, the Division sent the sponsor a telephone facsimile expressing concern that in a series of nonclinical reproduction toxicology studies, there was no unequivocal toxicity demonstrated in parent animals in the high dose groups. The sponsor responded (IND [redacted] Serial 170, June 29, 2001) that higher animal doses are generally not feasible because of saturation of absorption of the drug via the subcutaneous injection route of administration. A brief teleconference was held on July 10, 2001. The Division indicated that the toxicokinetic data did not clearly show saturation and that if the sponsor would submit results from an ongoing [redacted] study and a Segment II study using higher T-20 doses, that further nonclinical reproductive studies will not be required. The sponsor agreed. Those studies (References 2307 and 3303, respectively) were submitted and are included in this NDA.

Segment II Fertility

42. NDA 21-481 Reference 3300, Volume 26. IND [redacted] Serial 047, 323.
The effects of T-20 peptide on embryo-fetal development in rats via subcutaneous administration. [redacted] Study 99-4163. Final Report. November 18, 1999, amended, April 25, 2002.

[redacted] conducted this study during February 1999 to assess the maternal and embryo-fetal developmental toxicity following T-20 administration twice daily via subcutaneous injection in the pregnant rat. This was a GLP study except that Trimeris, Inc. did not analyze the dose solutions for the concentration, stability, and homogeneity of the study drug as indicated in the original study protocol. The concentrations of those solutions were assumed to be [redacted] pure for the purposes of interpreting the study results. The rats used on this study were Albino Rats Crl:CD®(SD) IGS BR from [redacted]

Female rats (25/group) were treated for 10 days on days 6-15 of gestation, and all rats were necropsied on gestation day 20. Dose levels were 0, 2.4, 9.0, and 30 mg/kg/day administered as 0, 1.2, 4.5, and 15 mg/kg/dose twice a day by subcutaneous injection. Placebo was [redacted] and [redacted] buffer. Dose volumes were 1 mL/kg for all doses.

All animals were observed twice a day for mortality and for toxicologic effects. Detailed physical observations were recorded before randomization and daily throughout the study. Maternal body weights and feed consumption values were recorded for all animals on days 6, 9, 12, 16, 18, and 20. Necropsy examinations included corpora lutea/uterine implantation counting. Fetuses were removed from the uterus, weighed, and evaluated for malformations. Half the fetuses were preserved in Bouin's fixative and examined for malformations and variations using a dissecting microscope. The remaining fetuses were processed and stained using [redacted] and evaluated for skeletal malformations and ossification variations.

Results:
Clinical observations during gestation and necropsy included alopecia at extremities or snout, chromodacryorrhea, incisors broken, missing or maloccluded, and decreased fecal volume. These observations were not dose-related. Alopecia was by far the most frequent observation (3 animals in the low T-20-dose group and 2 animals each in the middle and high T-20 dose groups) though it was not observed in control animals. One animal in the high dose group had decreased fecal volume and an extreme bilateral distended renal pelvis at necropsy.

Twenty-four of 25 rats became pregnant in each of the four groups. There was a slight dose-related increase in the number of early resorptions in the T-20-treated groups but these were not statistically significant.

Total counts of cesarean section data

	Group dose (mg/kg/day)			
	0	2.4	9.0	30
Early resorptions	7	6	14	17
Late resorptions	0	0	0	0
Dead fetuses	0	0	0	0
Postimplantation losses (total)	7	6	14	17
Number of dams affected	6	5	6	7

There were no adverse effects of treatment on mortality, pregnancy rates, maternal body weights, or macroscopic postmortem findings.

—— reported that T-20 did not produce maternal toxicity, fetal toxicity, or developmental effects in this study.

Reviewer comments:
—— attributed the increased early resorption and post-implantation losses to "minor variations" and stated that there were not "corresponding changes in implantation loss" accompanying these increases. It is not clear what they mean. —— did not consider the increased early resorptions to be toxicologically significant. However, like the previous Segment I reproduction study (—— 99-4162), the dose range in this study does not include a high dose that induces unequivocal maternal toxicity. Had they included a higher dose in the study design, the dose related trend of increased early resorptions might be shown to be statistically significant.

There were no observed effects of treatment on postmortem fetal body weights, visceral observations, or skeletal observations. Incidences of malformations in fetuses of dams treated with T-20 were similar to controls.

——concluded that a no observed effect level in this study was 30 mg/kg/day for maternal toxicity and reproductive toxicity, including fetal malformations.

The high dose in this study should have been chosen to demonstrate unequivocal maternal toxicity, but it was not. The protocol for the study does not address the justification for the dose range selected. The dose related trend of increased incidence of early resorptions might or might not be biologically significant. This study cannot resolve the question of biological significance because the dose range selected was too low.

Please refer to the final paragraph of the review to Reference 3200 (above) for a summary of the communication between the Division and the sponsor and the resolution concerning the dose selection for reproductive studies.

43. NDA 21-481 Reference 3301, Volume 27. IND —— Serial 047, 324.
A range-finding study of the effects of T-20 peptide on embryo-fetal development in rabbits via subcutaneous administration. ——, Study 99-4164. Final Report. November 18, 1999, amended, April 25, 2002.

——, conducted this range-finding study during February and March 1999 to provide data on the potential for T-20 to induce maternal or fetal developmental toxicity in the pregnant rabbit. This was a GLP study except that Trimeris, Inc. did not analyze the dose solutions for the concentration, stability, and homogeneity of the study drug as indicated in the original study protocol. The concentrations of those solutions were assumed to be —— pure for the purposes of interpreting the study results. The rabbits used on this study were New Zealand White Rabbits Hra:(NZW)SPF from——

Twenty rabbits were mated and treated (4/group) with placebo or T-20 for 13 days (on days 6-18 of gestation), and all rabbits were necropsied on gestation day 29. T-20 dose levels were 1.0, 3.0, 10, and 30 mg/kg/day; placebo controls received mannitol and carbonate buffer. All animals were dosed at a volume of 1 mL/kg/dose, twice a day (T-20 doses of 0.5, 1.5, 5, and 15 mg/kg/dose), 12 hours apart. The day of mating was considered day 0 of gestation.

All animals were observed twice a day for mortality and for toxicologic effects. Detailed physical observations were recorded daily throughout the study beginning on gestation day 1. Maternal body weights were recorded for all animals on days 0 (from the supplier), 4, 6, 9, 12, 15, 19, 24, and 29. Necropsy examinations (gestation day 29) included corpora lutea/uterine implantation counting. Fetuses were removed from the uterus, weighed, and evaluated for malformations.

Results:
Three of four rabbits became pregnant in the control group and the mid-high T-20 dose group; four of four rabbits became pregnant in the other three groups.

Alopecia and decreased fecal volume were noted in some animals in 3 of 4 of the T-20 groups, particularly in the last 3 or 4 days of the study. These findings were not dose-related. There were no recorded gross necropsy findings in the control animals.

There were no obvious T-20 treatment effects (compared with controls) on maternal body weights, maternal body weight gain, preimplantation loss, postimplantation loss, numbers of early resorptions, late resorptions, or live or dead fetuses. There were no dead fetuses. Fetal body weights from T-20 treated rabbits were similar to controls. Apart from resorptions, none of the fetuses exhibited any gross external malformations.

concluded that T-20 did not produce maternal or fetal toxicity or teratogenic effects when administered to rabbits subcutaneously two times a day at doses up to 30 mg/kg/day.

Like the two previous reproduction studies in rats (99-4162 and 99-4163), this study did not include a high enough dose to induce maternal toxicity. The protocol for this study does not address the reasons for the dose range chosen.

Please refer to the final paragraph of the review to Reference 3200 (above) for a summary of the communication between the Division and the sponsor and the resolution concerning the dose selection for reproductive studies.

44. NDA 21-481 Reference 3302, Volume 28. IND Serial 047, 324.
The effects of subcutaneous administration of T-20 peptide on embryo-fetal development in rabbits. Study 99-4165. Final Report. November 18, 1999, amended, April 25, 2002.

conducted this study during March and April 1999 to assess the potential for T-20 to induce maternal or fetal developmental toxicity in the pregnant rabbit. This was a GLP study except that Trimeris, Inc. did not analyze the dose solutions for the concentration, stability, and homogeneity of the study drug as indicated in the original study

protocol. The concentrations of those solutions were assumed to be [redacted] pure for the purposes of interpreting the study results. The rabbits used on this study were New Zealand White Rabbits Hra:(NZW)SPF from [redacted]

Eighty rabbits were mated and treated (20/group) with placebo or T-20 for 13 days (on days 6-18 of gestation), and all rabbits were necropsied on gestation day 29. T-20 doses were 3.0, 10, and 30 mg/kg/day; placebo controls received mannitol and carbonate buffer. All animals were dosed at a volume of 1 mL/kg/dose, twice a day (T-20 doses of 0.5, 1.5, 5, and 15 mg/kg/dose), 12 hours apart. The day of mating was considered day 0 of gestation.

All animals were observed twice a day for mortality and for toxicologic effects. Detailed physical observations were recorded daily throughout the study beginning on gestation day 1. Maternal body weights were recorded for all animals on days 4, 6, 9, 12, 15, 19, 24, and 29. Feed consumption was recorded daily. Necropsy examinations (gestation day 29) included corpora lutea/uterine implantation counting. Fetuses were removed from the uterus, weighed, and evaluated for malformations using microdissection. Fetuses were processed for microscopic evaluation of skeletal malformations and ossification variations.

Reviewer comment: This is essentially a repeat of the previous study ([redacted] 99-4164) except that the lowest dose (1 mg/kg/day) from that study is not used in this study, feed consumption data and microscopic fetal observations are recorded in this study, and 20 animals per group are used instead of 4 animals per group.

Results:
Nineteen of 20 rabbits became pregnant in the low and high T-20 dose groups; 20 of 20 rabbits became pregnant in the placebo control group and the middle T-20 dose group.

Alopecia, ano-genital staining, and decreased fecal volume were noted in some animals in all groups. These findings were not dose-related. At necropsy there were discolored foci in the lungs of one animal each in the low and middle T-20 dose groups and 3 animals in the high T-20 dose group. Discoloration of the liver occurred in one rabbit in the high dose group. Cystic ovaries were noted in two rabbits from each group (including controls).

There were no obvious T-20 treatment effects (compared with controls) on maternal body weights, maternal body weight gain, feed consumption, preimplantation loss, or live or dead fetuses. There were no dead fetuses.

There were more early resorptions in all the T-20 dose groups than in the control group and more late resorptions in two of the T-20 groups than in the controls, but these differences are not dose related and not statistically significant.

Fetal incidence (litters affected) of cesarean section data

	Group dose (mg/kg/day)			
	0	3	10	30
Fetuses (litters)	151 (20)	160 (19)	159 (20)	169 (19)
Early resorptions	2 (2)	6 (4)	5 (4)	4 (3)
Late resorptions	1 (1)	6 (5)	1 (1)	2 (2)
Dead fetuses	0 (0)	0 (0)	0 (0)	0 (0)
Postimplantation losses (total)	3 (3)	12 (8)	6 (4)	6 (5)

Fetal body weights from T-20 treated rabbits were similar to controls. There was one dark blue fetus in the control group among the live fetuses. All other fetuses appeared within normal limits during external exams. Visceral examinations revealed two malformations: One fetus from the low T-20-dose group had a diaphragmatic hernia and another from the same group but different litter had a left ectopic kidney. Other visceral findings, such as additional subclavian artery, distended ureter, underdeveloped renal papilla, cysts, or hemorrhagic ring around the iris, were considered variations rather than malformations. The incidence of these findings was in the single digits (mostly below 5). However, discolored thymus occurred 8 or 9 times in each of the T-20 dose groups and 2 times in the control group.

There were a few incidences of skeletal malformations (e.g., fused sternebrae) and none of these showed a dose relation. There were many more skeletal variations (e.g., hyoid arches bent or ossified), but none showed dose dependence. There were considerably more incidences of "27 presacral vertebra(e)" and "13th full rib(s)" in the T-20 dose groups than in the controls, however. These findings are not statistically significant; it is unknown whether they are biologically significant.

Fetal incidence (litters affected) of two types of skeletal variations

	Group dose (mg/kg/day)			
	0	3	10	30
27 Presacral vertebra(e)	12 (8)	29 (10)	26 (10)	28 (13)
Rib(s) 13th full	37 (15)	77 (17)	65 (15)	56 (16)

—— concluded that T-20 did not produce maternal or fetal toxicity or teratogenic effects when administered to rabbits subcutaneously two times a day at doses up to 30 mg/kg/day, and the no observable effect level (NOEL) for maternal or developmental toxicity under the conditions of this study was 30 mg/kg/day.

In this study there is some evidence, however slight, that T-20 caused an increase in early resorptions, reddened thymuses in viable fetuses, and perhaps minor skeletal abnormalities. None of these effects was statistically significant, but the study used a maximum dose based on a previous dose-finding study (—— 99-4164) that was too low (it did not generate unequivocal toxicity in the pregnant rabbits). Had the high dose caused some maternal toxicity in the earlier study, the findings in this study could be considered inconsequential. However, that was not the

case. Therefore, it remains uncertain whether the effects seen in this study are part of the toxicity profile of T-20.

Please refer to the final paragraph of the review to Reference 3200 (above) for a summary of the communication between the Division and the sponsor and the resolution concerning the dose selection for reproductive studies.

45. NDA 21-481 Reference 3303, Volumes 29-30. IND ——— Serial 319.
Ro 29-9800 (T-20; Fusion Inhibitor): A teratology and plasma concentration study of Ro 29-9800 administered subcutaneously (b.i.d.) to pregnant Crl:CD®(SD) BR rats. Roche Study 07666. Final Report RR 1006929, April 26, 2002.

Hoffmann-La Roche Inc. conducted this GLP study during October and November 2001 to assess the embryo-fetal development in the rat. This study was conducted because previous reproductive studies in rats and rabbits produced no frank toxicity in the dams at the high dose (30 mg/kg/day). The sponsor agreed to conduct this study in which the doses are extended up to 500 mg/kg/day, to partially address the Division's concern that the animals in previous reproductive toxicology studies were underdosed (IND ——— Serial 170, June 29, 2001).

Mated female Sprague-Dawley rats (———— were dosed subcutaneously, twice a day, approximately 12 hours apart, with Ro 29-9800 at doses of 0, 125, 250, or 500 mg/kg/day from Gestation Day 6 to 11 for toxicokinetic evaluations (n=12/group), and Gestation Day 6 to 17 for teratological evaluations (n=22/group). Clinical observations and body weights of all rats were monitored during the study and food consumption was measured for the teratology study rats. Teratology study rats were Cesarean sectioned on Gestation Day 21 and their litters were evaluated for teratological examinations. Toxicokinetic rats were bled before and after the first dosing on Gestation Days 6 and 11. ————, analyzed the plasma samples. Thoracic and abdominal organs of teratology study dams were examined macroscopically at necropsy. Fetuses were weighed, sexed and examined. Every other fetus was stained by ——— for skeletal examination. The other fetuses were examined using the ———

Results:
Dosing solutions were between 96% and 99% of nominal dose.

One female in the mid-dose group was found dead for unknown reasons. At necropsy, all organs and tissues appeared normal. Maternal food consumption and body-weight gains were similar across all groups throughout the treatment and post-treatment periods. The only clinical observations in the teratology rats were thickening of skin at injection sites. Gross necropsy findings in dams included discoloration of liver, pale mass on the spleen, nodule on the diaphram, mammary tumor, and fibrous adhesions between the stomach, spleen and intestines. None of the findings were observed in more than one animal per group.

All dams were pregnant. The number of dams with resorptions, resorption rate, and average litter size were similar across all groups. There were no dead fetuses. The number of viable male fetuses, the percent of litters with resorptions, the percent of resorptions, and the average body weight of viable fetuses were similar across all groups. Among the fetuses, there were no

significant differences between those whose mothers were dosed with Ro 29-9800 and those whose mothers were controls. Convoluted and/or dilated ureters and renal pelvises were seen in visceral tissues. Skeletal findings included short or misshapen ribs, vertebrae or sternums and ossified metacarpals and phalanges. The mean numbers of treated litters and fetuses with findings of any type were never greater than controls, and were occasionally lower than controls.

Toxicokinetic parameters for Ro 29-9800 and its metabolite Ro 50-6343 were as follows:

Gestation Day	Dose mg/kg/day	AUC ng-h/mL	AUC/dose (ng-h/mL)/(mg/kg/day)	Cmax ng/mL	Tmax hours
Ro 29-9800					
6	125	63,900	511	18,400	1
	250	78,400	314	16,700	2
	500	91,300	183	23,800	0.5
11	125	325,000	2,600	49,400	4
	250	546,000	2,180	64,000	4
	500	499,000	998	62,200	6
Ro 50-6343					
6	125	23,900	191	2,830	2
	250	30,800	123	4,010	4
	500	29,100	58.2	3,790	4
11	125	93,300	746	11,700	6
	250	134,000	536	14,100	6
	500	56,100	112	7,230	6

Exposure to Ro 29-9800 and Ro 50-6343 in the female rat increases in a less than dose-proportional manner over the dose range 125 to 500 mg/kg/day. Metabolite Ro 50-6343 reaches a maximum plasma concentration on the first day of dosing (Gestation Day 6). Parent drug (Ro 29-9800) saturation of plasma levels is attained by Gestation Day 11. The variation in Tmax values reflects variability in absorption and indicates generally a decrease in absorption over time.

These toxicokinetic and toxicology results indicate that Ro 29-9800 and its metabolites are nontoxic to the dam and to the developing fetus throughout organogenesis (Gestation Days 6-17) when administered subcutaneously at doses up to 500 mg/kg/day. The NOAEL in this study is 500 mg/kg/day for both the treated dams and fetuses.

In this study enfuvirtide was detected in control rat plasma samples. This appears to be a laboratory error.

46. NDA 21-481 Reference 3304, Volume 31. IND [redacted] Serial 284.
Ro 29-9800 ([T-20] Fusion Inhibitor): A pilot plasma concentration study of Ro 29-9800 administered subcutaneously (b.i.d.) to pregnant New Zealand white rabbits. Roche Study 07487. Final Report RR 1004950, February 12, 2002.

Hoffmann-La Roche conducted this non-GLP study during December 2000 and January and February 2001 to determine the pharmacokinetics of Ro 29-9800 and Ro 50-6343 in the plasma of pregnant rabbits under the conditions of a subcutaneous teratology study. Six pregnant rabbits (New England White, from [redacted] per group received

daily subcutaneous doses of 10, 30, 60 or 100 mg/kg/day administered as two approximately equal doses approximately 12 hours apart from gestation day 6 through 11. Four injection sites were selected on the dorsal surface and injections were rotated so that each site was treated every other day. The rabbits were bled prior to and following the first dose on gestation day 6 and the last dose on gestation day 11. Body weights were collected on gestation day 0 and daily during dosing. Clinical observations were recorded approximately 30 to 60 minutes after dosing. Does were sacrificed within 1 to 3 days after the final bleeding. Pregnancy status was determined and injection site tissue was collected for histopathologic evaluation. ———————, ———————analyzed the plasma samples for this study using an ———————assay.

The two lower doses of this study were chosen to be identical to the two highest doses in the —— Study 99-4165 to compare the toxicokinetic data. In the earlier study, —— concluded that T-20 did not produce maternal or fetal toxicity or teratogenic effects when administered to rabbits subcutaneously two times a day at doses up to 30 mg/kg/day.

Results:
There were no unscheduled deaths on this study. All but one rabbit was pregnant at necropsy.

Clinical signs related to injection site reactions were seen in the three higher dose groups. The most frequent injection site finding was hematoma, seen in at least half of all animals in these groups. Other injection site findings seen only in the two highest dose groups include lesion, erythema, swelling and eschar.

Group mean body weight losses were recorded in all dose groups throughout the study (-5%, -48%, -42%, and -95% for doses of 10, 30, 60, and 100 mg/kg/day, respectively, over the duration of the dosing period).

The most frequent observation at necropsy was red foci at injection sites (2/6, 5/6, 1/6, and 2/6 animals for dose groups of 10, 30, 60, and 100 mg/kg/day, respectively). The only other finding, in half the high dose group animals only, was injection site thickening.

Microscopic changes were seen in all dose groups at all injection sites. The changes consisted of a serocellular crust on the dermis of some animals, hemorrhage in the dermis and subcutis, degeneration and chronic inflammation in the subcutaneous muscle, and granulomas in the subcutaneous tissues. The granulomas had centers containing necrotic material surrounded by fibrous stoma containing a mixture of macrophages, eosinophils, and giant cells. The incidence and severity of granuloma formation and necrosis tended to increase with dose. The incidence and severity of other changes tended to be distributed across all dose groups. The areas of hemorrhage in the dermis and subcutis corresponded to the macroscopic observation of red foci.

The toxicokinetic parameters are presented as follows:

Dose mg/kg/day	AUC µg-h/mL	AUC/dose (µg-h/mL) /(mg/kg/day)	C_{max} µg/mL	t_{max} hours
Ro 29-9800 (T-20)				
Gestation day 6 (1st day of dosing)				
10	32	6	9	1
30	247	17	51	1
60	349	12	68	1
100	702	14	130	2
Gestation day 11 (last day of dosing)				
10	33	7	8	2
30	177	12	27	1
60	123	4	46	2
100	204	4	62	2
Ro 50-6343				
Gestation day 6 (1st day of dosing)				
10	5	1	1	4
30	23	2	3	4
60	30	1	4	6
100	41	1	5	6
Gestation day 11 (last day of dosing)				
10	8	2	1	2
30	43	3	3	4
60	68	2	9	4
100	145	3	14	2

Plasma concentrations of Ro 29-9800 (T-20) peaked at 1 to 2 hours post dosing. Plasma concentrations of metabolite Ro 50-6343 peaked between 2 and 6 hours post dosing.

Theoretical and observed increases in exposure (AUC, Cmax) with increased dose from the low dose (10 mg/kg/day) are presented below:

Dose mg/kg/day	Theoretical increases in exposure compared to low dose (fold)	Observed increases in Ro 29-9800 $AUC_{0\rightarrow 12h}$ (fold)	Observed increases in Ro 29-9800 Cmax (fold)	Observed increases in Ro 50-6343 $AUC_{0\rightarrow 12h}$ (fold)	Observed increases in Ro 50-6343 Cmax (fold)
Gestation Day 6 (1st day of dosing)					
30	3	7.6	5.6	4.6	5.8
60	6	10.8	7.4	6.0	7.8
100	10	21.7	14.2	8.1	9.1
Gestation Day 11 (last day of dosing)					
30	3	5.4	3.2	5.4	3.1
60	6	3.8	5.5	8.6	9.3
100	10	6.2	7.4	18.4	15.5

Greater than dose-proportional increases in exposure of Ro 29-9800 occurred on gestation day 6 and both greater-than and less-than dose-proportional increases in exposure of Ro 29-9800 occurred on gestation day 11. For Ro 50-6343, both greater-than and less-than dose-proportional increases in exposure occurred on gestation day 6, and greater than dose-proportional increases in exposure occurred on gestation day 11.

Comparing exposure parameters (AUC and Cmax) at gestation day 11 with those at gestation day 6 provides a measure of the change in the pharmacokinetics over time. The sponsor refers to the AUC and Cmax ratios (day 11/day 6) as "repeated dosing factors" and describes them as a measure of the change in the "disposition process" but did not provide a mechanistic explanation for them. The "repeated dosing factors" of both Ro 29-9800 and Ro 50-6343 combined are presented below.

Dose mg/kg/day	AUC(day 11)/ AUC(day 6)	Cmax(day11)/ Cmax(day 6)
10	1.01	0.92
30	0.72	0.53
60	0.35	0.68
100	0.29	0.48

These data indicate that (except for the lowest dose) exposures decrease over time with repeated dosing. This trend is most pronounced at the highest dose.

Segment III Peri Post Natal

47. NDA 21-481 Reference 3400, Volumes 32-34. IND [redacted] Serial 047, 311.
Study of effects of T-20 peptide on pre- and postnatal development, including maternal function, in rats. [redacted] Study 99-4176. Final report, April 3, 2002.

[redacted], conducted this GLP study for Trimeris, Inc. The in-life portion of the study ran from October 1999 to March 2000.

Twenty-five mated female albino rats [Crl:CD®(SD)IGS BR] per dose group received subcutaneous injections of T-20 at doses of 0, 2.4, 9, or 30 mg/kg/day at approximately half the daily dose in twice daily injections at approximately 12-hour intervals. The diluent was sterile water. Rats were dosed from Gestation Day 6 through Lactation Day 20. F_0 females were checked twice daily for mortality or severe toxicity, and detailed physical observations were made daily. F_1 pups were given gross examinations, counted and tattooed as soon as possible after delivery. Litters were culled to 5/sex on Day 4 of lactation. F_1 pups were observed for pinna detachment beginning on Lactation Day 4 and eye opening on Lactation Day 13. At weaning (Lactation Day 21), 1 pup/sex/litter (25 pups/sex/group) was (were) randomly selected to continue on study.

Selected F_1 pups were given detailed physical exams weekly. Body weights were recorded weekly. Female F_1 pups were observed for vaginal opening beginning on Postnatal Day 28. Male F_1 pups were observed for preputial separation beginning on Postnatal Day 37. A functional observation battery including gait, posture, vocalization, and abnormal behavior was performed of on all F_1 pups on Postnatal Day 22 or 23. Locomotor behavior was measured on all F_1 pups on Postnatal Days 28 and 55 (± 2 days each). Learning and memory recall were evaluated on all F_1 selected pups beginning on Postnatal Day 50. Auditory startle habituation response was measured on Postnatal Day 31 (± 1 day). Estrous smearing of female F_1 pups began ten days prior to initiation of mating (cohabitation) and evaluations continued daily until there was

evidence of mating or the 20-day cohabitation period ended. F_1 males from the same treatment group were mated with F_1 females. F_1 females were weighed every three days during pregnancy.

F_2 litters were observed, counted, sexed, and tattooed as soon as possible after delivery and again on Lactation Day 4.

F_0 females were euthanized on Lactation Day 21. All F_0 and F_1 animals were examined macroscopically at necropsy. Postmortem examination of females included a count of uterine implantation scars, if present. F_1 and F_2 pups found dead at lactation were examined externally and internally, and presence or absence of milk in stomach was noted. F_1 females were euthanized on Lactation Day 4 or Gestation Day 25 and pregnancy status and/or implantation data recorded. F_1 males were euthanized after the F_1 females were euthanized and given macroscopic postmortem examinations.

Results:
All but one F_0 rat in the 9 mg/kg/day group became pregnant. The duration of gestation was statistically significantly increased in the 30 mg/kg/day group (increased by 10 hours or +2%) compared with controls. Gestation duration for all groups remained within normal limits (21-22 days). All pregnant rats delivered pups. No dams had all pups stillborn. During lactation, red exudate from the ano/genital area was observed in 2 animals from the high dose group. Alopecia was observed in 2 to 5 animals from each group. There were no statistically significant differences from controls in F_0 mean body weights and body weight gains during gestation and lactation. Feed consumption was similar to or greater than controls throughout the study. There were no clinical observations that the ——— considered treatment-related. All F_0 rats survived to Lactation Day 21 (euthanasia). There were no post-mortem findings in T-20 treated F_0 rats.

The number of stillborn F_1 pups in the high dose group (30 mg/kg/day) was greater than that in the controls (6 vs. 2) but the difference was not statistically significant. There were no statistically significant differences from controls in numbers of F_1 pups born live, stillborn, dying, missing, or cannibalized, surviving 4 days, or surviving 21 days. Sex distribution, viability and lactation indices of F_1 pups were comparable across all dose groups and controls. Observations of abnormalities in F_1 pups (e.g., red exudate from ano/genital area, soft protrusion from abdomen, skin irregularity, ulceration, necrosis tip of tail, deformed right paw, domed head) were not dose dependent or statistically significant. F_1 pup body weights on Day 1 in the high dose group were statistically significantly elevated (+8.6%) compared with controls. Body weight gains of the low dose group were depressed (-24%) for the Lactation Days 1-4 interval compared with controls.

There were no treatment-related clinical observations of the F_1 pups during growth, development, premating, mating, and postmating periods. There were no post-mortem findings in T-20 group culled, mated, or surviving through lactation.

All F_1 pups met the test criteria for pinna detachment and for eye opening, though there was a statistically significant decrease in age of eye opening for the 30 mg/kg/day group compared with controls, which may have been a result of increased duration of gestation. There were no significant differences in mean age or body weight at preputial separation between the T-20 treated male rats and controls, although one male rat (9 mg/kg/day) did not meet the criteria for preputial separation (separation by day 50) during the study. There were no significant

differences in mean age or body weight at vaginal opening between the low dose and control female F_1 pups, but there were significant increases in body weights in the 9 and 30 mg/kg/day females (+8.4%, both groups) when vaginal opening occurred. This may have been a result of increased duration of gestation.

There were no obvious effects of treatment from the open field assessments of F_1 pups. There were no differences between T-20 treated and control F_1 pups in auditory startle response, or in performance in the Biel water maze. There were no treatment-related changes in the locomotor activity of F_1 pups assessed at 28 and 55 days of age. In the locomotor activity test, F_1 females in the 30 mg/kg/day group had a statistically significantly higher total number of beam breaks (over one hour, total numbers not given) than controls, but the difference was not interval-dependent and the [redacted] did not consider the difference treatment-related.

Female F_1 rats had normal estrous cycles. Male F_1 rats fertility indices were comparable to controls. Female F_1 rats fertility indices (pregnancy rates) decreased with dose (100%, 96%, 95.8%, and 84%, respectively). Female F_1 rats that became pregnant had normal clinical observations and normal feed consumption during gestation and lactation. Body weight gains during gestation Days 0-3 were decreased (-26%) across all treated groups compared with controls during gestation.

Mean litter size at birth for the T-20 treated group F_2 pups was comparable to controls. The number of liveborn pups, stillborn pups, viability and lactation indices, and pup sex distribution were all comparable for all T-20 group F_2 pups compared with the control group. One dam in the 2.4 mg/kg/day group had a total litter loss by Lactation Day 2, but this was considered normal in laboratory rats. It resulted in a decrease (statistically significant) in pup survivability index (-5%) in the 2.4 mg/kg/day group compared with controls. The pup survivability index was also significantly decreased in the 9 mg/kg/day group (-4%), but it was not significantly reduced in the 30 mg/kg/day group. Because the reduction in pup survivability did not include the high dose pups, it was not considered treatment-related.

Fifteen F_2 pups (total) from three litters (all 9 mg/kg/day group lineage) were observed to have subcutaneous hemorrhage (hematomas) and many had what appeared to be a loose extra layer of skin. The [redacted] explained that this syndrome had been observed in other rat studies of this strain in its laboratory. The pups are born alive and then either die or are cannibalized before Lactation Day 4. Examinations for skeletal malformations in four pups were conducted and no skeletal malformations were found. Since the T-20 high dose group F_2 pups were not involved, this observation was not considered treatment-related.

Mean body weights and body weight gains of T-20 group F_2 pups were not different from controls during lactation, and there were no postmortem findings in F_2 pups that were considered treatment-related.

There [redacted] concluded that T-20 administered subcutaneously up to and including 30 mg/kg/day during gestation and throughout lactation did not produce maternal or offspring toxicity.

Reviewer comments:
The [redacted]'s explanation that the statistically significant increase in total beam breaks among F_1 females in the 30 mg/kg/day group (compared with controls) was not treatment-related because

the difference was not interval-dependent is not reasonable. The ——did not indicate the magnitude of the difference. Beam breaks are the endpoint of this locomotor activity test and without a plausible alternative hypothesis to explain the result, the difference suggests a treatment-related effect. The biological significance of increased locomotor activity in F_1 females of T-20 treated rats may be difficult to assess, but that does not obviate the results.

The doses administered to F_0 dams in this study are the same as those administered to rats in the 6-month repeat-dose toxicity study, and are the same or similar to those administered in other reproductive and behavioral studies that Trimeris/Hoffmann-La Roche conducted in rats, mice, and rabbits. The high dose (30 mg/kg/day) produced little or no maternal toxicity in this study. This dose in rats is a human equivalent dose of approximately 4.8 mg/kg/day (on a body surface area basis). The maximum clinical dose (90 mg b.i.d.) is approximately 3 mg/kg/day (assuming a 60 kg human). Thus, the rat dose is greater than the human dose, although the safety margin is less than a factor of 2.

Please refer to the final paragraph of the review to NDA Reference 3200 (above) for a summary of the communication between the Division and the sponsor and the resolution concerning the dose selection for reproductive studies.

Genetic Toxicology

48. NDA 21-481 Reference 3500, Volume 35. IND —— Serial 000.
Ames/Salmonella-E. coli reverse mutation assay on test article T-20. ——— Study 0301FT14.001, August 26, 1996.

——————————————————— conducted this study for Trimeris, Inc. It was a GLP study except that ——— did not verify the homogeneity, stability, or accuracy of preparation of the test article and control (————— saline) dosing solutions. Trimeris provided the test article as a clear frozen liquid sterile solution.

The test article was tested in *Salmonella typhimurium* – TA1535, TA1537, TA98, TA100 and TA 102 strains from ——————————— The test article was also tested with *Escherichia coli* – WP2 uvrA from ————————————————

Triplicate cultures of each strain were evaluated with the solvent DMSO, with and without S9. Positive controls (without S9) were ——— (10.0 µg/plate, for TA1535 and TA100), ——— (5.00 µg/plate, for TA98, ——— (2.5 µg/plate, for TA102), —— (2.00 µg/plate, for WP2 uvrA). ————(30.0 µg/plate) was evaluated in TA102, and —— (2.50 µg/plate) was evaluated in the remaining Salmonella strains and in WP2 uvrA (80.0 µg/plate) in the presence of S9.

Toxicity testing was conducted at doses of 50.0, 167, 500, 1670, and 5000 µg/plate. Test article was found to be freely soluble at all doses tested and non-toxic to all tester strains at all doses evaluated under either treatment. Mutagenicity testing was conducted in duplicate at doses of 16.7, 50.0, 167, 500, 1670, and 5000 µg/plate, with and without S9. Revertant frequencies for all doses of T-20 in all tester strains with and without S9, under both treatment conditions,

approximated or were less than negative controls. All positive and negative control values in both assays were within acceptable ranges. Therefore, the results were all negative and T-20 demonstrated no mutagenicity in this study.

49. NDA 21-481 Reference 3501, Volume 35. IND —— Serial 000, 004. —— mammalian cell forward gene mutation assay on T-20 ——Study 0314FT14.001. Final report, February 18, 1997.

—— performed this study for Trimeris, Inc. The assay was completed August 28, 1996. The study was designed to comply with the guidelines for mammalian cell mutagenicity testing as set forth by various international regulatory authorities (e.g., OECD, 1981; US EPA, 1982; US FDA, 1992), and it complies fully with proposed revised testing guidelines developed by the Organization for Economic Co-operation and Development (1994).

This assay measures the ability of a test article to induce mutations at the xanthine-guanine phosphoribosyl transferase (XPRT) locus in AS52 Chinese hamster ovary cells. The mutation restricts the cell from metabolizing 6-thioguanine (TG) to toxic products. Therefore, the presence of the mutation results in survival of the host cell in the presence of 6-thioguanine. Such mutant cells (xanthine-guanine phosphoribosyl transferase-free, XPRT$^-$) form colonies in the presence of 10 µM TG.

Cytotoxicity of T-20 was evaluated by treating AS52 Chinese hamster ovary cells (clone 1.3) with T-20 for 5 hours in the presence and absence of S9. T-20 was evaluated at concentrations of —— and —— µg/mL. The highest concentration is 1000x the clinical target plasma concentration. ——. T-20 was somewhat cytotoxic in the presence and absence of S9. Relative survivals at a concentration of —— µg/mL were 53% and 70%, with and without S9, respectively. However, dose-dependent cytotoxicity was not evident.

Mutagenicity of T-20 was evaluated by treating AS52 Chinese hamster ovary cells with T-20 at concentrations of ——, and ——µg/mL, with positive and negative controls, and with and without S9 (all with duplicate cultures). ——

—— The mutant frequency is expressed as TG^r mutants/10^6 clonable cells, and is calculated by dividing the total number of (mutant) clones observed by the number of cells plated, and corrected for the cloning efficiency (average mutant frequencies from the negative controls). The 8 negative control plates (buffered saline and no treatment) averaged 34.6 ± 9.3 TG^r mutants/10^6 clonable cells. The T-20 plates ranged from 19.7 to 50.4 TG^r mutants/10^6 clonable cells, though there was no dose dependence, and no statistically significant increases in mutants from controls. Therefore, the mutagenicity assay was rerun. In the confirmatory assay, the results were similar: The negative controls averaged 30.6 ± 8.1 TG^r mutants/10^6 clonable cells. The T-20 plates ranged from 23.3 to 48.5 TG^r mutants/10^6 clonable cells. A statistically significant increase in average mutant frequency was observed at 500 µg/mL T-20, but it was not linear and did not represent a two-fold or net increase of 30 TG^r mutants/10^6 clonable cells compared to negative controls. Therefore, this result (at 500 µg/mL T-20) did not meet the assay criteria for an

equivocal response. The average mutant frequencies for all other cultures, with and without S9, approximated or were less than control values. All positive and negative control values were within acceptable ranges. Therefore the result at 500 µg/mL T-20 is considered a statistical aberration due to the fluctuation of the spontaneous mutant frequency.

[redacted] concluded that T-20 is not mutagenic in the [redacted]XPRT mammalian cell forward gene mutagenicity assay.

Reviewer comment:
One of the three guidance criteria for a valid assay—namely, that the spontaneous mutant frequency in the solvent control must be between 0 and 25 mutants per 10^6 clonable cells, was exceeded in both the original mutagenic assay and the confirmatory assay. The means were 34.6 and 30.6 TG^r mutants/10^6 clonable cells, respectively. In this case, the contravention does not likely invalidate the results. The other validation criteria were met for these assays.

50. NDA 21-481 Reference 3502, Volume 35. IND [redacted] Serial 000, 168.
In vivo micronucleus test with T-20 in mouse bone marrow erythropoietic cells.
[redacted]Study 0309FT14.001. September 4, 1996.

[redacted] conducted this study for Trimeris, Inc. (Today, [redacted] is [redacted]) It was a GLP study except that [redacted] did not verify the homogeneity, stability, or accuracy of preparation of the test article and control ([redacted] saline) dosing solutions. Trimeris provided the test article as a clear frozen liquid sterile solution.

This micronucleus test (MNT) was conducted to evaluate the potential of T-20 to induce micronuclei in newly formed polychromatic erythrocytes in mouse bone marrow. The MNT detects both clastogens and aneugens (kinetochore and/or spindle poisons).

In a preliminary toxicity screening, two mice/sex/group were administered a single intravenous injection of T-20 at doses of 0, 100, 200, 300, 600, and 1000 mg/kg. [redacted] saline was used as a negative control. Mice were observed for mortality and pharmacotoxic signs (200, 600, 1000 mg/kg groups only) immediately, and at 24, 48, and 72 hours post dosing. All mice survived to 72 hours. Only one mouse (a male) showed any abnormal signs beyond the initial post dosing observation (writhing at 24 hours in the 600 mg/kg group).

At 72 hours all mice were sacrificed, bone marrow was collected from their femurs, and smears were prepared for microscopic analysis. Slides were stained and scored for the ratio of polychromatic to normochromatic erythrocytes (PCE/NCE) per 1000 total erythrocytes, as an index of toxicity.

Results indicated that T-20 produced a significant depression in PCE/NCE at 200 mg/kg (combined sex and males only) compared with controls, and a significant increase in PCE/NCE at 600 mg/kg in female mice.

From these initial results (preliminary toxicity screen), T-20 doses of 100, 500, and 1000 mg/kg were selected for the primary study. Doses were administered intravenously to nine groups of

mice (five/sex/group) plus 5 male and 5 female controls for bone marrow harvest at 24, 48, and 72 hours after injection. The positive control was triethylenemelamine (TEM, 0.5 mg/kg); all positive control animals were sacrificed at 24 hours post dosing.

Observations for mortality and pharmacotoxic signs were made in the first two 2 hours and at approximately 24, 48, and 72 hours post dosing. Bone marrow slides were prepared and scored for the number of micronucleated polychromatic erythrocytes in 2000 PCEs/mouse and the PCE/NCE per 1000 erythrocytes for each mouse.

At this point in the original report summary, ——— writes,

> "Due to unexpected (and unacceptably high) mortality in the males treated at 1000 mg/kg for harvest 48 hours after treatment, ten additional males subsequently were treated with T-20 at a dose of 1000 mg/kg, while two groups received the concurrent positive and negative controls (five male mice/control group)."

Essentially, there were too many early deaths among the 1000 mg/kg males in the 48-hour sacrifice group to meet the minimum requirements (4 mice/sex/group) of the protocol, and that part of the study was repeated.

In total, 27 male mice and 19 female mice were treated with T-20 at 1000 mg/kg. Replacement animals were introduced into the study when mice were found dead and the male 1000 mg/kg group intended for sacrifice at 48 hours was repeated with new animals. In all, 9 males and 6 females were found dead, but finally all groups were represented by either 4 or 5 animals/sex/sacrifice time.

Micronucleated polychromatic erythrocytes (MPCE) frequencies (the primary endpoint of this study) for all negative controls were within acceptable ranges, and the positive controls produced statistically significant increases in MPCE frequencies in this study. T-20 did not induce any statistically significant or dose-dependent increases in MPCE frequencies at any dose or harvest time evaluated, compared with concurrent negative controls.

Statistically significant decreases in PCE/NCE ratios were observed for the positive control (combined sex and by-sex comparisons with the negative controls). Analysis of the combined sex data showed that the T-20 mice did not demonstrate any significant depression in PCE/NCE at any dose or at any harvest time evaluated. However, males treated with 500 or 1000 mg/kg and sacrificed at 72 hours post dosing showed significant decreases in PCE/NCE.

Under the evaluation criteria of the study protocol, these results indicate T-20 is non-clastogenic.

Reviewer comments:
The design of this study (including choice of species, sex, doses, frequency of dosing, frequency of bone marrow harvest, concurrence of positive controls, number of analyzable animals, number of cells per animal scored, and attention to artifacts) appears to be adequate. Therefore, this study appears to be valid and T-20 demonstrated no clastogenesis.

Hypersensitivity Studies

51. NDA 21-481 Reference 3600, Volume 35. IND [redacted] Serial 017.
T-20 skin sensitization in the guinea-pig ([redacted] method). [redacted] Study 98-2397, May 26, 1998.

Trimeris, Inc. sponsored this study after the Division recommended (facimile, January 15, 1998) that Trimeris conduct a dermal irritancy study prior to initiating clinical trials using a continuous infusion form of drug delivery. The experimental phase of this study occurred between February 13, 1998 and March 15, 1998. Seventeen human patients had received T-20 by intravenous injection under clinical protocol TRI-001 prior to this study. However, studies TRI-002 and TRI-003, both subcutaneous infusion studies, were not begun until after this study was completed.

[redacted] conducted this GLP study for Trimeris, Inc. to assess the skin sensitization potential of T-20.

Fifteen male albino guinea pigs (10 test animals and 5 controls; Dunkin/Hartley strain from [redacted]) were tested with T-20 dissolved in sterile water for injection. The protocol method used was the [redacted] test described by Magnusson and A.M. Kligman in *Allergic Contact Dermititis in the Guinea-pig: Identification of contact allergens*, Thomas, C.C., Springfield, Illinois, 1970.

Positive controls were tested in a previous experiment (between December 15, 1997 and January 8, 1998) using 15 guinea pigs from the same supplier. The positive control article was [redacted] administered in a [redacted] solution (v/v) of [redacted] for intradermal injection, neat for topical application, and both neat and in a [redacted] solution (v/v) of [redacted] for the allergic challenge. Control animals (5) received [redacted] Slight irritation was seen in all test and control animals following intradermal injections and topical applications. Dermal reactions in the 10 test animals following the challenge were more marked than the dermal reactions in control animals, thus indicating a delayed contact hypersensitivity in the test animals and supporting the experimental method.

A preliminary test on other animals from the same supplier was conducted to test the dose levels. Animals were pre-treated with an intradermal injection of Freund's complete adjuvant, 50:50 with water for irrigation, approximately 2 weeks prior to the start of the preliminary investigation. Trimeris indicated to [redacted] that the maximum solubility of T-20 was 50 mg/mL. Two animals were administered intradermal injections of T-20 in sterile water for injection at doses of 0, 1, 5, 10, 25, and 50 mg/mL. Site responses were rated for diameter (mm), erythema (0-4), and edema (0-4) at 24, 48 and 72 hours. Four animals were administered T-20 topically in doses of 5, 10, 25, and 50 mg/mL and rated for erythema (0-4), edema (0-4) and localized dermal reaction at 0, 24, and 48 hours. The intradermal injections resulted in a dose response in all measures. The topical applications resulted in a minor localized response in two of the four animals at the high dose only, through 24 hours only. As a result of this experiment, [redacted] selected the following dose levels for the primary experiment: 50 mg/mL for intradermal injection and topical application, and 50 and 25 mg/mL for the challenge application.

For the primary experiment, three pairs of intradermal injections were made in 10 animals on the dorsal skin (scapular region) where the fur had been clipped. The injections (for induction) were

Freund's complete adjuvant (50:50, v:v with water for irrigation), T-20, 50 mg/mL, and a 50:50 mixture of T-20, 50 mg/mL, and Freund's complete adjuvant. All animals showed necrosis at the intradermal injection sites where Freund's complete adjuvant was applied, and slight irritation where T-20 solution alone was applied. (The study report does not indicate when these observations were made.)

One week after the injections, the fur of the animals was clipped again and a patch of [redacted] paper was saturated with approximately 0.4 mL of T-20, 50 mg/mL and placed on the skin of the test animals, and covered by a length of plastic adhesive tape. The patch was further secured with a bandage and left in place for 48 hours. Control animals (5) received similar treatments without the T-20 solutions. Slight erythema was noted in all animals after the topical application. (The study report does not indicate when these observations were made.)

Two weeks after the topical applications were made, the hair was clipped and shaved on two separate sites on the 10 test animals and 5 control animals and a patches with 50 mg/mL and 25 mg/mL T-20 were applied and secured to the test animals for 24 hours. (Freund's complete adjuvant was applied to the controls.) The challenge sites were evaluated at 24, 48, and 72 hours after removal of the patches.

There was slight erythema in 9 of 10 high-dose animals at 24 hours, and 8 of 10 animals at 48 and 72 hours. With the 25 mg/mL patches, slight erythema occurred in 3 of 10 animals at 24 hours and 1 animal at 48 and 72 hours. No edema was observed in any of the animals, and no reactions were observed in the 5 control animals.

Body weights increased (+54%, control means; +43% test means) in all animals during the study (4 weeks). (One control animal gained 306 grams, whereas the control mean weight gain without that animal was 190 grams.) No clinical signs of ill health or toxicity were noted in any animal during the study.

[redacted] concluded that T-20 is associated with the development of delayed contact hypersensitivity and recommended that the labeling of the product should reflect the same (e.g., "May cause sensitization by skin contact.").

Reviewer comment:
This study seems to have been properly conducted and the results are unambiguous. T-20 tested positive for delayed contact hypersensitivity.

52. NDA 21-481 Reference 3601, Volume 35. IND [redacted] Serial 056, 211.
T-20 skin sensitization in the guinea pig ([redacted]. Evaluation of 50 mg/ml and 100 mg/ml T-20 carbonate formulations, and 50 mg/ml and 100 mg/ml [redacted] formulations. Trimeris study [redacted] 005. Final report [redacted] 005/002348/SS, September 28, 2001.

[redacted] conducted this study for Trimeris during October 1999. The study was a GLP study except [redacted] did not assay the test article for stability, homogeneity, and concentration.

[redacted] obtained 35 healthy male albino guinea pigs (Dunkin/Hartley strain) from [redacted]

[redacted] conducted a preliminary study to identify intradermal and topical concentrations of test substance that would produce irritation suitable for the induction phase and the maximum nonirritant topical concentrations for the challenge phase.

For the main study induction phase, three pairs of intradermal injections (0.1 mL each) were administered to the shaved scapular areas of test animals (n=20): Freud's adjuvant (50:50 with water), buffered T-20 at 100 mg/mL in sterile water, and buffered T-20 at 100 mg/mL in Freund's adjuvant (50:50 with water). The buffered T-20 solutions included either carbonate (Na_2CO_3) or Tris buffer. Three control groups (n=5 each) received either sterile water, carbonate buffer, or Tris buffer in place of T-20 solutions.

Six days after the injections, the previously treated areas were shaved again and the skins were topically rubbed with 0.5 mL of [redacted] in petrolatum. Twenty-four hours later, patches of [redacted] paper ([redacted]) saturated with 0.4 mL buffered T-20 in sterile water (100 mg/mL) or placebo solutions were applied to the guinea pigs' skin, covered, and secured. After about 48 hours, the patches were removed and the skins were assessed for erythema and edema.

The challenge phase was initiated two weeks after the topical induction phase in a similar manner to the topical induction phase.

Group	Induction treatment	Challenge treatment	Number of animals
1 (Group 2 controls	Carbonate placebo	100 & 50 mg/mL T-20 carbonate	5
2	100 mg/mL T-20 carbonate	100 & 50 mg/mL T-20 carbonate	10
3 (Group 4 controls)	Tris placebo	100 & 50 mg/mL T-20 Tris	5
4	100 mg/mL T-20 Tris	100 & 50 mg/mL T-20 Tris	10
5 (Tris buffer controls)	Sterile water	100 & 50 mg/mL T-20 Tris & Tris placebo	5

Approximately two weeks after the induction phase, both flanks of the animals were exposed to the challenge applications on separate areas of the skin. The higher concentration test material was applied at an anterior site on the flank and the lower concentration material at a posterior site on the flank.

Results:
One test animal from Group 2 was sacrificed prior to the challenge phase because it was thin and had hunched posture, pale eyes, and exhibited difficulty breathing. Necropsy revealed pale spleen and pale liver. [redacted] did not attribute these findings to the treatment. There were no other animals that exhibited signs of ill health. Body weights increased for all other animals during the study.

Intradermal injections: Necrosis was observed in test and control animals receiving [redacted] Slight irritation was seen in test animals at sites receiving either carbonate or Tris buffer formulations of T-20 and in control animals receiving carbonate or Tris placebo. No dermal reactions were seen in Group 5 (sterile water) control animals.

Topical applications: Slight to well-defined erythema was observed in all groups of animals including controls.

In the challenge phase, test animals with the T-20 carbonate formulation (Group 2) exhibited more marked and persistent reactions than controls (Group 1). Four of nine test animals gave positive responses (thickening, dryness, and sloughing of the epidermis). Two test animals also had edema. One animal gave an inconclusive response (slight erythema and edema only to 24 hours) and the other four animals were negative.

Topical challenge with T-20 Tris formulation resulted in well-defined erythema (persistent for 48 to 72 hours), thickening, dryness and sloughing of the epidermis, and slight edema (persistent for at least 72 hours) in all ten animals (group 4 compared with group 3).

No animals in Group 5 (sterile water induction) exhibited dermal reactions.

Overall, T-20 is considered to have the potential to cause skin sensitization (delayed contact hypersensitivity), with the Tris formulation causing a stronger reaction than the carbonate formulation.

In February 2001 (Serial 126), Hoffmann-La Roche indicated that the T-20 Tris formulation would be discontinued clinically.

Special Toxicity Studies

53. NDA 21-481 Reference 3703, Volume 36. IND [redacted] Serial 211.
Exploratory assessment of injection site lesions after subcutaneous administration of T-20 Fusion Inhibitor in minipigs. Roche Study 07385, [redacted] Study 6131-315. Final report RR 1003491 September 18, 2001.

[redacted] conducted this non-GLP study for Hoffmann-La Roche during March and April 2000. The study was conducted to assess the minipig as a model for studying localized injection site lesions following subcutaneous injections of T-20. This was a 14-day repeat-dose study with multiple subcutaneous injections per day, and included a 14-day post dosing observation period. One male and two female minipigs received all four treatments (presented below) twice daily for 14 days.

Injection site	Material	Volume (mL)	Injections/site/ dose	Total injections/site/ day
1	Vehicle 1	1	2	4
2	Vehicle 2	1	1	2
3	T-20 (50 mg/mL)	1	2	4
4	T-20 (100 mg/mL)	1	1	2

The vehicles were mannitol in carbonate solutions:

Mannitol (25 mg/mL), Na_2CO_3 (5.3 mg/mL); reconstituted volume 1 mL. Mannitol (50 mg/mL), Na_2CO_3 (10.6 mg/mL); reconstituted volume 0.5 mL.

The 50 mg/mL T-20 solution was reconstituted in the first vehicle and the 100 mg/mL T-20 solution was reconstituted in the second vehicle. The four injection sites were on the backs of each animal.

The animals were observed twice daily for mortality and morbundity. Daily detailed observations were made for each animal. Dermal irritation was scored for erythema, edema, atonia, desquamation, fissuring, eschar, and exfoliation at each injection site daily, before the first daily injection and during recovery. Palpable subcutaneous masses were measured and recorded. Body weights were recorded pre-study and weekly. Blood samples (3 mL) were collected for antibody analyses before treatment and on Days 15 and 29.

Full-thickness skin samples were taken from each animal at each treatment site and an untreated site on Days 8, 15, and 29 (during recovery). Samples were taken by punch biopsy and divided into approximate quarters. Two quarter samples were fixed in formalin for microscopic examination and the other two quarter samples were fixed in [redacted] fixative for possible electron microscopic evaluation. (However, the em samples were not examined.)

There were no mortalities or moribund animals. All three animals gained weight during the course of the treatment period. Subcutaneous masses ranging from 1 to 5 cm at one or both test material injection sites were noted for each animal beginning on Day 8. The masses dissipated upon cessation of treatment and were no longer apparent by the end of the recovery period. Skin biopsies revealed minimal to moderate edema, hemorrhage, congestion, necrosis, inflammation, and degenerated collagen in both T-20 treated and vehicle treated sites. Two distinct patterns of microscopic changes were observed. On Day 15, changes in the dermis tended to be more evident at sites administered four daily injections of vehicle or 50 mg/mL of T-20 compared with two daily injections of vehicle or 100 mg/mL of T-20. The most prominent microscopic findings in subcutaneous tissue occurred primarily at sites treated daily with four 50 mg/mL or two 100 mg/mL T-20 solutions compared with vehicles. A well-formed granuloma was evident at injection site 3 in one female on Days 8 and 15, which persisted after recovery.

One female exhibited T-20 reactive antibodies at baseline (titer 1:100); her titer increased by Day 15 (1:400) and persisted after treatment (Day 29, 1:800). The other two animals had minimal titers (1:100) at Day 29 only.

54. NDA 21-481 Reference 3704, Volume 36. IND——— Serial 211.
Assessment of injection site lesions in minipigs after subcutaneous administration of three formulations of T-20 Fusion Inhibitor. Roche Study 07421, ——————— Study 6131-320. Final report RR 1003492, September 18, 2001.

——————— conducted this non-GLP study for Hoffmann-La Roche during August and September 2000. This study was conducted to assess injection site lesions following multiple daily subcutaneous injections of three different formulations of T-20 in the minipig. This was a 14-day study with a 14-day post dosing observation period. Three male minipigs received all treatments (presented below) daily for 14 days.

Treatment	Material	Concentration T-20 (mg/mL)	Volume (mL)	Injections/site/ dose	Total injections/site/ day
1	Vehicle carbonate	0	1	2	4
2	Vehicle carbonate	0	1	1	2
3	Vehicle Tris	0	1	1	2
4	T-20 carbonate lyophilized	50	1	2	4
5	T-20 carbonate precipitated	100	1	1	2
6	T-20 Tris	100	1	1	2
7	Untreated site	N/A	0	N/A	N/A

Treatments 1, 2 and 3 were vehicle matches for treatments 4, 5, and 6, respectively.

One or more treatments were suspended based on observations of dose sites as follows: Treatment 4 was discontinued starting at the p.m. dose on Day 7 for animal M01180 and starting at the a.m. dose on Day 8 for the other two animals. Animal M01180 did not receive Treatment 6 on Study Day 9, nor any doses after the morning of Day 9. The other two animals did not receive Treatment 1 after the morning dose of Day 9.

The animals were observed twice daily for mortality and morbundity. Daily detailed observations were made for each animal. Dermal irritation was scored for erythema, edema, atonia, desquamation, fissuring, eschar, and exfoliation at each injection site daily, before the first daily injection and during recovery. Palpable subcutaneous masses were noted beginning on Day 4 and were measured and recorded beginning on Day 8. Body weights were recorded pre-study and weekly. Blood samples (3 mL) were collected for antibody analyses before treatment and on Days 15 and 29.

Skin samples were taken from each animal at some or all the treatment sites on Days 8, 10, 24, and 29. Samples were taken by punch biopsy, surgical or full excision biopsy and divided into approximate halves. Two portions of each biopsy were fixed in formalin for microscopic examination and two more portions were processed for ——————— evaluation.

Results:
There were no mortalities or moribund animals. All three animals gained weight during the course of the treatment period. Subcutaneous masses were observed at the sites of Treatments 4, 5, and 6 beginning on Day 4 that increased in size on Days 5, 6, and 7. Palpable masses ranged

from 1 to 5 cm at injection sites 4, 5, and 6 beginning on Day 8. In addition, a cloudy white discharge from injection site 6 was noted for animal M01178 on Day 25, and erythema was noted for animal M01181 at injection site 4 on Days 8 through 10. The masses dissipated upon cessation of treatment and were generally not apparent by the end of the recovery period.

Well-defined granulomas were evident at injection sites 4, 5, and 6 as early as Day 8 and persisted until Day 29. Granulomas were absent from the vehicle injection sites throughout the study. T-20 with Tris appeared to induce the most severe granulomas and the most number of granulomas compared with the T-20 carbonate formulations. The Tris site granulomas were characterized by a necrotic center with amorphous eosinophilic, proteinaceous debris, and surrounded by multinucleated giant cells and foamy macrophages with distended cytoplasm. Positive staining for T-20 peptide was noted only in biopsy sections with granulomas. Granulomas on Day 29 were smaller than when noted at the end of the treatment period.

One animal exhibited T-20 reactive antibodies on Days 15 and 29 (titers 1:200 and 1:800, respectively). The other two animals had no titers.

In February 2001 (IND —— Serial 126), Hoffmann-La Roche indicated that the T-20 Tris formulation would be discontinued clinically.

Other Nonclinical Toxicology

55. NDA 21-481 Reference 3700, Volume 37. IND — Serial 047.
Effect of subcutaneously administered T-20 on locomotor activity in mice. ——— Study TMS 002/99-4088. Compliance statement signed and report issued January 14, 2000.

———, conducted this study for Trimeris, Inc. at ——— ———, on August 17, 1999 to assess the effects of subcutaneous administration of T-20 on spontaneous locomotor activity in the mouse. — conducted the study under — GLP conditions except that neither Trimeris, Inc. nor — confirmed the concentrations or quality of the dosing solutions.

Boissier and Simon (1965) described the methodology utilized in this study: Boissier JR, Simon P, The action of caffeine on the spontaneous motility of the mouse, Arch Int Pharmacodyn ——— The 50 ICR CD-1 male mice used on this study came from ———

T-20 was administered as a single subcutaneous dose to mice (10/group) at doses of 5, 15, and 50 mg/kg. Two control groups received either placebo (——— and carbonate buffer) or 5 mg/kg diazepam. All doses were administered at a dose volume of 10 mL/kg.

Fifteen minutes following subcutaneous administration, the mice were placed individually into cages on an activity meter. The mobility of the animals was recorded at 10-minute intervals over the following one-hour period. At the end of the one-hour period, the animals were killed.

Results:

There were no statistically significant differences in activity counts between the T-20 treated mice and the placebo controls. Diazepam (the positive control) administered subcutaneously at 5 mg/kg produced statistically significant decreases in activity counts compared to placebo controls between 15 and 45 minutes post dosing.

[redacted] concluded that T-20 administered subcutaneously at doses up to 50 mg/kg did not affect the spontaneous locomotor activity of mice under the conditions of this study. Positive controls showed a marked decrease in spontaneous locomotor activity compared with placebo controls.

Reviewer comment:
This study report does not describe the basis for the dose range selection, except to state that the doses were based on results from previous studies. On a body surface area basis, a mouse dose of 50 mg/kg is a human equivalent dose (to a 60 kg human) of approximately 4 mg/kg. At the time of this study, T-20 had been administered to humans at 200 mg/day, or approximately 3.3 mg/kg/day. Therefore the high dose in this study allows for a safety margin of only about 1.2. It is possible that Trimeris was using proportional pharmacokinetics data and not a body surface area conversion to select doses. Trimeris, Inc. has not previously submitted mouse pharmacokinetics data.

56. NDA 21-481 Reference 3701, Volume 37. IND [redacted] Serial 047.
Irwin general behavior analysis in mice following subcutaneous administration of T-20. [redacted] Study TMS 003/99-4087. Compliance statement signed and report issued January 14, 2000.

[redacted] conducted this study for Trimeris, Inc. at [redacted], during August 1999 to assess the effects of subcutaneous administration of T-20 on the Irwin general behavior in the mouse. [redacted] conducted the study under [redacted] GLP conditions except that neither Trimeris, Inc. nor [redacted] confirmed the concentrations or quality of the dosing solutions.

Irwin (1964) described the methodology utilized in this study: Irwin S, Comprehensive observational assessment: 1a. A systematic quantitative procedure for assessing the behavioral and physiological state in the mouse, Psychopharmacologia, (1964) 13:222-57. The 16 ICR CD-1 male mice used on this study came from [redacted]

T-20 was administered as a single subcutaneous dose to mice (4/group) at doses of 5, 15, and 50 mg/kg. A control group received placebo (mannitol and carbonate buffer). All doses were administered at a dose volume of 10 mL/kg.

At 5, 15, 30, 60, and 120 minutes and 24 hours after dosing, the behavior of T-20 dosed mice was scored to assess changes in behavior or physiological state. Animals were observed daily for mortalities and gross signs of toxicity during a 7-day post-dosing period. At the end of the 7-day period, the animals were killed.

Results:
There were no abnormalities recorded for any animal in any group at any observation time during this study. However, there were no data recorded in the report tables, either. Several

parameters, such as locomotor activity, alertness, startle response, and pupil diameter (and others) have normal score values that are positive integers, but no integers (including zeros) were recorded in the tables for this study report.

[redacted] stated that there were no mortalities or gross signs of toxicity observed during this study or during the 7-day follow-up period and there were no differences in behavior or physiological state between the T-20 treated mice and the placebo control group.

[redacted] concluded that T-20 does not cause Irwin-scale changes in behavior or physiological state in mice under the conditions of this study.

Reviewer comment:
The data tables presented suggest that this study was not conducted in compliance with GLPs. Also, the doses selected for this study appear to be low. See comment in the review for the previous study ([redacted] 002/99-4088).

57. NDA 21-481 Reference 3702, Volume 37. IND [redacted] Serial 047.
Effect of intravenously administered T-20 on cardiovascular and respiratory function in the anaesthetised dog. [redacted] Study [redacted] 004/99-4526. Compliance statement signed and report issued March 24, 2000.

[redacted] conducted this study for Trimeris, Inc. at [redacted], [redacted] during September and October 1999 to assess the effects of intravenous administration of T-20 on various cardiovascular and respiratory parameters in the anaesthetized beagle dog. [redacted] conducted the study under [redacted] conditions except that neither Trimeris, Inc. nor [redacted] confirmed the concentrations or quality of the dosing solutions.

The 4 beagle dogs (1 male, 3 females) used on this study were obtained from [redacted] (no other information provided).

The dogs were deprived of food for a minimum of sixteen hours prior to commencement of the experiment. Anesthesia was induced by intravenous injection of sodium thiopentone and maintained intravenously by a mixture of α–chloralose and pentobarbitone sodium (20 mL 1% α-chloralose + 1 mL Sagatal, 60 mg/mL) given as required. Body temperature was maintained by means of a homeothermic blanket and rectal probe.

[redacted]

[ ]

All four beagles received placebo, and T-20 injections at 5, 15, and 50 mg/kg, in that order. Dosing with placebo occurred at 30 minutes after measurements were begun, and each succeeding T-20 dose occurred 30 minutes after the previous dosing. At the end of the observation period each animal was killed with an overdose of pentobarbitone sodium by intravenous injection.

Results:
T-20 administration at 15 and 50 mg/kg produced a slight mean transient decrease in diastolic pressure and a slight mean increase in systolic pressure. There was an overall slight mean transient decrease in mean arterial blood pressure after administration of T-20 at 50 mg/kg. Over the course of the experiments (150 minutes) there was a gradual decrease in mean arterial blood pressure (-10% to -15%). The blood pressure of one animal (the male) was unaffected by administration of T-20 or placebo.

T-20 administration at 15 mg/kg and 50 mg/kg produced a slight transient mean increase in heart rate. The heart rate of one animal (the male) was unaffected by administration of placebo or T-20.

T-20 administration at 50 mg/kg produced a slight mean decrease left ventricular systolic pressure. T-20 administration at 15 mg/kg and 50 mg/kg induced a small, dose-dependent, transient increase in mean left ventricular dp/dt maximum.

Mean femoral blood flow declined and femoral resistance increased during the course of these experiments. Tidal volume gradually increased over the course of these experiments, but tidal volume decreased transiently after administration of T-20 at 50 mg/kg.

T-20 administration at 15 mg/kg and 50 mg/kg induced a slight, transient dose-related mean increase in respiration rate and a concurrent increase in mean minute volume. Respiration rate and minute volume were unaffected in one animal (the male).

Normal electrocardiograms were obtained throughout the experiments. Two female animals exhibited transient reductions in QRS amplitude after administration of T-20 at 15 mg/kg and 50 mg/kg. A third female exhibited a transient reduction in QRS amplitude accompanied by ST-elevation after administration of 50 mg/kg only. The ECG waveform of the male animal was unaffected by administration of T-20 or placebo.

There were no marked changes in the QTc interval in any animal during this study. There was a slight transient decrease in mean PR interval that coincided with the slight heart rate increase after administration of 50 mg/kg T-20.

[ ] concluded that intravenous administration of T-20 or placebo did not produce any overt cardiorespiratory effects in beagles under the conditions of this study. Slight, transient effects

were noted, but one animal (the male) was unaffected by administration of T-20 or placebo. [redacted] attributed gradual changes over the course of the experiment to the anesthetic.

Reviewer comment:
Slight transient cardiorespiratory effects were noted in anesthetized female beagles after administration of T-20. T-20 was administered three times in increasing doses in 30-minute intervals, but there are no pharmacokinetic data showing that T-20 (at various doses) is fully metabolized within 30 minutes in dogs. Under these conditions, it is not possible to extrapolate the effects to humans who are dosed daily (or twice daily) by subcutaneous injection or infusion. Even the placebo data are not true controls because they do not control for stress or anesthetic for 2.5 hours (the duration of the experiment). Therefore, although the effects noted were small, it is unknown whether they represent a risk in humans.

58. NDA 21-481 Reference 3705, Volume 37. IND [redacted], Serial 319.
Ro 29-9800/000 (T-20 Fusion Inhibitor): A pharmacokinetic study with escalating single intravenous doses administered to fed dogs. Roche Study 07660, [redacted] Project 27610_1. Final Report RR 1007599, April 24, 2002.

In the previous study ([redacted] Study [redacted]004/99-4526, Reference 3702 in this NDA), Ro 29-9800 was administered by intravenous injection to anesthetized beagle dogs to evaluate the effect of Ro 29-9800 on cardiovascular and respiratory function. However, plasma concentrations of Ro 29-9800 were not assessed in that study. Therefore, Hoffmann-La Roche conducted this non-GLP study to collect those data.

The same doses of Ro 29-9800 (0, 5, 15, and 50 mg/kg) were administered in the same dose escalation manner to 3 beagle dogs per sex, and at the same dosing intervals (every 30 minutes) as was done in [redacted] Study [redacted]004/99-4526. However, in this study the dogs were not anesthetized. Blood samples were collected according to the following schedule:

	Injection time (minutes)	Sample collection times (minutes)
Placebo	0	20
5 mg/kg	30	35, 40, 55
15 mg/kg	60	65, 70, 85
50 mg/kg	90	95, 100, 115, 130, 150

[redacted] analyzed the plasma samples for Ro 29-9800 using a validated [redacted] assay. The lower limit of quantitation was [redacted].

Results:
The highest measured Ro 29-9800 plasma concentrations occurred in the first samples following dosing at each administered dose level (Cmax values: 0, 108, 396, and 1240 µg/mL, respectively) and concentrations tapered slowly downward in samples collected at subsequent collection times between dosing. There were no marked gender differences in the measured plasma levels of Ro 29-9800. AUC and half-life values were not calculated since doses were administered sequentially in the same animals.

In ——Study —— 004/99-4526, minor transient decreases in blood pressure and increases in heart rate and respiration rate were noted in females dosed at 15 mg/kg and 50 mg/kg, but not in the single male animal on that study. There were no effects noted in beagles of either sex when dosed at 5 mg/kg.

The sponsor notes that in clinical trial T20-208, the peak plasma concentration after subcutaneous injection of the recommended human clinical dose (100 mg) was approximately 5 µg/mL and the highest plasma concentration attained in the present dog study from the 5 mg/kg administered dose was 108 µg/mL. Therefore, the NOAEL dose in dogs in the cardiovascular and respiratory study resulted in a plasma concentration of Ro 29-9800 (in this study) that was 22 times greater than the plasma concentration in humans following the standard clinical dose.

In this study, low levels of Ro 29-9800 were detected in plasma samples after injection of vehicle (0 mg/kg), although there was no record of misdosing. This observation occurred in other studies for which ——————— conducted the plasma analyses. The sponsor provided no explanation for these observations.

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