MODULE 2.6 NONCLINICAL SUMMARY

2.6.6 TOXICOLOGY WRITTEN SUMMARY

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LIST OF ABBREVIATIONS

ALP	alkaline phosphatase
ALT	alanine aminotransferase
APTT	activated partial thromboplastin time
AST	aspartate aminotransferase
AUC	area under plasma concentration-time curve
b.i.d.	twice daily
C_{max}	peak plasma concentration
DMSO	dimethyl sulfoxide
ECG	electrocardiogram
F	female
F1	first generation
FCA	Freund's complete adjuvant
GD	gestation day
GLP	good laboratory practice
HBr	hydrogen bromide
HPMC	hydroxypropyl-methylcellulose
HR	heart rate
KLH	keyhole limpet hemocyanin
LLNA	local lymph node assay
M	male
MCC	microcrystalline cellulose
MFD	maximum feasible dose
MI	mitotic index
NOAEL	no observed adverse effect level
PEG400	polyethylene glycol 400
PT	prothrombin time
T3	triiodothyronine
T4	tetraiodothyronine/thyroxine
TK	thymidine kinase
TSH	thyroid stimulating hormone

TPGS	alpha-tocopheryl polyethylene glycol succinate
UVA	ultraviolet A

1 BRIEF SUMMARY

TMC125 was studied in single and repeated dose toxicity studies up to and including 3 months in mice, 6 months in rats and 12 months in Beagle dogs, a series of genetic toxicity studies and reproductive and developmental toxicity studies covering fertility to postnatal development. In all pivotal repeated dose toxicity studies plasma levels of TMC125 have been determined. The toxicity studies conducted are listed in the Overview Table 2.6.7.1. The nonclinical toxicity program and design of the studies was consistent with the best scientific practices and international guidelines. The pivotal studies have been conducted according to good laboratory practice (GLP) standards (OECD Principles of GLP[1], which conform to FDA GLP regulations[2]). The individual study details are given in the Tabulated Summaries in Module 2.6.7.

The drug substance, TMC125, has been used in 3 forms in the nonclinical studies, initially in base-form (which was used in a limited number of studies) and subsequently as the hydrogen bromide (HBr) salt which showed better bioavailability. Throughout this document, these forms are referred to as TMC125 base and TMC125 HBr. Finally a spray-dried form of the base became available at a later stage of development. In this form, TMC125 base is amorphorized by spray-drying technology using hydroxypropyl-methylcellulose (HPMC) as a stabilizing polymer and microcrystalline cellulose (MCC) as an inert carrier. Two different ratios of active to polymer were used ([redacted] and [redacted]). The spray-dried formulation was developed for the production of an oral tablet for clinical use. Throughout this document, the spray-dried form is referred to as spray-dried TMC125. In order to assess nonclinical safety of TMC125 at higher exposure levels, some studies originally conducted with the base or the HBr form were repeated with the spray-dried form. These include 1- and 6-month studies in the dog, a fertility and early embryonic development study in the rat and an embryo-fetal development study in the rabbit. The pre- and postnatal development study in the rat was directly performed with the spray-dried form. In an attempt to further increase the exposure, TMC125 was also tested via dietary admixture in rodents using HBr and spray-dried forms (see Module 2.6.4, Pharmacokinetics Written Summary). No improvement in exposure was found by using dietary administration in comparison to gavage administration of TMC125 HBr, a finding which limited the use of dietary administration for further nonclinical testing. The dose levels used in all studies are expressed as the base equivalent and appropriate correction factors were used when the HBr salt and the spray-dried forms were used.

Single dose oral (gavage) and subcutaneous studies were conducted in mice and rats, with TMC125 formulated in polyethylene glycol 400 (PEG400). Single escalating dose oral studies in dogs were conducted with spray-dried TMC125 formulated as tablets or in aqueous suspension.

In **mice**, there were no relevant effects following oral administration of TMC125 base or TMC125 HBr at doses up to 1000 mg/kg. Similarly, there was no effect of TMC125 base administered by subcutaneous injection at doses up to 320 mg/kg.

In **rats**, there was no relevant effect following oral administration of TMC125 base or TMC125 HBr at doses up to 1000 mg/kg. Similarly, there was no effect of TMC125 base administered by subcutaneous injection at doses up to 320 mg/kg, other than skin irritation/necrosis at the site of injection in 1 female treated at the high dose.

In **dogs**, the single dose oral toxicity was determined using spray-dried TMC125 ([redacted] active to polymer ratio) formulated as tablets or as an aqueous suspension in a study comprised of

3 phases. A dose-escalation phase up to 160 mg/kg was followed by a 5-day repeated dose phase at 120 mg/kg/day using tablets. In the third phase, dogs were given 350 mg/kg/day of spray-dried TMC125 in an aqueous suspension by oral gavage for 5 consecutive days in fasted or fed condition. No relevant toxicity findings were observed. The highest exposure was seen in the 350 mg/kg/day fed animals where mean peak plasma concentration (C_{max}) and area under plasma concentration-time curve (AUC_{0-24h}) values on Day 5 were 11 and 7.6 µg/mL and 135 and 92 µg.h/mL in males and females, respectively.

Repeated dose studies evaluated the effects of TMC125 HBr, formulated in PEG400, in mice in studies of 2-week and 3-month durations and these served as dose range finding studies for the carcinogenicity study. Spray-dried TMC125 was tested via dietary admixture in a 3-month study in mice. In Wistar rats TMC125 base was tested in a 2-week study but subsequently TMC125 HBr formulated in PEG400 was used in studies of 1-, 3- and 6-month durations. An additional 3-month study with TMC125 HBr in PEG400 was performed in Sprague-Dawley rats in preparation for the carcinogenicity study. Also in Sprague-Dawley rats, spray-dried TMC125 was tested via the dietary route in a 3-month study. In dogs TMC125 base was tested in a 2-week and 1-month study but subsequently TMC125 HBr formulated in PEG400 was used in studies of 3-, 6- and 12-month durations. When the spray-dried form became available it was tested as an aqueous suspension in dogs in studies of 1- and 6-month durations. In all studies control animals were administered the vehicle and toxicokinetic parameters were evaluated (using satellite animals in the rodent studies).

In **mice,** the oral (gavage) toxicity of TMC125 HBr in PEG400 vehicle was evaluated in a 2-week and 3-month study up to 1200 mg/kg/day. The main target organ in these studies was the liver which showed increases in serum transaminases and histopathological changes, mainly hepatocellular hypertrophy and necrosis. The no observed adverse effect level (NOAEL) was not established due to limited liver changes at the lower dose levels. Systemic exposures (AUC_{0-24h}) observed at the highest dose in the 3-month study were 7.8 and 10.1 µg.h/mL in males and females, respectively. A dietary 3-month study in mice with spray-dried TMC125 resulted in mortality in males from a dose level of 450 mg/kg/day. The cause of mortality was attributed to a disturbance in blood coagulation leading to cardiomyopathy, hemothorax and bleedings. These findings were further investigated in 2 mechanistic studies (1 with TMC125 HBr given via oral gavage and another with spray-dried TMC125 given via the diet) and were shown to be mediated via the vitamin K pathway. Cardiac lesions and hemorrhages were only observed in the male mice and not in females and were only seen with dietary dosing. The changes in blood coagulation were less pronounced in female mice relative to males and they were more pronounced after dietary administration relative to gavage. Liver changes were more apparent after dietary dosing. The highest systemic exposures (AUC_{0-24h}) in mice in the dietary 3-month study were 10.2 and 8.12 µg.h/mL in males and females, respectively.

In **rats,** the oral (gavage) toxicity of TMC125 in PEG400 vehicle was evaluated in Wistar rats in a study of 2 weeks duration, at doses up to 320 mg/kg/day using TMC125 base and in studies up to 6 months duration and 600 mg/kg/day using TMC125 HBr. As dose range finding studies for the carcinogenicity study, a 3-month gavage study with TMC125 HBr was performed, as well as a 3-month dietary study with spray-dried TMC125 in Sprague-Dawley rats. There were no deaths associated with TMC125 in any study, but there were a limited number of accidental deaths that were considered related to the viscous and irritant nature of the vehicle (PEG400) used in the gavage studies. There were no relevant clinical signs or effects on body weight or

food consumption and no abnormalities detected during ophthalmoscopy. Adaptive histopathological liver changes (hypertrophy) were observed in all rat studies of 3-month duration and longer. These changes were not associated with increases in liver transaminases. Thyroid changes, indicative of an increased activity, were also observed in studies of 3-month duration and longer and were considered to be a rodent-specific response to liver enzyme induction and increased elimination of thyroid hormones. The NOAEL in the 6-month study with TMC125 HBr following oral gavage was 70 mg/kg/day, where exposure ($AUC_{0\text{-last}}$) was 0.21 and 0.66 µg.h/mL, in males and females, respectively. No NOAEL was determined in the 3-month dietary study due to limited changes observed at the lowest dose level. Systemic exposure ($AUC_{0\text{-}24h}$) at 330 mg/kg/day was 1.21 and 2.95 µg.h/mL in males and females, respectively. At the maximum tested dose, exposure values obtained with dietary administration of spray-dried TMC125 (2.96-5.34 µg.h/mL at 1300 mg/kg/day) were similar to those obtained with gavage administration with TMC125 HBr (2.29-6.82 µg.h/mL at 600 mg/k/day).

In **dogs,** the oral (gavage) toxicity of TMC125 base formulated in PEG400 was evaluated in 2-week and 1-month studies at dose levels up to 160 mg /kg/day, and using TMC125 HBr in studies up to 12 months and 240 mg/kg/day. Recovery from possible effects was also studied in the 12-month study. TMC125 was well tolerated and no target organs of toxicity were observed. The NOAEL after 12 months of dosing was 240 mg/kg/day corresponding to an $AUC_{0\text{-}24h}$ of 35.6 and 23.2 µg.h/mL in males and females, respectively. Additional 1- and 6-month dog studies with spray-dried TMC125 suspended in water were conducted with dose levels up to 500 mg/kg/day given over 2 equal doses separated by 5 hours. The highest dose level was associated with decreases in food consumption and relevant decreases in body weight. From a dose level of 160 mg/kg/day hepatic changes (microgranuloma) were observed, while gall bladder changes (inspissated bile) were seen after a dose of 500 mg/kg/day. The occurrence of these changes after dosing the spray-dried form is probably related to a higher systemic exposure ($AUC_{0\text{-}24h}$) which amounted to 61.8 and 61.5 µg.h/mL in males and females, respectively, after 6 months of dosing at 500 mg/kg/day.

Genotoxicity tests, in vitro and in vivo, have shown TMC125 to be free of genotoxic potential. These studies included an in vitro mutation assay (Ames test), and in vitro clastogenicity assays (mouse lymphoma test and cytogenetic test in human lymphocytes) with and without liver metabolic activation system and an in vivo clastogenicity assay (mouse bone marrow micronucleus test; at a single dose of 2000 mg/kg in PEG400). In the latter test, C_{max} and $AUC_{0\text{-}\infty}$ were 7.85 µg/mL and 111 µg.h/mL, respectively.

Carcinogenicity studies are ongoing and no information on the potential carcinogenic risk of TMC125 is yet available. Dosing of animals in the 2-year carcinogenicity studies in mice and rats with TMC125 HBr has been completed and histopathological examination is being performed. Dose range finding studies in the mouse and rat have been completed, with TMC125 HBr via gavage or spray-dried TMC125 via the diet. Gavage administration using TMC125 HBr was chosen for the carcinogenicity studies as spray-dried TMC125 administered via the diet did not increase the exposure and was not tolerated in male mice. In addition, the use of transgenic RasH2 mice has been explored in a dose range finding study, but a classical 2-year study in conventional mice is being conducted.

Reproductive and developmental effects of TMC125 HBr in PEG400 vehicle were evaluated in a fertility and early embryonic development study in rats and in embryo-fetal development studies in rat and rabbit. In order to maximize exposure, a fertility study in the rat and an

embryo-fetal development study in the rabbit were repeated with spray-dried TMC125 suspended in water. Also a pre- and postnatal development study in rats was performed with this form.

A first **fertility and early embryonic development study** in rats was performed with TMC125 HBr in PEG400. No relevant effects on male or female fertility were observed in this study up to a dose level of 506 mg/kg/day. The study was repeated with spray-dried TMC125 suspended in water up to a dose level of 500 mg/kg/day. No relevant effects were observed in either study and the NOAEL was 506 or 500 mg/kg/day. Exposure in the spray-dried study amounted to ($AUC_{0\text{-}24h}$) 4.54 and 9.09 μg.h/mL in males and females, respectively.

In an **embryo-fetal developmental toxicity study** pregnant rats were dosed with TMC125 HBr in PEG400 up to 1000 mg/kg/day and this did not result in maternal or fetal toxicity. The fetal NOAEL was 1000 mg/kg/day and TMC125 is therefore considered not to be teratogenic in the rat. Maternal exposure ($AUC_{0\text{-}24h}$) at this dose level amounted to 8.27 μg.h/mL.

In an **embryo-fetal developmental toxicity study** TMC125 HBr formulated in alpha-tocopheryl polyethylene glycol succinate (TPGS)/HPMC was dosed in pregnant rabbits up to 750 mg/kg/day. TMC125 has shown no teratogenic potential, however, exposure ($AUC_{0\text{-}24h}$) at end of treatment at the highest dose level was only 3.76 μg.h/mL. Therefore, an additional study was conducted using spray-dried TMC125 suspended in water and at doses up to 375 mg/kg/day. The maternal NOAEL was 125 mg/kg/day ($AUC_{0\text{-}24h}$ 6.09 μg.h/mL) and fetal NOAEL was 375 mg/kg/day ($AUC_{0\text{-}24h}$ 9.67 μg.h/mL). It is concluded that TMC125 is not teratogenic in rabbits.

No adverse effects were observed in a **pre- and postnatal development study** in the rat using spray-dried TMC125 at dose levels up to 500 mg/kg/day. Clinical condition, sensory function/reflexes, behavior and reproductive performance of F1 pups were unaffected by treatment with spray-dried TMC125. There were no delayed effects of maternal treatment with TMC125 on the selected offspring allowed to develop to adulthood. Similarly, the maternal NOAEL was 500 mg/kg/day. The highest maternal exposure ($AUC_{0\text{-}24h}$) achieved at end of treatment was 7.60 μg.h/mL.

Local tolerance has been evaluated in a range of in vivo and in vitro studies. TMC125 was classified as "nonsensitizing" based on an in vivo guinea pig skin sensitization study and was also found to be unlikely to cause skin sensitization in a mouse local lymph node assay (LLNA). TMC125 was also classified as "nonirritant" based on an in vivo rabbit skin irritation study. In an in vitro eye irritation assay, TMC125 base was considered as a "mild" eye irritant, whereas TMC125 HBr was considered as a "very severe" eye irritant. No effects were observed in an in vitro phototoxicity study.

Immunotoxicity has been evaluated in rats. TMC125 HBr formulated in PEG400 was administered at a dose of 600 mg/kg/day for 4 weeks. There were no relevant effects of treatment with TMC125 and the immune response, as measured by IgM production, was not affected by treatment. Exposures ($AUC_{0\text{-}24h}$) amounted to 1.54 and 5.44 μg.h/mL, in males and females respectively, at the end of the study.

The toxicity of several **drug substance impurities and intermediates** of TMC125 was investigated. The impurities were qualified above their specified levels and all tested compounds were considered nongenotoxic.

In conclusion, the key target organs in rats were the liver, the thyroid and the coagulation system. Changes in the liver and thyroid were considered adaptive or rodent-specific. No target organs of toxicity were identified in dogs dosed with TMC125 HBr up to 12 months at 240 mg/kg/day. Dosing with spray-dried TMC125 further increased exposure in the dog and this resulted in hepatic and gall bladder changes in the 1- and 6-month studies. The main target organs in the mouse were the liver, the coagulation system and the heart as a secondary target to changes in coagulation (only after dietary dosing). Degenerative/inflammatory changes were observed in the liver following oral gavage and dietary administration. Dietary dosing disturbed blood coagulation leading to hemorrhages, diathesis and subsequent cardiac lesions in male mice. The disturbance in coagulation parameters was mediated via vitamin K pathway. Cardiac lesions were not observed in female mice, or in male and female rats or dogs.

There was no evidence of a genotoxic effect. Carcinogenicity studies are currently ongoing and no information on the potential carcinogenic risk is yet available.

TMC125 was not associated with effects on fertility or early embryonic development and showed no teratogenic potential. Pre- and postweaning functions were unaffected.

2 SINGLE-DOSE TOXICITY

Single dose oral (gavage) and subcutaneous studies were conducted in mice and rats, with TMC125 base and TMC125 HBr formulated in PEG400. An escalating single dose oral study in dogs was conducted with spray-dried TMC125 as tablets or in aqueous suspension.

2.1 MOUSE STUDIES

2.1.1 Oral

TMC125 base formulated in PEG400 was administered once, by oral gavage, at 0 (vehicle), 40, 160 and 640 mg/kg in a dose volume of up to 10 mL/kg, to 2 CD1 mice/sex/dose[3]. For 3 days after dosing, regular observations were made for clinical signs and body weight. All animals were subject to macroscopic examination at necropsy.

All animals survived. There were no noteworthy clinical observations or effects on body weight and no relevant macroscopic findings were observed at necropsy. The NOAEL was considered to be 640 mg/kg. No toxicokinetic determinations were made in this study. See Tabulated Summary 2.6.7.5.

2.1.2 Oral (GLP)

TMC125 HBr formulated in PEG400 was administered once, by oral gavage, at 0 (vehicle), 500 and 1000 mg/kg in a dose volume of 10 mL/kg to 5 CD1 mice/sex/dose[4,5]. For 2 weeks after dosing, regular observations were made for clinical signs and body weight. Blood samples were collected during the first 8 hours after dosing for toxicokinetic analysis. All animals were subject to macroscopic examination at necropsy.

All animals survived. There were no noteworthy clinical observations, apart from slight body weight loss (8% and 12%) on Day 3 in 2 females given 1000 mg/kg, and no macroscopic findings were observed at necropsy. The NOAEL was considered to be 500 mg/kg based on the body weight effect at the highest dose. See Tabulated Summary 2.6.7.5.

Toxicokinetic analysis indicated nonlinear pharmacokinetics. Systemic exposure expressed as C_{max} and $AUC_{0\text{-}8h}$ values are given in Table 1 below.

Table 1: C_{max} and $AUC_{0\text{-}8h}$ Values of TMC125 HBr After Single Oral Dose in Mice

TMC125 Dose (mg/kg)	C_{max} (µg/mL)		$AUC_{0\text{-}8h}$ (µg.h/mL)	
	M	F	M	F
500	2.80	1.47	10.4	8.70
1000	7.69	4.50	28.1	24.0

2.1.3 Subcutaneous

TMC125 base formulated in PEG400 was administered once, by subcutaneous injection, at 0 (vehicle), 20, 80 and 320 mg/kg in a dose volume of up to 5 mL/kg, to 2 CD1 mice/sex/dose[6].

For 3 days after dosing, regular observations were made for clinical signs and body weight. All animals were subject to macroscopic examination at necropsy.

All animals survived and there were no noteworthy clinical observations or effects on body weight. The only macroscopic finding observed at necropsy was powdery white deposits at the site of injection. The NOAEL was considered to be 320 mg/kg. No toxicokinetic determinations were made in this study. See Tabulated Summary 2.6.7.5.

2.2 RAT STUDIES

2.2.1 Oral

TMC125 base formulated in PEG400 was administered once, by oral gavage, at 0 (vehicle), 40, 160 and 640 mg/kg in a dose volume of up to 10 mL/kg, to 2 Wistar rats/sex/dose[7]. For 3 days after dosing, regular observations were made for clinical signs and body weight. All animals were subject to macroscopic examination at necropsy and gross lesions were examined microscopically.

All animals survived. There were no noteworthy clinical observations or effects on body weight and no relevant macroscopic or microscopic findings were observed at necropsy. The NOAEL was considered to be 640 mg/kg, based on the absence of effects. No toxicokinetic determinations were made in this study. See Tabulated Summary 2.6.7.5.

2.2.2 Oral (GLP)

TMC125 HBr formulated in PEG400 was administered once, by oral gavage, at 0 (vehicle), 500 and 1000 mg/kg in a dose volume of 5 mL/kg, to 5 Wistar rats/sex/dose[8,9]. For 2 weeks after dosing, regular observations were made for clinical signs and body weight. Blood samples were collected during the first 8 hours after dosing for toxicokinetic analysis. All animals were subject to macroscopic examination at necropsy.

All animals survived. There were no noteworthy clinical observations or effects on body weight and no macroscopic findings were observed at necropsy. The NOAEL was considered to be 1000 mg/kg, based on the absence of effects. See Tabulated Summary 2.6.7.5.

Toxicokinetic analysis indicated nonlinear pharmacokinetics. Systemic exposure expressed as C_{max} and $AUC_{0-\infty}$ values are given in Table 2 below.

Table 2: C_{max} and $AUC_{0-\infty}$ Values of TMC125 HBr After Single Oral Dose in Rats

TMC125 Dose (mg/kg)	C_{max} (µg/mL)		$AUC_{0-\infty}$ (µg.h/mL)	
	M	F	M	F
500	0.57	0.93	2.68	4.50[a]
1000	0.60	0.96	2.92[a]	5.06

[a] AUC_{0-8h}

2.2.3 Subcutaneous

TMC125 base formulated in PEG400 was administered once, by subcutaneous injection, at 0 (vehicle), 20, 80 and 320 mg/kg in a dose volume of up to 5 mL/kg, to 2 Wistar rats/sex/dose[10].

For 3 days after dosing, regular observations were made for clinical signs and body weight. All animals sacrificed at the end of the study were subject to macroscopic examination at necropsy.

All animals survived and there were no effects on body weight. At all dose levels, powdery white deposits at the site of injection were noted. At 320 mg/kg 1 of the 2 females showed skin irritation and necrosis at the injection site. The NOAEL was considered to be 80 mg/kg, based on the effects at the injection site. No toxicokinetic determinations were made in this study but in a similar study[11] toxicokinetics were determined at 320 mg/kg. In the latter study, C_{max} was 0.04 µg/mL and the corresponding $AUC_{0-\infty}$ was 1 µg.h/mL in males and females. See Tabulated Summary 2.6.7.5.

2.3 DOG STUDIES

2.3.1 Oral (Spray-dried)

This study was done in 3 phases using spray-dried TMC125 ([redacted] active to polymer ratio) formulated as 100 mg tablets or as an aqueous suspension[12]. The first part was a dose-escalation phase. Escalating doses were administered once, every 3 or 4 days as tablets, to 1 male and 1 female Beagle dog at 0 (vehicle), 40, 80, 120 and 160 mg/kg. In the second phase, 1 male and 1 female were dosed at the maximum feasible dose (MFD), 120 mg/kg/day (12 tablets), for 5 consecutive days. In a third phase, to explore increasing exposure, 2 male and 2 female dogs were given spray-dried TMC125 aqueous suspension by oral gavage at 350 mg/kg/day for 5 consecutive days after overnight fasting, followed by a 5-day wash-out period and a 5-day administration with the same formulation, 30 minutes after gavage of a mashed diet. The dose of 350 mg/kg, given in a volume of 10 mL/kg, was considered the MFD for this formulation. In all phases, regular observations were made for clinical signs, body weight, hematology and serum biochemistry. Electrocardiogram (ECG) and heart rate (HR) were determined during the third phase. Blood samples for toxicokinetic analysis were collected during the 24 hours after each single dose in the dose-escalation phase and at Day 1 and Day 5 in each of the repeated dose Phase 2 (tablet) and Phase 3 (suspension). The 2 animals used in all phases were sacrificed at the end of the dosing period and subjected to macroscopic examination at necropsy followed by histopathological examination. The 2 other animals used only in phase 3 were retained.

All animals survived the study. There were no effects on body weight, hematological or serum parameters in any phase of the study, nor changes in ECG or HR during the third study phase. Although there were no TMC125-related adverse effects in the dose escalation phase, for practical reasons a repeated dose of 120 mg/kg/day (12 tablets) was selected. In the first repeated dose phase (tablets), mucoid feces were noted in the female animal and a slight increase (27%) in bilirubin in the male. In the second repeated dose phase (suspension), mucoid feces were again present in the fasted animals. In animals fed before dosing, reversible ataxia, spasms and mydriasis occurred in 1 male dog and bilirubin was again slightly increased in both male animals. As no central nervous system symptoms have been observed in any other study in dogs with TMC125, it is likely that this animal suffered from a spontaneous epileptic attack. No relevant changes were observed at macro- and microscopical examination. See Tabulated Summary 2.6.7.5.

Toxicokinetic analysis during the dose escalation phase indicated nonlinear pharmacokinetics. Systemic exposure expressed as C_{max} and AUC values are given in Table 3 below. Highest

exposures were achieved with spray-dried TMC125 suspension, especially when given under fed condition.

Table 3: C_{max} and AUC Values of Spray-dried TMC125 After Oral Administration in Dogs

TMC125 Dose (mg/kg)	C_{max} (µg/mL)		AUC[a] (µg.h/mL)	
Escalation phase	**M (n=1)**	**F (n=1)**	**M (n=1)**	**F (n=1)**
40	1.09	0.90	25.2	14.9
80	1.69	1.20	31.2	14.4
120	1.35	1.54	27.1	17.5
160	1.84	2.43	28.5	42.1
Repeat dose not fasted (tablet)	**M (n=1)**	**F (n=1)**	**M (n=1)**	**F (n=1)**
120 (1st dose)	1.74	5.48	15.9[b]	72.9
120 (5th dose)	2.87	3.82	42.1	62.5
Repeat dose (suspension)	**M (n=2)**	**F (n=2)**	**M (n=2)**	**F (n=2)**
350 fasted (1st dose)	4.57	3.91	54.1[b]	40.9[b]
350 fasted (5th dose)	6.01	5.15	83.5	69.4
350 fed (1st dose)	9.51	8.97	77.6[b]	100[b]
350 fed (5th dose)	11.1	7.61	135	92.4

[a] $AUC_{0-\infty}$ after single dose and AUC_{0-24h} after repeat dose; [b] AUC_{0-24h}

3 REPEAT-DOSE TOXICITY

A 2-week repeated dose study using TMC125 base was performed in the Wistar rat. TMC125 HBr was used in 1-month, 3-month and 6-month studies in the same strain. A 2-week repeated dose study using TMC125 base was performed in the Beagle dog. TMC125 HBr was used in 3-month, 6-month and 12-month studies in the dog. TMC125 spray-dried ([redacted] active to polymer ratio) was tested in a 4-day pilot study in the dog followed by a 1-month and 6-month repeated dose study. In all studies control animals were administered the vehicle and toxicokinetic parameters were evaluated (using satellite animals in the rat studies).

3.1 RAT STUDIES

3.1.1 14-Day Oral

TMC125 base formulated in PEG400 was administered once daily, by oral gavage, for 14 days[13]. Two control groups and 3 treated groups were given, 0 (water), 0 (vehicle), 20, 80 and 320 mg/kg/day in a dose volume of up to 6 mL/kg. Each group consisted of 5 male and 5 female Wistar rats (main animals) together with 4 male and 4 female satellite animals (treated groups) for toxicokinetic analysis. Regular observations were made for clinical signs, body weight and food consumption, together with ophthalmoscopic examination and clinical laboratory investigations (hematology, blood chemistry and urine analysis) at termination. Blood samples were collected on Day 13 during the first 24 hours after dosing for toxicokinetic analysis. All animals found dead or sacrificed during or at the end of the study were subject to macroscopic examination at necropsy, and a number of organs were weighed from the main animals. Samples of a range of tissues and organs were preserved for histological examination. Tissues and organs from main animals in both control groups and the high dose group and all gross lesions were examined microscopically.

There were no mortalities associated with TMC125, but there were 5 accidental deaths associated with the irritant and viscous nature of the vehicle. These were in main animals: 1 female and 2 females in groups given 80 and 320 mg/kg/day, respectively, and in satellite animals: 1 male and 1 female in the group given 80 mg/kg/day. There were no relevant clinical signs or changes in the eyes, or effects on body weight, food consumption, clinical laboratory investigations or organs weights. Macroscopic and microscopic examination revealed no treatment-related changes. The NOAEL was considered to be 320 mg/kg/day based on the absence of any relevant changes. See Tabulated Summary 2.6.7.6.A.

Toxicokinetic analysis indicated nonlinear pharmacokinetics. Systemic exposure expressed as C_{max} and $AUC_{0\text{-}24h}$ values are given in Table 4 below.

Table 4: C_{max} and AUC_{0-24h} Values of TMC125 Base on Day 13 After Repeated Oral Administration (2 Weeks) in Rats

TMC125 Dose (mg/kg/day)	C_{max} (µg/mL)		AUC_{0-24h} (µg.h/mL)	
	M	F	M	F
20	0.09	0.07	0.31[a]	0.30[a]
80	0.27	0.12	0.65[a]	0.49[a]
320	0.18	0.15	0.99	0.96

[a] AUC_{0-8h}

3.1.2 1-Month Oral (GLP)

TMC125 HBr formulated in PEG400 was administered once daily, by oral gavage, for at least 28 days[14,15]. One control group and 3 treated groups were given, 0 (vehicle), 20, 80 and 320 mg/kg/day in a dose volume of 5 mL/kg. Each group consisted of 10 male and 10 female Wistar rats (main animals) together with 5 male and 5 female satellite animals (treated groups) or 2 male and 2 female satellite animals (control group) for toxicokinetic analysis. Regular observations were made for clinical signs, body weight and food consumption, together with ophthalmoscopic examination and clinical laboratory investigations (hematology, blood chemistry and urine analysis) at termination. Blood samples were collected on Day 1 and Day 29 during the 24 hours after dosing for toxicokinetic analysis. All main animals were subject to macroscopic examination at necropsy, and a number of organs were weighed. Samples of a range of tissues and organs were preserved for histological examination. Tissues and organs from main animals in the control and high dose group and a limited list of organs in the low and intermediate dose groups, as well as from all main animals that died or were sacrificed preterminally, and all gross lesions were examined microscopically.

There were no mortalities associated with TMC125, but there were 2 accidental deaths associated with the blood collection procedure in the satellite animals: 1 in each of the groups given vehicle or 80 mg/kg/day. There were no relevant clinical signs or changes in the eyes, or effects on body weight or food consumption. There were some minor changes in urinary electrolytes (sodium and potassium). Macroscopic evaluation, including organs weights, and microscopic examination revealed no treatment-related changes. The NOAEL was considered to be 320 mg/kg/day based on the absence of any relevant histopathological changes. See Tabulated Summary 2.6.7.7.A.

Toxicokinetic analysis indicated nonlinear pharmacokinetics. Systemic exposure expressed as C_{max} and AUC values are given in Table 5 below.

Table 5: C_{max} and AUC Values of TMC125 HBr After Repeated Oral Administration (4 Weeks) in Rats

TMC125 Dose (mg/kg/day)	Sampling Day	C_{max} (µg/mL)		AUC[a] (µg.h/mL)	
		M	F	M	F
20	Day 1	0.07	0.07	0.28[b]	0.31[b]
	Day 29	0.06	0.05	0.24[b]	0.27[b]
80	Day 1	0.07	0.11	0.41[b]	0.59[b]
	Day 29	0.05	0.09	0.44	1.01
320	Day 1	0.21	0.26	1.53	1.16[b]
	Day 29	0.20	0.18	1.16	1.52

[a] $AUC_{0-\infty}$ after single dose (Day 1) or AUC_{0-24h} after repeated dose; [b] AUC_{0-8h}

3.1.3 3-Month Oral (GLP)

TMC125 HBr formulated in PEG400 was administered once daily, by oral gavage, for at least 13 weeks[16,17]. One control group and 3 treated groups were given, 0 (vehicle), 70, 200 and 600 mg/kg/day in a dose volume of 5 mL/kg. Each group consisted of 20 male and 20 female Wistar rats (main animals) together with 5 male and 5 female satellite animals (treated groups only) or 2 male and 2 female (vehicle group) for toxicokinetic analysis. Regular observations were made for clinical signs, body weight and food consumption, together with ophthalmoscopic examination and clinical laboratory investigations (hematology, blood chemistry and urine analysis) in week 6 and 13. Blood samples were collected on Day 1 and Week 13 during the 24 hours (and again at 48 hours in Week 13) after dosing for toxicokinetic analysis. All main animals found dead or sacrificed during or at the end of the study were subject to macroscopic examination at necropsy, and a number of organs were weighed. Samples of a range of tissues and organs were preserved for histological examination. Tissues and organs from main animals in the control and high dose group, selected tissues in low and intermediate dose group, as well as from all main animals that died or were sacrificed early, and all gross lesions were examined microscopically. Staging of spermatogenesis was done on extra sections of testes in 10 control and 10 high dose animals.

There were no mortalities associated with TMC125, but there were 5 deaths in the study. These were in main animals: 2 control females and 1 male given 70 mg/kg and in satellite animals: 1 male and 1 female given 600 mg/kg. Death was due to gavage accidents and the irritant and viscous nature of the vehicle in 3 of the cases. However, the cause of death was not established for 1 control female and 1 male given 70 mg/kg but was considered unrelated to treatment. No changes in the eyes, or effects on body weight or food consumption were observed. Hematology was not affected by treatment but small increases (up to 11%) in serum potassium and total protein were noted in animals given 600 mg/kg/day. Similarly there were increases in urine electrolytes (sodium (2.1-fold), calcium (96%)) in animals given 600 mg/kg/day. Macroscopic evaluation revealed no treatment-related changes, but liver weight was slightly increased (up to 9%) in females given 600 mg/kg/day. Microscopic examination revealed treatment-related changes in liver, kidney, thyroid and pituitary. The basophilic stippling of the liver cytoplasm in high dose females (together with increased weight) reflects enzyme induction as an adaptive response after administration of a xenobiotic, rather than a direct adverse effect of TMC125. The increased incidence of thyroid hypertrophy and/or hyperplasia of follicular cells at 200 mg/kg/day and above, and the slight increase in the severity of hypertrophic/vacuolated cells

in the pituitary pars anterior in males at 600 mg/kg/day, probably represent a rodent specific adaptive response as a consequence of liver enzyme induction and enhanced elimination of thyroid hormones. The increased incidence of tubular basophilia in the kidneys of males at 200 mg/kg/day and above and in females at 600 mg/kg/day may be associated with the urinary changes. Staging of spermatogenesis revealed normal testicular function. The NOAEL was considered to be 70 mg/kg/day based on the absence of any relevant histopathological changes. See Tabulated Summary 2.6.7.7.B.

Toxicokinetic analysis indicated nonlinear pharmacokinetics. Systemic exposure expressed as C_{max} and AUC values are given in Table 6 below.

Table 6: C_{max} and AUC Values of TMC125 HBr After Repeated Oral Administration (13 Weeks) in Rats

TMC125 Dose (mg/kg/day)	Sampling Day	C_{max} (µg/mL)		AUC[a] (µg.h/mL)	
		M	F	M	F
70	Day 1	0.07	0.08	0.46	0.55[b]
	Day 84	0.04	0.09	0.58	1.03
200	Day 1	0.15	0.15	0.82[b]	0.92[b]
	Day 84	0.06	0.12	0.69	1.40
600	Day 1	0.28	0.33	1.81	3.32
	Day 84	0.12	0.32	1.69	4.40[c]

[a] $AUC_{0-\infty}$ after single dose (Day 1) or AUC_{0-24h} after repeated dose; [b] AUC_{0-8h}; [c] AUC_{0-48h}

3.1.4 6-Month Oral (GLP)

TMC125 HBr formulated in PEG400 was administered once daily, by oral gavage, for at least 26 weeks[18,19]. One control group and 3 treated groups were given, 0 (vehicle), 70, 200 and 600 mg/kg/day in a dose volume of 5 mL/kg. Each group consisted of 20 male and 20 female Wistar rats (main animals) together with 9 male and 9 female satellite animals for toxicokinetic analysis. Regular observations were made for clinical signs, body weight and food consumption, together with ophthalmoscopic examination and clinical laboratory investigations (hematology, blood chemistry and urine analysis) in week 13 and 26. Blood samples were collected on Day 1, Week 13 and Week 26 during the 24 hours (and again at 48 hours in Week 26) after dosing for toxicokinetic analysis. All main animals found dead or sacrificed during or at the end of the study were subject to macroscopic examination at necropsy, and a number of organs were weighed. Samples of a range of tissues and organs were preserved for histological examination. Tissues and organs from main animals in the control and high dose group and a limited number of organs in the low and intermediate dose groups, as well as from all main animals that died or were sacrificed early, and all gross lesions were examined microscopically.

There were no mortalities associated with TMC125. One female given 600 mg/kg/day died as a result of a pyogranulomatous skin lesion. There was no evidence of toxicity or treatment-related effects contributing to the animal's morbidity. There were no relevant clinical signs or changes in the eyes, or relevant effects on body weight or food consumption. Hematology was not affected by treatment but a dose-related increase (up to 11%) in total protein was noted at 200 and 600 mg/kg in females. Other than an increase in urinary volume (up to 58%) in animals given 600 mg/kg/day, there were no effects of treatment on urinary parameters. Macroscopic evaluation revealed no treatment-related changes, but liver weight was slightly increased (up to

10%) in females given 600 mg/kg/day and thyroid weights were increased in males (15%) at the same dose. Microscopic examination revealed treatment-related changes in thyroid glands only and no changes in liver, kidney and pituitary. There was an increased incidence and severity of thyroid follicular cell hypertrophy in animals given 200 mg/kg/day and above. These changes probably represent a rodent specific adaptive response to enhanced elimination of thyroid hormones. The NOAEL was considered to be 70 mg/kg/day based on the absence of any relevant histopathological changes. See Tabulated Summary 2.6.7.7.C.

Toxicokinetic analysis indicated nonlinear pharmacokinetics. Systemic exposure expressed as C_{max} and AUC values are given in Table 7 below.

Table 7: C_{max} and AUC Values of TMC125 HBr After Repeated Oral Administration (26 Weeks) in Rats

TMC125 Dose (mg/kg/day)	Sampling Period	C_{max} (µg/mL)		AUC[a] (µg.h/mL)	
		M	F	M	F
70	Day 1	0.02	0.05	0.13[b]	0.27[b]
	Week 13	0.05	0.07	0.41	0.69
	Week 26	0.06	0.06	0.21[b]	0.66
200	Day 1	0.04	0.07	0.63	0.92
	Week 13	0.04	0.07	0.23[b]	0.89
	Week 26	0.05	0.14	0.56	1.29
600	Day 1	0.33	0.24	1.97	1.95
	Week 13	0.14	0.31	1.84	4.53
	Week 26	0.12	0.28	1.73	3.56

[a] $AUC_{0-\infty}$ after single dose (day 1) or AUC_{0-24h} after repeated dose; [b] AUC_{0-8h}

3.2 DOG STUDIES

3.2.1 4-Day Oral (Spray-dried)

Spray-dried TMC125 ([redacted] active to polymer ratio) formulated as a suspension in demineralized water was administered twice daily, by oral gavage, for 4 days[20]. One male and 1 female beagle dog were given 500 mg/kg/day, divided over 2 equal doses, 5 hours apart, in a dose volume of 10 mL/kg per dose. Regular observations were made for clinical signs and body weight. Blood samples were collected on Day 4 for toxicokinetic analysis but not analyzed, as the dose was considered sufficiently safe to proceed into a 1-month study. The animals were not sacrificed after dosing but were retained.

Both animals survived. In both dogs mydriasis was observed on the first day at 4 hours after the first and at 1, 2 and 4 hours after the second dose, as well as on the next 2 days of dosing but not at the last day. No relevant effects were seen on body weight. This study demonstrated the tolerability of spray-dried TMC125 at 500mg/kg/day, which was used subsequently in longer duration studies. Toxicokinetic evaluations were not determined in this study. See Tabulated Summary 2.6.7.6.B.

3.2.2 14-Day Oral

TMC125 base formulated in PEG400 was administered twice daily, by oral gavage, for 14 days[21]. One control group and 3 treated groups were given 0 (vehicle), 10, 40 and

160 mg/kg/day, divided over 2 equal doses, 6 hours apart, in a dose volume of 1 mL/kg/dose. Each group consisted of 2 male and 2 female Beagle dogs. Regular observations were made for clinical signs, body weight and food consumption, together with ophthalmoscopic examination, ECG and clinical laboratory investigations (hematology, blood chemistry at Day 7 and Day 13, urine analysis at Day 13). Blood samples were collected on day 13 during the first 24 hours after dosing for toxicokinetic analysis. All animals sacrificed at the end of the study were subject to macroscopic examination at necropsy and a number of organs were weighed. Samples of a range of tissues and organs were preserved for histological examination. All tissues and organs from animals in all dose groups and all gross lesions were examined microscopically.

There were no mortalities in the study and there were no relevant clinical signs, or effects on body weight, food consumption, ophthalmoscopy or ECG. In the laboratory investigations, hematological, serum chemistry and urinalysis parameters were unchanged after TMC125 treatment. Macroscopic examination at necropsy, organ weights and histopathological examination indicated no relevant changes that could be attributable to treatment. The NOAEL was considered to be 160 mg/kg/day based on the absence of any relevant changes. See Tabulated Summary 2.6.7.6.C.

Systemic exposure expressed as C_{max} and $AUC_{0\text{-}24h}$ values are given in Table 8 below. Exposure increased less than dose proportionally.

Table 8: C_{max} and $AUC_{0\text{-}24h}$ Values of TMC125 Base on Day 13 After Repeated Oral Administration (2 Weeks) in Dogs

TMC125 Dose (mg/kg/day)	C_{max} (µg/mL)	$AUC_{0\text{-}24h}$ (µg.h/mL)
	M + F	M + F
10 (5 b.i.d.)	0.36[a] 0.52[b]	8.01
40 (20 b.i.d.)	0.98[a] 1.13[b]	19.4
160 (80 b.i.d.)	0.76[a] 2.18[b]	30.1

[a] C_{max}: after the first dosing; [b] C_{max}: after the second dosing
b.i.d.: twice daily

3.2.3 1-Month Oral (GLP)

TMC125 base formulated in PEG400 was administered twice daily, by oral gavage, for at least 28 days[22,23]. One control group and 3 treated groups were given 0 (vehicle), 40, 80 and 160 mg/kg/day, divided over 2 equal doses, 7 hours apart, in a dose volume of initially 3 mL/kg and reduced to 1 mL/kg on Day 9. Each group consisted of 4 male and 4 female Beagle dogs. Regular observations were made for clinical signs, body weight and food consumption, together with ophthalmoscopic examination, ECG and clinical laboratory investigations (hematology, blood chemistry and urine analysis) at termination. Blood samples were collected on Day 1 during the 7 hours after the first dose and Day 28 during the 24 hours after dosing for toxicokinetic analysis. All animals found dead or sacrificed during or at the end of the study were subject to macroscopic examination at necropsy and a number of organs were weighed. Samples of a range of tissues and organs were preserved for histological examination. All tissues and organs from animals in all dose groups and all gross lesions were examined microscopically.

There were no mortalities associated with TMC125, but 1 male given 80 mg/kg/day and 1 female given 160 mg/kg/day were sacrificed after having shown signs related to the large volume of PEG400 given. At necropsy these dogs presented dark red foci in the lungs and foamy contents in lungs and oral cavity related to abnormal passage of test formulation. Vomiting was noted in all groups including controls and therefore the dose volume of vehicle was reduced from 3 mL/kg to 1 mL/kg from the second dose on Day 9. There were no other relevant clinical signs, effects on food consumption, ophthalmoscopy or ECG related to TMC125. Body weight gain was variable with an apparent reduction in some females given 80 and 160 mg/kg/day. There were no relevant effects on laboratory investigations. Macroscopic examination at necropsy and histopathological examination indicated no changes that could be attributed to treatment. The NOAEL was considered to be 160 mg/kg/day based on the absence of any functional disturbances or morphological changes. See Tabulated Summary 2.6.7.7.D.

Toxicokinetic analysis indicated nonlinear pharmacokinetics. Systemic exposure expressed as C_{max} and AUC values are given in Table 9 below.

Table 9: C_{max} and AUC Values of TMC125 Base After Repeated Oral Administration (4 Weeks) in Dogs

TMC125 Dose (mg/kg/day)	Sampling Day	C_{max}[a] (µg/mL)		AUC[d] (µg.h/mL)	
		M	F	M	F
40 (20 b.i.d.)	Day 1	0.61	0.48	3.33	2.25
	Day 28	0.96	0.78	6.09	4.35
		1.19[b]	0.81[b]	18.6[e]	12.7[e]
80 (40 b.i.d.)	Day 1	0.65	0.55	3.39	2.74
	Day 28	1.15	0.80	6.92[c]	4.70
		1.27[b]	0.92[b]	20.4[c,e]	13.8[e]
160 (80 b.i.d.)	Day 1	1.03	0.68	5.33	3.49
	Day 28	1.33	1.13	8.23	6.56[c]
		1.50[b]	1.23[b]	24.4[e]	18.4[c,e]

[a] C_{max} after the first dosing; [b] C_{max} after the second dosing; [c] 3 animals only; [d] $AUC_{0\text{-}7h}$; [e] $AUC_{0\text{-}24h}$; b.i.d.: twice daily

3.2.4 1-Month Oral (Spray-dried, GLP)

Spray-dried TMC125 ([redacted] active to polymer ratio) formulated as a suspension in water was administered twice daily, by oral gavage, for at least 28 days[24]. One control group and 3 treated groups were given, 0 (vehicle), 40, 160 and 500 mg/kg/day, divided over 2 equal doses, 5 hours apart, in a dose volume of 10 mL/kg/dose. Each group consisted of 3 male and 3 female Beagle dogs. The dogs were dosed in fed condition. Regular observations were made for clinical signs, body weight and food consumption, together with ophthalmoscopic examination, ECG and clinical laboratory investigations (hematology, blood chemistry and urine analysis, at day 15 and Day 29). Blood samples were collected for toxicokinetic analysis on Day 1 and Day 24 during the 24 hours after the first dose administration. All animals sacrificed at the end of the study were subject to macroscopic examination at necropsy and a number of organs were weighed. Samples of a range of tissues and organs were preserved for histological examination. All tissues and organs from animals in all dose groups and all gross lesions were examined microscopically.

All animals survived. Transient vomiting was noted mainly during the first week in animals given 160 and 500 mg/kg/day. One high dose female showed signs of deteriorating condition (dehydration, decreased activity, conjunctivitis) in the last week of dosing. There were no effects

on ophthalmoscopy or ECG related to TMC125. There was a decrease in food consumption (up to 60%) and a dose-related decrease in body weight (up to 31%) and weight gain from 20 mg/kg/day onwards. Slight increases in red blood cells, hemoglobin and hematocrit (up to 18%) were observed in high dose males after 4 weeks of dosing. Although some of these changes were also observed in females after 2 weeks of dosing they were absent at the end of the study. A decrease in reticulocytes was noted in both sexes (up to 44%). White blood cells (27%), neutrophils (41%) and monocytes (33%) were decreased in males. Alkaline phosphatase (ALP) was increased (66%) in males at the high dose level. This coincided with an increase in total bilirubin (up to 2.9-fold) and a decrease (up to 19%) in inorganic phosphorus. One or 2 animals in the high dose group influenced all of these changes. Macroscopic examination and organ weights at necropsy revealed a reduction (up to 52%) in mean thymus weight in animals given 500 mg/kg/day. This correlated with thymus involution which was found at histopathological examination. A moderately decreased (39%) weight of the popliteal lymph nodes was seen in some high dose females, as well as sternal bone marrow atrophy. The thymic involution, decrease in lymph node weight and bone marrow atrophy were likely a consequence of the body weight loss that was observed in these dogs. Other microscopic changes included inspissated bile in the gall bladder in 2 females and microgranuloma's in the liver of 1 male (moderate) and female (minimal), given 500 mg/kg/day. One high dose female showed mineralization within goblet cells in the colon. Given the dose-related effects on body weight and food consumption a NOAEL could not be established. See Tabulated Summary 2.6.7.7.E.

Toxicokinetic analysis indicated nonlinear pharmacokinetics. Systemic exposure expressed as C_{max} and AUC values are given in Table 10 below.

Table 10: C_{max} and AUC Values of Spray-dried TMC125 After Repeated Oral Administration (4 Weeks) in Dogs

TMC125 Dose (mg/kg/day)	Sampling Day	C_{max} (µg/mL)				AUC[a] (µg.h/mL)	
		M		F			
		C_{max1}	C_{max2}	C_{max1}	C_{max2}	M	F
40 (20 b.i.d.)	Day 1	1.70	3.17	1.92	2.92	49.8	45.8
	Day 24	1.92	2.47	1.79	2.08	37.3	31.7
160 (80 b.i.d.)	Day 1	4.52	5.08	5.71	7.06	71.7[b]	111
	Day 24	2.85	3.70	3.17	4.11	51.9	55.7
500 (250 b.i.d.)	Day 1	4.24	7.56	5.68	8.27	109	105[b]
	Day 24	1.56	3.64	3.75	5.60	42.2	75.1

[a] $AUC_{0-\infty}$ after single dose (day 1) or AUC_{0-24h} after repeated dose; [b] AUC_{0-24h}
C_{max1} : after the first dosing; C_{max2} : after the second dosing; b.i.d.: twice daily

3.2.5 3-Month Oral (GLP)

TMC125 HBr formulated in PEG400 was administered twice daily, by oral gavage, for at least 13 weeks[25,26]. One control group and 3 treated groups were given, 0 (vehicle), 20, 40 and 80 mg/kg/day, divided over 2 equal doses, 7 hours apart, in a dose volume of 1 mL/kg/dose. Each group consisted of 4 male and 4 female Beagle dogs. Regular observations were made for clinical signs, body weight and food consumption, together with ophthalmoscopic examination, ECG and clinical laboratory investigations (hematology, blood chemistry and urine analysis, at Week 6 and Week 13). Blood samples were collected on Day 1, Day 30 and Day 59 during the 7 hours after the first dose and on Day 87 during the 24 hours after the first dosing for

toxicokinetic analysis. All animals sacrificed at the end of the study were subject to macroscopic examination at necropsy and a number of organs were weighed. Samples of a range of tissues and organs were preserved for histological examination. All tissues and organs from animals in all dose groups and all gross lesions were examined microscopically.

All animals survived. From Week 6 onwards, erythema, piloerection and alopecia occurred in 1 female at 40 mg/kg/day and 1 male and 1 female at 80 mg/kg/day. There were no relevant effects on body weight (and weight gain), food consumption, ophthalmoscopy or ECG related to TMC125. There were a number of marginal changes on laboratory investigations none of which were considered related to TMC125. Macroscopic and histopathological examination revealed no effect of TMC125 treatment. In the absence of any relevant changes the NOAEL was considered to be 80 mg/kg/day. See Tabulated Summary 2.6.7.7.F.

Toxicokinetic analysis indicated nonlinear pharmacokinetics. Systemic exposure, expressed as C_{max} and AUC values, are given in Table 11 below.

Table 11: C_{max} and AUC Values of TMC125 HBr After Repeated Oral Administration (13 Weeks) in Dogs

TMC125 Dose (mg/kg/day)	Sampling Day	C_{max}[a] (µg/mL)		AUC[c] (µg.h/mL)	
		M	F	M	F
20 (10 b.i.d.)	Day 1	0.57	0.35	2.69	1.66
	Day 30	0.74	0.69	4.24	3.78
	Day 59	0.76	0.54	4.38	3.03
	Day 87	0.67	0.66	4.10	3.59
		0.83[b]	0.64[b]	12.6[d]	10.2[d]
40 (20 b.i.d.)	Day 1	0.65	0.45	3.02	2.33
	Day 30	1.03	0.73	5.97	4.38
	Day 59	1.01	0.61	5.91	3.39
	Day 87	1.06	0.73	6.43	4.22
		1.38[b]	0.74[b]	21.8[d]	11.6[d]
80 (40 b.i.d.)	Day 1	1.02	0.89	5.10	4.50
	Day 30	1.18	1.15	7.20	6.88
	Day 59	1.43	0.86	8.09	5.16
	Day 87	1.22	0.85	7.60	5.35
		1.50[b]	1.06[b]	24.1[d]	15.9[d]

[a] C_{max} after the first dosing; [b] C_{max} after the second dosing; [c] $AUC_{0\text{-}7h}$; [d] $AUC_{0\text{-}24h}$; b.i.d.: twice daily

3.2.6 6-Month Oral (GLP)

TMC125 HBr formulated in PEG400 was administered twice daily (to Day 72) then once daily, by oral gavage, for at least 26 weeks[27,28]. One control group and 3 treated groups were given 0 (vehicle), 20, 40 and 80 mg/kg/day, divided over 2 equal doses, 7 hours apart, in a dose volume of 1 mL/kg up to and including Day 72 after which the dogs were dosed once daily with the same total dose. Each group consisted of 4 male and 4 female Beagle dogs. Regular observations were made for clinical signs, body weight and food consumption, together with ophthalmoscopic examination, ECG and clinical laboratory investigations (hematology, blood chemistry and urine analysis, at Week 6, Week 13 and Week 26). Blood samples were collected on Day 1 during the 7 hours after the first dose and on Day 86 and Day 177 during the 24 hours after dosing for toxicokinetic analysis. All animals sacrificed at the end of the study were subject to macroscopic examination at necropsy and a number of organs were weighed. Samples of a range of tissues

and organs were preserved for histological examination. All tissues and organs from animals in all dose groups and all gross lesions were examined microscopically.

All animals survived. From Week 5 onwards, erythema and alopecia occurred in 2 animals given either 40 or 80 mg/kg/day. These findings were reversible except for erythema in 1 high dose male which lasted until study termination. There were no relevant effects on body weight (and weight gain), food consumption, ophthalmoscopy or ECG related to TMC125. There were a number of marginal changes on laboratory investigations none of which were considered related to TMC125 except for a slight decrease (22%) in inorganic phosphate in high dose females. Macroscopic examination, including organ weights at necropsy and histopathological examination revealed no relevant effect of TMC125 treatment. In the absence of any relevant changes the NOAEL was considered to be 80 mg/kg/day. See Tabulated Summary 2.6.7.7.G.

Toxicokinetic analysis indicated nonlinear pharmacokinetics. Systemic exposure, expressed as C_{max} and AUC values, are given in Table 12 below.

Table 12: C_{max} and AUC Values of TMC125 HBr After Repeated Oral Administration (26 Weeks) in Dogs

TMC125 Dose (mg/kg/day)	Sampling Day	C_{max} (µg/mL)		AUC[a] (µg.h/mL)	
		M	F	M	F
20 (10 b.i.d.)	Day 1	0.33[c]	0.35[c]	1.62	1.70
20 (q.d.)	Day 86	0.98	0.89	5.86	5.07
				14.2[b]	11.2[b]
20 (q.d.)	Day 177	1.01	0.86	6.02	4.75
				15.7[b]	10.8[b]
40 (20 b.i.d.)	Day 1	0.83[c]	0.49[c]	4.29	2.62
40 (q.d.)	Day 86	1.10	1.06	6.51	6.03
				15.2[b]	13.6[b]
40 (q.d.)	Day 177	1.45	0.75	8.47	4.08
				19.8[b]	9.69[b]
80 (40 b.i.d.)	Day 1	1.45[c]	0.71[c]	7.23	3.13
80 (q.d.)	Day 86	2.03	1.95	11.6	10.7
				26.7[b]	22.0[b]
80 (q.d.)	Day 177	2.20	1.69	11.8	7.80
				28.6[b]	17.2[b]

[a] $AUC_{0\text{-}7h}$; [b] $AUC_{0\text{-}24h}$; [c] C_{max} : after the first dosing
q.d.: once daily; b.i.d.: twice daily

3.2.7 6-Month Oral (Spray-dried, GLP)

Spray-dried TMC125 ([redacted] active to polymer ratio) formulated as a suspension in water was administered twice daily, by oral gavage, for 6 consecutive months[29]. One control group and 2 treated groups were given, 0 (vehicle), 160 and 500 mg/kg/day, divided over 2 equal doses, 5 hours apart, in a dose volume of 10 mL/kg/dose. Each group consisted of 3 male and 3 female Beagle dogs. The dogs were dosed in fed condition. Regular observations were made for clinical signs, body weight and food consumption, together with ophthalmoscopic examination, ECG and clinical laboratory investigations (hematology, blood chemistry after 1, 3 and 6 months and urine analysis after 3 and 6 months). Blood samples were collected on Days 1, 30 and 181 during the 24 hours after dosing for toxicokinetic analysis. All animals sacrificed at the end of the study were subject to macroscopic examination at necropsy and a number of organs were weighed.

Samples of a range of tissues and organs were preserved for histological examination. All tissues and organs from animals in all dose groups and all gross lesions were examined microscopically.

All animals survived. Vomiting was noted in a dose-related frequency and severity, mainly after the second dose. There were no effects on ophthalmoscopy or ECG related to TMC125. A decrease in food consumption (14%) in females dosed at 500 mg/kg/day led to a decrease in body weight (11%) and weight gain (73%). No relevant hematological changes were seen. Total bilirubin (79%), ALP (3-fold) and alanine aminotransferase (ALT) (2.6-fold) were increased in males dosed at 500 mg/kg/day while triglycerides were decreased (up to 50%) in males at both dose levels. Microscopic examination showed microgranuloma in the liver in males only at both dose levels. Other minor inflammatory changes were seen in both sexes at both doses. Bile inspissation in the gall bladder was present at the high dose only. Given these effects a NOAEL was not determined. See Tabulated Summary 2.6.7.7.H.

Toxicokinetic analysis indicated nonlinear pharmacokinetics. Systemic exposure expressed as C_{max} and AUC values are given in Table 13 below.

Table 13: C_{max} and AUC Values of Spray-dried TMC125 After Repeated Oral Administration (26 Weeks) in Dogs

TMC125 Dose (mg/kg/day)	Sampling Day	C_{max} (µg/mL)				AUC[a] (µg.h/mL)	
		M		F		M	F
		C_{max1}	C_{max2}	C_{max1}	C_{max2}		
160 (80 b.i.d.)	Day 1	3.30	5.91	2.91	5.99	74.3	62.6
	Day 30	2.51	4.14	2.81	4.66	54.9	45.6
	Day 181	2.22	3.85	2.79	3.99	54.5	44.6
500 (250 b.i.d.)	Day 1	2.46	5.34	2.55	6.72	72.5[b]	70.2[b]
	Day 30	3.59	5.43	3.58	6.32	68.6	72.9
	Day 181	2.95	4.54	3.12	5.77	61.8	61.5

[a] $AUC_{0-\infty}$ after single dose (Day 1) or AUC_{0-24h} after repeated dose; [b] AUC_{0-24h}
C_{max1}: after the first dosing; C_{max2}: after the second dosing; b.i.d.: twice daily

3.2.8 12-Month Oral (GLP)

After a pilot study to assess tolerability to the high dose, TMC125 HBr formulated in PEG400 was administered once daily, by oral gavage, for at least 52 weeks[30,31]. One control group and 3 treated groups were given 0 (vehicle), 30, 80 and 240 mg/kg/day in a dose volume of 1 mL/kg. Each group consisted of 4 male and 4 female Beagle dogs. In addition, from pretest to Week 5, 3 male and 3 female animals were assigned to 3-month recovery groups given 80 and 240 mg/kg/day. From Week 6, onwards, however, former recovery animals of the mid dose group were re-assigned to become recovery animals of the high dose group. Former recovery animals of the high dose group were assigned to a 3-month interim sacrifice. Regular observations were made for clinical signs, body weight and food consumption, together with ophthalmoscopic examination, ECG and clinical laboratory investigations (hematology, blood chemistry and urine analysis, in all animals at Weeks 13, 26, 39 and 52, and in Week 65 for recovery animals). Blood samples were collected on Days 1, 21, 91, 182, 273 and 366 during the 24 hours after dosing for toxicokinetic analysis. All animals sacrificed at the end of their respective treatment period or recovery were subject to macroscopic examination at necropsy and a number of organs were weighed. Samples of a range of tissues and organs were preserved

for histological examination. All tissues and organs from animals in all dose groups and all gross lesions were examined microscopically.

There was no mortality associated with TMC125, however, 2 animals died (1 female given 80 mg/kg/day and 1 male given 240 mg/kg/day). These deaths were considered to be associated with the irritant and viscous nature of the vehicle and possible gavage errors as evidenced by lesions in the lungs and trachea. There were no relevant effects related to TMC125 on any of the measured parameters, clinical signs, body weight (and weight gain), food consumption, ophthalmoscopy, ECG or laboratory investigations. Macroscopic examination, including organ weights at necropsy and histopathological examination, revealed no effect of TMC125 treatment. In the absence of any relevant changes the NOAEL was considered to be 240 mg/kg/day. See Tabulated Summary 2.6.7.7.I.

Toxicokinetic analysis indicated nonlinear pharmacokinetics. Systemic exposure expressed as C_{max} and AUC values are given in Table 14 below.

Table 14: C_{max} and AUC Values of TMC125 HBr After Repeated Oral Administration (52 Weeks) in Dogs

TMC125 Dose (mg/kg/day)	Sampling Day	C_{max} (µg/mL)		AUC[a] (µg.h/mL)	
		M	F	M	F
30	Day 1	0.90	0.66	12.5	7.14[b]
	Day 21	1.03	0.81	13.0	10.5
	Day 91	1.12	0.92	14.6	9.77
	Day 182	1.26	0.77	17.1	9.98
	Day 273	1.12	1.00	15.8	10.6
	Day 366	0.96	0.81	13.5	11.9
80	Day 1	2.04	1.58	22.9[b]	17.9[b]
	Day 21	2.60	2.18	30.1	23.9
	Day 91	2.21	1.76	30.8	20.8
	Day 182	1.98	0.76	26.7	10.2
	Day 273	1.89	1.10	26.9	10.4
	Day 366	2.71	1.71	33.9	19.3
240	Day 1	2.78	2.29	34.8[b]	27.0[b]
	Day 21	2.58	2.20	33.4	29.0
	Day 91	2.43	2.04	36.5	24.6
	Day 182	2.19	1.53	33.4	21.1
	Day 273	2.54	2.14	40.0	27.4
	Day 366	2.16	1.77	35.6	23.2

[a] $AUC_{0-\infty}$ after single dose (Day 1) or AUC_{0-24h} after repeated dose; [b] AUC_{0-24h}

4 GENOTOXICITY

Genotoxicity tests included in vitro nonmammalian cell (Ames test) and mammalian cell (mouse lymphoma test) mutation assays, an in vitro chromosome aberration assay (with human lymphocytes), all with and without a liver metabolic activation system, and an in vivo clastogenicity assay (mouse bone marrow micronucleus test up to a dose of 2000 mg/kg).

4.1 IN VITRO NONMAMMALIAN CELL SYSTEM (GLP)

TMC125 base formulated in dimethyl sulfoxide (DMSO) was tested in 2 bacterial reverse mutation assays (Ames test). The first[32] with 5 histidine-requiring strains of *Salmonella typhimurium* (TA98, TA100, TA102, TA1535 and TA1537) and in the second[33], TA102 was replaced by a tryptophan-requiring strain *Escherichia coli* WP2uvrA. Following a suitable dose range finding in each study, TMC125 was tested in 2 independent experiments, in the tester strains of *Salmonella typhimurium* or in tester strain WP2uvrA of *Escherichia coli*, in the absence or presence of rat liver S9 metabolic activation (Aroclor-induced rat liver S9 mix) up to 500 µg/plate in the first study and 333 µg/plate in the second study. The upper concentration in each assay was limited by precipitation. TMC125 did not induce a dose-related, more than 2- or 3-fold increase in the number of revertant colonies in any of the tester strains in the absence or presence of S9 metabolic activation. The vehicle control and appropriate positive control reference articles confirmed the adequacy of the test system. It was concluded that TMC125 was not mutagenic under the condition of these assays. See Tabulated Summary 2.6.7.8.A and 2.6.7.8.B.

4.2 IN VITRO MAMMALIAN CELL SYSTEM

4.2.1 In Vitro Mammalian Forward Mutation Assay, Mouse Lymphoma Cells (GLP)

TMC125 base formulated in DMSO was tested in an in vitro mammalian forward mutation assay at the thymidine kinase (TK) locus in mouse lymphoma L5178Y cells[34]. Following 2 dose range finding tests, TMC125 was tested up to 75 µg/mL for 3 and 24 hour exposure periods (without metabolic activation) and up to 100 µg/mL for a 3 hour exposure period (with metabolic activation). The tests were performed to the limit of toxicity and there was no biologically significant induction in mutation frequency either in the absence (3 and 24 hour exposure), or in the presence of a metabolic activation system (3 hour exposure). The vehicle control and appropriate positive control reference articles confirmed the adequacy of the test system. It was concluded that TMC125 was not mutagenic under the conditions of this assay. See Tabulated Summary 2.6.7.8.C.

4.2.2 In Vitro Mammalian Chromosome Aberration Assay (GLP)

In the in vitro clastogenicity assay, the effect of TMC125 base formulated in DMSO was tested for the ability to induce chromosome aberrations in cultured peripheral human lymphocytes, in the absence or presence of S9 metabolic activation system (Aroclor-induced rat liver S9 mix)[35]. In a first assay, TMC125 was tested up to 100 µg/mL for a 3 hour exposure time with a 24 hour fixation time in the absence and presence of S9 mix. TMC125 precipitated in the culture medium

at this dose level. In a second assay, TMC125 was tested up to 10 μg/mL for a 24 or 48 hour continuous exposure with a 24 or 48 hour fixation time in the absence of S9 mix. Appropriate toxicity (mitotic index (MI) = 44% - 45%) was observed at 10 μg/mL. In the presence of S9 fraction, TMC125 was tested up to 56 μg/mL for a 3 hour exposure time with a 48 hour fixation time. TMC125 precipitated in the culture medium at a concentration of 56 μg/mL. TMC125 did not induce a statistically or biologically significant increase in the number of cells with chromosome aberrations in the absence or presence of S9 mix, in 2 independently repeated experiments. The vehicle control and appropriate positive control articles confirmed the adequacy of the test system. It was concluded that TMC125 was not clastogenic in human lymphocytes under the conditions of this assay. See Tabulated Summary 2.6.7.8.D.

4.3 IN VIVO MAMMALIAN CELL SYSTEM

4.3.1 In Vivo Mammalian Micronucleus Test (GLP)

To evaluate the clastogenic effect on early forms of erythrocytes in bone marrow, TMC125 HBr formulated in PEG400 was administered to mice in an in vivo micronucleus test[36,37]. Single oral (gavage) doses of 0 (vehicle control), 2000 mg/kg TMC125 and 50 mg/kg cyclophosphamide (positive control) were administered to NMRI BR mice (5 males/time point) and bone marrow was sampled 24 hour (vehicle control and TMC125) or 48 hour (TMC125 and positive control) after dosing. Toxicokinetic evaluation was performed. No increase in the frequency of micronucleated cells was observed in the polychromatic erythrocytes of the bone marrow of animals tested with TMC125. There was no effect on the ratio of polychromatic to normochromatic erythrocytes compared to vehicle controls, indicating a lack of toxicity to the bone marrow. The vehicle and positive control confirmed the adequacy of the test system. TMC125 C_{max} and $AUC_{0-\infty}$ were 7.85 μg/mL and 111 μg.h/mL, respectively. It was concluded that TMC125 was not clastogenic under the conditions of this assay. See Tabulated Summary 2.6.7.9.A.

5 CARCINOGENICITY

No information on the potential carcinogenic risk of TMC125 is yet available. Dosing of animals in the 2-year carcinogenicity studies in mice and rats with TMC125 HBr has been completed and histopathological examination is currently being performed. Dose range finding studies in the mouse with TMC125 HBr, formulated in PEG400, were performed by oral gavage in 2 studies, of 2 weeks and 3 months duration. In addition, the use of transgenic RasH2 mice has been explored in a dose range finding study, but this approach was not considered further. An additional 3-month study by oral gavage has been undertaken in the rat strain used in the carcinogenicity study (Sprague-Dawley). The potential of using the spray-dried TMC125 via the dietary route to increase exposure in mice and rats was also investigated in 3-month studies. This route, however, did not yield higher exposures compared to gavage dosing and therefore it was not used in the 2-year carcinogenicity studies.

5.1 LONG-TERM STUDIES

5.1.1 Mouse Studies

5.1.1.1 2-WEEK ORAL DOSE RANGE FINDING

TMC125 HBr formulated in PEG400 was administered once daily, by oral gavage, for 2 weeks[38]. One control group and 4 treated groups were given, 0 (vehicle), 100, 300, 900 and 1200 mg/kg/day in a dose volume of 10 mL/kg. Each group consisted of 5 male and 5 female CD1 mice. Regular observations were made for clinical signs, body weight and food consumption, together with clinical laboratory investigations (hematology and blood chemistry, at termination). All animals found dead or sacrificed during or at the end of the study were subject to macroscopic examination at necropsy, and a number of organs were weighed. Samples of a range of tissues and organs were preserved for histological examination. A selection of tissues and organs from all animals in the control group and groups dosed at 900 and 1200 mg/kg, and a limited number of organs in low and intermediate dose groups, as well as all gross lesions were examined microscopically.

There were no mortalities associated with TMC125, but there were 2 accidental deaths associated with the irritant and viscous nature of the vehicle. Both were males in the group given 1200 mg/kg/day. There were no relevant clinical signs, or effects on body weight or food consumption. Hematological parameters were broadly unchanged with TMC125 treatment but an increase in white blood cells (up to 60%) was noted in mice given 1200 mg/kg/day. In serum, increases in ALP (up to 88%), aspartate aminotransferase (AST) (up to 4.8-fold), ALT (up to 7-fold), triglyceride (up to 2-fold), and cholesterol (females; up to 57%) were generally dose related from 100 mg/kg/day onwards. A number of other serum chemistry parameters changed in response to treatment with TMC125 and are not considered further because they either did not show a dose response or were decreases and/or were not associated with histopathological changes (e.g., glucose, total protein, urea nitrogen). Macroscopic examination and organ weights at necropsy indicated an increase in liver weight in animals from a dose of 300 mg/kg onwards. The relative liver weights increased up to 68%. In addition, heart weight was increased in males given 900 or 1200 mg/kg/day (up to 15%) and pancreas weight was decreased in females given 900 and 1200 mg/kg/day (up to 22%). There was no histological correlate for the weight changes

in heart and pancreas. Microscopically the liver changes were confirmed as dose-related hepatocellular hypertrophy with single cell and/or (multi)focal necrosis. No relevant histopathological changes were observed at the dose level of 100 mg/kg. A NOAEL was not established in this preliminary study. Toxicokinetic determinations were not made in this study. See Tabulated Summary 2.6.7.10.A.

5.1.1.2 3-Month Oral Gavage Dose Range Finding (GLP)

TMC125 HBr formulated in PEG400 was administered once daily, by oral gavage, for 3 months[39]. One control group and 4 treated groups were given, 0 (vehicle), 10, 50, 200 and 800 mg/kg/day in a dose volume of 10 mL/kg. Each group consisted of 15 male and 15 female CD1 mice (main animals), of which 5 males and 5 females from each group were sacrificed at 1 month, together with 15 male and 15 female satellite animals (treated animals only) for toxicokinetic analysis. Regular observations were made for clinical signs, body weight and food consumption, together with clinical laboratory investigations (hematology and blood chemistry, at 1-month interim sacrifice and at termination). Blood samples were collected on Day 1, Day 29 and Day 91 during the 24 hours after dosing for toxicokinetic analysis. All main animals found dead or sacrificed during or at the end of the study were subject to macroscopic examination at necropsy, and a number of organs were weighed from the main animals. Samples of a range of tissues and organs were preserved for histological examination. Tissues and organs from main animals in the vehicle, 200 and 800 mg/kg/day dose groups and a limited number of organs in the low and intermediate groups, as well as from all main animals that died or were sacrificed early, and all gross lesions were examined microscopically.

There were no mortalities associated with TMC125, but there were a number of accidental deaths associated with the irritant and viscous nature of the vehicle. These were in main animals: 1 male, 2 females and 1 male, 1 female and 1 male in the groups given vehicle, 50, 200 and 800 mg/kg/day, respectively, and in satellite animals: 1 male, 1 female and 4 males in the groups given 50, 200 and 800 mg/kg/day, respectively. There were no relevant clinical signs or effects on food consumption. Body weight gain was variable and there was an indication of a reduction in body weight in males in all treated groups (up to 27%), but this was not dose related. Hematological parameters were broadly unchanged with TMC125 treatment but an increase in thrombocytes (up to 21%) was noted in male mice given 50 mg/kg/day and above. This was not associated with macro- or microscopic thrombi. Reticulocytes were decreased at the high dose (16%). Other changes in hematological parameters were small (up to 5%). As in the 2-week dose range finding study, there were changes in serum chemistry. Increases in ALP (up to 2-fold), AST (up to 2.6-fold), ALT (up to 9-fold), triglyceride (up to 42%) and cholesterol (females; up to 37%) were generally dose related from 200 mg/kg/day. No relevant serum changes were observed at 10 mg/kg, while cholesterol and triglycerides were only affected at the highest dose level. Macroscopic examination and organ weights at interim and terminal necropsy consisted of paleness, swelling and more pronounced lobulation at 800 mg/kg/day and an increase in liver weight in animals given 50 mg/kg/day or above. The terminal relative liver weights increased up to 51%. Microscopically the liver changes were confirmed as dose-related hepatocellular hypertrophy with single cell necrosis, hydropic appearance and vacuolation. Electron microscopy of 2 high dose mice demonstrated accumulation of lipid globules in the hepatocytes. Degeneration of hepatocytes was not observed. The weights of the adrenal glands increased (58%) in males but not in a dose-related manner. The increase in adrenal weight was associated with marginally swollen or eosinophilic cortical cells from 200 mg/kg/day, onwards. A similar

histological finding, without associated change in adrenal weight, was seen in females. A NOAEL was not established in this study but at 10 mg/kg/day the changes were minimal and consisted of slight liver related changes in serum parameters and histopathology. See Tabulated Summary 2.6.7.10.B. Based on the outcome of this study, the dose levels selected for the 2-year carcinogenicity study in mice were 0 (vehicle), 50, 200 and 400 mg/kg/day.

Toxicokinetic analysis indicated nonlinear pharmacokinetics. Systemic exposure expressed as C_{max} and AUC values are given in Table 15 below.

Table 15: C_{max} and AUC Values of TMC125 HBr After Repeated Oral Administration (13 Weeks) in Mice

TMC125 Dose (mg/kg/day)	Sampling Day	C_{max} (µg/mL)		AUC[a] (µg.h/mL)	
		M	F	M	F
10	Day 1	0.34	0.23	1.62[b]	1.27[b]
	Day 29	0.35	0.30	1.62	1.48
	Day 91	0.24	0.22	1.44	1.09[b]
50	Day 1	0.60	0.67	4.04[b]	4.13[b]
	Day 29	0.50	0.39	2.44[b]	2.76
	Day 91	0.56	0.37	3.14	1.70[b]
200	Day 1	1.71	1.27	9.73[b]	9.38
	Day 29	0.50	0.69	3.96	3.42
	Day 91	0.41	0.50	3.93	4.60
800	Day 1	5.21	4.60	43.4	41.1
	Day 29	1.77	2.58	12.2	11.3
	Day 91	1.50	1.48	7.80	10.1

[a] $AUC_{0-\infty}$ after single dose (Day 1) or AUC_{0-24h} after repeated dose; [b] AUC_{0-8h}

5.1.1.3 3-Month Oral Dose Range Finding (Spray-dried, GLP)

In an attempt to increase exposure in mice, a 3-month study with 1-month interim sacrifice was conducted using spray-dried TMC125 ([redacted] active to polymer ratio) admixed with diet[40]. Initially, 2 control groups and 3 treated groups were given, 0 (untreated diet), 0 (untreated diet mixed with spray-dried polymers), 450, 1620 and 2320 mg/kg/day; diet was available ad libitum. The diet substitution factor was 5% in the highest dose group which is the maximum recommended value for rodents. Each group consisted of 30 male and 30 female CD1 mice (main animals), of which 10 males and 10 females from each group were sacrificed at 1 month, together with 36 male and 36 female satellite animals (except for the polymer vehicle group where 18 male and 18 female satellite animals were included) for toxicokinetic analysis. Due to marked adverse effects including deaths, the animals given 2320 mg/kg/day were sacrificed after 7 weeks treatment and the 1620 mg/kg/day dose was reduced to 800 mg/kg/day on Day 50. A new group, comprised of 10 male and 10 female main animals and 6 male and 6 female satellite animals from the vehicle groups, was formed at Week 9 and was treated at 200 mg/kg/day until Week 13. Regular observations were made for clinical signs, body weight and food consumption, together with ophthalmoscopic examination and clinical laboratory investigations (hematology, blood chemistry and urine analysis at Week 5/7 and 13). Blood samples were collected on Day 7, and in Weeks 4, 7 (2320 mg/kg/day group only) and 13, during the 24 hours after dosing for toxicokinetic analysis. All main animals found dead or sacrificed during or at the end of the study were subject to macroscopic examination at necropsy, and a number of organs were weighed. Samples of a range of tissues and organs were preserved for histological examination.

Tissues and organs from main animals in both control groups and the high dose group and a limited number of organs in the other groups, as well as from all main animals that died or were sacrificed early, and all gross lesions were examined microscopically.

Treatment-related deaths occurred in male mice only. Investigations revealed that hemorrhagic cardiomyopathy, often associated with hemothorax at necropsy, was determined as the cause of death in 1 male at 450 mg/kg, 6 males at 1620 mg/kg/day and 11 males at 2320 mg/kg/day. Several male satellite animals (1 animal at 450, 3 animals at 1620/800 and 7 animals at 2320 mg/kg/day) died as well but as no macroscopic post-mortem examinations were performed, the cause of death was not established. Myocarditis was also observed in some male animals given 1620 or 2320 mg/kg at the scheduled 1-month interim sacrifice. Mainly at the highest dose level, some of the affected males showed also sporadic macroscopic hemorrhages in other tissues such as testes or thymus. No mortality, hemorrhage or cardiac changes were observed in females, although the exposure to TMC125 was comparable between both sexes. No cardiac changes were noted in male mice dosed during 5 weeks at 200 mg/kg/day. The relevant clinical signs were those of a deteriorating clinical condition in a few animals given 1620/800 or 2320 mg/kg/day. There were no effects on the eyes. There was no relevant effect on body weight or body weight gain, other than a lower body weight gain in the subset of males given 2320 mg/kg/day for 4 weeks (the weight gain of the equivalent females was increased). Food consumption was unaffected by treatment. Hematological parameters were broadly unchanged with TMC125 treatment at 4, 7 or 13 weeks but the platelet count in males given 1620/800 mg/kg/day was increased (29%) after 13 weeks. Increases in ALP (up to 6.3-fold), ALT (up to 6.5-fold), AST (up to 2.1-fold) and triglycerides (up to 2.4-fold) were noted in all treated groups at all dose levels. Macroscopic examination and organ weights at interim and terminal necropsy indicated an increase in liver weight (up to 2.4-fold) in animals from 200 mg/kg/day. These liver weight changes correlated with an enlarged liver size. Microscopically, minimal to marked centrilobular hepatocellular hypertrophy, associated with degenerative/necrotic changes were seen. The NOAEL was not determined in this study due to the hemorrhagic cardiomyopathy and liver changes. See Tabulated Summary 2.6.7.10.C.

Toxicokinetic analysis indicated nonlinear pharmacokinetics. Systemic exposure expressed as C_{max} and $AUC_{0\text{-}24h}$ values are given in Table 16 below.

Table 16: C_{max} and $AUC_{0\text{-}24h}$ Values of Spray-dried TMC125 After Repeated Oral Administration (13 Weeks) in Mice

TMC125 Dose (mg/kg/day)	Sampling Day	C_{max} (µg/mL)		$AUC_{0\text{-}24h}$ (µg.h/mL)	
		M	F	M	F
450	Day 7	0.23	0.35	3.20	4.50
	Day 29	0.26	0.19	2.95	2.59
	Day 90	0.13	0.20	1.98	2.59
1620/800[a]	Day 7	0.50	0.71	9.76	12.3
	Day 29	0.97	0.42	8.79	7.98
	Day 90	0.35	0.23	4.54	3.47
2320	Day 7	1.29	1.18	18.9	16.8
	Day 29	0.95	0.45	11.5	7.89
	Day 49	0.98	0.61	10.2	8.12
200	Day 34	0.20	0.10	2.35	1.53

[a] the dose level was reduced from 1620 to 800 mg/kg/day from Day 57 onwards

The outcome of this study elicited the conduct of 2 mechanistic toxicity studies in mice to explore the mechanism behind the hemorrhagic/cardiac pathology and its relevance to humans. The results are described in Section 8.2.

5.1.1.4 24-Month Oral Carcinogenicity (GLP)

Dosing in the 2-year oral gavage carcinogenicity study in CD1 mice with TMC125 HBr at 0 (vehicle), 50, 200 and 400 mg/kg/day has been completed and histopathological examination is currently being performed. The results will be reported upon completion.

5.1.2 Rat Studies

5.1.2.1 3-Month Oral Gavage Dose Range Finding (GLP)

An additional 3-month rat study, with 1-month interim sacrifice, was conducted in the strain of rat to be used in the 2-year carcinogenicity study. TMC125 HBr, formulated in PEG400 was administered once daily, by oral gavage, for 3 months[41]. One control group and 3 treated groups were given, 0 (vehicle), 70, 200 and 600 mg/kg/day in a dose volume of 5 mL/kg. Each group consisted of 20 male and 20 female Sprague-Dawley rats (main animals), of which 10 males and 10 females from each group were assigned to a 1-month interim sacrifice, together with 6 male and 6 female satellite animals (except for the vehicle group which had no satellites) for toxicokinetic analysis. Regular observations were made for clinical signs, body weight and food consumption, together with ophthalmoscopic examination and clinical laboratory investigations (hematology, blood chemistry, endocrinology and urine analysis at Day 29/30 and Day 91/92). Blood samples were collected on Day 1, Day 31 and Day 86 during the 24 hours after dosing for toxicokinetic analysis. All main animals found dead or sacrificed during the study or at scheduled necropsy were subject to macroscopic examination at necropsy, and a number of organs were weighed. Samples of a range of tissues and organs were preserved for histological examination. Tissues and organs from main animals in the control and high dose group and a limited number of organs in the low and intermediate dose groups, as well as from all main animals that died or were sacrificed early, and all gross lesions were examined microscopically.

There were no mortalities associated with TMC125, but there were a small number of accidental deaths associated with the irritant and viscous nature of the vehicle: 1 main vehicle female and 1 main male animal given 70 mg/kg/day and 1 male satellite animal given 200 mg/kg/day. There were no relevant clinical signs or effects on the eyes. Body weight (8%) and food consumption (9%) were slightly reduced in males given 600 mg/kg/day. Hematological parameters were broadly unchanged but an increase in neutrophils (up to 52%) and monocytes (up to 48%) was observed from 70 mg/kg/day in females. In males, neutrophils were also increased (up to 89%) from 200 mg/kg/day and this was associated with a decrease in eosinophils (40%) at the high dose. No apparent dose relationship was observed for these changes in white blood cells. The changes in serum chemistry were limited and consisted of a slight increase in total protein (up to 16%) and albumin (up to 10%, high dose females only). Examination of thyroid stimulating hormone (TSH), triiodothyronine (T3) and thyroxine (T4) levels indicated changes in thyroid homeostasis. T4 (up to 60%) and T3 (up to 29%) were decreased and there was a compensatory rise in TSH (up to 2-fold) in a dose-related fashion. At necropsy, increases in liver weight (up to 23%) and liver lobulation were noted in some animals at the high dose level. Microscopically the liver changes were confirmed as hepatocellular fatty-like vacuolization from 200 mg/kg in

females and in both sexes at the high dose. Hepatocellular hypertrophy was noted in 1 male and female at 600 mg/kg/day. In the thyroid gland, increases in high follicular epithelium and small follicles were noted from 200 mg/kg/day. The changes in liver and thyroid were considered to reflect an adaptive response to liver enzyme induction rather than an adverse effect of TMC125. The weight of the adrenal glands increased (25%) in animals given 600 mg/kg/day and in females this was associated with minimal and diffusely swollen zona fasciculata cells. No NOAEL was determined in this study, although the findings at the lowest dose level were limited and not associated with histopathological changes. See Tabulated Summary 2.6.7.10.D.

Based on the outcome of this study, the dose levels selected for the 2-year carcinogenicity study in rats were 0 (vehicle), 70, 200 and 600 mg/kg/day.

Toxicokinetic analysis indicated nonlinear pharmacokinetics. Systemic exposure expressed as C_{max} and AUC values are given in Table 17 below.

Table 17: C_{max} and AUC Values of TMC125 HBr After Repeated Oral Administration (13 Weeks) in Rats

TMC125 Dose (mg/kg/day)	Sampling Day	C_{max} (µg/mL)		AUC[a] (µg.h/mL)	
		M	F	M	F
70	Day 1	0.14	0.16	0.79	1.14
	Day 31	0.09	0.14	0.53	1.05
	Day 86	0.06	0.20	1.00	1.41
200	Day 1	0.25	0.28	1.64	1.93
	Day 31	0.15	0.37	0.99	2.01
	Day 86	0.12	0.46	0.74	2.28
600	Day 1	0.44	1.28	2.61	5.93
	Day 31	0.20	0.84	1.47	6.85
	Day 86	0.26	0.80	2.29	6.82

[a] $AUC_{0-\infty}$ after single dose (Day 1) or AUC_{0-24h} after repeated dose

5.1.2.2 3-Month Oral Dietary Dose Range Finding (Spray-dried, GLP)

In an attempt to increase exposure in rats, a 3-month rat study with 1-month interim sacrifice was conducted using spray-dried TMC125 ([redacted] active to polymer ratio) admixed with diet[42]. Two control groups and 3 treated groups were given, 0 (untreated diet), 0 (untreated diet mixed with spray-dried polymers), 330, 990 and 1300 mg/kg/day; diet was available ad libitum. Each group consisted of 30 male and 30 female Sprague-Dawley rats (main animals), of which 10 males and 10 females from each group were assigned to a 1-month interim sacrifice, together with 6 male and 6 female satellite animals (except for the polymer vehicle group where 3 male and 3 female satellite animals were included) for toxicokinetic analysis. Regular observations were made for clinical signs, body weight and food consumption, together with ophthalmoscopic examination and clinical laboratory investigations (hematology, blood chemistry and urine analysis at Week 4 and 12). Blood samples were collected on Day 7, Day 26 and Day 84 during the 24 hours after dosing for toxicokinetic analysis. All main animals sacrificed at the end of the study were subject to macroscopic examination at necropsy, and a number of organs were weighed. Samples of a range of tissues and organs were preserved for histological examination. Tissues and organs from main animals in both control groups and the high dose group and a limited number of organs in the low and intermediate dose groups and all gross lesions were examined microscopically.

There were no mortalities associated with TMC125, but there were 2 accidental deaths associated with the blood collection procedure: 2 female satellite animals given 990 mg/kg/day. There were no relevant clinical signs or effects on the eyes. Food consumption was unaffected by treatment but body weight gain was slightly reduced in males given 990 mg/kg/day (9%) and in males and females given 1300 mg/kg/day (up to 12%). At the end of the treatment period, there was an increase in prothrombin time (PT) in all treated males (up to 22%), however, all treated females showed a reduction in PT (up to 13%). Activated partial thromboplastin time (APTT) was also increased in all treated males (up to 61%). Only females given 990 mg/kg/day had an elevated APTT value (10%). The prolongation in clotting times was not associated with macro- or microscopical bleedings. The changes in serum chemistry were limited. Macroscopic examination and organ weights at interim and terminal necropsy indicated an increase in liver weight in animals from a dose of 330 mg/kg/day. The terminal relative liver weights increased by up to 27% but this was not associated with histological changes.

Morphological changes indicative of increased thyroid activity, i.e. follicular cell hypertrophy and small follicles, occurred in all treated groups. These changes were accompanied by increases in thyroid weights (up to 30%) in animals given 900 or 1300 mg/kg/day and were considered as an adaptive response to increased elimination of thyroid hormones. The NOAEL was not determined in this study although the changes at the lowest dose level were limited. See Tabulated Summary 2.6.7.10.E.

Toxicokinetic analysis indicated nonlinear pharmacokinetics. Systemic exposure expressed as C_{max} and $AUC_{0\text{-}24h}$ values are given in Table 18 below. In spite of the high dose levels given, systemic exposure was not increased with the dietary administration compared to gavage dosing. As a result, the 2-year carcinogenicity study was performed using oral gavage with TMC125 HBr.

Table 18: C_{max} and $AUC_{0\text{-}24h}$ Values of Spray-dried TMC125 After Repeated Oral Administration (13 Weeks) in Rats

TMC125 Dose (mg/kg/day)	Sampling Day	C_{max} (µg/mL)		$AUC_{0\text{-}24h}$ (µg.h/mL)	
		M	F	M	F
330	Day 7	0.07	0.14	0.91	2.37
	Day 26	0.07	0.20	1.07	3.02
	Day 84	0.06	0.17	1.21	2.95
990	Day 7	0.14	0.44	1.51	4.73
	Day 26	0.09	0.43	1.35	4.87
	Day 84	0.11	0.39	2.09	6.73
1300	Day 7	0.19	0.69	2.14	7.12
	Day 26	0.15	0.35	2.07	4.09
	Day 84	0.16	0.35	2.96	5.34

5.1.2.3 24-Month Oral Carcinogenicity (GLP)

Dosing in the 2-year oral, gavage, carcinogenicity study in rats with TMC125 HBr at 0 (vehicle), 70, 200 and 600 mg/kg/day has been completed and histopathological examination is currently being performed. The results will be reported upon completion.

5.2 SHORT- OR MEDIUM-TERM STUDIES

No short- or medium-term carcinogenicity studies have been conducted.

5.3 OTHER STUDIES

5.3.1 Transgenic Mouse Studies

5.3.1.1 1-MONTH ORAL DOSE RANGE FINDING (GLP)

TMC125 HBr formulated in PEG400 was administered once daily, by oral gavage, for 4 weeks[43]. One control group and 4 treated groups were given, 0 (vehicle), 50, 200, 400 and 800 mg/kg/day in a dose volume of 10 mL/kg. Each group consisted of 10 male and 10 female CB6F1-nonTgrasH2 mice (main animals) together with 13 male and 13 female satellite animals (except for the vehicle groups where 9 males and 9 females were included). The animals were nontransgenic littermates of transgenic rasH2 mice, produced by Mendelian segregation. Regular observations were made for clinical signs, body weight and food consumption, together with clinical laboratory investigations (hematology and blood chemistry, at termination). Satellite animals were sampled for assessment of TMC125 exposure and APTT and PT. Blood samples were collected on Day 1 and Day 28 during the 24 hours after dosing for toxicokinetic analysis. All animals found dead or sacrificed during or at the end of the study were subject to macroscopic examination at necropsy, and a number of organs were weighed. Samples of a range of tissues and organs were preserved for histological examination. A selection of tissues and organs from control and high dose main animals, liver and adrenal from low and intermediate dose groups and all gross lesions were examined microscopically.
There were no mortalities associated with TMC125, but there were 6 accidental deaths associated with the irritant and viscous nature of the vehicle: 1 satellite female in each of the groups given 50, 200 or 400 mg/kg/day, 1 satellite male given 400 or 800 mg/kg/day, and 1 main female given 800 mg/kg/day. There were no relevant clinical signs, or effects on body weight or food consumption. Platelet count was increased (up to 23%) in females from 400 mg/kg/day and APTT was increased (22%) in satellite males given 800 mg/kg/day. Increases in ALT (up to 6-fold) and cholesterol (up to 25%) were noted in females from 400 mg/kg/day, and triglycerides (up to 95%) in both sexes from 400 mg/kg/day. Macroscopic examination and organ weights at necropsy indicated an increase in liver weight (up to 62%) from a dose of 200 mg/kg/day. Microscopically the liver changes were confirmed as dose-related hepatocellular hypertrophy associated with vacuolated hepatocytes and in females with necrosis. In addition, minimal to moderate adrenal cortical cell hypertrophy was observed from a dose of 200 mg/kg/day onwards. The NOAEL was considered to be 50 mg/kg/day based on the absence of any effect. See Tabulated Summary 2.6.7.10.F

Toxicokinetic analysis indicated nonlinear pharmacokinetics. Systemic exposure expressed as C_{max} and AUC values are given in Table 19 below.

Table 19: C_{max} and AUC Values of TMC125 HBr After Repeated Oral Administration (4 Weeks) in Transgenic Mice

TMC125 Dose (mg/kg/day)	Sampling Day	C_{max} (µg/mL)		AUC[a] (µg.h/mL)	
		M	F	M	F
50	Day 1	0.37	0.32	2.41[b]	2.45
	Day 28	0.20	0.22	1.15[b]	1.22
200	Day 1	0.59	0.67	7.04	4.06
	Day 28	0.50	0.22	2.39	1.86
400	Day 1	2.00	1.25	13.4	8.91
	Day 28	0.55	0.40	3.88	3.26
800	Day 1	3.03	2.51	33.2	21.2
	Day 28	0.73	1.16	6.11	6.58

[a] $AUC_{0-\infty}$ after single dose (Day 1) or AUC_{0-24h} after repeated dose; [b] AUC_{0-12h}

6 REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Reproduction and developmental effects of TMC125 were initially examined using TMC125 HBr in a fertility and early embryonic development study in rats and in embryo-fetal development studies in rats and rabbits. To investigate potential effects at higher exposures, a fertility and early embryonic development study in rats and an embryo-fetal development study in rabbits were repeated using spray-dried TMC125 suspended in water. This formulation was also used in the pre- and postnatal development study in the rat.

6.1 FERTILITY AND EARLY EMBRYONIC DEVELOPMENT

6.1.1 Fertility and Early Embryonic Development (GLP)

TMC125 HBr formulated in PEG400 was administered once daily, by oral gavage, for 4 weeks (males) or 2 weeks (females) after which time the animals were mated[44]. One control group and 3 treated groups were given, 0 (vehicle), 127, 253 and 506 mg/kg/day in a dose volume of 5 mL/kg. Each group consisted of 24 male and 24 female Wistar rats. Regular observations were made for clinical signs, body weight and food consumption. Vaginal smears were taken for vaginal cytology and to determine when mating occurred. The dams were sacrificed on Gestation Day (GD) 21 (GD0 was the day mating was confirmed) and males were sacrificed after the outcome of mating was confirmed. All animals found dead or sacrificed during or at the end of the study were subject to macroscopic examination at necropsy. Samples of a range of reproductive tissues and organs and gross lesions were preserved. Pregnancy rate and uterine data (e.g., number of corpora lutea, implantations and live fetuses) were collected and analyzed. No toxicokinetic evaluations were performed in this study. However the same strain of animals and similar dose levels were used in study TMC125-NC129[16] and the toxicokinetic data (Table 6) are considered to be representative of values expected in this present study.

There were no mortalities associated with TMC125, but there were a number of accidental deaths associated with the irritant and viscous nature of the vehicle: 1 male, 1 male, 2 males and 2 animals (1 male and 1 female) in groups given 0 (vehicle), 127, 253 and 506 mg/kg/day, respectively. There were no relevant clinical signs or effects on body weight and food consumption or on gross pathology. There was no effect on estrus cycle, mating or pregnancy rate and the number of corpora lutea, implantations and live fetuses and derived indices were similar for treated and control groups. It was concluded that there was no effect on male or female fertility at doses up to 506 mg/kg/day. Therefore, the NOAEL was considered to be 506 mg/kg/day. See Tabulated Summary 2.6.7.12.A.

6.1.2 Fertility and early embryonic development (Spray-dried, GLP)

Spray-dried TMC125 ([redacted] active to polymer ratio) formulated as a suspension in water was administered once daily, by oral gavage, for 4 weeks prior to mating, during mating and up to termination (males) or 2 weeks prior to mating, during mating and up to GD7 (GD0 was the day mating was confirmed) (females)[45]. One control group and 3 treated groups were given, 0 (vehicle), 125, 250 or 500 mg/kg/day in a dose volume of 20 mL/kg. Each group consisted of 24 male and 24 female Sprague-Dawley rats. Regular observations for clinical signs, body weight and food consumption were made throughout the study. Estrous cycles were recorded and

the precoital interval was noted. The females were sacrificed on Day 14 of pregnancy for evaluation of pregnancy status whereas the males were sacrificed after fertility rate in females was established. Blood samples were collected on the first day of dosing (Day 0) and at the start of the mating period (Day 28 for males, Day 14 for females) during the 24 hours after dosing for toxicokinetic analysis.

There were no mortalities associated with TMC125, but there were a number of accidental deaths due to gavage errors: 3 males in the vehicle group and 1 male in the 500 mg/kg/day group. No adverse clinical signs were observed in males or females in any of the treated groups. No relevant effects on body weight performance and food consumption were noted throughout the study. There was no relevant effect on the estrous cycle, the precoital interval, the copulation index or the fertility index at all dose levels. No toxicologically relevant effects were observed on the number of corpora lutea of pregnancy, implantations, resorptions, the extent of pre- and post-implantation losses or the number of live embryos.
It was concluded that there was no effect on male or female fertility up to 500 mg/kg/day. Therefore, the NOAEL was considered to be 500 mg/kg/day. See Tabulated Summary 2.6.7.12.B.
Systemic exposure expressed as C_{max} and AUC are given in Table 20 below.

Table 20: C_{max} and AUC Values of Spray-dried TMC125 After Repeated Oral Administration in Rats

TMC125 Dose (mg/kg/day)	Sampling Day	C_{max} (µg/mL)		AUC[a] (µg.h/mL)	
		M	F	M	F
125	Day 0	0.57	1.39	2.72	7.79
	Day 14/28[b]	0.38	1.12	1.53	5.45
250	Day 0	0.93	1.72	5.48	9.77
	Day 14/28[b]	0.57	1.21	2.77	5.02
500	Day 0	1.58	2.13	10.7	18.1
	Day 14/28[b]	0.76	1.44	4.54	9.09

[a] $AUC_{0-\infty}$ after single dose (Day 1) or AUC_{0-24h} after repeated dose; [b] Day 14 for females, Day 28 for males

6.2 EMBRYO-FETAL DEVELOPMENT

6.2.1 Studies in Rats

6.2.1.1 EMBRYO-FETAL DEVELOPMENT (GLP)

TMC125 HBr formulated in PEG400 was administered once daily, by oral gavage, during the period of GD6 to GD16 (GD0 is the day mating was confirmed)[46,47]. Following a small pilot study where 6 pregnant females were dosed up to 1000 mg/kg/day, 1 control group and 3 treated groups were given, 0 (vehicle), 250, 500 and 1000 mg/kg/day in a dose volume of 5 mL/kg in the main study. Each group consisted of 20, 22 or 24, sexually mature female Wistar rats (main animals), together with 2 to 6 female satellite animals for toxicokinetic analysis. Stock males were used for mating with the females. Regular observations were made for clinical signs, body weight and food consumption. Blood samples were collected on Day 11 of dosing (GD16) during the 24 hours after dosing for toxicokinetic analysis. The dams were sacrificed on GD21 and all animals found dead or sacrificed during or at the end of the study were subject to macroscopic examination at necropsy. The uterus (with cervix and ovaries) was excised,

weighed and examined. Uterine data, the numbers of corpora lutea, implantations, live fetuses, fetal body weight and fetal sex distribution, were collected and analyzed. All live fetuses were examined externally. Alternate live fetuses were preserved, eviscerated and stained with Alizarin red S for skeletal examinations. The remaining live fetuses were preserved in Bouin's fluid for visceral examination using a modified Wilson technique.

There were no mortalities associated with TMC125, but there were a number of accidental deaths mainly associated with the irritant and viscous nature of the vehicle: 3 in main animals given 500 mg/kg/day and 1 in each of the satellite groups given 500 or 1000 mg/kg/day. There were no relevant clinical signs or effects on body weight and food consumption or on gross pathology. There was no effect on gravid uterine weight, pregnancy rate, number of corpora lutea, number of pre-implantation loss, post-implantation loss or live implantations, fetal body weight, sex ratio or fetal abnormalities. A small number of fetuses, in all treated groups, showed minor morphological changes for which the majority lacked a consistent relationship to treatment. At the high dose level there was a slight increase in the incidence of skeletal variants. The incidence of fetuses with anomalous thoracic vertebral centrum/centra (47%) and anomalous (wavy/thickened/bent) ribs (23%) appeared to be slightly increased versus the control group data for both findings (33% and 10%, respectively). These changes are commonly seen in the Wistar rat and are indicative of a transient effect on development.

The maternal and embryo fetal NOAEL was considered to be 1000 mg/kg/day based on the absence of any effect. There was no evidence that TMC125 was teratogenic in rats. See Tabulated Summary 2.6.7.13.A.

Toxicokinetic analysis indicated nonlinear pharmacokinetics. Systemic exposure expressed as C_{max} and AUC_{0-24h} values are given in Table 21 below.

Table 21: C_{max} and AUC_{0-24h} Values of TMC125 HBr After Repeated Oral Administration (GD6 to GD16) in Female Rats

TMC125 Dose (mg/kg/day)	Sampling Day	C_{max} (µg/mL)	AUC_{0-24h} (µg.h/mL)
250	Day 11 (GD16)	0.51	3.26
500	Day 11 (GD16)	0.58	7.94
1000	Day 11 (GD16)	0.57	8.27

GD: day of gestation

6.2.2 Studies in Rabbits

6.2.2.1 Tolerability Study in Nonpregnant Animals

TMC125 HBr formulated in TPGS and HPMC was administered once daily, by oral gavage, for 5 days[48,49]. Three groups were given 210, 500 and 750 mg/kg/day in various dose volumes from 2.1 to 10 mL/kg. The dose level of 750 mg/kg was considered the MFD since a formulation of 100 mg/mL resulted in a thick and foamy suspension. Each group consisted of 2 nonpregnant female New Zealand white rabbits. Regular observations were made for clinical signs, body weight and food consumption. Blood samples were collected on Day 5 during the 24 hours after dosing for toxicokinetic analysis. At the end of the study the animals were subject to macroscopic examination at necropsy.

There were no mortalities associated with TMC125. There were no consistent clinical signs or changes in body weight or food consumption and no gross pathology findings. The toxicokinetic data indicated similar exposure across all dose levels. The average systemic exposure expressed as C_{max} and AUC values, was 0.14 µg/mL and 1.24 µg.h/mL, respectively. See Tabulated Summary 2.6.7.11.

6.2.2.2 EMBRYO-FETAL DEVELOPMENT (GLP)

TMC125 HBr formulated in TPGS/HPMC was administered once daily, by oral gavage, during the period of GD6 to GD18 (GD0 is the day mating was confirmed)[50,51]. One control group and 3 treated groups were given, 0 (vehicle), 50, 200 and 750 mg/kg/day in a dose volume of 10 mL/kg. Each group initially consisted of at least 19 time-mated, sexually mature female New Zealand white rabbits but due to an insufficient number of litters additional animals were added to provide dose groups of 25, 22, 22 and 29 animals, respectively. Regular observations were made for clinical signs, body weight and food consumption. Blood samples were collected on Day 13 of dosing (GD18) during the 24 hours after dosing for toxicokinetic analysis. The dams were sacrificed on GD28 and all animals found dead or sacrificed during or at the end of the study were subject to macroscopic examination at necropsy. The uterus (with cervix and ovaries) was excised, weighed and examined. Uterine data, the numbers of corpora lutea, implantations, live fetuses, fetal body weight and fetal sex distribution, were analyzed. All live fetuses were examined externally and viscerally by fresh tissue examination. The fetuses were then eviscerated, preserved, stained with Alizarin Red S, and examined skeletally for abnormalities. The heads of one half of the fetuses were fixed in Bouin's fixative for Wilson sectioning.

There were no mortalities associated with TMC125, but there was 1 death in the vehicle group associated with the dosing procedure and there were 2 deaths at 750 mg/kg/day where the cause of death was not established. There was no effect on gross pathology, gravid uterine weight, pregnancy rate, number of corpora lutea, number of pre-implantation loss, post-implantation loss or live implantations, fetal body weight, sex ratio or fetal abnormalities. The maternal and fetal NOAEL was considered to be 750 mg/kg/day. There is no evidence that TMC125 is teratogenic in rabbits. See Tabulated Summary 2.6.7.13.B.

Systemic exposure expressed as C_{max} and $AUC_{0\text{-}24h}$ values are given in Table 22 below.

Table 22: C_{max} and $AUC_{0\text{-}24h}$ Values of TMC125 HBr After Repeated Oral Administration (GD6 to GD18) in Female Rabbits

TMC125 Dose (mg/kg/day)	Sampling Day	C_{max} (µg/mL)	$AUC_{0\text{-}24h}$ (µg.h/mL)
50	Day 13 (GD18)	0.13	1.29
200	Day 13 (GD18)	0.24	2.24
750	Day 13 (GD18)	0.59	3.76

GD: day of gestation

6.2.2.3 TOLERABILITY STUDY IN NONPREGNANT ANIMALS (HIGHER DOSES)

In this study, the effect of the maximum feasible concentration (75 mg/mL) of TMC125 HBr in TPGS/HPMC at increased dose volume (up to 15 mL/kg for single daily administration) or increased dosing frequency (b.i.d. at a volume of 10 mL/kg) was investigated. The formulation was administered, by oral gavage, for 5 days[52]. One control and 3 treated groups were given 0 (vehicle), 750 or 1125 mg/kg/day as a single dose, or 1500 mg/kg/day divided over 2 equal

doses, 4 hours apart. Each group consisted of 4 nonpregnant female New Zealand white rabbits. Regular observations were made for clinical signs, body weight and food consumption. Blood samples were collected on Day 5 during the 24 hours after dosing for toxicokinetic analysis. At the end of the study the animals were subject to macroscopic examination at necropsy.

There were no mortalities associated with TMC125 but 1 control female died on Day 1. Necropsy revealed congested lungs and kidneys, a dark liver and little intestinal tract content in this animal. There were no relevant clinical signs or changes in body weight or food consumption and no gross pathology. See Tabulated Summary 2.6.7.11.

In this study, higher daily dose levels were tolerated with TMC125 HBr (in TPGS/HPMC), relative to the original embryo-fetal study (described in section 6.2.2.2), as a result of increasing dose volumes and/or frequency of dose administration[50,51]. However, it was decided to perform an additional embryo-fetal development study with spray-dried TMC125, on the basis of a comparative pharmacokinetics study which concluded that higher exposures could be obtained with the spray-dried form[53].

Systemic exposure expressed as C_{max} and AUC_{0-24h} values are given in Table 23 below.

Table 23: C_{max} and AUC_{0-24h} Values of TMC125 HBr After Repeated Oral Administration (5 Days) in Female Rabbits

TMC125 Dose (mg/kg/day)	Sampling Day	C_{max} (µg/mL)	AUC_{0-24h} (µg.h/mL)
750	Day 5	1.12	9.05
1125	Day 5	2.12	20.1
1500 (750 b.i.d.)	Day 5	C_{max1} = 1.38 C_{max2} =1.84	21.0

C_{max1}: after the first dosing, C_{max2}: after the second dosing
b.i.d.: twice daily

6.2.2.4 Dose Range Finding Study of Embryo-Fetal Developmental Toxicity (Spray-Dried)

Spray-dried TMC125 ([redacted] active to polymer ratio) formulated as a suspension in water was administered once daily, by oral gavage, during the period of GD6 to GD18 (GD0 is the day mating was confirmed)[54]. One control group and 3 treated groups were given, 0 (vehicle), 125, 250 and 525 mg/kg/day in a dose volume of 15 mL/kg. Each group consisted of 5 time-mated, sexually mature female New Zealand white rabbits. Regular observations were made for clinical signs, body weight and food consumption. Blood samples were collected on the Day 1 (GD6) and Day 13 (GD18) from 3 females in each group during 24 hours after dosing for toxicokinetic analysis. The dams were sacrificed on GD28 and all animals were subject to macroscopic examination at necropsy. The uterus was examined and uterine data, the numbers of corpora lutea, implantations, live fetuses, fetal body weight and fetal sex distribution, were analyzed. All live fetuses were examined externally.

All animals survived and there were no relevant clinical signs or changes in body weight or food consumption. There was no effect on gross pathology, gravid uterine weight, pregnancy rate, number of corpora lutea, live implantations, fetal body weight, sex ratio or fetal abnormalities. At 525 mg/kg/day, there was an apparent increase in pre- (19%) and post-implantation (24%) losses, mainly related to 1 animal. These values, however, fell within the respective background

data ranges (upper limits 44% and 26%, respectively). The etiology of this finding, in relation to treatment, is unclear given the small group sizes. A similar finding was not observed in the main study with spray-dried TMC125 in rabbits[55]. The maternal NOAEL was considered to be 525 mg/kg/day, based on the absence of any relevant findings and the fetal NOAEL was considered to be 250 mg/kg/day.

Due to practical difficulties with formulating the test material at 35 mg/mL, the maximum practical concentration of spray-dried TMC125 was considered to be 25 mg/mL. At the maximum dose volume of 15 mL/kg, the highest achievable dose was therefore 375 mg/kg/day, which was subsequently used in the main embryo-fetal development study in the rabbit (see Section 6.2.2.4)[55].
See Tabulated Summary 2.6.7.11.

Toxicokinetic analysis indicated nonlinear pharmacokinetics. The C_{max} and AUC_{0-24h} values are given in Table 24 below.

Table 24: C_{max} and AUC_{0-24h} Values of Spray-dried TMC125 After Repeated Oral Administration (GD6 to GD18) in Female Rabbits

TMC125 Dose (mg/kg/day)	Sampling Day	C_{max} (µg/mL)	AUC_{0-24h} (µg.h/mL)
125	Day 1 (GD6)	0.58	7.58
	Day 13 (GD18)	0.47	5.25
250	Day 1 (GD6)	0.89	15.0
	Day 13 (GD18)	0.71	8.95
525	Day 1 (GD6)	1.37	19.6
	Day 13 (GD18)	0.76	7.98

GD: day of gestation

6.2.2.5 EMBRYO-FETAL DEVELOPMENT (SPRAY-DRIED, GLP)

Spray-dried TMC125 ([redacted] active to polymer ratio) formulated in water was administered once daily by oral gavage during the period of GD6 to GD19 (GD0 was the day mating was confirmed)[55]. One control group and 3 treated groups were given, 0 (vehicle), 125, 250 and 375 mg/kg/day in a dose volume of 15 mL/kg. Each group consisted of 20 time-mated, sexually mature female New Zealand white rabbits. Regular observations were made for clinical signs, body weight and food consumption. Blood samples were collected on Day 1 (GD6) and Day 13 (GD18) during the 24 hours after dosing for toxicokinetic analysis. The dams were sacrificed on GD28 and all animals sacrificed at the end of the study were subject to macroscopic examination at necropsy. The uterus was examined and uterine data, the numbers of corpora lutea, implantations, live fetuses, fetal body weight and fetal sex distribution were analyzed. All live fetuses were examined externally and viscerally (including the brain) by fresh tissue examination. The fetuses were then eviscerated, preserved, stained with Alizarin Red S, and examined skeletally for abnormalities.

All animals survived and there were no relevant clinical signs. At 250 and 375 mg/kg/day body weight was decreased after the start of dosing until GD9 (up to 96 g of body weight loss versus 21 g in vehicle animals). At 375 mg/kg/day this resulted in a marginally reduced body weight gain over the entire treatment period. Food consumption was similarly reduced until GD8 (up to 32%). There was no effect on gross pathology, gravid uterine weight, pregnancy rate, number of corpora lutea, number of pre-implantation loss, post-implantation loss or live implantations, fetal

body weight, sex ratio or fetal abnormalities. The maternal NOAEL was considered to be 125 mg/kg/day, based on the effect on body weight and food consumption at the higher dose levels and the fetal NOAEL was considered to be 375 mg/kg/day based on the absence of any relevant findings. There is no evidence that TMC125 is teratogenic in rabbits up to maternally toxic dose levels. Tabulated Summary 2.6.7.13.C.

Systemic exposure expressed as C_{max} and $AUC_{0\text{-}24h}$ values are given in Table 25 below.

Table 25: C_{max} and $AUC_{0\text{-}24h}$ Values of Spray-dried TMC125 After Repeated Oral Administration (GD6 to GD19) in Female Rabbits

TMC125 Dose (mg/kg/day)	Sampling Day	C_{max} (µg/mL)	$AUC_{0\text{-}24h}$ (µg.h/mL)
125	Day 1 (GD6)	0.51	7.58
	Day 13 (GD18)	0.38	6.09
250	Day 1 (GD6)	0.63	11.6
	Day 13 (GD18)	0.41	5.27
375	Day 1 (GD6)	0.70	12.3
	Day 13 (GD18)	0.54	9.67

GD: day of gestation

6.3 PRENATAL AND POSTNATAL DEVELOPMENT, INCLUDING MATERNAL FUNCTION

6.3.1 Dose Range Finding Study of Prenatal and Postnatal Developmental Toxicity (Spray-dried, GLP)

Spray-dried TMC125 ([redacted] active to polymer ratio) formulated in water was administered once daily by oral gavage to time-mated female rats from Day 7 of gestation to Day 7 of lactation (GD1 is day mating was confirmed and Day 1 postpartum is day on which parturition occurred)[56]. One control group and 3 treated groups were given, 0 (vehicle), 125, 250 and 500 mg/kg/day in a dose volume of 20 mL/kg. Each group consisted of 6 time-mated female Sprague Dawley rats (main animals), together with 6 time-mated female satellite animals for toxicokinetic analysis. Regular observations were made for maternal clinical signs, body weight and food consumption. Blood samples were collected on Day 1 (GD7) and Day 11 (GD17) during the 24 hours after dosing for toxicokinetic analysis. The females were allowed to litter, and nesting and nursing behavior were observed and any abnormalities recorded. Only dams that died or were sacrificed before the scheduled termination were subject to macroscopic examination. Pups were not examined macroscopically.

In the dams there were no mortalities associated with TMC125, but 1 dam (250 mg/kg/day) was found dead on Day 22 and the cause of death was attributed to difficulties at parturition. In addition, there were 3 accidental deaths associated with the dosing procedure: 1 main animal given 125 mg/kg/day and 2 satellite animals given 250 mg/kg/day. There were no relevant clinical signs or effects on body weight or food consumption of the dams and no effect of maternal treatment on parturition or the numbers of pups born, the proportion of male pups born, or pup survival, clinical condition or body weight.

The maternal and developmental NOAEL was considered to be 500 mg/kg/day based on the absence of any effect. There were no apparent effects on the pups at this dose. See Tabulated Summary 2.6.7.11.

Systemic exposure, expressed as C_{max} and AUC values are given in Table 26 below.

Table 26: C_{max} and AUC Values of Spray-dried TMC125 After Repeated Oral Administration (11 Days) in Female Rats

TMC125 Dose (mg/kg/day)	Sampling Day	C_{max} (µg/mL)	AUC[a] (µg.h/mL)
125	Day 1 (GD7)	0.89	6.59
	Day 11 (GD17)	1.02	7.22
250	Day 1 (GD7)	1.11	10.4
	Day 11 (GD17)	1.08	8.64
500	Day 1 (GD7)	1.62	23.6
	Day 11 (GD17)	0.99	12.8

[a] $AUC_{0-\infty}$ after single dose (GD7) or AUC_{0-24h} after repeated dose
GD: day of gestation

6.3.2 Prenatal and Postnatal Developmental Toxicity (Spray-dried, GLP)

Spray-dried TMC125 ([redacted] active to polymer ratio) formulated in water was administered once daily by oral gavage to time-mated females from Day 7 of gestation to Day 21 of lactation (GD1 is day mating was confirmed and Day 1 postpartum is day on which parturition occurred)[57]. The first generation (F1) was allowed to mature untreated. One control group and 3 treated groups were given, 0 (vehicle), 125, 250 and 500 mg/kg/day in a dose volume of 20 mL/kg. Each group consisted of 28 time-mated female Sprague-Dawley rats (main animals), together with 6 time-mated female satellite animals for toxicokinetic analysis. Regular observations were made for maternal clinical signs, body weight and food consumption. Blood samples were collected on Day 1 (GD7) and Day 11 (GD17) during the 24 hours after dosing for toxicokinetic analysis. The females were allowed to litter and nesting and nursing behavior were observed. Parturition, litter size and numbers of each sex were recorded, as were clinical observations in pups and pup body weight. Developmental landmarks (the number with ears open, eyes open, preputial separation, vagina open) and sensory function/reflexes (static righting reflex, startle response and pupillary light reflex, tail flick test) and behavior (learning and memory and locomotor activity) and assessment of reproductive performance of offspring were recorded. The F0 females were sacrificed at weaning of their litters. All principal females were subject to macroscopic examination, including counting the number of implantation scars in each uterine horn. Samples of a range of tissues and organs were preserved but were not examined microscopically. F1 offspring were subject to macroscopic examination and samples of a range of tissues and organs were preserved but were not examined microscopically.

In the dams there were no mortalities associated with TMC125. There were no relevant clinical signs or effects on body weight or food consumption of the dams and no effect of maternal treatment on gestation, parturition or the numbers of pups born, the sex ratio, pup survival, or clinical condition. Pup body weight was higher in TMC125 treated animals than in controls, although without clear dose-response. As a consequence of this, the developmental landmarks occurred earlier in comparison with the lighter control pups. Clinical condition, sensory function/reflexes, behavior and reproductive performance of F1 pups were unaffected by

maternal treatment with TMC125. There was no evidence of an effect of TMC125 on the macroscopic findings of dams or F1 offspring.

The maternal NOAEL was considered to be 500 mg/kg/day based on the absence of any effect. There were no apparent effects on the pups at this dose and there were no delayed effects of maternal treatment with TMC125 on the selected offspring allowed to develop to adulthood for assessment of physical development, sensory functions, reflexes, behavior, sexual maturation or reproductive performance. Therefore the NOAEL for pup development after maternal treatment with TMC125 was considered to be 500 mg/kg/day. See Tabulated Summary 2.6.7.14.A

Systemic exposure expressed as C_{max} and AUC values are given in Table 27 below.

Table 27: C_{max} and AUC Values of Spray-dried TMC125 After Repeated Oral Administration (11 Days) in Female Rats

TMC125 Dose (mg/kg/day)	Sampling Day	C_{max} (µg/mL)	AUC[a] (µg.h/mL)
125	Day 1(GD7)	1.03	6.15
	Day 11 (GD17)	0.65	5.29
250	Day 1(GD7)	1.30	9.42
	Day 11 (GD17)	0.85	7.60
500	Day 1(GD7)	1.66	12.1
	Day 11 (GD17)	0.64	3.63

[a] $AUC_{0-\infty}$ after single dose (GD7) or AUC_{0-24h} after repeated dose
GD: day of gestation

6.4 STUDIES IN WHICH THE OFFSPRING ARE DOSED

No studies in offspring were performed.

7 LOCAL TOLERANCE

Both TMC125 base and TMC125 HBr were tested in a range of local tolerance studies. Skin sensitization and irritation were evaluated using in vivo models, whereas eye irritation and phototoxicity were evaluated using in vitro models.

7.1.1 In Vitro Studies

7.1.1.1 Eye Irritation; Bovine Corneal Opacity-Permeability Assay

The bovine corneal opacity-permeability (BCOP) was used to investigate the ocular irritation potential of TMC125 base by measuring the opacity and permeability after exposure of the corneal epithelium. TMC125 base was applied for 4 hours as a 20% w/w suspension in saline[58]. TMC125 induced a small increase in opacity and no increase in permeability and was classified as a "mild" eye irritant. See Tabulated Summary 2.6.7.16.

An identical test, as described above, was performed with TMC125 HBr, which induced a very strong increase in opacity and a small increase in permeability when applied as a 20% w/w suspension in saline. As a result of this reaction TMC125 HBr was classified as a "very severe" eye irritant[59]. See Tabulated Summary 2.6.7.16.

7.1.1.2 Phototoxicity; Balb/c Mouse Fibroblast Assay

The Balb/c mouse fibroblast assay was used to investigate in vitro phototoxic and cytotoxic potential of TMC125 HBr. This is measured by a reduction in Neutral Red uptake following exposure to ultraviolet (UVA) light. Balb/c 3T3 fibroblast cells were treated with concentrations of TMC125 HBr from 0.002 to 6.42 mg/L (in Dulbecco's phosphate-buffered saline/DMSO) in the presence and absence of UVA light[60]. No effect was observed and TMC125 HBr was considered to be nonphototoxic. See Tabulated Summary 2.6.7.16.

7.1.2 In Vivo Studies

7.1.2.1 Skin Sensitization; Local Lymph Node Assay (GLP)

The LLNA determines the level of T lymphocyte proliferation in the lymph node draining the site of chemical application. This is done by measuring the amount of radiolabeled thymidine incorporated into the dividing cells in the lymph node after intravenous injection of ^{3}H thymidine. TMC125 base tested negative in this assay[61] and it was concluded that it is unlikely that TMC125 causes skin sensitization or delayed hypersensitivity. See Tabulated Summary 2.6.7.16.

7.1.2.2 Skin Sensitization; Delayed Hypersensitivity (GLP)

The potential for TMC125 base to induce delayed contact hypersensitivity was evaluated using the maximization test of Magnusson and Kligman[62]. Following a preliminary study, 30 naïve guinea pigs were allocated to 2 groups: a control group of 5 animals/sex/concentration and a treated group of 10 animals/sex/concentration. On Day 1, intradermal injections were performed in the interscapular region of all animals: 50% Freund's complete adjuvant (FCA) in saline, TMC125 (0.1% w/w) in corn oil (treated group) or vehicle (control group), TMC125 (0.1%) in

FCA/saline (treated) or 50% vehicle in FCA/saline. On Day 7, sodium lauryl sulfate in vaseline was applied to induce local irritation. On Day 8, TMC125 (50% w/w ethanol/water) or vehicle was applied topically to the same site and covered with an occlusive dressing for 48 hours. On Day 22 all animals were challenged by cutaneous application of the test substance (50% w/w TMC125 in acetone) to the right flank. Skin reactions were evaluated 24 and 48 hours after removal of dressing. Regular observations were made for clinical signs and body weight. At the end of the study the animals were sacrificed and not examined at necropsy.

All animals survived and there were no noteworthy clinical observations or effects on body weight. In the treated group, grade 1 erythema was observed in 2 animals at 24 hours only. TMC125 did not induce delayed contact hypersensitivity in guinea pigs. TMC125 was classified as "nonsensitizing" to the skin. See Tabulated Summary 2.6.7.16.

7.1.2.3 Skin Irritation (GLP)

The possible skin irritation potential of TMC125 base was evaluated in New Zealand white rabbits[63]. TMC125 (0.5 g) was applied for 4 hours by topical semi-occlusive application to the intact clipped skin of 3 rabbits. The scoring of skin reactions was performed 1, 24, 48 and 72 hours after removal of the dressing.

All animals survived and there were no noteworthy clinical observations or effects on body weight. No skin reactions, staining of skin or corrosive effects were observed. TMC125 was classified as "nonirritant" to the skin. See Tabulated Summary 2.6.7.16.

8 OTHER TOXICITY STUDIES

8.1 IMMUNOTOXICITY

8.1.1 4-Week Oral Rat Study (GLP)

TMC125 HBr formulated in PEG400 was administered once daily, by oral gavage, for 4 weeks[64]. One control group and 3 treated groups were given, 0 (vehicle), 70, 200 and 600 mg/kg/day in a dose volume of 5 mL/kg. Each group consisted of 8 male and 8 female Sprague-Dawley rats (main animals) together with 6 male and 6 female satellite animals (treated groups only) for toxicokinetic analysis. Regular observations were made for clinical signs, body weight and food consumption, together with hematology (blood and bone marrow, at termination). The antibody response to the T-cell dependent antigen keyhole limpet hemocyanin (KLH) was evaluated by subcutaneous administration of KLH on Day 22, followed by enzyme-linked immunosorbent assay (ELISA) determination of IgM levels in serum samples taken at necropsy. Blood samples were collected on Day 26 during the 24 hours after dosing for toxicokinetic analysis. All main animals sacrificed at the end of the study were subject to macroscopic examination at necropsy, and a limited number of organs were weighed. Samples of a range of lymphoid tissues and organs and all gross lesions were preserved and were examined microscopically.
There were no mortalities associated with TMC125, but there was 1 accidental death in the satellite animals (1 female given 600 mg/kg/day). There were no relevant clinical signs, or effects on food consumption. Body weight gain was slightly lower in high dose males (6%) and in females from 200 mg/kg (up to 13%) but there was no clear relationship to dose level. The hematology, myelograms and immune response of treated animals, as measured by IgM production, were not affected by treatment. Macroscopic evaluation, including organ weights, and microscopic examination revealed no treatment-related changes. No immunotoxicological response to TMC125 HBr administered at doses up to 600 mg/kg/day was found. The NOAEL was considered to be 600 mg/kg/day based on the absence of any hematological or histopathological changes. See Tabulated Summary 2.6.7.17.A

Systemic exposure, expressed as C_{max} and $AUC_{0\text{-}24h}$ values are given in Table 28 below:

Table 28: C_{max} and $AUC_{0\text{-}24h}$ Values of TMC125 HBr After Repeated Oral Administration (4 Weeks) in Rats

TMC125 Dose (mg/kg/day)	Sampling Day	C_{max} (µg/mL)		$AUC_{0\text{-}24h}$ (µg.h/mL)	
		M	F	M	F
70	Day 26	0.09	0.13	0.57	0.59[a]
200	Day 26	0.15	0.21	0.85	1.32
600	Day 26	0.25	0.58	1.54	5.44

[a] $AUC_{0\text{-}8h}$

8.2 MECHANISTIC STUDIES

Two studies were conducted to mechanistically address the mortality caused by hemorrhagic cardiomyopathy that was observed in male mice in the 3-month study with spray-dried TMC125

([redacted] active to polymer ratio) administered by dietary admixture (Section 5.1.1.3)[40]. More specifically, the studies evaluated the role of vitamin K in the etiology of hemorrhagic cardiomyopathy. Literature describes a similar syndrome in cases of vitamin K deficiencies, which was more specific to male mice than females[65,66].

8.2.1 6-Week Oral Dietary Mouse Study (Spray-dried)

In this study, spray-dried TMC125 ([redacted] active to polymer ratio) was admixed with diet[67]. Two control groups and 2 treated groups were given, 0 (untreated diet mixed with spray-dried polymers), 0 (vitamin K_1 and untreated diet mixed with spray-dried polymers), 2320 (spray-dried TMC125) and 2320 (spray-dried TMC125 and vitamin K_1) mg/kg/day; diet was available ad libitum. Vitamin K_1 was given subcutaneously at 15 mg/kg/day. Each group consisted of 20 male and 20 female CD1 mice (main animals), together with 8 male and 8 female satellite animals for toxicokinetic analysis. Regular observations were made for clinical signs, body weight and food consumption, together with clinical laboratory investigations (coagulation times, clotting factors and cardiac troponin, Week 6). The clotting factors investigated were II and VII (vitamin K dependent), and VIII and XI (nonvitamin K dependent). Blood and liver samples were collected on Day 44 during the 24 hours after dosing for toxicokinetic analysis. All main animals found dead or sacrificed during or at the end of the study were subject to macroscopic examination at necropsy, and a number of organs were weighed. Necropsies were performed with a detailed assessment for hemorrhages. Samples of a range of tissues and organs were preserved for histological examination. Tissues and organs from all main animals, and all gross lesions, were examined microscopically.

There was only 1 death in male mice given spray-dried TMC125 without vitamin K_1. The animal was sacrificed during the study (Day 16) due to the poor clinical condition. This animal had marked prolongation in clotting times, hemothorax, hemorrhagic cardiomyopathy and elevated cardiac troponin. Compared to the vehicle group without vitamin K_1, PT was prolonged by 3.9-fold and APTT was >100 sec in this animal. Furthermore, all clotting factors investigated were markedly decreased: II (-90%), VII (-65%), VIII (-73%) and XI (–93%).

Since only 1 animal developed hemorrhagic cardiomyopathy, treatment duration was prolonged from 4 to 6 weeks to allow sufficient time for development and assessment of any potential cases. There were no relevant clinical signs in any of the animals with the exception of the one animal that died. In the TMC125-treated groups, there was a slightly lower body weight (4%) and weight gain (32%) in males. In females, there was an increase in body weight and weight gain in all groups compared to the vehicle group that did not receive vitamin K. In general, male animals dosed with TMC125-alone showed prolongation of APTT (68%), PT (99%) and decreases in the vitamin K-dependent clotting factors II (69%) and VII (76%). These changes were counteracted by daily administration of Vitamin K_1. The effects on coagulation parameters were less pronounced in females, in which APTT was increased by 51%, PT by 34%, and factors II and VII decreased by 30% and 44%, respectively. These findings were in accordance with the findings described by Allen *et al*[65]. which demonstrated that male mice are more sensitive to vitamin K deficiencies relative to females. Factor VIII (nonvitamin K dependent) was increased in mice dosed with TMC125 and vitamin K_1 compared to both vehicle groups. The other nonvitamin K dependent clotting factor (XI) was decreased in groups dosed with TMC125. This, however, has been reported previously in rodents with vitamin K deficiency[68] and the observed

decrease in factor XI was partly counteracted by administration of vitamin K_1. The values for clotting times and clotting factors are summarized in Table 29 below.

Table 29: Coagulation Parameters in Male and Female Mice After Repeated Oral Dietary Administration (6 Weeks) in Mice

Parameter	Sex	Vehicle HPMC/MCC	TMC125 + Vit K_1 2320 mg/kg/day + 15 mg/kg/day	TMC125 2320 mg/kg/day	Vehicle + Vit K_1 HPMC/MCC + 15 mg/kg/day
PT (sec)	M	8.63 ± 0.11	8.00 ± 0.08***	17.17 ± 1.42***	7.93 ± 0.13***
	F	8.52 ± 0.10	8.26 ± 0.09*	11.38 ± 0.28***	8.07 ± 0.10**
APTT (sec)	M	39.26 ± 1.53	45.01 ± 1.92*	66.04 ± 3.70***	33.67 ± 1.13**
	F	35.60 ± 1.31	40.57 ± 1.24**	53.71 ± 2.22***	33.48 ± 0.87
Factor II (% of normal)	M	120.36 ± 4.42	150.86 ± 6.18***	36.97 ± 3.89***	122.10 ± 4.81
	F	105.87 ± 3.73	141.79 ± 5.45***	73.71 ± 2.95***	116.15 ± 3.39*
Factor VII (% of normal)	M	111.56 ± 2.20	131.74 ± 2.84***	26.88 ± 4.34***	120.04 ± 2.98*
	F	108.78 ± 2.19	124.43 ± 3.06***	61.41 ± 4.25***	119.56 ± 2.03**
Factor VIII (% of normal)	M	144.74 ± 7.98	212.91 ± 12.04***	135.07 ± 12.95	155.80 ± 11.43
	F	152.77 ± 8.66	210.54 ± 10.04***	158.18 ± 10.76	144.09 ± 5.67
Factor XI (% of normal)	M	98.39 ± 7.48	67.56 ± 7.17**	37.82 ± 4.92***	101.13 ± 12.81
	F	102.88 ± 9.33	75.29 ± 6.63	54.15 ± 9.18***	100.84 ± 9.50

Values represent the mean ± SE (n=20); * p<0.05; ** p<0.01; *** p<0.001: statistical comparison with the vehicle group was performed using Mann-Whitney U test (two-tailed).

No cardiac lesions nor increases in cardiac troponin were observed in any animal in the study other than the one animal that died of cardiomyopathy. Macroscopic examination and organ weights at necropsy indicated an increase in liver weight in animals in both TMC125 treated groups. The relative liver weights increased by up to 2- to 3-fold. Dosing with TMC125 resulted in slight to marked centrilobular to diffuse hepatocellular hypertrophy with hydropic appearance/vacuolation and minimal to marked single cell and (multi)focal necrosis. The hepatic necrosis was often associated with hemorrhages in different degrees of severity in males only, but the incidence and severity of the TMC125-induced focal hemorrhagic necrosis was more pronounced in males dosed with TMC125-alone compared to males dosed with TMC125 and vitamin K_1. These findings indicate that co-administration of vitamin K_1 reduces the hepatic hemorrhagic necrosis observed as a result of TMC125 treatment in male animals. In contrast to the 3-month dietary study (Section 5.1.1.3), no macro- or microscopic hemorrhages in other organs were observed in males in this study.

To conclude, this study confirms the hypothesis that the hemorrhagic cardiomyopathy observed in male mice after dietary dosing was mediated via a vitamin K pathway. These findings are in agreement with those reported by Allen *et al.* who showed that cardiomyopathy can develop in male mice as a result of coagulopathy mediated by vitamin K deficiency[65]. Allen *et al.* also reported that female mice are less sensitive to these effects, which was also evident in the above mechanistic study. See Tabulated Summary 2.6.7.17.B.

Systemic exposure, expressed as C_{max} and $AUC_{0\text{-}24h}$ values, are given in Table 30 below. No clear pharmacokinetic differences between male and female mice were seen due to a large

interindividual variability. The liver to plasma AUC_{0-24h} ratio ranged from 12 to 15 for both males and females in all TMC125-treated groups.

Table 30: C_{max} and AUC_{0-24h} Values of Spray-dried TMC125 After Repeated Oral Dietary Administration (6 Weeks) in Mice

TMC125 Dose (mg/kg/day)	Sample Day 44	C_{max} (µg/mL or g)		AUC_{0-24h} (µg.h/mL or g)	
		M	F	M	F
2320 (+ vitamin K_1 sc)	Plasma	0.49	0.28	8.34	5.76
	Liver	7.39	4.24	121	70.5
2320	Plasma	0.56	0.35	9.00	6.41
	Liver	9.21	5.25	139	95.6

sc: subcutaneous

8.2.2 4-Week Oral Gavage Mouse Study

TMC125 HBr formulated in PEG400 (vehicle) was administered once daily, by oral gavage, for at least 4 weeks[69]. Two control groups and 2 treated groups were given, 0 (vehicle), 0 (vehicle and vitamin K_1), 1000 TMC125 HBr and 1000 (TMC125 HBr and vitamin K_1) mg/kg/day. Vitamin K_1 was given subcutaneously at 15 mg/kg/day. Each group consisted of 20 male and 20 female CD1 mice (main animals), together with 8 male and 8 female satellite animals for toxicokinetic analysis. Regular observations were made for clinical signs, body weight and food consumption, together with clinical laboratory investigations (coagulation times, clotting factors and cardiac troponin, Week 4). The clotting factors investigated were II and VII (vitamin K dependent), and VIII and XI (nonvitamin K dependent). Blood and liver samples were collected on Day 24 during the 24 hours after dosing for toxicokinetic analysis. All main animals found dead or sacrificed during or at the end of the study were subject to macroscopic examination at necropsy, and a number of organs were weighed. Necropsies were performed with a detailed assessment for hemorrhages. Samples of a range of tissues and organs were preserved for histological examination. Tissues and organs from all main animals, and all gross lesions, were examined microscopically.

There were no mortalities associated with TMC125, but in main group animals there were 3 accidental deaths associated with the viscous and irritant nature of the vehicle, 1 male and 1 female given TMC125 HBr with vitamin K_1 and 1 female given TMC125 HBr. There were no relevant clinical signs. In females given TMC125 HBr with Vitamin K_1 there was an increase in body weight (6%) and weight gain (74%) compared to the vehicle group that did not receive vitamin K_1. In general, male animals dosed with TMC125 HBr showed prolongation of APTT (56%) and PT (72%) and decreases in the vitamin K dependent clotting factors II (40%) and VII (42%). This was counteracted by daily administration of vitamin K_1. These changes were almost (clotting times: increase up to 31% in APTT and 10% in PT) or totally (vitamin K-dependent factors) absent in females dosed with TMC125 without vitamin K_1. Factor VIII (nonvitamin K dependent) was increased in male and female mice dosed with TMC125 HBr and vitamin K_1. The other nonvitamin K dependent clotting factor (XI) was decreased in the male group dosed with TMC125 alone. This, however, has been reported previously in rodents with vitamin K deficiency[68]. The decrease in factor XI observed in the current study was counteracted by administration of vitamin K_1. The values for clotting times and clotting factors are summarized in Table 31 below.

Table 31: Coagulation Parameters in Male and Female Mice After Repeated Oral Gavage Administration (4 Weeks) in Mice

Parameter	Sex	Vehicle PEG400	TMC125 + Vit K_1 1000 mg/kg/day + 15 mg/kg/day	TMC125 1000 mg/kg/day	Vehicle + Vit K_1 PEG400 + 15 mg/kg/day
PT (sec)	M	8.20 ± 0.10	7.61 ± 0.11***	14.12 ± 1.64***	7.74 ± 0.08**
	F	8.31 ± 0.12	7.68 ± 0.10***	9.12 ± 0.17***	7.86 ± 0.10**
APTT (sec)	M	36.77 ± 1.00	43.31 ± 1.82**	57.39 ± 3.21***	34.25 ± 0.96
	F	38.64 ± 1.45	47.74 ± 1.37***	50.63 ± 2.19***	36.98 ± 1.08
Factor II (% of normal)	M	106.42 ± 3.12	143.04 ± 5.16***	63.85 ± 7.01***	113.41 ± 3.15
	F	91.70 ± 3.46	133.73 ± 4.17***	93.42 ± 3.06	96.24 ± 3.94
Factor VII (% of normal)	M	100.48 ± 3.35	122.48 ± 3.36***	58.01 ± 8.04***	110.66 ± 3.31*
	F	94.93 ± 3.36	117.78 ± 2.99***	86.65 ± 4.18	100.20 ± 3.39
Factor VIII (% of normal)	M	103.48 ± 8.55	136.09 ± 9.31*	82.02 ± 7.25	112.73 ± 10.09
	F	91.73 ± 9.98	148.76 ± 9.99***	113.14 ± 12.22	88.16 ± 5.51
Factor XI (% of normal)	M	73.27 ± 7.59	65.83 ± 6.00	39.76 ± 4.89***	67.63 ± 4.46
	F	65.27 ± 8.30	63.61 ± 4.54	57.30 ± 6.00	61.16 ± 4.43

Values represent the mean ± SE (n=20); * p<0.05; ** p<0.01; *** p<0.001: statistical comparison with the vehicle group was performed using Mann-Whitney U test (two-tailed).

Macroscopic examination and organ weights at necropsy indicated an increase in liver weight (1.5- to 2-fold) in both TMC125 treated groups. This change was associated with minimal to moderate centrilobular to diffuse hepatocellular hypertrophy and hydropic appearance/vacuolation with single cell/focal necrosis. In contrast to the dietary study, there was no hemorrhagic necrosis in the liver in this study. Hemorrhagic cardiomyopathy was not seen in any animal and this correlated with an absence of changes in cardiac troponin.

To conclude, this mechanistic study demonstrated that the effect of TMC125 on clotting parameters in mice was mediated via vitamin K. This study, however, did not result in hemorrhagic cardiomyopathy in any animal and the effects on coagulation parameters seen were less pronounced relative to those reported after dietary dosing. See Tabulated Summary 2.6.7.17.B.

Systemic exposure expressed as C_{max} and $AUC_{0\text{-}24h}$ values are given in Table 32 below. The liver to plasma $AUC_{0\text{-}24h}$ ratio ranged from 10 to 15 for both males and females in all TMC125-treated groups.

Table 32: C_{max} and AUC_{0-24h} Values of TMC125 HBr After Repeated Oral Gavage Administration (4 weeks) in mice

TMC125 Dose (mg/kg/day)	Sample Day 25	C_{max} (µg/mL or g)		AUC_{0-24h} (µg.h/mL or g)	
		M	F	M	F
1000	Plasma	1.67	1.51	9.25	11.2
	Liver	26.1	19.9	113	114
1000 (+ vitamin K_1 sc)	Plasma	1.73	1.59	8.42	9.41[a]
	Liver	25.3	17.7	130	98.6[a]

[a]: AUC_{0-8h}
sc: subcutaneous

8.2.3 Assessment of Mechanistic Studies

The literature describes cases of hemorrhagic cardiomyopathy and hemothorax in vitamin K-deficient mice[65,66]. The disorder affects primarily the heart muscle by producing myofiber degeneration, necrosis, and hemorrhage. Inflammation was considered secondary to the extensive myocardial injury. In all these cases males were much more sensitive to coagulopathy and hemorrhagic cardiomyopathy than females. It has been hypothesized that females are less susceptible to vitamin K deficiencies since they are able to form the active form of vitamin K more rapidly and at a more effective concentration based on an induced epoxidase activity by estrogens[70]. The mechanistic studies conducted with TMC125 demonstrated that the effect of TMC125 on clotting times and clotting factors in mice is mediated via a vitamin K pathway. Although the hemorrhagic cardiomyopathy and hemothorax in the dietary mechanistic study could not be reproduced to the same extent as in a previous 3-month dietary study, the findings were in line with those described by Allen et al[65]. The underlying mechanism for the cardiac lesions can be explained by the severely disturbed coagulation resulting in interstitial hemorrhagic diathesis within the myocardium and subsequent muscle degeneration and inflammation. The mouse is believed to be more sensitive to develop hemorrhagic cardiomyopathy due to a higher heart rate and thinner ventricular and atrial wall, relative to other species including humans where such pathology associated with vitamin K deficiency has not been reported. The effects of TMC125 on coagulation times were more pronounced after dietary dosing than after gavage, without any differences in liver and plasma exposures. The exact reason for this discrepancy is not clear. Also in rat studies changes in coagulation time, without hemorrhages, were noted only after dietary administration and not after gavage[42]. Gavage dosing in animals is, obviously, much more comparable to oral administration of tablets in humans than continuous dietary dosing. No changes in coagulation parameters were observed in dogs and no direct cardiac changes were noted in female mice or in male and female rats or dogs. Based on the above, the findings observed in male mice are considered to be of no relevance to humans.

8.3 STUDIES ON DRUG SUBSTANCE IMPURITIES

8.3.1 Impurity A* and Impurity B*

8.3.1.1 In Vitro Bacterial Reverse Mutation Assay, Ames Test Impurity A* (GLP)

TMC125 base spiked with 2% Impurity A* formulated in DMSO, was tested in a classical bacterial reverse mutation assay (Ames test) with 5 histidine-requiring strains of *Salmonella typhimurium*

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(TA98, TA100, TA102, TA1535 and TA1537)[71]. In this study, concentrations up to 1000 µg/plate were tested in 2 independent assays, in the absence or presence of metabolic activation (Aroclor-induced rat liver S9 mix); precipitation occurred at 250 µg/plate and above. Weak bacteriotoxic effects were observed (as a decrease in the number of revertant colonies) with strains TA102 and TA1535 in the absence of S9 at 125 and 500 µg/plate and above, respectively. In the presence of S9, weak bacteriotoxic effects were observed in TA102 and TA1537 at 250 and 500 µg/plate and above, respectively. TMC125 spiked with 2% Impurity A* did not induce a dose-related, more than 2- or 3-fold increase in the number of revertant colonies in any of the tester strains in the absence or presence of S9 metabolic activation. The vehicle control and appropriate positive control reference articles confirmed the adequacy of the test system. It was concluded that TMC125 spiked with 2% Impurity A* was not mutagenic under the conditions of these assays. See Tabulated Summary 2.6.7.17.C.

8.3.1.2 In Vitro Bacterial Reverse Mutation Assay, Ames Test Impurity B *(GLP)

TMC125 base spiked with 2% Impurity B* formulated in DMSO, was tested in a bacterial reverse mutation assay (Ames test) with 5 histidine-requiring strains of *Salmonella typhimurium* (TA98, TA100, TA102, TA1535 and TA1537)[72]. In this study, concentrations up to 1000 µg/plate were tested in 2 independent assays, in the absence or presence of metabolic activation (Aroclor-induced rat liver S9 mix). Precipitation occurred at 250 µg/plate and above. In the first experiment only, weak bacteriotoxic effects were observed (as a decrease in the number of revertant colonies) with strain TA1535 in the presence of S9 from 500 µg/plate onwards and with TA1537 in the presence and absence of S9 at 1000 µg/plate. TMC125 spiked with 2% Impurity B* did not induce a dose-related, more than 2- or 3-fold increase in the number of revertant colonies in any of the tester strains in the absence or presence of S9 metabolic activation. The vehicle control and appropriate positive control reference articles confirmed the adequacy of the test system. It was concluded that TMC125 spiked with 2% Impurity B* was not mutagenic under the conditions of these assays. See Tabulated Summary 2.6.7.17.C.

8.3.1.3 In Vitro Mammalian Forward Mutation Assay, Mouse Lymphoma Cells Impurity A* (GLP)

TMC125 base spiked with 2% Impurity A* and formulated in DMSO, was tested in an in vitro mammalian forward mutation assay at the thymidine kinase (TK) locus in mouse lymphoma L5178Y cells[73]. Following a dose range finding test, TMC125 spiked with 2% Impurity A* was tested, in the absence of metabolic activation (Aroclor-induced rat liver S9 mix) up to 14 µg/mL following 3 or 24 hours treatment and with S9 metabolic activation up to 50 µg/mL following 3 hours treatment. The tests were performed at the limit of toxicity and there was no biologically significant induction in mutation frequency either in the absence (3 and 24 hours exposure), or in the presence of a metabolic activation system (3 hours exposure). The vehicle control and appropriate positive control reference articles confirmed the adequacy of the test system. It was concluded that TMC125 spiked with 2% Impurity A* was not mutagenic or clastogenic under the conditions of this assay. See Tabulated Summary 2.6.7.17.C.

8.3.1.4 In Vitro Mammalian Forward Mutation Assay, Mouse Lymphoma Cells Impurity B* (GLP)

TMC125 base spiked with 2% Impurity B* and formulated in DMSO, was tested in an in vitro mammalian forward mutation assay at the TK locus in mouse lymphoma L5178Y cells[74].

TMC125 spiked with 2% Impurity B* was tested, in the absence of metabolic activation (Aroclor-induced rat liver S9 mix) up to 12 µg/mL following 3 or 24 hours treatment and with S9 metabolic activation up to 50 µg/mL following 3 hours treatment. The tests were performed at the limit of toxicity and there was no biologically significant induction in mutation frequency either in the absence (3 and 24 hours exposure), or in the presence of a metabolic activation system (3 hours exposure). The vehicle control and appropriate positive control reference articles confirmed the adequacy of the test system. It was concluded that TMC125 spiked with 2% Impurity B* was not mutagenic or clastogenic under the conditions of this assay. See Tabulated Summary 2.6.7.17.C.

8.3.1.5 3-MONTH ORAL DOG STUDY (GLP)

The potential toxicity of impurities Impurity A* and Impurity B* was tested in a 3-month dog study[75]. One control group and 2 treated groups were given, 0 (vehicle), 10 and 20 mg/kg/day TMC125 HBr. Both impurities were dosed at 0.2 and 0.4 mg/kg/day, respectively, and thus at 2% of the TMC125 HBr dose. The total dose volume was 2 mL/kg/day. Both TMC125 HBr and the impurities were formulated in the vehicle, PEG400. Each group consisted of 3 male and 3 female Beagle dogs. Regular observations were made for clinical signs, body weight and food consumption. Clinical laboratory investigations (hematology, blood chemistry and urine analysis) were performed after 1 month and at the end of the study. All animals were subject to macroscopic examination at necropsy and a number of organs were weighed. Samples of a range of tissues and organs were preserved for histological examination. All tissues and organs from animals in all dose groups and all gross lesions, were examined microscopically.

There were no mortalities in the study and there were no clinical signs, or relevant effects on body weight or food consumption. Hematological, serum chemistry and urinalysis parameters were not relevantly changed after TMC125 treatment. Macroscopic examination at necropsy, organ weights and histopathological examination indicated no changes that could be attributed to treatment. The NOAEL was therefore 20 mg/kg/day TMC125 combined with 0.4 mg/kg of each Impurity A* and Impurity B*. See Tabulated Summary 2.6.7.17.C.

8.3.2 IMPURITY C*

8.3.2.1 IN VITRO BACTERIAL REVERSE MUTATION ASSAY, AMES TEST IMPURITY C* (GLP)

Impurity C* (also referred to [redacted] formulated in DMSO, was tested in a bacterial reverse mutation assays (Ames test) with 5 histidine-requiring strains of *Salmonella typhimurium* (TA98, TA100, TA102, TA1535 and TA1537)[76]. Concentrations from 4.88 up to 312.5 µg/plate were tested in 4 independent assays, in the absence or presence of metabolic activation (Aroclor-induced rat liver S9 mix). Precipitation occurred at 19.53 µg/plate and above. The test material did not induce a dose-related, more than 2- or 3-fold increase in the number of revertant colonies in any of the tester strains in the absence or presence of S9 metabolic activation. The vehicle control and appropriate positive control reference articles confirmed the adequacy of the test system. It was concluded that the test material was not mutagenic under the conditions of these assays. See Tabulated Summary 2.6.7.17.C.

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8.4 GENOTOXICITY OF INTERMEDIATES

The Quality of TMC125 is described in the Quality Overall Summary and in Module 3. Genotoxicity studies were conducted on intermediate products (Intermediate A* and Intermediate B*). These studies are discussed below and summarized in Tabulated Summary 2.6.7.17.D.

8.4.1 INTERMEDIATE A*

8.4.1.1 IN VITRO BACTERIAL REVERSE MUTATION ASSAY, AMES TEST (GLP)

Intermediate A* formulated in DMSO was tested in a bacterial reverse mutation study (Ames test)[77]. Four histidine-requiring strains of *Salmonella typhimurium* (TA98, TA100, TA1535 and TA1537) and *Escherichia coli* WP2uvrA were evaluated. Concentrations up to 5000 µg/plate were tested in 2 independent assays, in the absence or presence of metabolic activation (Aroclor-induced rat liver S9 mix). No decrease in the number of revertants was observed. Intermediate A* did not induce a dose-related, more than 2- or 3-fold increase in the number of revertant colonies in any of the tester strains in the absence or presence of S9 metabolic activation. The vehicle control and appropriate positive control reference articles confirmed the adequacy of the test system. It was concluded that Intermediate A* was not mutagenic under the conditions of these assays. See Tabulated Summary 2.6.7.17.D.

8.4.1.2 IN VITRO MAMMALIAN CHROMOSOMAL ABERRATION ASSAY (SCREENING AND GLP)

Intermediate A* was tested in a screening in vitro mammalian chromosome aberration test in human lymphocytes[78]. Intermediate A* was tested, in the absence or presence of metabolic activation (Aroclor-induced rat liver S9 mix) up to 470 µg/mL following 4 hours treatment with a 20-hour expression period and also for a 24-hour continuous exposure in the absence of S9 up to 470 µg/mL. Precipitation occurred at 470 µg/mL and above. The test material did not induce biologically relevant or statistical increases in the number of cells with chromosome aberrations in the absence or presence of metabolic activation. The vehicle control and appropriate positive control reference article confirmed the adequacy of the test system. Intermediate A* was considered to be nonclastogenic to human lymphocytes in vitro. Tabulated Summary 2.6.7.17.D.

Intermediate A* formulated in tetra hydrofurane was also tested in an in vitro mammalian chromosome aberration test in Chinese hamster V79 cells[79]. Following a dose range finding test, Intermediate A* was tested, in the absence or presence of S9 metabolic activation (Aroclor-induced rat liver S9 mix) at up to 40 µg/mL, following 4, 18 or 28 hours treatment. Precipitation occurred at 10 µg/mL and above. The chromosomes were prepared 18 or 28 hours after the start of treatment. There was no biologically relevant or statistical increase in the number of cells with chromosomal aberrations in the absence or presence of metabolic activation system. The vehicle control and appropriate positive control reference article confirmed the adequacy of the test system. It was concluded that Intermediate A* was not clastogenic in the absence or presence of S9. See Tabulated Summary 2.6.7.17.D.

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8.4.1.3 ASSESSMENT OF INTERMEDIATE A* GENOTOXICITY

Intermediate A* does not induce mutagenicity in bacteria, nor clastogenicity in human lymphocytes or Chinese hamster V79 cells in the absence or presence of metabolic activation system. Therefore, Intermediate A* is considered to be a nongenotoxic intermediate.

8.4.2 INTERMEDIATE B*

8.4.2.1 IN VITRO BACTERIAL REVERSE MUTATION ASSAY, AMES TEST (GLP)

Intermediate B* formulated in DMSO was tested in a bacterial reverse mutation assays (Ames test)[80]. Four histidine-requiring strains of *Salmonella typhimurium* (TA98, TA100, TA1535 and TA1537) and *Escherichia coli* WP2uvrA were evaluated. Concentrations up to 5000 µg/plate were tested in 2 independent assays, in the absence or presence of metabolic activation (Aroclor-induced rat liver S9 mix). Precipitation occurred at 100 µg/plate and above. The bacterial background lawn was reduced with TA1535 and TA1537 but not in any of the other strains tested. No decrease in the number of revertants was observed with the exception of TA1537 at 2500 µg/plate and above. Intermediate C* did not induce a dose-related, more than 2- or 3-fold increase in the number of revertant colonies in any of the tester strains in the absence or presence of S9 metabolic activation. The vehicle control and appropriate positive control reference articles confirmed the adequacy of the test system. It was concluded that Intermediate B* was not mutagenic under the conditions of these assays. See Tabulated Summary 2.6.7.17.D.

8.4.2.2 IN VITRO MAMMALIAN CHROMOSOMAL ABERRATION ASSAY (SCREENING AND GLP)

Intermediate B* was tested in a screening in vitro mammalian chromosome aberration test in human lymphocytes in the absence or presence of metabolic activation (Aroclor-induced rat liver S9 mix)[81]. Preliminary toxicity evaluations indicated that the test material exhibited a steep toxicity curve. Intermediate B* was tested, up to 41.77 µg/mL following 4 hours exposure with a 20-hour expression period without S9, up to 55.69 µg/mL following 4 hours exposure with a 20-hour expression period with S9, and up to 27.85 µg/mL following a 24 hour exposure without S9. The test material did not induce biologically relevant and statistically significant increases in the number of cells with chromosome aberrations in the absence or presence of metabolic activation. The vehicle control and appropriate positive control reference article confirmed the adequacy of the test system. Intermediate B* was considered to be nonclastogenic to human lymphocytes in vitro. Tabulated Summary 2.6.7.17.D.

Intermediate B* formulated in DMSO was tested in an in vitro mammalian chromosome aberration test in Chinese hamster V79 cells[82]. Intermediate B* was evaluated up to 12.5 and 25 µg/mL in the absence or presence of metabolic activation (Aroclor-induced rat liver S9 mix), respectively, following 4 hours treatment with precipitation encountered at 25 µg/mL. Cytogenetic evaluation at higher concentration levels was limited by cytotoxicity. The chromosomes were prepared following an 18-hour preparation interval. Preliminary range finding experiments without S9 indicated a steep cytotoxicity response, even with the lowest evaluated concentration level of 21.5 µg/mL (2%-18% cell number versus vehicle control). A similar, sharp cytotoxicity curve was observed in the main experiment, with surviving cell numbers dropping from 60.3% to 0.2% between 12.5 µg/mL and 25 µg/mL, indicating that concentrations near or above 12.5 µg/mL had high toxic potential. Following 4 hours exposure at 25 µg/mL, with S9, T002327 did not induce any

increase in cells with chromosomal aberrations. At the single, highest concentration of 12.5 µg/mL, after 4 hours exposure without S9, a statistically significant increase in cells with chromosomal aberrations was observed. The vehicle control and appropriate positive control reference article confirmed the adequacy of the test system.

Under the conditions of this assay, Intermediate B* did not induce clastogenic effects in the presence of metabolic activation. In the absence of metabolic activation, the test material did demonstrate a potential to increase the number of aberrant cells, but only at the highest concentration level evaluated. See Tabulated Summary 2.6.7.17.D.

8.4.2.3 IN VIVO MAMMALIAN MICRONUCLEUS TEST (GLP)

To evaluate the clastogenic effect in vivo, Intermediate B* formulated in corn oil was administered to mice in an in vivo micronucleus test[83]. Single oral (gavage) doses of 0 (vehicle), 500, 1000 and 2000 mg/kg Intermediate B* and 50 mg/kg cyclophosphamide (positive reference article) were administered to NMRI BR mice (6 males and 6 females per sampling time point for each dose) and bone marrow was sampled 24 or 48 hours (2000 mg/kg only) after dosing. In a separate study[84], plasma samples were taken for toxicokinetic evaluation. Clinical signs included a reduction of spontaneous activity and ruffled fur, which increased in incidence with increasing dose levels. No increase in the frequency of micronucleated cells was observed in the polychromatic erythrocytes of the bone marrow of animals tested with Intermediate B*. There was no effect on the ratio of polychromatic to normochromatic erythrocytes compared to vehicle controls. The vehicle control and the positive reference articles confirmed the adequacy of the test system. It was concluded that Intermediate B* was not clastogenic in vivo up to the limit dose of 2000 mg/kg. At 2000 mg/kg C_{max} values were 0.058 and 0.074 ng/mL in males and females, respectively and corresponding $AUC_{0\text{-}7h}$ were 0.22 and 0.19 µg.h/mL.

8.4.2.4 ASSESSMENT OF INTERMEDIATE B* GENOTOXICITY

Intermediate B* was not mutagenic in bacteria, nor did it induce micronuclei in mice exposed to an oral limit dose of 2000 mg/kg.

Although an isolated observation of increased chromosomal aberrations was noted in Chinese hamster V79 cells, this finding contrasts to negative in vitro clastogenicity results obtained with peripheral human lymphocytes at higher concentrations. Rodent cell lines, in comparison to human lymphocytes, have been noted to exhibit an unacceptably high false positive response rate in genotoxicity assays[85]. Therefore, the clastogenic activity observed in V79, but not human cells, should be treated with caution.

An increase in chromosomal aberrations in V79 cells was only seen at a single treatment condition and treatment level (absence of S9 and at the highest concentration level evaluated (12.5 µg/mL)). Although the achieved cell number and MI values at this dose level were above the recommended limit of 50%, cytotoxicity exhibited a sharp increase (60.3% to 0.2% cell count) between 12.5 and 25 µg/mL. Therefore the nature of the clastogenic response could not be substantiated at multiple concentrations above 12.5 µg/mL. This steep cytotoxicity slope is a hallmark of positive in vitro chromosomal aberration responses that often have limited relevance for in vivo exposure scenarios[86]. In comparison, chromosomal aberration analysis of human lymphocytes treated up to higher concentrations of 41.77 µg/mL (4 hour exposure) and 27.85 µg/mL (24 hour exposure) without metabolic activation failed to induce clastogenicity. Using a

weight-of-evidence approach, such a positive finding, uncorroborated by genotoxic activity in complementary exposure regimens, is suggestive of a lack of true genotoxic hazard[87].

In conclusion, Intermediate B* is not considered as a genotoxic intermediate based on the weight of evidence analysis including all available data.

8.5 BATCH QUALIFICATION STUDIES

8.5.1 In Vitro Bacterial Reverse Mutation Assay, Ames Test (GLP)

Batch [redacted] ([redacted]) of TMC125 HBr formulated in DMSO was tested in a bacterial reverse mutation assay (Ames test) with 5 histidine-requiring strains of *Salmonella typhimurium* (TA98, TA100, TA102, TA1535 and TA1537)[88]. Concentrations from 0.61 up to 5000 µg/plate were tested in 2 independent assays, in the absence or presence of metabolic activation (Aroclor-induced rat liver S9 mix); precipitation occurred at 156.25 µg/plate and above. With some strains in the absence of S9 mix, weak bacteriotoxic effects were observed, visualized by a decrease in the number of revertants at the highest concentrations. TMC125 HBr [redacted] did not induce a dose-related, more than 2- or 3-fold increase in the number of revertant colonies in any of the tester strains in the absence or presence of S9 metabolic activation. The vehicle control and appropriate positive control reference articles confirmed the adequacy of the test system. It was concluded that TMC125 HBr [redacted] was not mutagenic under the conditions of these assays. See Tabulated Summary 2.6.7.17.E.

8.5.2 In Vitro Bacterial Reverse Mutation Assay, Ames Test (GLP)

The stressed capsule content of clinical trial formulations of TMC125 base Batch [redacted] formulated in DMSO was tested in a bacterial reverse mutation assay (Ames test) with 5 histidine-requiring strains of *Salmonella typhimurium* (TA98, TA100, TA102, TA1535 and TA1537)[89]. Concentrations from 10 up to 1000 µg/plate were tested in 2 independent assays, in the absence or presence of metabolic activation (Aroclor-induced rat liver S9 mix); precipitation occurred at all concentrations but it did not interfere with scoring the number of revertant colonies. The test material did not induce a dose-related, more than 2- or 3-fold increase in the number of revertant colonies in any of the tester strains in the absence or presence of S9 metabolic activation. The vehicle control and appropriate positive control reference articles confirmed the adequacy of the test system. It was concluded that the test material was not mutagenic under the conditions of these assays. See Tabulated Summary 2.6.7.17.E.

8.5.3 2-Week Repeated Dose Toxicity – Dog (GLP)

The toxicity of TMC125 HBr batch [redacted] ([redacted]) was compared with Batch [redacted] ([redacted]). Both test materials were formulated in PEG400 and were administered daily, by oral gavage, for at least 14 days[90]. One control group and 4 treated groups were given, 0 (vehicle), 20 ([redacted]), 240 ([redacted]), 20 ([redacted]) and 240 ([redacted]) mg/kg/day, in a dose volume of 2 mL/kg. Each group consisted of 3 male and 3 female Beagle dogs. Regular observations were made for clinical signs, body weight and food consumption, together with ophthalmoscopic examination, and ECG. At the end of the study clinical laboratory investigations (hematology, blood chemistry and urine analysis) was undertaken. Blood samples were collected on Days 10 during the first 24 hours after dosing for toxicokinetic analysis. All animals sacrificed at the end of the study were subject to macroscopic examination at necropsy and a number of organs were weighed.

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Samples of a range of tissues and organs were preserved for histological examination. All tissues and organs from animals in all dose groups and all gross lesions were examined microscopically.

There were no mortalities in the study and there were no relevant clinical signs, or effects on body weight, food consumption, ophthalmoscopy or ECG. In the laboratory investigations, hematological, serum chemistry and urinalysis parameters were unchanged with TMC125 treatment. Macroscopic examination at necropsy, organ weights and histopathological examination indicated no relevant changes that could be attributable to treatment. It was concluded that the overall toxicological profile of the 2 batches was comparable. See Tabulated Summary 2.6.7.17.E.

Toxicokinetic analysis indicated nonlinear pharmacokinetics. Systemic exposure expressed as C_{max} and $AUC_{0\text{-}24h}$ values are given in Table 33 below.

Table 33: C_{max} and $AUC_{0\text{-}24h}$ Values of TMC125 HBr After Repeated Oral Administration (14 Days) in Dogs

TMC125 Dose (mg/kg/day)	Sampling Day	C_{max} (µg/mL)		$AUC_{0\text{-}24h}$ (µg.h/mL)	
		M	F	M	F
20 ([redacted])	Day 10	0.19	0.83	3.12	8.70
240 ([redacted])	Day 10	1.17	1.12	18.9	18.4
20 ([redacted])	Day 10	1.07	0.37	10.8	4.39
240 ([redacted])	Day 10	1.02	2.48	18.5	27.3

9 DISCUSSION AND CONCLUSIONS

TMC125 was originally tested in its base form in a limited number of studies. Later on, exposure was improved by the use of the HBr salt (TMC125 HBr) and as a result most nonclinical safety studies were conducted with this form. The improvement in exposure by using the HBr salt was more obvious in rats than in dogs. Further improvement in exposure was also noticed with development of the spray-dried formulation, which became available at a later stage of drug development. To assess nonclinical safety of TMC125 at the highest possible exposure, some studies were repeated with the spray-dried form. These included, 1- and 6-month repeated dose studies in dogs, fertility and early embryonic development study in the rat and an embryo-fetal development study in the rabbit. The pre- and postnatal developmental toxicity study in the rat was directly performed with spray-dried TMC125.

Toxicokinetic data in all species used in the nonclinical safety evaluation program indicated that the absorption of TMC125 after oral administration is limited and dependent on the TMC125 form used. Saturation of absorption, due to poor solubility and permeability across the intestine, was frequently noted over the dose ranges tested. In addition, at high dose or exposure levels, C_{max} and AUC values decreased after repeated administration due to metabolic auto-induction. This was observed in all species, mainly with TMC125 HBr and spray-dried forms. This phenomenon somewhat limited the use of the various tested TMC125 forms in the overall safety assessment, as the increase in exposure observed after single administration with a particular form was not sustained upon repeated administration. In an effort to increase exposure in view of the carcinogenicity studies, 3-month dietary studies with spray-dried TMC125 were performed in rat and mouse. This approach, however, yielded no increase in exposure versus gavage administration with the HBr salt and was poorly tolerated in male mice. Therefore, the dietary route was not considered further and the carcinogenicity studies were performed with gavage administration.

At the recommended clinical dose regimen (200 mg twice daily; formulation A*) in treatment-experienced HIV-1 infected subjects, the exposure is equivalent to a C_{max} value of 0.45 µg/mL and an $AUC_{0\text{-}24h}$ of 7.4 µg.h/mL (Clinical trial TMC125-C228). These values were used for comparison with animal toxicokinetic data.

The acute toxicity of TMC125 in mouse, rat or dog was limited. No TMC125-related mortality was noted in the different single dose studies performed by either oral gavage or subcutaneous injection.

No TMC125-related mortality was noted in repeated dose toxicity studies up to 6 months in rats or up to 12 months in dogs, but on some occasions animals were found dead or needed to be prematurely sacrificed. In most cases this was confirmed to be due to gavage errors, mainly caused by the irritant and viscous nature of the vehicle (PEG400) used.

In the **rat**, the key target organs identified in most of the repeated dose studies were the liver and the thyroid. The NOAEL in the 6-month gavage study with TMC125 HBr in the rat was 70 mg/kg/day, corresponding to a C_{max} of 0.06 and 0.06 µg/mL and an $AUC_{0\text{-}last}$ of 0.21 and 0.66 µg.h/mL at the end of the study, in males and females, respectively. Based on the above, the exposure at the NOAEL level after 6 months of dosing in the rat was below that anticipated in the clinic at the recommended dose (C_{max} 0.45 µg/mL; $AUC_{0\text{-}24h}$ 7.4 µg.h/mL), although the findings were limited to adaptive liver and thyroid changes which are regarded as of no

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relevance to humans. No NOAEL was detected in the dietary 3-month study with spray-dried TMC125 due to limited changes observed at the lowest dose level. Systemic exposure (AUC_{0-24h}) at the highest dietary dose level (1300 mg/kg/day) was 2.96 and 5.34 µg.h/mL in males and females, respectively.

Liver weight was slightly increased (up to 23%) in the 3- and 6-month studies with TMC125 HBr in Sprague-Dawley or Wistar rats at the highest tested dose level only (600 mg/kg/day). A dietary 3-month study in the Sprague-Dawley rat with spray-dried TMC125 resulted in an increased liver weight (up to 27%) from a dose of 330 mg/kg/day (AUC_{0-24h} 1.21 and 2.95 µg.h/mL in males and females, respectively). In all these studies, the liver changes were not associated with relevant changes in liver transaminases. No histopathological liver changes were seen in the 6-month gavage study with TMC125 HBr (Wistar) or in the 3-month dietary study with spray-dried TMC125 (Sprague-Dawley). In the two 3-month gavage studies with TMC125 HBr, however, liver changes were limited to basophilic stippling (Wistar) or fatty-like vacuolization (Sprague-Dawley). Ex-vivo metabolic studies following 3-month administration of TMC125 HBr in Sprague-Dawley rats indicated that TMC125 was an inducer of hepatic microsomal CYP3A, CYP2B and other CYP subfamily forms (Module 2.6.4 Pharmacokinetic Written Sumnmary, Section 5.5.1). Based on all above, the described liver changes in rats are considered to reflect an adaptive response to liver enzyme induction after administration of a xenobiotic, rather than a direct adverse effect of TMC125[91].

Thyroid changes, indicative of an increased activity, were observed in all rat studies of 3-month duration and longer from a dose of 200 mg/kg/day, (AUC_{0-24h} 0.74 and 2.28 µg.h/mL in males and females, respectively). Measurement of T3, T4 and TSH in the 3-month gavage study with TMC125 HBr in Sprague-Dawley rats demonstrated decreases in T3 and T4 and a compensatory rise in TSH and in the same study, induction of microsomal thyroxine UDP-GT activity was reported ex-vivo (Module 2.6.4 Pharmacokinetic Written Sumnmary, Section 5.5.1). In another 3-month study with TMC125 HBr in the Wistar rat, the thryoid findings were associated with a slight increase in severity of hypertrophy and cell vacuolation of the TSH-producing cells in the pars anterior of the pituitary gland in high dose males only. Hypertrophy/ hyperplasia of thyrotrophs in the pituitary gland is a well-known histological finding related to a compensatory increased TSH demand in the rat. No thyroid or pituitary changes have been observed in studies in mice or dogs. Similar to the liver findings, the thyroid and pituitary changes seen in rats are considered to be rodent-specific related to adaptive response to enhanced thyroid hormone elimination and, therefore, pose no safety risk to humans[92].

Urinary changes consisting of an altered elimination of some electrolytes (calcium, sodium, chloride) were noted in a 1-month and 3-month study in Wistar rats with TMC125 HBr from a dose of 80 or at 600 mg/kg/day, respectively. However, no such changes were observed in a dietary 3-month study with spray-dried TMC125 (despite similarities in exposure between gavage and dietary administration) or in the chronic 6-month study with TMC125 HBr. No renal changes have been observed in mice or dogs. Therefore, these changes are considered to be of no relevance to humans.

Increases in coagulation times in rat studies with TMC125 were only observed during a 3-month dietary study. An increase in PT was seen in all treated males (up to 22%), however, all treated females showed a reduction in PT (up to 13%). APTT was also increased in all treated males (up to 61%) but only females in the 990 mg/kg/day dose group had a marginally elevated APTT (10%). Systemic exposure (AUC_{0-24h}) at the lowest dose level (330 mg/kg/day) was 1.21 and

2.95 µg.h/mL in males and females, respectively. Given the fact that these changes were only observed after dietary dosing and not in any gavage study and were not associated with any macro- or microscopic hemorrhages, their relevance is low. Also in mice, dietary dosing had a higher impact on coagulation (see further) compared with dosing by gavage.

In the **dog**, no target organs of toxicity were observed in studies up to 12 months duration with TMC125 HBr. The NOAEL after 12 months of dosing in the dog was 240 mg/kg corresponding to a C_{max} of 1.77 and 2.16 µg/mL and an $AUC_{0\text{-}24h}$ of 23.2 and 35.6 µg.h/mL in females and males, respectively. In the dog, exposure at the NOAEL after 12 months of dosing was at least 3-fold higher for both C_{max} and AUC compared to the anticipated human exposure (C_{max} 0.45 µg/mL; $AUC_{0\text{-}24h}$ 7.4 µg.h/mL). However, in a 1-month and 6-month repeated dose toxicity study with spray-dried TMC125 in the dog, changes were observed in the liver (microgranuloma and minor inflammatory changes) and gall bladder (inspissated bile). In the 6-month study, these changes were noted from a dose level of 160 mg/kg/day, corresponding to an $AUC_{0\text{-}24h}$ of 54.5 and 44.6 µg.h/mL in males and females, respectively. The occurrence of these changes after dosing TMC125 spray-dried in the dog is therefore likely related to a higher systemic exposure compared to dosing with TMC125 HBr.

In the 1-month study with spray-dried TMC125 in dogs, liver changes consisting of inspissated bile in the gall bladder and microgranulomas were observed in 2 high dose (250 mg/kg b.i.d.) animals with associated increases in serum bilirubin and ALP. This dose level was considered to exceed the maximum tolerated dose based on a significant decrease in body weight and subsequent effects on hematological parameters and lymphoid organ atrophy. In the 6-month study, hepatic changes were noted from a dose level of 160 mg/kg/day, while gall bladder changes were noted at a dose level of 500 mg/kg/day. The dose level of 160 mg/kg/day corresponds to an $AUC_{0\text{-}24h}$ of 54.5 and 44.6 µg.h/mL in males and females, respectively, while values after 500 mg/kg/day were 61.8 and 61.5 µg.h/mL in males and females, respectively. The occurrence of these changes after dosing with TMC125 spray-dried in the dog is therefore likely related to a higher systemic exposure compared to dosing with TMC125 HBr. This exposure is up to 3-fold higher than that observed at the highest dose used in dog studies with TMC125 HBr (240 mg/kg) and at least 8-fold higher than the anticipated human exposure (C_{max} 0.45 µg/mL; $AUC_{0\text{-}24h}$ 7.4 µg.h/mL).

In **mice**, no NOAEL was determined in gavage studies up to 3 months duration with TMC125 HBr due to limited hepatic effects seen at the lowest dose levels tested. Systemic exposures ($AUC_{0\text{-}last}$) observed at the lowest dose in the 3-month study were 1.44 and 1.09 µg.h/mL in males and females, respectively. The liver was the main target organ after gavage and dietary dosing in mice and the changes were more pronounced compared to those in other species. In addition, dietary dosing of spray-dried TMC125 resulted in mortality in male mice caused by coagulopathy with subsequent cardiac effects and hemorrhages in different organs.

Liver weight was increased (up to 50%) in a 3-month gavage study in mice with TMC125 HBr from a dose of 50 mg/kg/day ($AUC_{0\text{-}24h}$ 3.14 µg.h/mL). In the 3-month dietary study with spray-dried TMC125, the liver weight increases were most pronounced (up to 2.4-fold) and seen at all dose levels. At autopsy the liver changes consisted of paleness, swelling and more pronounced lobulation. Histopathologically, hepatocellular hypertrophy was associated with degenerative and necrotic changes in all studies. These changes were associated with pronounced increases in liver transaminases, while in most studies increases in cholesterol and/or triglycerides were also observed although mostly only at the highest dose levels. Electron microscopic examination at

the high dose (800 mg/kg) in the 3-month study with TMC125 HBr in mice indeed demonstrated accumulation of lipid globules in the hepatocytes as compared to that in control mice. In mice, a dose-related induction (up to 11-fold) in the microsomal activities of CYP3A and CYP2B was noted following repeated administration of TMC125 HBr for 3 months (Module 2.6.4 Pharmacokinetic Written Sumnmary, Section 5.5.1). This strong enzymatic induction may have contributed to the liver changes seen in mice.

A 3-month dietary study with spray-dried TMC125 in the mouse resulted in unexpected high mortality in male mice from a dose of 450 mg/kg/day ($AUC_{0\text{-}24h}$ 1.98 µg.h/mL). The cause of the death was hemorrhagic cardiomyopathy, often associated with hemothorax and hemorrhages in several organs. No mortality, hemorrhages or cardiac changes were observed in females, although systemic exposure was comparable between both sexes. Despite similarity in exposure obtained after gavage and dietary repeated dosing (9-10 µg.h/mL), the myocarditis-related mortality was only observed after dietary dosing. Literature describes identical cases of hemorrhagic cardiomyopathy and hemothorax caused by a coagulopathy in vitamin K-deficient mice[65]. Two mechanistic studies were performed and demonstrated that the effect of TMC125 on coagulation times and clotting factors was mediated via a vitamin K pathway. The mouse is believed to be more sensitive to develop hemorrhagic cardiomyopathy due to a higher heart rate and thinner ventricular and atrial wall, relative to other species, including humans where such pathology associated with vitamin K deficiency has not been reported. The effects of TMC125 on coagulation times were much more pronounced after dietary dosing than after gavage dosing without any differences in liver and plasma exposures. The exact cause of this discrepancy is not clear. However, also in rat studies changes in coagulation times (without subsequent hemorrhages) were only noted after dietary administration and not after gavage (see above). Gavage administration is, obviously, much more comparable to human dosing than continuous dietary dosing. No changes in coagulation parameters have been observed in dog studies. Based on the above, the findings observed in male mice are considered to be of no relevance to humans.

Genotoxicity tests, in vitro and in vivo, have shown TMC125 to be free of genotoxic potential. TMC125 was not genotoxic in the Ames test, an in vitro mammalian cell mutation assay (mouse lymphoma), an in vitro chromosomal aberration assay (human lymphocytes) and an in vivo micronucleus test up to a dose of 2000 mg/kg (C_{max} and AUC of 7.9 µg/mL and 111 µg.h/mL, respectively). The information available to date does not suggest potential carcinogenic risk based on a genotoxic mechanism. The use of transgenic RasH2 mice has been explored in a dose range finding study, but it was decided to conduct a classical 2-year carcinogenicity study in CD1 mice. Exposure in the dose range finding study in littermate RasH2 mice was clearly lower than that obtained in studies in CD1 mice at identical dose levels[93].

Several **drug substance impurities and intermediates** of TMC125 were investigated. The impurities were qualified above their specified levels and all tested compounds were considered nongenotoxic.

Reproduction and developmental toxicity studies demonstrated that TMC125 did not affect reproductive parameters. The effects of TMC125 HBr in PEG400 or TPGS/HPMC vehicle were evaluated in a fertility and early embryonic development study in rats and in embryo-fetal development studies in rat and rabbit. In order to maximize exposure, a fertility study in the rat and an embryo-fetal development study in the rabbit were repeated with spray-dried TMC125 suspended in water. Also a pre- and postnatal development study in rats was performed with this formulation.

The first fertility and early embryonic development study in rats was performed with TMC125 HBr in PEG400. No relevant effects on male or female fertility were observed in this study up to a dose level of 506 mg/kg/day. No exposure assessment was performed but data from another study in the same rat strain dosed at 600 mg/kg/day are considered representative (C_{max} 0.12 and 0.32 µg/mL and $AUC_{0\text{-last}}$ 1.69 and 4.40 µg.h/mL in males and females, respectively). The study was repeated with spray-dried TMC125 suspended in water up to a dose level of 500 mg/kg/day and toxicokinetic assessment was performed. Again no relevant effects were observed and exposure amounted to C_{max} 0.76 and 1.44 µg/mL, and $AUC_{0\text{-}24h}$ 4.54 µg.h/mL and 9.09 µg.h/mL, males and females, respectively. These values are approximately equivalent to the exposure observed in patients at the recommended therapeutic dose (C_{max} 0.45 µg/mL; $AUC_{0\text{-}24h}$ 7.4 µg.h/mL).

Dosing pregnant rats with TMC125 HBr up to 1000 mg/kg/day did not result in maternal toxicity. At the high dose level, there was a slight increase in the incidence of fetuses with minor skeletal variants. Anomalous thoracic vertebral centrum (47%) and anomalous (wavy/thickened/bent) ribs (23%) appeared to be increased versus the control group data for both findings (33% and 10%, respectively). These findings are commonly seen in the Wistar rat. The thoracic region is known to be a particularly labile region and susceptible to slight disturbances during development. It has been documented that anomalous thoracic vertebral centrum and wavy/thickened/bent ribs are frequently observed in the Wistar rat and that 1) there is species specificity in the induction of wavy ribs, 2) wavy ribs are repairable in that they do not persist postnatally and 3) wavy ribs are related to fetal growth rather than teratogenicity[94,95]. These changes are therefore an indication of a transient effect on development and it was considered that there was no evidence that TMC125 is teratogenic in rats. The fetal NOAEL was 1000 mg/kg/day. Maternal exposure at this dose level amounted to 0.57 µg/mL (C_{max}) and 8.27 µg.h/mL ($AUC_{0\text{-}24h}$), which was equivalent to the anticipated exposure in the clinic at the recommended dose.

TMC125 HBr has shown no teratogenic potential or maternal toxicity in rabbits at dose levels up to 750 mg/kg. However, exposure ($AUC_{0\text{-}24h}$) at end of treatment at this dose level was only 3.76 µg.h/mL. Therefore an additional study was conducted using spray-dried TMC125 suspended in water and dosed up to 375 mg/kg. The maternal NOAEL was 125 mg/kg (C_{max} 0.38 µg/mL; $AUC_{0\text{-}24h}$ 6.09 µg.h/mL) while no fetal effects were noted up to the highest dose tested (C_{max} 0.54 µg/mL; $AUC_{0\text{-}24h}$ 9.67 µg.h/mL). Maternal exposure in the beginning of dosing (Day 6), and thus during the period of organogenesis, was higher (C_{max} 0.70 µg/mL; $AUC_{0\text{-}24h}$ 12.3 µg.h/mL), but levels declined subsequently due to enzymatic auto-induction. It was therefore concluded that TMC125 is not teratogenic in rabbits up to exposures equivalent or higher than those observed in the clinic.

No adverse effects were observed in a pre- and postnatal development study in the rat using spray-dried TMC125 at dose levels up to 500 mg/kg/day. Clinical condition, sensory function/reflexes, behavior and reproductive performance of F1 pups were unaffected by maternal treatment with spray-dried TMC125. There were no delayed effects of treatment with TMC125 on the selected offspring allowed to develop to adulthood. The highest maternal exposure ($AUC_{0\text{-}24h}$) achieved at end of treatment was 7.60 µg.h/mL, while the highest C_{max} was 0.85 µg/mL.

Local tolerance has been evaluated in a range of in vivo and in vitro studies. TMC125 is classified as "nonsensitizing" based on an in vivo guinea pig skin sensitization study and a

mouse LLNA. TMC125 is also classified as "nonirritant" to the skin based on the rabbit skin irritation test. In an in vitro eye irritation assay, TMC125 base was considered to be a "mild" eye irritant, whereas the HBr-salt form was considered a "very severe" eye irritant. No effects were observed in an in vitro phototoxicity study.

To evaluate the potential for **immunotoxicity**, TMC125 HBr has been evaluated in rats in a dedicated 4-week study up to a dose level of 600 mg/kg/day. There were no relevant effects of treatment with TMC125 and the immune response, as measured by IgM production, was not affected by treatment at exposures ($AUC_{0\text{-}24h}$) of up to 1.54 and 5.44 µg.h/mL, in males and females, respectively.

In conclusion, the key target organs in rats were the liver, the thyroid and the coagulation system. Changes in the liver and thyroid were considered adaptive or rodent-specific. Dosing TMC125 HBr up to 12 months was not associated with target organ toxicity in the dog. Dosing spray-dried TMC125 further increased exposure in the dog and this resulted in hepatic and gall bladder changes in a 1- and 6-month study. The main target organs in the mouse after gavage dosing were the liver and the coagulation system and the heart as a secondary target to changes in coagulation (only after dietary dosing).

There was no evidence of a genotoxic effect but carcinogenicity studies are currently ongoing.

TMC125 was not associated with effects on fertility or early embryonic development and showed no teratogenic potential. Pre- and postweaning functions were unaffected. Exposures in the reprotoxicology studies were equivalent to or exceeded those anticipated in the clinic.

10 TABLES AND FIGURES

In-text tables and figures are included at appropriate points throughout the summary. This excludes the Tabulated Summaries, which are located in Section 2.6.7.

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2.6.7.1 Toxicology: Overview

Test Article: etravirine

Type of Study	Species/ Strain	Route/Method of Administration	Duration of dosing	Doses (mg/kg[a])	GLP compliance	Testing Facility	Study/Report number	Location in CTD
Single Dose Toxicity	Mouse/ CD1	Oral/Gavage	Single dose	0 (vehicle), 500, 1000 (HBr salt)	Yes	[redacted]	TMC125-NC131	4.2.3.1
	Mouse/ CD1	Oral/Gavage	Single dose	0 (vehicle), 40, 160, 640 (Base)	No	J&J PRD	TMC125-Exp5092	4.2.3.1
	Mouse/ CD1	Subcutaneous/ Injection	Single dose	0 (vehicle), 20, 80, 320 (Base)	No	J&J PRD	TMC125-Exp5094	4.2.3.1
	Rat/ Wistar	Oral/Gavage	Single dose	0 (vehicle), 500, 1000 (HBr salt)	Yes	[redacted]	TMC125-NC132	4.2.3.1
	Rat/ Wistar	Oral/Gavage	Single dose	0 (vehicle), 40, 160, 640 (Base)	No	J&J PRD	TMC125-Exp5093	4.2.3.1
	Rat/ Wistar	Subcutaneous/ Injection	Single dose	0 (vehicle), 20, 80, 320 (Base)	No	J&J PRD	TMC125-Exp5095	4.2.3.1
	Dog/ Beagle	Oral/Tablet Oral/Gavage	Single dose escalation followed by 5 day repeated dose 5 days fasted/ wash-out/ 5 days fed	0 (vehicle), 40, 80, 120, 160 (Escalating) 350 (Spray-dried)	No	J&J PRD	TMC125-NC224	4.2.3.1
Repeat Dose Toxicity	Rat/ Wistar	Oral/Gavage	2 weeks	0 (control), 0 (vehicle), 20, 80, 320 (Base)	No	J&J PRD	TMC125-Exp5096	4.2.3.2
	Rat/ Wistar	Oral/Gavage	28 days	0 (vehicle), 20, 80, 320 (HBr salt)	Yes	[redacted]	TMC125-NC113	4.2.3.2
	Rat/ Wistar	Oral/Gavage	3 months	0 (vehicle), 70, 200, 600 (HBr salt)	Yes	[redacted]	TMC125-NC129	4.2.3.2
	Rat/ Wistar	Oral/Gavage	26 weeks	0 (vehicle), 70, 200, 600 (HBr salt)	Yes	[redacted]	TMC125-NC133	4.2.3.2
	Dog/ Beagle	Oral/Gavage	4 days	250 b.i.d. (Spray-dried)	No	J&J PRD	TMC125-NC243	4.2.3.2

[a] unless otherwise stated
b.i.d.: twice daily

2.6.7.1 Toxicology: Overview (Continued)

Test Article: etravirine

Type of Study	Species/ Strain	Route/Method of Administration	Duration of dosing	Doses (mg/kg[a])	GLP compliance	Testing Facility	Study/Report number	Location in CTD
Repeat Dose Toxicity (Continued)	Dog/ Beagle	Oral/Gavage	2 weeks	0 (vehicle), 5, 20, 80 b.i.d. (Base)	No	J&J PRD	TMC125-Exp5097	4.2.3.2
	Dog/ Beagle	Oral/Gavage	28 days	0 (vehicle), 20, 40, 80 b.i.d. (Base)	Yes	[redacted]	TMC125-NC107	4.2.3.2
	Dog/ Beagle	Oral/Gavage	1 month	0 (vehicle), 20 , 80, 250 b.i.d. (Spray-dried)	Yes	J&J PRD	TMC125-NC242	4.2.3.2
	Dog/ Beagle	Oral/Gavage	3 months	0 (vehicle), 10, 20, 40 b.i.d. (HBr salt)	Yes	[redacted]	TMC125-NC116	4.2.3.2
	Dog/ Beagle	Oral/Gavage	6 months	0 (vehicle), 10, 20, 40 b.i.d. (HBr salt)	Yes	[redacted]	TMC125-NC117	4.2.3.2
	Dog/ Beagle	Oral/Gavage	6 months	0 (vehicle), 80, 250 b.i.d. (Spray-dried)	Yes	J&J PRD	TMC125-NC321	4.2.3.2
	Dog/ Beagle	Oral/Gavage	12 months (+13 week interim 3 months reversibility)	0 (vehicle), 30, 80, 240 (HBr salt)	Yes	[redacted]	TMC125-NC134	4.2.3.2
Genotoxicity – In Vitro	*Salmonella typhimurium* TA98, TA100, TA102, TA1535, TA1537	In vitro	---	0 (vehicle), 5, 10, 25, 50, 100, 250, 500 µg/plate (Base)	Yes	J&J PRD	TMC125-Exp5081	4.2.3.3.1

[a] unless otherwise stated
b.i.d.: twice daily

2.6.7.1 Toxicology: Overview (Continued)

Test Article: etravirine

Type of Study	Species/ Strain	Route/Method of Administration	Duration of dosing	Doses (mg/kg[a])	GLP compliance	Testing Facility	Study/Report number	Location in CTD
Genotoxicity – In Vitro (Continued)	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537 *Escherichia coli* WP_2uvrA	In vitro	---	0 (vehicle), 3, 10, 33, 100, 333 µg/plate (Base)	Yes	[redacted]	TMC125-NC130	4.2.3.3.1
	Mouse lymphoma cells L5178Y	In vitro	---	Up to 100 µg/mL Vehicle (Base)	Yes	J&J PRD	TMC125-Exp5091	4.2.3.3.1
	Human peripheral lymphocytes	In vitro	---	Up to 100 µg/mL Vehicle (Base)	Yes	[redacted]	TMC125-NC122	4.2.3.3.1
Genotoxicity – In Vivo	Mouse/ NMRI BR	Oral/Gavage	Single dose	0 (vehicle), 2000 (HBr salt)	Yes	[redacted]	TMC125-NC120	4.2.3.3.2
Carcinogenicity	Mouse/ CD1	Oral/Gavage	2 weeks	0 (vehicle), 100, 300, 900, 1200 (HBr salt)	No	J&J PRD	TMC125-NC179	4.2.3.4.1
	Mouse/ CD1	Oral/Gavage	3 months + 1 month interim	0 (vehicle), 10, 50, 200, 800 (HBr salt)	Yes	J&J PRD	TMC125-NC146	4.2.3.4.1
	Mouse/ CD1	Oral/Dietary admixture	13 weeks	0 (vehicle), 450, 1620/800*, 2320/-*, -/200** (Spray-dried)	Yes	[redacted]	TMC125-NC195	4.2.3.4.1
	Rat/ Sprague-Dawley	Oral/Gavage	3 months + 1 month interim	0 (vehicle), 70, 200, 600 (HBr salt)	Yes	J&J PRD	TMC125-NC140	4.2.3.4.1
	Rat/ Sprague-Dawley	Oral/Dietary admixture	13 weeks	0 (vehicle), 330, 990, 1300 (Spray-dried)	Yes	[redacted]	TMC125-NC196	4.2.3.4.1
	Mouse/ CB6F1-nonTgrasH2	Oral/Gavage	4 weeks	0 (vehicle), 50, 200, 400, 800 (HBr salt)	Yes	[redacted]	TMC125-NC197	4.2.3.4.3

[a] unless otherwise stated

*new dose levels from Day 50 onwards (because of unforeseen toxicity), **from Day 57 onwards

2.6.7.1 Toxicology: Overview (Continued)

Test Article: etravirine

Type of Study	Species/ Strain	Route/Method of Administration	Duration of dosing	Doses (mg/kg[a])	GLP compliance	Testing Facility	Study/Report number	Location in CTD
Reproductive and Developmental	Rat/ Wistar	Oral/Gavage	Males: 4 weeks before mating until termination Females: 2 weeks prior to mating up to GD8	0 (vehicle), 127, 253, 506 (HBr salt)	Yes	[redacted]	TMC125-NC125	4.2.3.5.1
	Rat/ Sprague-Dawley	Oral/Gavage	Males: 4 weeks before pairing until termination Females: 2 weeks prior to pairing up to GD7	0 (vehicle), 125, 250, 500 (Spray-dried)	Yes	J&J PRD	TMC125-NC337	4.2.3.5.1
	Rat/ Wistar	Oral/Gavage	GD6-16	0 (vehicle), 250, 500, 1000 (HBr salt)	Yes	[redacted]	TMC125-NC123	4.2.3.5.2
	Rabbit/ New Zealand white	Oral/Gavage	5 days/6 days	210, 500, 750 (HBr salt)	No	[redacted]	TMC125-NC127	4.2.3.5.2
	Rabbit/ New Zealand white	Oral/Gavage	5 days	0 (vehicle), 750, 1125, 750 (b.i.d.) (HBr salt)	No	J&J PRD	TMC125-NC178	4.2.3.5.2
	Rabbit/ New Zealand white	Oral/Gavage	GD6-18	0 (vehicle), 50, 200, 750 (HBr salt)	Yes	[redacted]	TMC125-NC124	4.2.3.5.2
	Rabbit/ New Zealand white	Oral/Gavage	GD6-18	0 (vehicle), 125, 250, 525 (Spray-dried)	No	[redacted]	TMC125-NC235	4.2.3.5.2
	Rabbit/ New Zealand white	Oral/Gavage	GD6-19	0 (vehicle), 125, 250, 357 (Spray-dried)	Yes	[redacted]	TMC125-NC150	4.2.3.5.2
	Rat/ Sprague-Dawley	Oral/Gavage	GD7-LD7	0 (vehicle), 125, 250, 500 (Spray-dried)	Yes	[redacted]	TMC125-NC236	4.2.3.5.3
	Rat/ Sprague-Dawley	Oral/Gavage	GD7-LD21	0 (vehicle), 125, 250, 500 (Spray-dried)	Yes	[redacted]	TMC125-NC145	4.2.3.5.3

[a] unless otherwise stated
b.i.d.: twice daily; GD: gestation day; LD: lactation day

2.6.7.1 Toxicology: Overview (Continued)

Test Article: etravirine

Type of Study	Species/ Strain	Route/Method of Administration	Duration of dosing	Doses (mg/kg[a])	GLP compliance	Testing Facility	Study/Report number	Location in CTD
Local Tolerance	Bovine corneas	Topical	---	20 g/g% 0.9% NaCl (vehicle)	No	J&J PRD	TMC125-NC298	4.2.3.6
	Bovine corneas	Topical	---	20 g/g% 0.9% NaCl (vehicle) (HBr salt)	No	J&J PRD	TMC125-NC159	4.2.3.6
	Balb/c mouse fibroblasts	In vitro	---	0 (vehicle), 0.002 to 6.42 mg/L	No	J&J LLC	TMC125-NC245	4.2.3.6
	Mouse/ CBA/J	Dermal application	3 days	0 (vehicle), 2.5%, 5%, 10%, 25%, 50% w/v TMC125 base in DMF	Yes	[redacted]	TMC125-NC324	4.2.3.6
	Guinea Pig/ Hartley	Dermal application	---	Intradermal injection: FCA 50% in saline, 0.1% TMC125 in corn oil, 0.1% TMC125 in FCA/saline Cutaneous route: 50% w/w	Yes	[redacted]	TMC125-[redacted] 20822	4.2.3.6
	Rabbit/ New Zealand white	Dermal application	4 hours	0.5 g	Yes	[redacted]	TMC125-NC160	4.2.3.6
Other Toxicity Studies	Rat/ Sprague-Dawley	Oral/Gavage	4 weeks	0 (vehicle), 70, 200, 600 (HBr salt)	Yes	[redacted]	TMC125-NC180	4.2.3.7.2
	Mouse/ CD1	TMC125: Oral/Diet Vitamin K_1: Subcutaneous injection	6 weeks	0 (vehicle), 0 (vehicle + 15 mg/kg/day vitamin K_1), 2320, 2320 + 15 mg/kg/day vitamin K_1 (Spay-dried)	No	J&J PRD	TMC125-NC240	4.2.3.7.3
	Mouse/ CD1	TMC125: Oral/Gavage Vitamin K_1: Subcutaneous injection	1 month	0 (vehicle), 0 (vehicle + 15 mg/kg/day vitamin K_1), 1000, 1000 + 15 mg/kg/day vitamin K_1 (HBr salt)	No	J&J PRD	TMC125-NC241	4.2.3.7.3
	Salmonella typhimurium TA98, TA100 TA102, TA1535, TA1537	In vitro	--	0 (vehicle), 15.63, 31.25, 62.5, 125, 250, 500, 1000 µg/plate (TMC125 spiked with 2% Impurity A*)	Yes	J&J PRD	TMC125-NC322	4.2.3.7.6

[a] unless otherwise stated
DMF: dimethyl formamide; FCA: Freund's complete adjuvant

＊：新薬承認情報提供時に置き換え

2.6.7.1 Toxicology: Overview (Continued)

Test Article: etravirine

Type of Study	Species/ Strain	Route/Method of Administration	Duration of dosing	Doses (mg/kg[a])	GLP compliance	Testing Facility	Study/Report number	Location in CTD
Other Toxicity Studies (Continued)	*Salmonella typhimurium* TA98, TA100 TA102, TA1535, TA1537	In vitro	---	0 (vehicle), 15.63, 31.25, 62.5, 125, 250, 500, 1000 µg/plate (TMC125 spiked with 2% Impurity B*)	Yes	J&J PRD	TMC125-NC247	4.2.3.7.6
	Mouse lymphoma cells L5178Y	In vitro	---	Concentrations up to 50 µg/mL (TMC125 spiked with 2% Impurity A*)	Yes	J&J PRD	TMC125-NC248	4.2.3.7.6
	Mouse lymphoma cells L5178Y	In vitro	---	Concentrations up to 50 µg/mL (TMC125 spiked with 2% Impurity B*)	Yes	J&J PRD	TMC125-NC323	4.2.3.7.6
	Dog/ Beagle	Oral/Gavage	3 months	0 (vehicle), 10, 20 (TMC125 HBr salt spiked with 2% Impurity A* or Impurity B*)	Yes	J&J PRD	TMC125-NC246	4.2.3.7.6
	Salmonella typhimurium TA98, TA100 TA102, TA1535, TA1537	In vitro	---	0 (vehicle), 4.88, 9.77, 19.53, 39.06, 78.13, 156.25, 312.5 µg/plate (Impurity C* impurity of HBr salt)	Yes	J&J PRD	TMC125-NC226	4.2.3.7.6
	Salmonella typhimurium TA98, TA100 TA102, TA1535, TA1537	In vitro	---	0 (vehicle), 10, 25, 50, 100, 250, 500, 1000 µg/plate (HBr salt)	Yes	J&J PRD	TMC125-NC163	4.2.3.7.7
	Salmonella typhimurium TA98, TA100 TA102, TA1535, TA1537	In vitro	---	0 (vehicle), 10, 25, 50, 100, 250, 500, 1000 µg/plate (Base)	Yes	J&J PRD	TMC125-NC211	4.2.3.7.7
	Dog/ Beagle	Oral/Gavage	2 weeks	0 (vehicle), 20, 240 (HBr salt)	Yes	J&J PRD	TMC125-NC175	4.2.3.7.7

[a] unless otherwise stated

＊：新薬承認情報提供時に置き換え

2.6.7.2 Toxicokinetics: Overview of Toxicokinetics Studies

Test Article: etravirine

Type of Study	Test System	Route/Method of Administration	Doses (mg/kg)	GLP Compliance	Study/Report Number	Location in CTD
Single-Dose Toxicity	Mouse/ CD1	Oral/Gavage	0 (vehicle), 500, 1000 (HBr salt)	No	TMC125-NC131	4.2.3.1
	Rat/ Wistar	Oral/Gavage	0 (vehicle), 500, 1000 (HBr salt)	No	TMC125-NC132	4.2.3.1
	Dog/ Beagle	Oral/Tablet Oral/Gavage	0 (vehicle), 40, 80, 120, 160 (Escalating) 350 (Spray-dried)	No	TMC125-NC224	4.2.3.1
Repeat-Dose Toxicity	Rat/ Wistar	Oral/Gavage	0 (control), 0 (vehicle), 20, 80, 320 (Base)	No	TMC125-Exp5096	4.2.3.2
	Rat/ Wistar	Oral/Gavage	0 (vehicle), 20, 80, 320 (HBr salt)	No	TMC125-NC113	4.2.3.2
	Rat/ Wistar	Oral/Gavage	0 (vehicle), 70, 200, 600 (HBr salt)	No	TMC125-NC129	4.2.3.2
	Rat/ Wistar	Oral/Gavage	0 (vehicle), 70, 200, 600 (HBr salt)	No	TMC125-NC133	4.2.3.2
	Dog/ Beagle	Oral/Gavage	0 (vehicle), 5, 20, 80 b.i.d. (base)	No	TMC125-Exp5097	4.2.3.2
	Dog/ Beagle	Oral/Gavage	0 (vehicle), 20, 40, 80 b.i.d.	No	TMC125-NC107	4.2.3.2
	Dog/ Beagle	Oral/Gavage	0 (vehicle), 20 , 80, 250 b.i.d. (Spray-dried)	Yes	TMC125-NC242	4.2.3.2
	Dog/ Beagle	Oral/Gavage	0 (vehicle), 10, 20, 40 b.i.d. (HBr salt)	No	TMC125-NC116	4.2.3.2

b.i.d. = twice daily

2.6.7.2 Toxicokinetics: Overview of Toxicokinetics Studies (Continued)

Test Article: etravirine

Type of Study	Test System	Route/Method of Administration	Doses (mg/kg)	GLP Compliance	Study/Report Number	Location in CTD
	Dog/ Beagle	Oral/Gavage	0 (vehicle), 10, 20, 40 b.i.d. (HBr salt)	No	TMC125-NC117	4.2.3.2
	Dog/ Beagle	Oral/Gavage	0 (vehicle), 80, 250 b.i.d. (Spray-dried)	Yes	TMC125-NC321	4.2.3.2
	Dog/ Beagle	Oral/Gavage	0 (vehicle), 30, 80, 240 (HBr salt)	Yes	TMC125-NC134	4.2.3.2
Carcinogenicity	Mouse/ NMRI BR	Oral/Gavage	0 (vehicle), 2000 (HBr salt)	No	TMC125-NC120	4.2.3.3.2
	Mouse/ CD1	Oral/Gavage	0 (vehicle), 10, 50, 200, 800 (HBr salt)	Yes	TMC125-NC146	4.2.3.4.1
	Mouse/ CD1	Oral/Dietary admixture	0 (vehicle), 450, 1620/800*, 2320/-*, -/200** (Spray-dried)	Yes	TMC125-NC195	4.2.3.4.1
	Rat/ Sprague-Dawley	Oral/Gavage	0 (vehicle), 70, 200, 600 (HBr salt)	Yes	TMC125-NC140	4.2.3.4.1
	Rat/ Sprague-Dawley	Oral/dietary admixture	0 (vehicle), 330, 990, 1300 (Spray-dried)	Yes	TMC125-NC196	4.2.3.4.1
	Mouse/ CB6F1-nonTgrasH2	Oral/Gavage	0 (vehicle), 50, 200, 400, 800 (HBr)	Yes	TMC125-NC197	4.2.3.4.3
Reproductive and Developmental	Rat/ Sprague-Dawley	Oral/Gavage	0 (vehicle), 125, 250, 500 (Spray-dried)	Yes	TMC125-NC337	4.2.3.5.1
	Rat/ Wistar	Oral/Gavage	0 (vehicle), 250, 500, 1000 (HBr salt)	No	TMC125-NC123	4.2.3.5.2
	Rabbit/ New Zealand white	Oral/Gavage	210, 500, 750 (HBr salt)	No	TMC125-NC127	4.2.3.5.2
	Rabbit/ New Zealand white	Oral/Gavage	0 (vehicle), 750, 1125, 750 (b.i.d.) (HBr salt)	No	TMC125-NC178	4.2.3.5.2
	Rabbit/ New Zealand white	Oral/Gavage	0 (vehicle), 50, 200, 750 (HBr salt)	No	TMC125-NC124	4.2.3.5.2

*new dose levels from Day 50 onwards (because of unforeseen toxicity), **from Day 57 onwards
b.i.d. = twice daily

2.6.7.2 Toxicokinetics: Overview of Toxicokinetics Studies (Continued)

Test Article: etravirine

Type of Study	Test System	Route/Method of Administration	Doses (mg/kg)	GLP Compliance	Study/Report Number	Location in CTD
	Rabbit/ New Zealand white	Oral/Gavage	0 (vehicle), 125, 250, 525 (Spray-dried)	No	TMC125-NC235	4.2.3.5.2
	Rabbit/ New Zealand white	Oral/Gavage	0 (vehicle), 125, 250, 357 (Spray-dried)	Yes	TMC125-NC150	4.2.3.5.2
	Rat/ Sprague-Dawley	Oral/Gavage	0 (vehicle), 125, 250, 500 (Spray-dried)	Yes	TMC125-NC236	4.2.3.5.3
	Rat/ Sprague-Dawley	Oral/Gavage	0 (vehicle), 125, 250, 500 (Spray-dried)	Yes	TMC125-NC145	4.2.3.5.3
Other Toxicity Studies	Rat/ Sprague-Dawley	Oral/Gavage	0 (vehicle), 70, 200, 600 (HBr salt)	Yes	TMC125-NC180	4.2.3.7.2
	Mouse/ CD1	TMC125: Oral/Gavage Vitamin K_1: Subcutaneous injection	0 (vehicle), 0 (vehicle + 15 mg/kg/day vitamin K_1),, 1000, 1000 + 15 mg/kg/day vitamin K_1 (HBr salt)	No	TMC125-NC241	4.2.3.7.3
	Mouse/ CD1	TMC125: Oral/Diet Vitamin K_1: Subcutaneous injection	0 (vehicle), 0 (vehicle + 15 mg/kg/day vitamin K_1),, 2320, 2320 + 15 mg/kg/day vitamin K_1 (Spay-dried)	No	TMC125-NC240	4.2.3.7.3

2.6.7.3 Toxicokinetics: Overview of Toxicokinetics Data

Test article: etravirine

C_{max} at steady state (µg/mL)										
Daily Dose (mg/kg/day)		Study Duration (days)	Mouse		Rat		Rabbit	Dog	
TMC125	Formulation[j]		M	F	M	F	F	M	F
10	HBr	91[a]	0.239	0.222					
30	HBr	366[f]						0.964	0.812
50	HBr	91[a]	0.557	0.366					
70	HBr	Week 26[d]			0.063	0.056			
80	HBr	366[f]						2.71	1.71
125	Spray-dried (1:2:0.5)	29/15[c]			0.381	1.12			
125 (pregnant)	Spray-dried (1:3:0.5)	11 (GD17)[g]				1.02			
125 (pregnant)	Spray-dried (1:3:0.5)	11 (GD17)[h]				0.648			
125 (pregnant)	Spray-dried (1:3:0.5)	13 (GD18)[i]					0.378		
160	Spray-dried (1:3:0.5)	181[e]						2.22	2.79
200	HBr	91[a]	0.414	0.501					
200 (diet)	Spray-dried (1:2:0.5)	34[b]	0.197	0.097					
200	HBr	Week 26[d]			0.051	0.140			
240	HBr	366[f]						2.16	1.77
250	Spray-dried (1:2:0.5)	29/15[c]			0.565	1.21			
250 (pregnant)	Spray-dried (1:3:0.5)	11 (GD17)[g]				1.08			
250 (pregnant)	Spray-dried (1:3:0.5)	11 (GD17)[h]				0.851			
250 (pregnant)	Spray-dried (1:3:0.5)	13 (GD18)[i]					0.408		
375 (pregnant)	Spray-dried (1:3:0.5)	13 (GD18)[i]					0.536		
450 (diet)	Spray-dried (1:2:0.5)	90[b]	0.126	0.195					
500	Spray-dried (1:2:0.5)	29/15[c]			0.763	1.44			
500	Spray-dried (1:3:0.5)	181[e]						2.95	3.12
500 (pregnant)	Spray-dried (1:3:0.5)	11 (GD17)[g]				0.991			
500 (pregnant)	Spray-dried (1:3:0.5)	11 (GD17)[h]				0.644			
600	HBr	Week 26[d]			0.124	0.275			
800	HBr	91[a]	1.50	1.48					
1620 /800 (diet)	Spray-dried (1:2:0.5)	90[b]	0.348	0.227					
2320 (diet)	Spray-dried (1:2:0.5)	49[b]	0.977	0.611					

Only the long-term and main studies in mice, rats, rabbits and dogs after oral administration of TMC125 were presented in this table.
a: NC146; b: NC195; c: NC337 (dosing for 1 month in males and 15 days in females); d: NC133; e: NC321; f: NC134; g: NC236: h: NC145; i: NC150
j: TMC125 HBr was formulated in polyethylene glycol 400; for spray-dried TMC125 ([redacted]) and ([redacted]) represent the active to polymer ratio; polymers used in spray-dried TMC125 are composed of hydroxypropyl-methylcellulose and microcrystalline cellulose.

2.6.7.3 Toxicokinetics: Overview of Toxicokinetics Data (Continued)

Test article: etravirine

AUC_{0-24h} at steady state (µg.h/ml)									
Daily Dose (mg/kg/day)		Study Duration (days)	Mouse		Rat		Rabbit	Dog	
TMC125	Formulation[j]		M	F	M	F	F	M	F
10	HBr	91[a]	1.44	1.09					
30	HBr	366[f]						13.5	11.9
50	HBr	91[a]	3.14	1.70					
70	HBr	Week 26[d]			0.210*	0.656			
80	HBr	366[f]						33.9	19.3
125	Spray-dried (1:2:0.5)	29/15[e]			1.53	5.45			
125 (pregnant)	Spray-dried (1:3:0.5)	11 (GD17)[g]				7.22			
125 (pregnant)	Spray-dried (1:3:0.5)	11 (GD17)[h]				5.29			
125	Spray-dried (1:3:0.5)	13 (GD18)[i]					6.09		
160	Spray-dried (1:3:0.5)	181[e]						54.5	44.6
200	HBr	91[a]	3.93	4.60					
200 (diet)	Spray-dried (1:2:0.5)	34[b]	2.35	1.53					
200	HBr	Week 26[d]			0.557	1.29			
240	HBr	366[f]						35.6	23.2
250	Spray-dried (1:2:0.5)	29/15[e]			2.77	5.02			
250 (pregnant)	Spray-dried (1:3:0.5)	11 (GD17)[g]				8.64			
250 (pregnant)	Spray-dried (1:3:0.5)	11 (GD17)[h]				7.60			
250	Spray-dried (1:3:0.5)	13 (GD18)[i]					5.27		
375	Spray-dried (1:3:0.5)	13 (GD18)[i]					9.67		
450 (diet)	Spray-dried (1:2:0.5)	90[b]	1.98	2.59					
500	Spray-dried (1:2:0.5)	29/15[e]			4.54	9.09			
500	Spray-dried (1:3:0.5)	181[e]						61.8	61.5
500 (pregnant)	Spray-dried (1:3:0.5)	11 (GD17)[g]				12.8			
500 (pregnant)	Spray-dried (1:3:0.5)	11 (GD17)[h]				3.63			
600	HBr	Week 26[d]			1.73	3.56			
800	HBr	91[a]	7.80	10.1					
1620/800 (diet)	Spray-dried (1:2:0.5)	90[b]	4.54	3.47					
2320 (diet)	Spray-dried (1:2:0.5)	49[b]	10.2	8.12					

Only the long-term and main studies in mice, rats, rabbits and dogs after oral administration of TMC125 were presented in this table.
a: NC146; b: NC195; c: NC337 (dosing for 1 month in males and 15 days in females); d: NC133; e: NC321; f: NC134; g: NC236: h: NC145; i: NC150
j: TMC125 HBr was formulated in polyethylene glycol 400; for spray-dried TMC125 ([redacted]) and ([redacted]) represent the active to polymer ratio; polymers used in spray-dried TMC125 are composed of hydroxypropyl-methylcellulose and microcrystalline cellulose.
* : AUC_{0-8h}

2.6.7.4 Toxicology: Drug Substance[a]

Test Article: etravirine

Batch No.	Purity (%)[b]	Specified Impurities (%)		Study No.	Type of Study
PROPOSED SPECIFICATION:		R405793 NMT0.30%	R230187 NMT 0.50%		
[redacted]	99.8%	ND	ND	TMC125-NC131	Acute oral toxicity study with TMC125K (HBr) in the mouse.
				TMC125-NC132	Acute oral toxicity study with TMC125K (HBr) in the rat.
				TMC125-NC129	3 Months oral toxicity study with TMC125K (HBr) by daily gavage in the rat.
				TMC125-NC116	3-Month toxicity study with TMC125K (HBr) by daily gavage in the dog.
				TMC125-NC117	6-Month toxicity study with TMC125K (HBr) by daily gavage in the dog.
				TMC125-NC120	Micronucleus test in bone marrow cells of the mouse.
				TMC125-NC125	Fertility and early embryonic toxicity study by oral gavage in Wistar rats.
				TMC125-NC123	Embryo-fetal developmental toxicity study by oral gavage in female Wistar rats.
				TMC125-NC127	Dose range finding study with TMC125K (HBr) by oral gavage in albino New Zealand white rabbits.
[redacted]	97%	ND	ND	TMC125-Exp5092	Single dose oral toxicity study in the Swiss mouse.
				TMC125-Exp5094	Single dose subcutaneous toxicity study in the Swiss mouse.
				TMC125-Exp5095	Single dose subcutaneous toxicity study in the Wistar rat.
				TMC125-Exp5096	2-Week repeated dose oral toxicity study in the Wistar rat.
				TMC125-Exp5097	2-Week repeated dose oral toxicity study in the Beagle dog.
				TMC125-Exp5081	In vitro bacterial reverse mutation test with *Salmonella typhimurium*.
				TMC125-Exp5091	In vitro mammalian forward mutation test with L5178Y mouse lymphoma cells (TK-locus) using the Microtitre® Fluctuation Technique.
[redacted][c]				TMC125-Exp5093	Single dose oral toxicity study in the Wistar Rat.
[redacted][c]				TMC125-NC224	Single dose escalation oral toxicity study followed by a 5-day repeated dose oral toxicity study in the Beagle dog (tolerance study).
[redacted]	100.6%	ND	0.06%	TMC125-NC224	Single dose escalation oral toxicity study followed by a 5-day repeated dose oral toxicity study in the Beagle dog (tolerance study).
				TMC125-NC243	4-Day repeated dose oral toxicity study of TMC125 in the Beagle dog.
				TMC125-NC242	1-Month repeated dose oral toxicity study of TMC125 in the Beagle dog.
				TMC125-NC235	Oral (gavage) development toxicity dose range finding study in the rabbit.
				TMC125-NC150	Oral (gavage) developmental toxicity study in the rabbit
				TMC125-NC145	Pre- and postnatal development study in rats.
				TMC125-NC236	Preliminary dosing (gavage) study in the rat.

[a] Data in this table is based on the information available in Module 3.2.S; [b] HPLC assay value if available, otherwise 100% - sum of impurities; [c] no information available
ND: not defined

2.6.7.4 Toxicology: Drug Substance (Continued)

Test Article: etravirine

Batch No.	Purity (%)[b]	Specified Impurities (%)		Study No.	Type of Study
PROPOSED SPECIFICATION:		R405793 NMT0.30%	R230187 NMT 0.50%		
[redacted]	99.6%	ND	ND	TMC125-NC113	Subacute 28-day oral toxicity study with TMC125K (HBr) by daily gavage in the rat.
[redacted]	100.7%	ND	ND	TMC125-NC129	3 Months oral toxicity study with TMC125K (HBr) by daily gavage in the rat.
				TMC125-NC133	26 Week oral (gavage study) administration toxicity study in the rat with TMC125K (HBr).
				TMC125-NC124	Embryo-fetal developmental toxicity study with TMC125K (HBr) by oral gavage in female albino New Zealand white rabbits.
[redacted]	99.8%	ND	ND	TMC125-NC133	26 Week oral (gavage study) administration toxicity study in the rat with TMC125K (HBr).
				TMC125-NC124	Embryo-fetal developmental toxicity study with TMC125K (HBr) by oral gavage in female albino New Zealand white rabbits.
[redacted]	98.6%	ND	ND	TMC125-NC107	Subacute 28-day oral toxicity study with TMC125 by daily gavage in the dog.
[redacted]	97.7%	ND	ND	TMC125-NC134	12-Month toxicity study with TMC125K (HBr) micronized by daily oral gavage followed by a 3-month recovery period.
[redacted]	100.0%	ND	ND	TMC125-NC134	12-Month toxicity study with TMC125K (HBr) micronized by daily oral gavage followed by a 3-month recovery period.
				TMC125-NC179	2-Week repeated dose oral toxicity study in the Swiss mouse.
				TMC125-NC175	2-Week repeated dose oral toxicity study in the Beagle dog.
[redacted]	99.7%	ND	ND	TMC125-NC130	Evaluation of the mutagenic activity of TMC125 in the *Salmonella typhimurium* reverse mutation assay and the *Escherichia coli* reverse mutation assay.
				TMC125-NC122	Evaluation of the ability of TMC125 to induce chromosome aberrations in cultured peripheral human lymphocytes.
[redacted]	99.2%	ND	ND	TMC125-NC146	3-Month repeated dose oral toxicity study in the Swiss Mouse.
				TMC125-NC140	3-Month repeated dose oral toxicity study with 1-month interim kill in the rat.
				TMC125-NC180	4-Week immunotoxicity study by oral route (gavage) in rats
[redacted]	98.9%	0.06%	0.05%	TMC125-NC195	13-Week toxicity study with 1-month interim kill by oral route (dietary admixture) in mice.
				TMC125-NC196	13-Week toxicity study with 1-month interim kill by oral route (dietary admixture) in rats.
				TMC125-NC240	6-Week repeated dose oral dietary mechanistic toxicity study of TMC125 spray-dried in the mouse.

[b] HPLC assay value if available, otherwise 100% - sum of impurities
ND: not defined

2.6.7.4 Toxicology: Drug Substance (Continued)

Test Article: etravirine

Batch No.	Purity (%)[b]	Specified Impurities (%)		Study No.	Type of Study
PROPOSED SPECIFICATION:		R405793 NMT0.30%	R230187 NMT 0.50%		
[redacted]	99.4%	ND	ND	TMC125-NC197	4-week toxicity study by oral route (gavage) in CB6F1-nonTgrasH2 mice
[redacted] (=[redacted])	99.8%	ND	ND	TMC125-NC127	Dose range finding study with TMC125K (HBr) by oral gavage in albino New Zealand white rabbits.
TMC125[redacted] (=[redacted])	100%	ND	ND	TMC125-NC178	5-Day repeated dose oral toxicity study in the female rabbit
[redacted]	99.2%	ND	ND	TMC125-[redacted] 20822	Skin sensitization test in guinea pigs.
[redacted]	100.0%	ND	ND	TMC125-NC159	In vitro bovine corneal opacity-permeability eye irritation test.
				TMC125-NC246	3-Month repeated dose oral toxicity study of TMC125K in the Beagle dog.
[redacted]	99.3%	0.08%	0.11%	TMC125-NC298	In vitro bovine corneal opacity-permeability eye irritation test.
[redacted]	96.6%	0.11%	0.07%	TMC125-NC175	2-Week repeated dose oral toxicity study in the Beagle dog.
				TMC125-NC163	In vitro bacterial reverse mutation test with *Salmonella typhimurium*.
[redacted]	99.0%	ND	ND	TMC125-NC211	In vitro bacterial reverse mutation test with *Salmonella typhimurium*.
[redacted]	98.4%	ND	ND	TMC125-NC241	1-Month repeated dose oral (gavage) mechanistic toxicity study of TMC125K in the mouse.
				TMC125-NC245	In vitro phototoxicity testing of Compound F* by the neutral red uptake assay using balb/c 3T3 mouse fibroblasts.
[redacted]	99.6%	< 0.05%	0.06%	TMC125-NC160	Skin irritation study of TMC125 in rabbits.
				TMC125-NC324	Local lymph node assay with TMC125.
[redacted]	99.3%	0.08%	0.11%	TMC125-NC248	In vitro mouse lymphoma test (incubation).
				TMC125-NC322	Ames test
				TMC125-NC247	Ames test (incubation).
				TMC125-NC323	In vitro mouse lymphoma test.
[redacted] (= [redacted])	99.8%	0.07%	0.09%	TMC125-NC321	6-Month repeated dose oral toxicity study of TMC125 in the Beagle dog
				TMC125-NC337	Oral fertility study of TMC125 in the male and female rat

[b] HPLC assay value if available, otherwise 100% - sum of impurities
DS = drug substance; ND: not defined

＊：新薬承認情報提供時に置き換え

2.6.7.5 Single-Dose Toxicity

Test Article: etravirine

Species/Strain No. and Gender/Group	Route/Method of Administration (Vehicle/Formulation)	Batch no.	Doses (mg/kg)	NOAEL (mg/kg)	Noteworthy Findings	Study No./ Location in CTD
Mouse/CD1 2M + 2F/group	Oral/Gavage[a] (PEG400)	[redacted]	Vehicle, 40, 160, 640 (Base)	640	No TMC125-related adverse effects	TMC125-Exp5092/ 4.2.3.1
Mouse/CD1 5M + 5F/group	Oral/Gavage[b] (PEG400)	[redacted]	Vehicle, 500, 1000 (HBr)	500	500: No mortality and no noteworthy findings 1000: No mortality, slight body weight loss in 2/5 females TK determined	TMC125-NC131[c]/ 4.2.3.1
Mouse/CD1 2M + 2F/group	Subcutaneous[a] (PEG400)	[redacted]	Vehicle, 20, 80, 320 (Base)	320	20, 80, 320: No TMC125-related adverse effects but subcutaneous powdery deposits at site of administration	TMC125-Exp5094/ 4.2.3.1
Rat/Wistar 5M + 5F/group	Oral/Gavage[b] (PEG400)	[redacted]	Vehicle, 500, 1000 (HBr)	1000	No TMC125-related adverse effects	TMC125-NC132[c]/ 4.2.3.1
Rat/Wistar 2M + 2F/group	Oral/Gavage[a] (PEG400)	[redacted]	Vehicle, 40, 160, 640 (Base)	640	No TMC125-related adverse effects	TMC125-Exp5093/ 4.2.3.1
Rat/Wistar 2M + 2F/group	Subcutaneous[a] (PEG400)	[redacted]	Vehicle, 20, 80, 320 (Base)	80	20, 80, 320: No mortality. Subcutaneous powdery deposits at site of administration 320: Skin irritation and necrosis at injection site in 1/2 females	TMC125-Exp5095/ 4.2.3.1
Dog/Beagle 1M + 1F/group	Oral/Gavage[c] (Tablets)	[redacted]	vehicle→40→80→120→160 (Spray-dried)	160	No TMC125-related adverse effects TK determined	TMC125-NC224 4.2.3.1
1M + 1F/group	Oral/Gavage[d] (Tablets)	[redacted]	120 (Spray-dried)	120	120: mucoid feces in female, slight increase in bilirubin in male; some vomiting TK determined	
2M + 2F/group	Oral/Gavage[d] (Aqueous suspension)	[redacted]	350 fasted, 350 fed (Spray-dried)		350 fasted: mucoid feces, some vomiting 350 fed: reversible ataxia, spasms and mydriasis in 1 male dog, slight increase in bilirubin in both male dogs, some vomiting TK determined	

[a] single dose followed by a 3 day observation period; [b] single dose followed by a 15 day observation period; [c] administered as single escalating doses every 3 or 4 days; [d] administered as a repeated dose for 5 consecutive days; [e] GLP compliant.
F: female; M: male; PEG400: polyethylene glycol 400; TK: toxicokinetic

2.6.7.6.A Repeat-Dose Toxicity

Report Title: 2 week repeated dose oral toxicity study in the Wistar rat **Test Article:** etravirine

Species/strain: Rat/Wistar
Age/weight at first dose: 5 - 6 weeks/mean 119 g (M) and 105 g (F)
Duration of dosing: 2 weeks
Duration of postdose: 0 days

Administration route/method: Oral/gavage
Doses: 0 (control), 0 (vehicle) 20, 80 and 320 mg/kg body weight/day as TMC125 base
Test article batch: [redacted]
Vehicle/formulation: Polyethylene glycol 400

Testing facility: Janssen Pharmaceutica
Study no.: TMC125-Exp5096
Location in CTD: 4.2.3.2
GLP compliance: No
Date start of study: [redacted] 19[redacted]

No Observed Adverse Effect Level: 320 mg/kg/day in the absence of any relevant changes

Toxicokinetics:

Dose (mg/kg/day)	0 (control)		0 (vehicle)		20		80		320	
No. of animals	M:0	F:0	M:0	F:0	M:4	F:4	M:4	F:4	M:4	F:4
Died or killed prematurely (unrelated to treatment)	0	0	0	0	0	0	1	1	0	0
$AUC_{0\text{-}8h}$ Day 13 (µg.h/mL)	-	-	-	0	0.31	0.30	0.65	0.49	0.68	0.65
$AUC_{0\text{-}24h}$ Day 13 (µg.h/mL	-	-	-	-	n/c	n/c	n/c	n/c	0.99	0.96

Noteworthy Findings:

Dose (mg/kg/day)	0 (control)		0 (vehicle)		20		80		320	
No. of animals	M:5	F:5	M:5	F:5	M:5	F:5	M:5	F:5	M:5	F:5
Died or killed prematurely (unrelated to treatment)	0	0	0	0	0	0	0	1	0	2
Clinical observations	-	-	-	-	-	-	-	-	-	-
Body weight (g)	-	-	-	-	-	-	-	-	-	-
Food consumption (g/week)	-	-	-	-	-	-	-	-	-	-
Ophthalmoscopy	-	-	-	-	NA	NA	NA	NA	-	-
Hematology	-	-	-	-	-	-	-	-	-	-
Serum chemistry	-	-	-	-	-	-	-	-	-	-
Urinalysis	-	-	-	-	-	-	-	-	-	-
Organ weights	-	-	-	-	-	-	-	-	-	-
Gross pathology	-	-	-	-	-	-	-	-	-	-
Histopathology	-	-	-	-	-	-	-	-	-	-

F: female; M: male; NA: not applicable; NC: not calculated; - no treatment related changes

2.6.7.6.B Repeat-Dose Toxicity

Report Title: 4-day repeated dose oral toxicity study of TMC125 in the Beagle dog

Test Article: etravirine

Species/strain: Dog/Beagle
Age/weight at first dose: 5.5 - 6 months/4.2 - 7.3 kg
Duration of dosing: 4 days
Duration of postdose: 0 days

Administration route/method: Oral/gavage
Doses: 500 mg/kg body weight/day (250 mg/kg/day b.i.d., 5 hours apart) as spray-dried TMC125 ([redacted] active to polymer ratio), dose correction factor was 4.5
Test article batch: [redacted]
Vehicle/formulation: hydroxypropyl methylcellulose + microcrystalline cellulose in demineralized water

Testing facility: J&J PRD
Study no.: TMC125-NC243
Location in CTD: 4.2.3.2
GLP compliance: No
Date start of study: [redacted] 20[redacted]

No Observed Adverse Effect Level: Not determined due to clinical observations

Toxicokinetics: Not applicable

Noteworthy Findings:

Dose (mg/kg/day)	500	
No. of animals	M:1	F:1
Died or killed prematurely	0	0
Clinical observations - mydriasis	1/1	1/1
Body weight (kg)	-	-
Gross pathology	NA	NA
Histopathology	NA	NA

b.i.d.: twice daily; F: female; M: male; NA: not applicable; - no treatment related changes

2.6.7.6.C Repeat-Dose Toxicity

Report Title: 2-week repeated dose oral toxicity study in the Beagle dog **Test Article:** etravirine

Species/strain: Dog/Beagle
Age/weight at first dose: 7 - 8 months/9.4 - 11.9 kg
Duration of dosing: 2 weeks
Duration of postdose: 0 days

Administration route/method: Oral/gavage
Doses: 0 (vehicle) 10, 40 and 160 mg/kg body weight/day (dose divided b.i.d., 6 hours apart) as TMC125 base
Test article batch: [redacted]
Vehicle/formulation: Polyethylene glycol 400

Testing facility: J&J PRD
Study no.: TMC125-Exp5097
Location in CTD: 4.2.3.2
GLP compliance: No
Date start of study: [redacted] 19[redacted]

No Observed Adverse Effect Level: 160 mg/kg/day in the absence of any relevant changes

Toxicokinetics:

Dose (mg/kg/day)	0 (vehicle)		10		40		160	
No. of animals	M:1	F:1	M:1	F:1	M:1	F:1	M:1	F:1
Died or killed prematurely	0	0	0	0	0	0	0	0
$AUC_{0\text{-}24h}$ Day 13 (µg.h/mL)	-		8.01		19.4		30.1	

Noteworthy Findings:

Dose (mg/kg/day)	0 (vehicle)		10		40		160	
No. of animals	M:2	F:2	M:2	F:2	M:2	F:2	M:2	F:2
Died or killed prematurely	0	0	0	0	0	0	0	0
Clinical observations	-	-	-	-	-	-	-	-
Body weight (kg)	-	-	-	-	-	-	-	-
Food consumption (g/week)	-	-	-	-	-	-	-	-
Ophthalmoscopy	-	-	-	-	-	-	-	-
ECG	-	-	-	-	-	-	-	-
Hematology	-	-	-	-	-	-	-	-
Serum chemistry	-	-	-	-	-	-	-	-
Urinalysis	-	-	-	-	-	-	-	-
Organ weights	-	-	-	-	-	-	-	-
Gross pathology	-	-	-	-	-	-	-	-
Histopathology	-	-	-	-	-	-	-	-

b.i.d.: twice daily; ECG: electrocardiogram; F: female; M: male; - no treatment related changes

2.6.7.7.A Repeat-Dose Toxicity

Report Title: Subacute 28-day oral toxicity study with TMC125K (HBr) by daily gavage in the rat **Test Article:** etravirine

Species/strain: Rat/Wistar
Age/weight at first dose: approximately 6 weeks/mean 190 g (M) and 155 g (F)
Duration of dosing: 4 weeks
Duration of postdose: 0 days

Administration route/method: Oral/gavage
Doses: 0 (vehicle) 20, 80 and 320 mg base eq./kg body weight/day as TMC125 HBr
Test article batch: [redacted]
Vehicle/formulation: Polyethylene glycol 400

Testing facility: [redacted]
Study no.: TMC125-NC113
Location in CTD: 4.2.3.2
GLP compliance: Yes
Date start of study: [redacted] 20[redacted]

No Observed Adverse Effect Level: 320 mg/kg/day in the absence of any relevant histopathological changes

Toxicokinetics:

Dose (mg base eq./kg/day)	0 (vehicle)		20		80		320	
No. of animals	M:2	F:2	M:5	F:5	M:5	F:5	M:5	F:5
Died or killed prematurely (unrelated to treatment)	0	1	0	0	0	1	0	0
$AUC_{0-\infty}$ Day 1 (µg.h/mL)	-	-	0.28[a]	0.31[a]	0.41[a]	0.59[a]	1.53	1.16[a]
AUC_{0-24h} Day 29 (µg.h/mL)	-	-	0.24[a]	0.27[a]	0.44	1.01	1.16	1.52

[a] AUC_{0-8h}

Noteworthy Findings:

Dose (mg base eq./kg/day)	0 (vehicle)		20		80		320	
No. of animals	M:10	F:10	M:10	F:10	M:10	F:10	M:10	F:10
Died or killed prematurely	0	0	0	0	0	0	0	0
Clinical observations	-	-	-	-	-	-	-	-
Body weight (g)	-	-	-	-	-	-	-	-
Food consumption (g/week)	-	-	-	-	-	-	-	-
Ophthalmoscopy	-	-	-	-	-	-	-	-
Hematology	-	-	-	-	-	-	-	-
Serum chemistry[a]								
- calcium (mmol/L)	2.61	2.52	0.97**	1.01	0.98	0.99	0.99	1.03*
- inorganic phosphorus (mmol/L)	2.43	1.95	0.96	1.01	0.98	1.03	1.05	1.14**

[a] for vehicle group means are shown. For treated groups, the multiples of vehicle/baseline are shown. Statistical significance based on actual data.
* $p \leq 0.05$; ** $p \leq 0.01$ (Dunnett test based on pooled variance)
F: female; M: male; - no treatment related changes

2.6.7.7.A Repeat-Dose Toxicity (Continued)

Report Title: Subacute 28-day oral toxicity study with TMC125K (HBr) by daily gavage in the rat **Test Article:** etravirine

Noteworthy Findings (Continued):

Dose (mg base eq./kg/day)	0 (vehicle)		20		80		320	
No. of animals	M:10	F:10	M:10	F:10	M:10	F:10	M:10	F:10
Urinalysis[a]								
- sodium (mmol/L)	9.6	37.7	1.92	0.78	2.39**	0.63*	1.82	0.62*
- potassium (mmol/L)	82.3	112.5	1.39	0.98	1.42	1.11	1.33	0.93
- chloride (mmol/L)	24	56	1.5	0.84	2.13*	0.84	1.83	0.63
Organ weights	-	-	-	-	-	-	-	-
Gross pathology	-	-	-	-	-	-	-	-
Histopathology	-	-	-	-	-	-	-	-

[a] for vehicle group means are shown. For treated groups, the multiples of vehicle/baseline are shown. Statistical significance based on actual data.
* $p \leq 0.05$; ** $p \leq 0.05$ (Steel test)
F: female; M: male; - no treatment related changes

2.6.7.7.B Repeat-Dose Toxicity

Report Title: 3 months oral toxicity study with TMC125K (HBr) by daily gavage in the rat

Test Article: etravirine

Species/strain: Rat/Wistar
Age/weight at first dose: 5 - 6 weeks/mean 193 g (M) and 163 g (F)
Duration of dosing: 13 weeks
Duration of postdose: 0 days

Administration route/method: Oral/gavage
Doses: 0 (vehicle) 70, 200 and 600 mg base eq./kg body weight/day as TMC125 HBr
Test article batch: [redacted] and [redacted]
Vehicle/formulation: Polyethylene glycol 400

Testing facility: [redacted]
Study no.: TMC125-NC129
Location in CTD: 4.2.3.2
GLP compliance: Yes
Date start of study: [redacted] 20[redacted]

No Observed Adverse Effect Level: 70 mg/kg/day in the absence of any relevant changes

Toxicokinetics:

Dose (mg base eq./kg/day)	0 (vehicle)		70		200		600	
No. of animals	M:2	F:2	M:5	F:5	M:5	F:5	M:5	F:5
Died or killed prematurely (unrelated to treatment)	0	0	0	0	0	0	1	1
$AUC_{0-\infty}$ Day 1 (µg.h/mL)	-	-	0.46	0.55[a]	0.82[a]	0.92[a]	1.81	3.32
AUC_{0-24h} Day 84 (µg.h/mL)	-	-	0.58	1.03	0.69	1.40	1.69	4.40[b]

[a] AUC_{0-8h}; [b] AUC_{0-48h}

Noteworthy Findings:

Dose (mg base eq./kg/day)	0 (vehicle)		70		200		600	
No. of animals	M:20	F:20	M:20	F:20	M:20	F:20	M:20	F:20
Died or killed prematurely (unrelated to treatment)	0	2	1	0	0	0	0	0
Clinical observations - chromodacryorrhea	0/20	2/20	2/20	1/20	2/20	1/20	6/20	5/20
Body weight (g)	-	-	-	-	-	-	-	-
Food consumption (g/week)	-	-	-	-	-	-	-	-
Ophthalmoscopy	-	-	-	-	-	-	-	-
Hematology	-	-	-	-	-	-	-	-
Serum chemistry[a]								
- potassium (mmol/L)	3.82	3.46	1.08*	1.04	1.03	1.05	1.09*	1.11**
- total protein (g/L)	61.6	69.2	0.99	0.99	1.03	1.00	1.07**	1.05*

[a] for vehicle group means are shown. For treated groups, the multiples of vehicle/baseline are shown. Statistical significance based on actual data.
* $p \leq 0.05$; ** $p \leq 0.01$ (Dunnett test based on pooled variance)
F: female; M: male; - no treatment related changes

2.6.7.7.B Repeat-Dose Toxicity (Continued)

Report Title: 3 months oral toxicity study with TMC125K (HBr) by daily gavage in the rat **Test Article:** etravirine

Noteworthy Findings (Continued):

Dose (mg base eq./kg/day)	0 (vehicle)		70		200		600	
No. of animals	**M:20**	**F:20**	**M:20**	**F:20**	**M:20**	**F:20**	**M:20**	**F:20**
Urinalysis[a]								
- sodium (mmol/L)	41.4	25.3	1.23	1.24	1.30	1.04	1.57*	2.08**
- calcium (mmol/L)	1.89	14.0	1.34	0.85	1.11	0.81	1.96*	0.99
Organ weights[a]								
- liver (%)	2.36	2.62	1.00	1.03	1.05	1.03	1.05	1.09**
Gross pathology	-	-	-	-	-	-	-	-
Histopathology								
Liver (no. examined)	**20**	**20**	**1**	**20**	**0**	**20**	**20**	**20**
- basophilic stippling grade 1	0/20	0/20	0/1	0/20	0/0	0/20	0/20	15/20
Kidneys (no. examined)	**20**	**20**	**20**	**20**	**20**	**20**	**20**	**20**
-tubular basophilia	2/20	2/20	2/20	0/20	10/20	2/20	9/20	8/20
grade 1	2	1	2	0	9	2	8	8
grade 2	0	1	0	0	1	0	1	0
Pituitary gland (no. examined)	**20**	**20**	**20**	**0**	**20**	**0**	**20**	**20**
- hypertrophy/vacuolated cells pars anterior	20/20	0/20	18/20	0/0	20/20	0/0	20/20	0/20
grade 1	14	0	9	0	11	0	4	0
grade 2	4	0	6	0	8	0	8	0
grade 3	2	0	3	0	1	0	8	0
Thyroid gland (no. examined)	**20**	**20**	**20**	**19**	**20**	**20**	**20**	**20**
-hypertrophy/hyperplasia follicular cells	6/20	2/20	6/20	2/19	14/20	6/20	19/20	18/20
grade 1	5	2	5	2	9	6	9	14
grade 2	1	0	1	0	5	0	7	4
grade 3	0	0	0	0	0	0	3	0

[a] for vehicle group means are shown. For treated groups, the multiples of vehicle/baseline are shown. Statistical significance based on actual data.
* $p \leq 0.05$; ** $p \leq 0.01$ (Dunnett test based on pooled variance)
F: female; M: male; - no treatment related changes

2.6.7.7.C Repeat-Dose Toxicity

Report Title: TMC125.HBr: 26 week oral (gavage) administration toxicity study in the rat **Test Article:** etravirine

Species/strain: Rat/Wistar
Age/weight at first dose: 5 - 6 weeks/160 - 207 g (M) and 119 - 175 g (F)
Duration of dosing: 26 weeks
Duration of postdose: 0 days

Administration route/method: Oral/gavage
Doses: 0 (vehicle) 70, 200 and 600 mg base eq./kg body weight/day as TMC125 HBr
Test article batch: [redacted] and [redacted]
Vehicle/formulation: Polyethylene glycol 400

Testing facility: [redacted]
Study no.: TMC125-NC133
Location in CTD: 4.2.3.2
GLP compliance: Yes
Date start of study: [redacted] 20[redacted]

No Observed Adverse Effect Level: 70 mg/kg/day in the absence of any relevant changes

Toxicokinetics:

Dose (mg base eq./kg/day)	0 (vehicle)		70		200		600	
No. of animals (3M/3F per time point)	M:9	F:9	M:9	F:9	M:9	F:9	M:9	F:9
Died or killed prematurely	0	0	0	0	0	0	0	0
$AUC_{0-\infty}$ Day 1 (µg.h/mL)	-	-	0.13[a]	0.27[a]	0.63	0.92	1.97	1.95
AUC_{0-24h} Week 13 (µg.h/mL)	-	-	0.41	0.69	0.23[a]	0.89	1.84	4.53
AUC_{0-24h} Week 26 (µg.h/mL)	-	-	0.21[a]	0.66	0.56	1.29	1.73	3.56

[a] AUC_{0-8h}

Noteworthy Findings:

Dose (mg base eq./kg/day)	0 (vehicle)		70		200		600	
No. of animals	M:20	F:20	M:20	F:20	M:20	F:20	M:20	F:20
Died or killed prematurely (unrelated to treatment)	0	0	0	0	0	0	0	1
Clinical observations	-	-	-	-	-	-	-	-
Body weight (g)	-	-	-	-	-	-	-	-
Food consumption (g/week)	-	-	-	-	-	-	-	-
Ophthalmoscopy	-	-	-	-	-	-	-	-
Hematology	-	-	-	-	-	-	-	-
Serum chemistry[a] - total protein (g/L)	72	72	1.01	1.04	1.01	1.07*	1.03	1.11***
Urinalysis[a] - volume (mL)	3.8	3.3	0.84	0.91	0.97	1.12	1.37*	1.58**

[a] for vehicle group means are shown. For treated groups, the multiples of vehicle/baseline are shown. Statistical significance based on actual data.
* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$ (Anova, regression and Dunnett test)
F: female; M: male; - no treatment related changes

2.6.7.7.C Repeat-Dose Toxicity (Continued)

Report Title: TMC125.HBr: 26 week oral (gavage) administration toxicity study in the rat **Test Article:** etravirine

Noteworthy Findings (Continued):

Dose (mg base eq./kg/day)	0 (vehicle)		70		200		600	
No. of animals	**M:20**	**F:20**	**M:20**	**F:20**	**M:20**	**F:20**	**M:20**	**F:20**
Organ weights[a]								
- liver (%)	10.05	6.11	0.99	1.02	0.98	1.03	1.00	1.10*
- thyroid (%)	0.02	0.02	1.05	1.13	1.00	1.13	1.15*	1.13
Gross pathology	-	-	-	-	-	-	-	-
Histopathology								
Thyroid gland (no. examined)	**20**	**20**	**20**	**20**	**20**	**20**	**20**	**19**
- follicular cell hypertrophy	3/20	2/20	4/20	3/20	9/20	10/20	17/20	14/19
grade 1	2	2	3	3	6	7	8	8
grade 2	1	0	1	0	3	3	9	6

[a] for vehicle group means are shown. For treated groups, the multiples of vehicle/baseline are shown. Statistical significance based on actual data.
* $p \leq 0.05$ (Anova and Dunnett test)
F: female; M: male; - no treatment related changes

2.6.7.7.D Repeat-Dose Toxicity

Report Title: Subacute 28-day oral toxicity study with TMC125 by daily gavage in the dog **Test Article:** etravirine

Species/strain: Dog/Beagle
Age/weight at first dose: 5 - 6 months/5.8 - 6.6 kg
Duration of dosing: 4 weeks
Duration of postdose: 0 days

Administration route/method: Oral/gavage
Doses: 0 (vehicle) 40, 80 and 160 mg/kg body weight/day (dose divided b.i.d., 7 hours apart) as TMC125 base
Test article batch: [redacted]
Vehicle/formulation: Polyethylene glycol 400

Testing facility: [redacted]
Study no.: TMC125-NC107
Location in CTD: 4.2.3.2
GLP compliance: Yes
Date start of study: [redacted] 20[redacted]

No Observed Adverse Effect Level: 160 mg/kg/day in the absence of any relevant changes

Toxicokinetics:

Dose (mg/kg/day)	0 (vehicle)		40		80		160	
No. of animals	M:4	F:4	M:4	F:4	M:4	F:4	M:4	F:4
Died or killed prematurely (unrelated to treatment)	0	0	0	0	1	0	0	1
$AUC_{0\text{-}7h}$ Day 1 (µg.h/mL)	-	-	3.33	2.25	3.39	2.74	5.33	3.49
$AUC_{0\text{-}7h}$ Day 28 (µg.h/mL)	-	-	6.09	4.35	6.92[a]	4.70	8.23	6.56[a]
$AUC_{0\text{-}24h}$ Day 28 (µg.h/mL)	-	-	18.6	12.7	20.4[a]	13.8	24.4	18.4[a]

[a] 3 animals only

Noteworthy Findings:

Dose (mg/kg/day)	0 (vehicle)		40		80		160	
No. of animals	M:4	F:4	M:4	F:4	M:4	F:4	M:4	F:4
Died or killed prematurely (unrelated to treatment)	0	0	0	0	1	0	0	1
Clinical observations - vomiting	+	+	+	+	+	+	+	+
Body weight (kg)[a]	7.1	6.4	0.99	1.02	1.01	0.94	1.01	0.98
Body weight gain (%)[a]	13	12	1.08	1.17	0.92	0.83	1.23	0.50
Food consumption (g/week)	-	-	-	-	-	-	-	-
Ophthalmoscopy	-	-	-	-	-	-	-	-
ECG	-	-	-	-	-	-	-	-
Hematology	-	-	-	-	-	-	-	-

[a] for vehicle group means are shown. For treated groups, the multiples of vehicle/baseline are shown. Statistical significance based on actual data.
b.i.d.: twice daily; ECG: electrocardiogram; F: female; M: male; - no treatment related changes; + vomiting noted for all groups to Day 9; as a result the volume of polyethylene glycol 400 was reduced from 3 to 1 mL/kg

2.6.7.7.D Repeat-Dose Toxicity (Continued)

Report Title: Subacute 28-day oral toxicity study with TMC125 by daily gavage in the dog **Test Article:** etravirine

Noteworthy Findings (Continued):

Dose (mg/kg/day)	0 (vehicle)		40		80		160	
No. of animals	M:4	F:4	M:4	F:4	M:4	F:4	M:4	F:4
Serum chemistry*								
- ALP (U/L)	375	413	0.90	0.74	0.72	0.80	0.65	0.52*
Urinalysis	-	-	-	-	-	-	-	-
Organ weights	-	-	-	-	-	-	-	-
Gross pathology	-	-	-	-	-	-	-	-
Histopathology	-	-	-	-	-	-	-	-

* $p \leq 0.05$ (Dunnett test based on pooled variance)
ALP: alkaline phosphatase; F: female; M: male; - no treatment related changes

2.6.7.7.E Repeat-Dose Toxicity

Report Title: 1-month repeated dose oral toxicity study of TMC125 in the Beagle dog **Test Article:** etravirine

Species/strain: Dog/Beagle
Age/weight at first dose: 6 - 6.5 months/3.9 - 7.0 kg
Duration of dosing: 4 weeks
Duration of postdose: 0 days

Administration route/method: Oral/gavage
Doses: 0 (vehicle) 40, 160 and 500 mg/kg body weight/day (dose divided b.i.d., 5 hours apart) as spray-dried TMC125 ([redacted] active to polymer ratio), dose correction factor was 4.5
Test article batch: [redacted]
Vehicle/formulation: hydroxypropyl methylcellulose + microcrystalline cellulose in demineralized water

Testing facility: J&J PRD
Study no.: TMC125-NC242
Location in CTD: 4.2.3.2
GLP compliance: Yes
Date start of study: [redacted] 20[redacted]

No Observed Adverse Effect Level: No level could be determined

Toxicokinetics:

Dose (mg/kg/day)	0 (vehicle)		40		160		500	
No. of animals	M:3	F:3	M:3	F:3	M:3	F:3	M:3	F:3
Died or killed prematurely	0	0	0	0	0	0	0	0
$AUC_{0-\infty}$ Day 1 (µg.h/mL)	-	-	49.8	45.8	71.7[a]	111	109	105[a]
AUC_{0-24h} Day 24 (µg.h/mL)	-	-	37.3	31.7	51.9	55.7	42.2	75.1

[a] AUC_{0-24h}

Noteworthy Findings:

Dose (mg/kg/day)	0 (vehicle)		40		160		500	
No. of animals	M:3	F:3	M:3	F:3	M:3	F:3	M:3	F:3
Died or killed prematurely	0	0	0	0	0	0	0	0
Clinical observations - vomiting (transient)	-	-	-	-	+	+	+	+
Body weight (kg)[a]	7.0	5.8	0.91	0.93	0.89	0.86	0.80	0.69
Body weight gain (kg)[a]	0.9	0.9	0.56	0.67	0.33	0.22	-0.56	-0.78
Food consumption (kg/week)[a]	1.97	1.64	0.77	0.89	0.68	0.73	0.43	0.40
Ophthalmoscopy	-	-	-	-	-	-	-	-
ECG	-	-	-	-	-	-	-	-

[a] for vehicle group means are shown. For treated groups, the multiples of vehicle/baseline are shown. No statistical analysis was performed on these data.
b.i.d.: twice daily; ECG: electrocardiogram; F: female; M: male; - no treatment related changes ; + transient vomiting noted in Week 1

2.6.7.7.E Repeat-Dose Toxicity (Continued)

Report Title: 1-month repeated dose oral toxicity study of TMC125 in the Beagle dog **Test Article:** etravirine

Noteworthy Findings (Continued):

Dose (mg/kg/day)	0 (vehicle)		40		160		500	
No. of animals	**M:3**	**F:3**	**M:3**	**F:3**	**M:3**	**F:3**	**M:3**	**F:3**
Hematology (terminal)[a]								
- white blood cells (10^3/µL)	9.9	8.1	0.95	1.01	0.78	0.93	0.73	0.99
- red blood cells (10^6/µL)	5.41	5.35	1.08	1.13	1.11	1.08	1.15	1.10
- hemoglobin (g/dL)	12.5	12.4	1.10	1.06	1.06	1.11	1.17	1.04
- hematocrit (%)	36.3	37.2	1.10	1.05	1.07	1.08	1.18	1.02
- reticulocytes (10^3/µL)	69.8	41.0	0.82	1.78	0.90	1.07	0.56	0.75
- neutrophils (10^3/µL)	6.61	5.03	0.85	1.04	0.59	0.93	0.67	1.01
- monocytes (10^3/µL)	0.66	0.41	0.92	0.98	0.79	0.98	0.67	0.93
Serum chemistry (terminal)[a]								
- ALP (U/L)	134	134	1.16	0.97	0.96	1.01	1.66	0.78
- inorganic phosphorus (mg/dL)	6.4	6.4	0.94	0.89	0.98	0.88	0.81	0.83
- total bilirubin (mg/dL)	0.08	0.10	1.13	1.10	1.00	1.20	2.88	1.30
Urinalysis	-	-	-	-	-	-	-	-
Organ weights[a]								
- popliteal lymph nodes (%)	0.01	0.01	0.96	0.99	0.72	0.95	0.88	0.61
- thymus (%)	0.16	0.17	0.86	1.08	1.04	0.89	0.48	0.65
Gross pathology	-	-	-	-	-	-	-	-
Histopathology (no. examined)	3	3	3	3	3	3	3	3
Bone marrow (femur)								
- atrophy	0	0	0	0	0	0	1	2
grade 2	0	0	0	0	0	0	1	1
grade 3	0	0	0	0	0	0	0	1
Bone marrow (sternum)								
- atrophy	0	0	0	0	0	0	1	2
grade 2	0	0	0	0	0	0	1	1
grade 3	0	0	0	0	0	0	0	1
Gall bladder								
- inspissated bile	0	0	0	0	0	0	0	2
Large intestine, colon								
- mineralization Goblet cells grade 3	0	0	0	0	0	0	0	1

[a] for vehicle group means are shown. For treated groups, the multiples of vehicle/baseline are shown. No statistical analysis was performed on these data
ALP: alkaline phosphatase; F: female; M: male; - no treatment related changes

2.6.7.7.E Repeat-Dose Toxicity (Continued)

Report Title: 1-month repeated dose oral toxicity study of TMC125 in the Beagle dog **Test Article:** etravirine

Noteworthy Findings (Continued):

Dose (mg/kg/day)	0 (vehicle)		40		160		500	
No. of animals	**M:3**	**F:3**	**M:3**	**F:3**	**M:3**	**F:3**	**M:3**	**F:3**
Histopathology (no. examined) (continued)	3	3	3	3	3	3	3	3
Liver								
- microgranuloma(s)	0	0	0	0	0	0	1	1
grade 1	0	0	0	0	0	0	0	1
grade 4	0	0	0	0	0	0	1	0
Thymus								
- involution	0	0	0	0	0	0	1	2
grade 2	0	0	0	0	0	0	1	0
grade 3	0	0	0	0	0	0	0	1
grade 4	0	0	0	0	0	0	0	1

F: female; M: male

2.6.7.7.F Repeat-Dose Toxicity

Report Title: 3-Month toxicity study with TMC125K (HBr) by daily gavage in the dog **Test Article:** etravirine

Species/strain: Dog/Beagle
Age/weight at first dose: 4 - 6 months/4.9 - 7.3 kg
Duration of dosing: 13 weeks
Duration of postdose: 0 days

Administration route/method: Oral/gavage
Doses: 0 (vehicle) 20, 40 and 80 mg base eq./kg body weight/day (dose divided b.i.d., 7 hours apart) as TMC125 HBr
Test article batch: [redacted]
Vehicle/formulation: Polyethylene glycol 400

Testing facility: [redacted]
Study no.: TMC125-NC116
Location in CTD: 4.2.3.2
GLP compliance: Yes
Date start of study: [redacted] 20[redacted]

No Observed Adverse Effect Level: 80 mg/kg/day in the absence of any relevant changes

Toxicokinetics:

Dose (mg base eq./kg/day)	0 (vehicle)		20		40		80	
No. of animals	M:4	F:4	M:4	F:4	M:4	F:4	M:4	F:4
Died or killed prematurely	0	0	0	0	0	0	0	0
$AUC_{0\text{-}7h}$ Day 1 (µg.h/mL)	-	-	2.69	1.66	3.02	2.33	5.10	4.50
$AUC_{0\text{-}7h}$ Day 30 (µg.h/mL)	-	-	4.24	3.78	5.97	4.38	7.20	6.88
$AUC_{0\text{-}7h}$ Day 59 (µg.h/mL)	-	-	4.38	3.03	5.91	3.39	8.09	5.16
$AUC_{0\text{-}7h}$ Day 87 (µg.h/mL)	-	-	4.10	3.59	6.43	4.22	7.60	5.35
$AUC_{0\text{-}24h}$ Day 87 (µg.h/mL)	-	-	12.6	10.2	21.8	11.6	24.1	15.9

Noteworthy Findings:

Dose (mg base eq./kg/day)	0 (vehicle)		20		40		80	
No. of animals	M:4	F:4	M:4	F:4	M:4	F:4	M:4	F:4
Died or killed prematurely	0	0	0	0	0	0	0	0
Clinical observations - erythema and alopecia	0/4	0/4	0/4	0/4	0/4	1/4	1/4	1/4
Body weight (kg)	-	-	-	-	-	-	-	-
Food consumption (g/week)	-	-	-	-	-	-	-	-
Ophthalmoscopy	-	-	-	-	-	-	-	-
ECG	-	-	-	-	-	-	-	-
Hematology	-	-	-	-	-	-	-	-
Serum chemistry	-	-	-	-	-	-	-	-
Urinalysis	-	-	-	-	-	-	-	-

b.i.d.: twice daily; ECG: electrocardiogram; F: female; M: male; - no treatment related changes

2.6.7.7.F Repeat-Dose Toxicity (Continued)

Report Title: 3-Month toxicity study with TMC125K (HBr) by daily gavage in the dog **Test Article:** etravirine

Noteworthy Findings (Continued):

Dose (mg base eq./kg/day)	0 (vehicle)		20		40		80	
No. of animals	**M:4**	**F:4**	**M:4**	**F:4**	**M:4**	**F:4**	**M:4**	**F:4**
Organ weights[a] - adrenals (%)	0.016	0.016	1.00	1.06	0.75*	1.00	0.75*	1.13
Gross pathology	-	-	-	-	-	-	-	-
Histopathology	-	-	-	-	-	-	-	-

[a] for vehicle group means are shown. For treated groups, the multiples of vehicle/baseline are shown. Statistical significance based on actual data.

* $p \leq 0.05$ (Dunnett test based on pooled variance)

F: female; M: male; - no treatment related changes

2.6.7.7.G Repeat-Dose Toxicity

Report Title: 6-month toxicity study with TMC125K (HBr) by daily gavage in the dog **Test Article:** etravirine

Species/strain: Dog/Beagle
Age/weight at first dose: 4 - 6 months/5.4 - 8.1 kg
Duration of dosing: 26 weeks
Duration of postdose: 0 days

Administration route/method: Oral/gavage
Doses: 0 (vehicle) 20, 40 and 80 mg base eq./kg body weight/day (dose divided b.i.d., 7 hours apart to day 72 then once daily) as TMC125 HBr
Test article batch: [redacted]
Vehicle/formulation: Polyethylene glycol 400

Testing facility: [redacted]
Study no.: TMC125-NC117
Location in CTD: 4.2.3.2
GLP compliance: Yes
Date start of study: [redacted] 20[redacted]

No Observed Adverse Effect Level: 80 mg/kg/day in the absence of any relevant changes

Toxicokinetics:

Dose (mg base eq./kg/day)	0 (vehicle)		20		40		80	
No. of animals	M:4	F:4	M:4	F:4	M:4	F:4	M:4	F:4
Died or killed prematurely	0	0	0	0	0	0	0	0
$AUC_{0\text{-}7h}$ Day 1 (µg.h/mL)	-	-	1.62	1.70	4.29	2.62	7.23	3.13
$AUC_{0\text{-}7h}$ Day 86 (µg.h/mL)	-	-	5.86	5.07	6.51	6.03	11.6	10.7
$AUC_{0\text{-}24h}$ Day 86 (µg.h/mL)	-	-	14.2	11.2	15.2	13.6	26.7	22.0
$AUC_{0\text{-}7h}$ Day 177 (µg.h/mL)	-	-	6.02	4.75	8.47	4.08	11.8	7.80
$AUC_{0\text{-}24h}$ Day 177 (µg.h/mL)	-	-	15.7	10.8	19.8	9.69	28.6	17.2

Noteworthy Findings:

Dose (mg base eq./kg/day)	0 (vehicle)		20		40		80	
No. of animals	M:4	F:4	M:4	F:4	M:4	F:4	M:4	F:4
Died or killed prematurely	0	0	0	0	0	0	0	0
Clinical observations - erythema and alopecia	0/4	0/4	0/4	0/4	1/4	0/4	1/4	0/4
Body weight (kg)	-	-	-	-	-	-	-	-
Food consumption (g/week)	-	-	-	-	-	-	-	-
Ophthalmoscopy	-	-	-	-	-	-	-	-
ECG	-	-	-	-	-	-	-	-
Hematology	-	-	-	-	-	-	-	-
Serum chemistry[a] - inorganic phosphate (mmol/L)	1.13	1.34	0.87	0.83	1.08	0.87	1.05	0.78*

[a] for vehicle group means are shown. For treated groups, the multiples of vehicle/baseline are shown. Statistical significance based on actual data.
* $p \leq 0.05$ (Dunnett test based on pooled variance)
b.i.d.: twice daily; ECG: electrocardiogram; F: female; M: male; - no treatment related changes

2.6.7.7.G Repeat-Dose Toxicity (Continued)

Report Title: 6-month toxicity study with TMC125K (HBr) by daily gavage in the dog **Test Article:** etravirine

Noteworthy Findings (Continued):

Dose (mg base eq./kg/day)	0 (vehicle)		20		40		80	
No. of animals	**M:4**	**F:4**	**M:4**	**F:4**	**M:4**	**F:4**	**M:4**	**F:4**
Urinalysis	-	-	-	-	-	-	-	-
Organ weights[a] - spleen (%)	0.26	0.29	1.08	0.98	1.00	0.92	1.32*	1.02
Gross pathology	-	-	-	-	-	-	-	-
Histopathology	-	-	-	-	-	-	-	-

[a] for vehicle group means are shown. For treated groups, the multiples of vehicle/baseline are shown. Statistical significance based on actual data.

* $p \leq 0.05$ (Dunnett test based on pooled variance)

F: female; M: male; - no treatment related changes

2.6.7.7.H Repeat-Dose Toxicity

Report Title: 6-Month repeated dose oral toxicity study in the Beagle dog **Test Article:** etravirine

Species/strain: Dog/Beagle
Age/weight at first dose: 8 months/4.8 - 7.0 kg
Duration of dosing: 26 weeks
Duration of post-dose: 0 days

Administration route/method: Oral/gavage
Doses: 0 (vehicle), 160 and 500 mg/kg body weight/day (dose divided b.i.d., 5 hours apart) as spray-dried TMC125 ([redacted] active to polymer ratio), dose correction factor was 4.5
Test article batch: [redacted]
Vehicle/formulation: hydroxypropyl methylcellulose + microcrystalline cellulose in demineralized water

Testing facility: J&J PRD
Study no.: TMC125-NC321
Location in CTD: 4.2.3.2
GLP compliance: Yes
Date start of study: [redacted] 20[redacted]

No Observed Adverse Effect Level: 80 mg/kg/day in the absence of any relevant changes.

Toxicokinetics:

Dose (mg/kg/day)	0 (vehicle)		160		500	
No. of animals	M:3	F:3	M:3	F:3	M:3	F:3
Died or killed prematurely (accidental)	0	0	0	0	0	0
$AUC_{0-\infty}$ Day 1 (µg.h/mL)	-	-	74.3	62.6	72.5[a]	70.2[a]
AUC_{0-24h} Day 30 (µg.h/mL)	-	-	54.9	45.6	68.6	72.9
AUC_{0-24h} Day 181 (µg.h/mL)	-	-	54.5	44.6	61.8	61.5

[a] AUC_{0-24h}

Dose (mg/kg/day)	0 (vehicle)		160		500	
No. of animals	M:3	F:3	M:3	F:3	M:3	F:3
Died or killed prematurely (accidental)	0	0	0	0	0	0
Mortality	0	0	0	0	0	0
Clinical observations - vomiting	0	0	2	1	3	3
Body weight (kg)[a]	8.1	6.3	1.05	1.08	1.0	0.89
Body weight gain (kg)[a]	1.6	1.1	1.44	1.36	0.94	0.27
Total Food consumption (kg)[a]	46.40	42.23	1.02	1.04	1.05	0.86
Ophthalmoscopy	-	-	-	-	-	-
ECG	-	-	-	-	-	-
Hematology	-	-	-	-	-	-

[a] for vehicle group means are shown. For treated groups, the multiples of vehicle/baseline are shown. Statistical significance based on actual data.
b.i.d.: twice daily; ECG: electrocardiogram; F: female; M: male; - no treatment related changes

2.6.7.7.H Repeat-Dose Toxicity (Continued)

Report Title: 6-Month repeated dose oral toxicity study in the Beagle dog **Test Article:** etravirine

Noteworthy Findings (Continued):

Dose (mg/kg/day)	0 (vehicle)		160		500	
No. of animals	M:3	F:3	M:3	F:3	M:3	F:3
Serum chemistry[a]						
- triglycerides (mg/dL)	36	43	0.50	0.67	0.64	0.74
- total bilirubin (mg/dL)	0.14	0.13	1.14	0.85	1.79	1.08
- ALP (U/L)	109	93	1.19	1.89	3.02	1.89
- ALT (U/L)	34	60	1.24	0.48	2.56	0.57
Urinalysis	-	-	-	-	-	-
Organ weights	-	-	-	-	-	-
Gross pathology	-	-	-	-	-	-
Histopathology (no. examined)	3	3	3	3	3	3
Gall bladder						
- inspissated bile	0	0	0	0	2	1
grade 1	0	0	0	0	2	0
grade 3	0	0	0	0	0	1
Liver						
- microgranulomas	0	0	2	0	3	0
grade 2	0	0	2	0	1	0
grade 3	0	0	0	0	2	0
- chronic inflammation	0	2	3	3	3	3
grade 1	0	1	2	3	1	1
grade 2	0	1	1	0	2	2
- granulocytic infiltration	0	1	2	0	3	1
grade 1	0	1	2	0	2	1
grade 2	0	0	0	0	1	0
- inflammation	0	0	1	2	1	2
grade 1	0	0	1	0	0	0
grade 2	0	0	0	2	1	2

[a] for vehicle group means are shown. For treated groups, the multiples of vehicle/baseline are shown. Statistical significance based on actual data.
ALP: alkaline phosphatase; ALT: alanine aminotransferase; F: female; M: male; - no treatment related changes

2.6.7.7.I Repeat-Dose Toxicity

Report Title: 12-Month toxicity study with TMC125K (HBr) micronised by daily gavage in the dog followed by a 3-month recovery period

Test Article: etravirine

Species/strain: Dog/Beagle
Age/weight at first dose: 4 - 6 months/4.6 - 6.2 kg
Duration of dosing: 13 weeks and 52 weeks
Duration of postdose: 13 weeks

Administration route/method: Oral/gavage
Doses: 0 (vehicle) 30, 80 and 240 mg base eq./kg body weight as TMC125 HBr micronized
Test article batch: [redacted]
Vehicle/formulation: Polyethylene glycol 400

Testing facility: [redacted]
Study no.: TMC125-NC134
Location in CTD: 4.2.3.2
GLP compliance: Yes
Date start of study: [redacted] 20[redacted]

No Observed Adverse Effect Level: 240 mg/kg/day in the absence of any relevant changes

Toxicokinetics:

Dose (mg base eq./kg/day)	0 (vehicle)		30		80		240	
No. of animals	M:4	F:4	M:4	F:4	M:4	F:4	M:10[a]	F:10[a]
Died or killed prematurely (unrelated to treatment)	0	0	0	0	0	1	1	0
$AUC_{0-\infty}$ Day 1 (µg.h/mL)	-	-	12.5	7.14[a]	22.9[a]	17.9[a]	34.8[a]	27.0[a]
AUC_{0-24h} Day 21 (µg.h/mL)	-	-	13.0	10.5	30.1	23.9	33.4	29.0
AUC_{0-24h} Day 91 (µg.h/mL)	-	-	14.6	9.77	30.8	20.7	36.5	24.6
AUC_{0-24h} Day 182 (µg.h/mL)	-	-	17.1	9.98	26.7	10.2	33.3	21.1
AUC_{0-24h} Day 273 (µg.h/mL)	-	-	15.8	10.6	26.9	10.4	40.0	27.4
AUC_{0-24h} Day 366 (µg.h/mL)	-	-	13.5	11.9	33.9	19.3	35.6	23.2

[a] AUC_{0-24h}

Noteworthy Findings:

Dose (mg base eq./kg/day)	0 (vehicle)		30		80		240	
No. of animals	M:4	F:4	M:4	F:4	M:4	F:4	M:10[a]	F:10[a]
Died or killed prematurely (unrelated to treatment)	0	0	0	0	0	1	1	0
Clinical observations	-	-	-	-	-	-	-	-
Body weight (kg)	-	-	-	-	-	-	-	-
Food consumption (g/week)	-	-	-	-	-	-	-	-
Ophthalmoscopy	-	-	-	-	-	-	-	-
ECG	-	-	-	-	-	-	-	-
Hematology	-	-	-	-	-	-	-	-

[a] In addition, from pretest to Week 5, 3M+3F were assigned to 3-month recovery groups given 80 and 240 mg/kg/day. From Week 6 onwards, the recovery animals of the 80 mg/kg/day group were re-assigned to become recovery animals of the 240 mg/kg/day group. The recovery animals of the 240 mg/kg/day group were assigned to a 3-month interim sacrifice.
ECG: electrocardiogram; F: female; M: male; - no treatment related changes

2.6.7.7.I Repeat-Dose Toxicity (Continued)

Report Title: 12-Month toxicity study with TMC125K (HBr) micronised by daily gavage in the dog followed by a 3-month recovery period **Test Article:** etravirine

Noteworthy Findings (Continued):

Dose (mg base eq./kg/day)	0 (vehicle)		30		80		240	
No. of animals	M:4	F:4	M:4	F:4	M:4	F:4	M:10[a]	F:10[a]
Serum chemistry (Week 52)[b] - albumin (g/L)	31	31	0.97	1.00	0.95	0.96	0.97	0.91**
Urinalysis	-	-	-	-	-	-	-	-
Organ weights	-	-	-	-	-	-	-	-
Gross pathology	-	-	-	-	-	-	-	-
Histopathology	-	-	-	-	-	-	-	-

[a] In addition, from pretest to Week 5, 3M+3F were assigned to 3-month recovery groups given 80 and 240 mg/kg/day. From Week 6 onwards, the recovery animals of the 80 mg/kg/day group were re-assigned to become recovery animals of the 240 mg/kg/day group. The recovery animals of the 240 mg/kg/day group were assigned to a 3-month interim sacrifice; [b] for vehicle group means are shown. For treated groups, the multiples of vehicle/baseline are shown. Statistical significance based on actual data.

** $p \leq 0.01$ (Dunnett test based on pooled variance)

F: female; M: male; - no treatment related changes

2.6.7.8.A Genotoxicity: In Vitro

Report Title: In vitro bacterial reverse mutation test with *Salmonella typhimurium* **Test Article:** etravirine

Test: Bacterial reverse mutation test (Ames test)
Species/strain: *Salmonella typhimurium* TA98, TA100, TA102, TA1535, TA1537
Metabolizing system: Aroclor induced rat liver S9 mix (assay # 1: (20 µL/plate), assay # 2: (50 µL/plate))
No. of independent assays: 2
No. of replicate cultures: 3

Treatment: Plate incorporation for 48 hours
Concentrations: 5, 10, 25, 50, 100, 250 and 500 µg/plate as TMC125 base
Test article batch: [redacted]
Vehicle test article: DMSO
Vehicle positive controls: Water, DMSO

Testing facility: J&J PRD
Study no.: TMC125-Exp5081
Location in CTD: 4.2.3.3.1
GLP compliance: Yes
Date start of study: [redacted] 20[redacted]

Cytotoxic effects: Yes
Genotoxic effects: TMC125 was not mutagenic in the *S. typhimurium* reverse mutation assay under the experimental conditions described

Metabolic Activation	Control, Test and Reference Articles	Concentration (µg/plate)	Assay # 1 Revertant Colony Counts (Mean ± SD)				
			TA98	TA100	TA102	TA1535	TA1537
Without S9	DMSO	-	21 ± 3.6	125 ± 20.5	368 ± 34.0	12 ± 5.5	7 ± 2.6
	TMC125	5	24 ± 4.6	149 ± 17.0	387 ± 21.5	13 ± 0.0	5 ± 1.2
		10	26 ± 1.2	168 ± 27.5	398 ± 26.8	10 ± 3.5	7 ± 3.1
		25	21 ± 3.0	127 ± 6.4	335 ± 4.7	6 ± 1.0	7 ± 0.6
		50	23 ± 6.9	153 ± 22.1	325 ± 48.0	8 ± 3.5	5 ± 2.6
		100	21 ± 1.0	125 ± 11.9	338 ± 29.3	9 ±5.9	4 ± 1.0
		250[b]	18 ± 4.0	153 ± 14.8	353 ± 17.7	7 ± 1.2	4 ± 2.0
		500	22 ± 4.9	145 ± 5.6	383 ± 7.9	9 ± 5.9	5 ± 1.2
	2-Nitrofluorene	5	1117 ± 104[a]	-	-	-	-
	Sodium azide	1	-	536 ± 1.5[a]	-	354 ± 30.6[a]	-
	4-Nitroquinoline-N-oxide	5	-	-	1244 ± 47.2[a]	-	-
	9-Aminoacridine	50	-	-	-	-	182 ± 16.9[a]
With S9 (20 µL/plate)	DMSO	-	29 ± 5.9	101 ± 2.3	400 ± 39.3	8 ± 0.6	7 ± 2.6
	TMC125	5	23 ± 6.6	101 ± 13.1	372 ± 39.9	10 ± 4.6	5 ± 1.5
		10	20 ± 5.3	109 ± 15.0	319 ± 23.1	9 ± 2.9	7 ± 0.6
		25	27 ± 5.5	102 ± 14.5	337 ± 33.3	9 ± 4.9	5 ± 1.2
		50	25 ± 6.4	105 ± 7.0	353 ± 11.1	7 ± 4.0	7 ± 2.1

[a] more than 2-fold increase with TA98, TA100 and TA102 compared to the vehicle control, more than 3-fold increase with TA1535 and TA1537 compared to the vehicle control; [b] precipitation at this dose level and above
DMSO: dimethyl sulfoxide; - not applicable

2.6.7.8.A Genotoxicity: In Vitro (Continued)

Report Title: In vitro bacterial reverse mutation test with *Salmonella typhimurium* **Test Article:** etravirine

Metabolic Activation	Control, Test and Reference Articles	Concentration (µg/plate)	Assay # 2 Revertant Colony Counts (Mean ± SD)				
			TA98	TA100	TA102	TA1535	TA1537
With S9 (20 µL/plate) (continued)	TMC125	100	27 ± 4.2	99 ± 25.7	335 ± 19.0	10 ± 4.0	6 ± 2.6
		250[b]	21 ± 5.7	94 ± 11.4	367 ± 19.7	7 ± 2.1	8 ± 1.5
		500	22 ± 4.5	100 ± 11.5	342 ± 29.5	7 ± 2.0	4 ± 1.2
	2-Aminoanthracene	1	942 ± 198[a]	445 ± 34.0[a]	-	135 ± 8.5[a]	-
		2.5	-	-	872 ± 59.2[a]	-	416 ± 35.4[a]
Without S9	DMSO	-	18 ± 0.6	151 ± 20.1	281 ± 28.8	10 ± 2.6	3 ± 0.6
	TMC125	5	20 ± 9.0	144 ± 8.2	310 ± 15.0	13 ± 1.0	4 ± 3.5
		10	19 ± 1.7	145 ± 15.3	319 ± 4.6	9 ± 3.1	3 ± 1.5
		25	20 ± 0.6	158 ± 12.1	278 ± 66.6	9 ± 0.6	4 ± 2.1
		50	21 ± 4.7	150 ± 5.5	362 ± 28.6	11 ± 1.5	5 ± 4.0
		100	19 ± 3.5	172 ± 8.7	339 ± 14.1	17 ± 2.0	6 ± 2.5
		250[b]	20 ± 2.6	148 ± 5.5	250 ± 77.0	10 ± 2.0	5 ± 3.6
		500	13 ± 5.5	175 ± 11.5	314 ± 54.7	11 ± 4.2	4 ± 2.9
	2-Nitrofluorene	5	994 ± 124.8[a]	-	-	-	-
	Sodium azide	1	-	583 ± 17.2[a]	-	135 ± 7.0[a]	-
	4-Nitroquinoline-N-oxide	5	-	-	2995 ± 502.8[a]	-	-
	9-Aminoacridine	50	-	-	-	-	116 ± 29.1[a]
With S9 (50 µL/plate)	DMSO	-	27 ± 6.9	102 ± 4.9	417 ± 6.5	9 ± 2.6	4 ± 2.5
	TMC125	5	18 ± 5.6	100 ± 10.2	452 ± 26.7	8 ± 3.0	3 ± 0.6
		10	19 ± 3.2	116 ± 11.9	418 ± 49.1	10 ± 2.1	5 ± 2.0
		25	19 ± 2.5	106 ± 24.4	415 ± 26.8	8 ± 2.9	8 ± 2.6
		50	18 ± 2.5	93 ± 12.5	395 ± 17.8	10 ± 4.6	2 ± 0.6
		100	20 ± 10.5	95 ± 5.1	399 ± 29.0	10 ± 4.0	4 ± 2.5
		250[b]	20 ± 1.0	91 ± 5.0	340 ± 109.3	11 ± 1.2	5 ± 2.1
		500	22 ± 4.0	106 ± 20.3	433 ± 31.4	10 ± 4.0	5 ± 1.2
	2-Aminoanthracene	1	364 ± 42.1[a]	632 ± 11.5[a]	-	200 ± 34.4[a]	-
		2.5	-	-	2262 ± 491.4[a]	-	400 ± 33.6[a]

[a] more than 2-fold increase with TA98, TA100 and TA102 compared to the vehicle control, more than 3-fold increase with TA1535 and TA1537 compared to the vehicle control; [b] precipitation at this dose level and above
DMSO: dimethyl sulfoxide; - not applicable

2.6.7.8.B Genotoxicity: In Vitro

Report Title: Evaluation of the mutagenic activity of TMC125 in the *Salmonella typhimurium* reverse mutation assay and the *Escherichia coli* reverse mutation assay (with independent repeat)

Test Article: etravirine

Test: Bacterial reverse mutation test (Ames test)
Species/strain: *Salmonella typhimurium* TA98, TA100, TA1535, TA1537, *Escherichia coli* WP_2uvrA
Metabolizing system: Aroclor induced rat liver S9 mix (assay # 1: 5% v/v, assay # 2: 10% v/v)
No. of independent assays: 2
No. of replicate cultures: 3

Treatment: Plate incorporation for 48 hours
Concentrations: 3, 10, 33, 100 and 333 µg/plate as TMC125 base
Test article batch: [redacted]
Vehicle test article: DMSO
Vehicle positive controls: Saline, DMSO

Testing facility: [redacted]
Study no.: TMC125-NC130
Location in CTD: 4.2.3.3.1
GLP compliance: Yes
Date start of study: [redacted] 20[redacted]

Cytotoxic effects: None
Genotoxic effects: TMC125 was not mutagenic in the *S. typhimurium* and *E. coli* reverse mutation assay under the experimental conditions described

Metabolic Activation	Control, Test and Reference Articles	Concentration (µg/plate)	Assay # 1 Revertant Colony Counts (Mean ± SD)				
			TA98	TA100	TA1535	TA1537	WP_2uvrA
Without S9	DMSO	-	18 ± 1	63 ± 3	11 ± 2	8 ± 3	13 ± 5
	TMC125	3	14 ± 4	66 ± 10	12 ± 6	11 ± 7	10 ± 3
		10	18 ± 7	61 ± 8	14 ± 4	8 ± 4	12 ± 4
		33	13 ± 2	68 ± 15	11 ± 4	5 ± 4	12 ± 3
		100	14 ± 1	62 ± 11	9 ± 3	8 ± 3	9 ± 1
		333[b]	19 ± 4	75 ± 3	11 ± 1	5 ± 2	12 ± 3
	Sodium azide	5	-	-	461 ± 24[a]	-	-
	9-Aminoacridine	60	-	-	-	449 ± 58[a]	-
	Daunomycine	4	422 ± 69[a]	-	-	-	-
	Methylmethanesulfonate	650	-	823 ± 47[a]	-	-	-
	4-Nitroquinoline-N-oxide	10	-	-	-	-	709 ± 99[a]
With S9 (5% v/v)	DMSO	-	19 ± 5	66 ± 12	8 ± 1	8 ± 1	9 ± 4
	TMC125	3	23 ± 1	73 ± 3	15 ± 4	7 ± 2	10 ± 3
		10	22 ± 1	69 ± 19	13 ± 2	6 ± 3	6 ± 2
		33	23 ± 4	64 ± 13	17 ± 5	9 ± 4	11 ± 3
		100	23 ± 2	72 ± 9	15 ± 6	6 ± 1	12 ± 2
		333[b]	23 ± 3	70 ± 10	10 ± 3	9 ± 2	11 ± 2
	2-Aminoanthracene	1	608 ± 43[a]	743 ± 129[a]	308 ± 16[a]	-	-
		2.5	-	-	-	143 ± 5[a]	-
		5	-	-	-	-	131 ± 18[a]

[a] more than 2-fold increase with TA98, TA100 and TA102 compared to the vehicle control, more than 3-fold increase with TA1535 and TA1537 compared to the vehicle control; [b] precipitation at this dose level and above
DMSO: dimethyl sulfoxide; - not applicable

2.6.7.8.B Genotoxicity: In Vitro (Continued)

Report Title: Evaluation of the mutagenic activity of TMC125 in the *Salmonella typhimurium* reverse mutation assay and the *Escherichia coli* reverse mutation assay (with independent repeat) **Test Article:** etravirine

Metabolic Activation	Control, Test and Reference Articles	Concentration (µg/plate)	Assay # 2 Revertant Colony Counts (Mean ± SD)				
			TA98	TA100	TA1535	TA1537	WP_2uvrA
Without S9	DMSO	-	27 ± 4	158 ± 14	21 ± 7	10 ± 1	12 ± 2
	TMC125	3	24 ± 3	164 ± 33	23 ± 4	8 ± 1	11 ± 3
		10	23 ± 8	160 ± 2	22 ± 3	8 ± 2	15 ± 2
		33	23 ± 6	161 ± 18	23 ± 4	8 ± 2	8 ± 3
		100	25 ± 4	158 ± 9	20 ± 4	7 ±1	11 ± 4
		333[b]	24 ± 3	157 ± 4	24 ± 4	7 ± 2	12 ± 1
	Sodium azide	5	-	-	600 ± 40[a]	-	-
	9-Aminoacridine	60	-	-	-	470 ± 273[a]	-
	Daunomycine	4	1243 ± 222[a]	-	-	-	-
	Methylmethanesulfonate	650	-	902 ± 45[a]	-	-	-
	4-Nitroquinoline-N-oxide	10	-	-	-	-	1046 ± 17[a]
With S9 (10% v/v)	DMSO	-	36 ± 7	129 ± 9	24 ± 7	8 ± 2	13 ± 3
	TMC125	3	36 ± 5	138 ± 8	23 ± 7	6 ± 2	15 ± 6
		10	30 ± 5	137 ± 9	28 ± 7	11 ± 5	13 ± 3
		33	32 ± 8	144 ± 14	21 ± 6	9 ± 1	13 ± 2
		100	37 ± 6	166 ± 16	20 ± 4	9 ± 1	17 ± 3
		333[b]	25 ± 2	156 ± 16	16 ± 2	10 ± 2	14 ± 0
	2-Aminoanthracene	1	495 ± 20[a]	470 ± 6[a]	196 ± 5[a]	-	-
		2.5	-	-	-	178 ± 17[a]	-
		5	-	-	-	-	186 ± 6[a]

[a] more than 2-fold increase with TA98, TA100 and TA102 compared to the vehicle control, more than 3-fold increase with TA1535 and TA1537 compared to the vehicle control; [b] precipitation at this dose level and above
DMSO: dimethyl sulfoxide; - not applicable

2.6.7.8.C Genotoxicity: In Vitro

Report Title: In vitro mammalian forward mutation test with L5178Y mouse lymphoma cells (TK-locus) using the microtitre® fluctuation technique

Test Article: etravirine

Test: In vitro mammalian gene mutation assay
Species/strain: Near-diploid L5178Y mouse lymphoma cells
Metabolizing System: Aroclor induced rat liver S9 mix (1.8% v/v)
No of independent assays: 1
No of replicate cultures: 1

Treatment: Assay # 1: 3 hours treatment (+/- S9), 24 hours treatment (- S9) 48 hours expression period.
Concentrations: 1 to 100 µg/mL as TMC125 base
Test article batch: [redacted]
Vehicle test article: DMSO

Testing facility: J&J PRD
Study no.: TMC125-Exp5091
Location in CTD: 4.2.3.3.1
GLP compliance: Yes
Date start of study: [redacted] 19[redacted]

Cytotoxic effects: Relative growth determination reduced under the experimental conditions described
Genotoxic effects: TMC125 was not mutagenic under the experimental conditions described

Metabolic Activation	Control, Test and Reference Articles	Concentration (µg/mL)[a]	Suspension Growth (% of control)	Plating Efficiency (% of control)	Relative Growth Determination	Mutation Frequency
Without S9	DMSO	0	100	100	-	41
3 hours	TMC125	1	99	108	107	44
		2.5	92	108	100	43
		5	39	84	32	-
		10	44	84	37	65
		20	40	90	36	56
		25	19	81	15	59
		37.5	15	80	12	47
		50	3	-	-	-
		75	0	-	-	-
	Methylmethanesulfonate	1.25	81	47	38	816[a]
With S9	DMSO	0	100	100	-	44
3 hours	TMC125	0.5	93	115	107	-
		1	105	95	100	39
		2.5	90	84	76	-
		5	97	88	86	41
		10	88	115	101	-
		25	61	105	64	54
		50	42	97	40	49
		75	19	100	19	49
		100	9	91	8	48
	N-nitrosodimethylamine	0.40	95	45	43	539[a]

[a] more than 2-fold increase as compared to the mutant frequency of the vehicle control.
DMSO: dimethyl sulfoxide; - not applicable

2.6.7.8.C Genotoxicity: In Vitro (Continued)

Report Title: In vitro mammalian forward mutation test with L5178Y mouse lymphoma cells (TK-locus) using the microtitre® fluctuation technique **Test Article:** etravirine

Metabolic Activation	Control, Test and Reference Articles	Concentration (µg/mL)[a]	Suspension Growth (% of control)	Plating Efficiency (% of control)	Relative Growth Determination	Mutation Frequency
Without S9	DMSO	0	100	100	-	55
24 hours	TMC125	1	104	93	96	51
		2.5	105	91	96	39
		5	71	120	85	-
		10	110	114	125	33
		25	63	127	80	-
		35	70	63	44	60
		40	58	69	40	58
		50	26	44	12	56
		75	1	-	-	-
	Methylmethanesulfonate	0.625	84	30	25	1183[a]

[a] more than 2-fold increase as compared to the mutant frequency of the vehicle control.

DMSO: dimethyl sulfoxide; - not applicable

2.6.7.8.D Genotoxicity: In Vitro

Report Title: Evaluation of the ability of TMC125 to induce chromosome aberrations in cultured peripheral human lymphocytes

Test Article: etravirine

Test: In vitro mammalian chromosome aberration test
Species/strain: Human peripheral lymphocytes
Metabolizing System: Aroclor induced rat liver S9 mix (1.8% v/v)
No of independent assays: 2
No of replicate cultures: 2
No of cells analyzed/culture: ≥ 100

Treatment: Assay # 1: 3 hours treatment (+/- S9), 24 hours fixation time.
Assay # 2: 24 hours treatment (- S9), 24 hours fixation; 48 hours treatment (- S9), 48 hours fixation; 3 hours treatment (+ S9), 48 hours fixation.
Concentrations: 1 to 100 µg/mL as TMC125 base
Test article batch: [redacted]
Vehicle test article: DMSO
Vehicle positive controls: Hank's balanced salt solution (HBSS) without Ca^{2+} and Mg^{2+}

Testing facility: [redacted]
Study no.: TMC125-NC122
Location in CTD: 4.2.3.3.1
GLP compliance: Yes
Date start of study: [redacted] 20[redacted]

Cytotoxic effects: None
Genotoxic effects: TMC125 was not clastogenic in human lymphocytes in vitro under the experimental conditions described

Assay # 1

Metabolic Activation	Control, Test and Reference Articles	Concentration (µg/mL)[a]	Mitotic Index (% of control)	Aberrant Cells (Mean %) (- gaps)	Abs/Cell (- gaps)	Total Polyploid Cells
Without S9 (3 hours treatment, 24 hours fixation)	DMSO	1.0% (v/v)	100	2.5	0.03	0
	TMC125	10	100	1.5	0.02	0
		33	40	1	0.01	4
		100[b]	48	2	0.03	1
	Mytomycin C	0.5	69	18***	0.22	0
With S9 (3 hours treatment, 24 hours fixation)	DMSO	1.0% (v/v)	100	0	0.00	0
	TMC125	10	80	1	0.01	0
		33	37	1	0.01	3
		100[b]	52	1.5	0.02	0
	Cyclophosphamide	15	40	16.5***	0.20	0

[a] unless stated otherwise; [b] precipitation at this dose level
*** $p < 0.001$ (Chi-square test)
DMSO: dimethyl sulfoxide

2.6.7.8.D Genotoxicity: In Vitro (Continued)

Report Title: Evaluation of the ability of TMC125 to induce chromosome aberrations in cultured peripheral human lymphocytes **Test Article:** etravirine

Assay # 2

Metabolic Activation	Control, Test and Reference Articles	Concentration (µg/mL)[a]	Mitotic Index (% of control)	Aberrant Cells (Mean %) (- gaps)	Abs/Cell (- gaps)	Total Polyploid Cells
Without S9 (24 hours treatment, 24 hours fixation)	DMSO	1.0% (v/v)	100	2	0.02	0
	TMC125	1	87	1	0.01	0
		5.6	107	3.5	0.04	0
		10	45	4.5	0.05	0
	Mytomycin C	0.2	61	31***	0.4	0
Without S9 (48 hours treatment, 48 hours fixation)	DMSO	1.0% (v/v)	100	1.5	0.02	0
	TMC125	3	81	0	0.00	0
		5.6	67	1.5	0.02	0
		10	44	0.5	0.01	0
	Mytomycin C	0.1	92	26***	0.41	0
With S9 (3 hours treatment, 48 hours fixation)	DMSO	1.0% (v/v)	100	1	0.01	0
	TMC125	17	143	1	0.01	0
		33	134	0.5	0.01	2
		56[b]	127	3	0.03	0
	Cyclophosphamide	15	-[c]	28***	0.38	0

[a] unless stated otherwise; [b] precipitation at this dose level; [c] cyclophosphamide was fixed after 24 hours. Therefore, the mitotic index could not be calculated as percentage of control.

*** $p < 0.001$ (Chi-square test)

DMSO: dimethyl sulfoxide

2.6.7.9.A Genotoxicity: In Vivo

Report Title: Micronucleus test in bone marrow cells of the mouse with TMC125K (HBr) **Test Article:** etravirine

Test: Mammalian erythrocyte micronucleus test
Species/strain: Mouse/NMRI BR
Age/weight at first dose: 6 - 8 weeks/30 - 31 g
Cells evaluated: Bone marrow polychromatic and normochromatic erythrocytes
No. of cells analyzed/animal: 2000 polychromatic erythrocytes for micronuclei screening; 1000 normochromatic erythrocytes for bone marrow toxicity

Treatment: Single dose with sampling after 24 and 48 hours
Administration route/method: Oral gavage
Doses: 2000 mg base eq./kg body weight as TMC125 HBr
Test article batch: [redacted]
Vehicle/formulation: Polyethylene glycol 400

Testing facility: [redacted]
Study no.: TMC125-NC120
Location in CTD: 4.2.3.3.2
GLP compliance: Yes
Date start of study: [redacted] 20[redacted]

Toxic/Cytotoxic effects: None
Genotoxic effects: TMC125 was not mutagenic in the micronucleus test under the experimental conditions described.

Toxicokinetics:

Dose (mg base eq./kg/day)	2000
No. of animals	M:5
Died or killed prematurely	0
C_{max} Day 1 (µg/mL)	7.847
$AUC_{0-\infty}$ Day 1 (µg.h/mL)	111.2

Noteworthy Findings

Control, Test and Reference Articles	Dose (mg base eq./kg body weight)	Number of Animals and Gender	Sampling Time (Hours)	# MNPCEs per 2000 PCEs (Mean ± SD)	Ratio PCEs / NCEs (Mean ± SD)
Polyethylene glycol 400	-	5M	24	1.6 ± 1.8	1.03 ± 0.02
TMC125	2000	5M	24	2.2 ± 1.5	1.01 ± 0.02
	2000	5M	48	0.8 ± 0.8	1.02 ± 0.03
Cyclophosphamide	50	5M	48	18.4 ± 3.4[a]	0.54 ± 0.18

[a] significant difference from corresponding control group; $p \leq 0.01$ (Wilcoxon rank sum test)
M: male; MNPCEs: micronucleated polychromatic erythrocytes; NCEs: normochromatic erythrocytes; PCEs: polychromatic erythrocytes; SD: standard deviation; # number

2.6.7.10.A Carcinogenicity - Non-pivotal

Report Title: 2-week repeated dose oral toxicity study in the Swiss mouse **Test Article:** etravirine

Species/strain: Mouse/CD1
Age/weight at first dose: 10 weeks/23.8 - 37.7 g
Duration of dosing: 2 weeks
Duration of postdose: 0 days

Administration route/method: Oral gavage
Doses: 0 (vehicle), 100, 300, 900 and 1200 mg base eq./kg body weight/day as TMC125 HBr
Test article batch: [redacted]
Vehicle/formulation: Polyethylene glycol 400

Testing facility: J&J PRD
Study no.: TMC125-NC179
Location in CTD: 4.2.3.4.1
GLP compliance: No
Date start of study: [redacted] 20[redacted]

No Observed Adverse Effect Level: was not established

Toxicokinetics: Not determined

Noteworthy Findings:

Dose (mg base eq./kg/day)	0 (vehicle)		100		300		900		1200	
No. of animals	**M:5**	**F:5**	**M:5**	**F:5**	**M:5**	**F:5**	**M:5**	**F:5**	**M:5**	**F:5**
Died or killed prematurely (unrelated to treatment)	0	0	0	0	0	0	0	0	2	0
Clinical observations	-	-	-	-	-	-	-	-	-	-
Body weight (g)	-	-	-	-	-	-	-	-	-	-
Food consumption (g/week)	-	-	-	-	-	-	-	-	-	-
Hematology[a]										
- white blood cells (10^3/µL)	7.2	3.5	0.97	1.31	0.83	1.31	0.85	1.51	1.36*	1.60
- lymphocytes (10^3/µL)	5.77	2.77	0.93	1.31	0.86	1.36	0.79	1.53	1.15	1.60
- monocytes (10^3/µL)	0.19	0.08	1.11	1.00	0.68	0.88	0.58	1.00	1.21	1.88*
Serum chemistry[a]										
- ALP (U/L)	71	95	1.49*	1.36*	1.32	0.93	1.46	0.88	1.49	1.88
- AST (U/L)	66	93	1.33	1.20	1.35*	1.34**	1.39	2.02**	4.79	2.41**
- ALT (U/L)	29	26	1.52	1.96	1.34	2.08*	1.86	4.23*	7.00	7.00**
- albumin (g/dL)	3.4	3.5	0.91*	1.00	0.88**	0.94	0.82**	0.97	0.71	0.91*
- cholesterol (mg/dL)	144	83	0.90	1.05	0.85*	1.33	0.78**	1.47**	0.71	1.57*
- triglyceride (mg/dL)	123	89	1.10	1.39	0.95	1.76	1.00	1.47	0.96	2.03**
- bilirubin (mg/dL)	0.17	0.12	0.53*	0.75**	0.53**	0.67**	0.35**	0.58**	0.29*	0.42**

[a] for vehicle group means are shown. For treated groups, the multiples of vehicle/baseline are shown. Statistical significance based on actual data.
* $p \leq 0.05$; ** $p \leq 0.01$ (Mann-Whitney U test)
ALP: alkaline phosphatase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; F: female; M: male; - no treatment related changes

2.6.7.10.A Carcinogenicity - Non-pivotal (Continued)

Report Title: 2-week repeated dose oral toxicity study in the Swiss mouse **Test Article:** etravirine

Noteworthy Findings (Continued):

Dose (mg base eq./kg/day)	0 (vehicle)		100		300		900		1200	
No. of animals	**M:5**	**F:5**	**M:5**	**F:5**	**M:5**	**F:5**	**M:5**	**F:5**	**M:5**	**F:5**
Organ weights[a]										
- heart (%)	0.54	0.60	1.01	0.95	1.04	0.95	1.13	0.90	1.15	0.94
- liver (%)	4.83	5.15	1.15*	1.08	1.20*	1.20*	1.38**	1.35**	1.51*	1.68**
- pancreas (%)	1.00	1.26	1.02	0.89	1.06	0.92	1.00	0.82	1.01	0.78**
Gross pathology										
Liver										
- yellow focus	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5
- pale	0/5	0/5	0/5	0/5	0/5	0/5	1/5	1/5	0/5	3/5
- more pronounced lobulation	0/5	0/5	0/5	0/5	0/5	0/5	1/5	1/5	2/5	2/5
- swollen	0/5	0/5	0/5	0/5	0/5	0/5	3/5	0/5	3/5	5/5*
Histopathology										
Liver (no. examined)	5	5	5	5	5	5	5	5	5	5
- hepatocellular hypertrophy (diffuse)	0/5	0/5	0/5	1/5	1/5	1/5	4/5*	4/5*	4/5*	5/5**
grade 1	0	0	0	1	1	1	3	4	4	2
grade 2	0	0	0	0	0	0	1	0	0	3
- necrosis (multi) focal	0/5	0/5	0/5	2/5	0/5	0/5	0/5	4/5*	1/5	3/5
grade 1	0	0	0	2	0	0	0	3	1	1
grade 2	0	0	0	0	0	0	0	1	0	2
- single cell necrosis (multi) focal	0/5	0/5	1/5	2/5	2/5	3/5	5/5**	3/5	3/5	5/5**
grade 1	0	0	1	2	2	3	5	2	2	1
grade 2	0	0	0	0	0	0	0	0	1	4
grade 3	0	0	0	0	0	0	0	1	0	0

[a] for vehicle group means are shown. For treated groups, the multiples of vehicle/baseline are shown. Statistical significance based on actual data.
* $p \leq 0.05$; ** $p \leq 0.01$ (Organ weight: Mann-Whitney U test; gross pathology: Chi-square test; histopathology: Fisher exact test)
F: female; M: male; - no treatment related changes

2.6.7.10.B Carcinogenicity

Report Title: 3-month repeated dose oral toxicity study in the Swiss mouse **Test Article:** etravirine

Species/strain: Mouse/CD1
Age/weight at first dose: 6 weeks/21.6 - 33.5 g
Duration of dosing: 4 or 13 weeks
Duration of postdose: 0 days

Administration route/method: Oral/gavage
Doses: 0 (vehicle), 10, 50, 200 and 800 mg base eq./kg body weight/day as TMC125 HBr
Test article batch: [redacted]
Vehicle/formulation: Polyethylene glycol 400

Testing facility: J&J PRD
Study no.: TMC125-NC146
Location in CTD: 4.2.3.4.1
GLP compliance: Yes
Date start of study: [redacted] 20[redacted]

No Observed Adverse Effect Level: not determined

Toxicokinetics:

Dose (mg base eq./kg/day)	0 (vehicle)		10		50		200		800	
No. of animals	M:0	F:0	M:45	F:45	M:45	F:45	M:45	F:45	M:45	F:45
Died or killed prematurely (unrelated to treatment)	0	0	0	0	1	0	0	1	4	0
$AUC_{0-\infty}$ Day 1 (µg.h/mL)	-	-	1.62[a]	1.27[a]	4.04[a]	4.13[a]	9.73[a]	9.38	43.4	41.1
AUC_{0-24h} Day 29 (µg.h/mL)	-	-	1.62	1.48	2.44[a]	2.76	3.96	3.42	12.2	11.3
AUC_{0-24h} Day 91 (µg.h/mL)	-	-	1.44	1.09[a]	3.14	1.70[a]	3.93	4.60	7.80	10.1

[a] AUC_{0-8h} reported

Noteworthy Findings:

Dose (mg base eq./kg/day)	0 (vehicle)		10		50		200		800	
No. of animals[a]	M:15	F:15	M:15	F:15	M:15	F:15	M:15	F:15	M:15	F:15
Died or killed prematurely (unrelated to treatment)	1	0	0	0	0	2	0	1	1	0
Clinical observations	-	-	-	-	-	-	-	-	-	-
Body weight (g) (Week 13)[b]	39.4	30.0	0.95*	1.01	0.94*	0.97	0.95	0.98	0.95	1.00
Body weight gain (g) (Week 1 - 13)[b]	8.5	5.8	0.82	1.02	0.79*	0.91	0.85	0.97	0.73**	0.93
Food consumption (g/week)	-	-	-	-	-	-	-	-	-	-

[a] includes 5M + 5F for interim kill at 1-month; [b] for vehicle group means are shown. For treated groups, the multiples of vehicle/baseline are shown. Statistical significance based on actual data.
* $p \leq 0.05$; ** $p \leq 0.01$ (Mann-Whitney U test)
F: female; M: male; - no treatment related changes

2.6.7.10.B Carcinogenicity (Continued)

Report Title: 3-month repeated dose oral toxicity study in the Swiss mouse **Test Article:** etravirine

Noteworthy Findings (Continued):

Dose (mg base eq./kg/day)	0 (vehicle)		10		50		200		800	
No. of animals[a]	M:15	F:15	M:15	F:15	M:15	F:15	M:15	F:15	M:15	F:15
Hematology (Week 13)[b]										
- reticulocytes (10^3/µL)	248.2	284.7	1.01	0.95	0.97	0.86	1.04	0.97	0.87	0.84*
- thrombocytes (10^3/µL)	1238	1180	1.10	1.01	1.11*	1.14	1.15**	1.07	1.21**	1.10
Serum chemistry (Week 13)[b]										
- ALP (U/L)	62	69	1.11	1.38*	1.27*	1.20	1.39	1.14	1.71*	2.04
- AST (U/L)	140	129	1.02	1.10	0.95	1.01	0.98	1.71	0.77	2.57**
- ALT (U/L)	54	40	1.09	1.63	1.52	1.45	1.28	4.83***	1.65*	8.75***
- cholesterol (mg/dL)	122	91	0.99	0.98	1.03	1.14	0.89	0.91	0.90	1.37**
- triglyceride (mg/dL)	142	124	1.03	1.11	1.25	1.24	0.93	1.11	1.42	1.31
- bilirubin (mg/dL)	0.13	0.10	0.85	0.90	0.54***	1.10	0.54***	0.90	0.31***	0.70**
Organ weights (Week 13)[b]										
- adrenal glands (%)	0.012	0.035	1.58*	1.00	1.25	0.91	1.17	1.09	1.33*	0.97
- liver (%)	5.15	5.11	1.08	1.07	1.11**	1.15***	1.16**	1.22***	1.47***	1.51***
- thymus (%)	0.103	0.147	0.82*	0.84	0.87	0.82	0.83	0.88	0.75**	0.82
Gross pathology (Week 13: terminal kill)										
Liver (no. examined)	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
- more pronounced lobulation	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	4/10	0/10
- pale	0/10	0/10	0/10	0/10	0/10	0/10	0/10	1/10	4/10	5/10*
- swollen	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	5/10*	3/10
Histopathology (Week 4: interim kill)[b]										
Adrenal glands (no. examined)	**5**	**5**	**5**	**0**	**5**	**0**	**5**	**5**	**5**	**5**
- swollen/eosinophilic cortical cells grade 1	1/5	0/5	2/5	0/5	2/5	0/5	4/5	1/5	4/5	0/5

[a] includes 5M + 5F for interim kill at 1-month; [b] for vehicle group means are shown. For treated groups, the multiples of vehicle/baseline are shown. Statistical significance based on actual data.

* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$ (Hematology + serum + organ weight: Mann-Whitney U test; gross pathology: Chi-square test; histopathology: Fisher Exact test)

ALP: alkaline phosphatase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; F: female; M: male

2.6.7.10.B Carcinogenicity (Continued)

Report Title: 3-month repeated dose oral toxicity study in the Swiss mouse **Test Article:** etravirine

Noteworthy Findings (Continued):

Dose (mg base eq./kg/day)	0 (vehicle)		10		50		200		800	
No. of animals[a]	M:15	F:15	M:15	F:15	M:15	F:15	M:15	F:15	M:15	F:15
Histopathology (Week 4: interim kill)[b] (continued)										
Liver (no. examined)	5	5	5	5	5	5	5	5	5	5
- hydropic appearance/vacuolation	0/5	0/5	0/5	0/5	0/5	2/5	4/5*	4/5*	4/5*	5/5**
grade 1	0	0	0	0	0	2	4	3	3	0
grade 2	0	0	0	0	0	0	0	1	1	4
grade 3	0	0	0	0	0	0	0	0	0	1
- hepatocellular hypertrophy (centrilobular) grade 1	1/5	0/5	1/5	0/5	1/5	1/5	0/5	1/5	1/5	0/5
- hepatocellular hypertrophy (diffuse)	0/5	0/5	0/5	0/5	0/5	0/5	2/5	2/5	4/5*	5/5**
grade 1	0	0	0	0	0	0	2	2	3	5
grade 2	0	0	0	0	0	0	0	0	1	0
- single cell necrosis (multi) focal	1/5	0/5	1/5	2/5	3/5	1/5	2/5	3/5	4/5	3/5
grade 1	1	0	1	2	3	1	2	3	0	1
grade 2	0	0	0	0	0	0	0	0	4	2
Histopathology (Week 13: terminal kill)										
Adrenal glands (no. examined)	10	10	10	10	10	10	10	10	10	10
- swollen/eosinophilic cortical cells grade 1	0/10	0/10	0/10	1/10	1/10	1/10	2/10	2/10	3/10	5/10*
Liver	10	10	10	10	10	10	10	10	10	10
- hydropic appearance/vacuolation	0/10	0/10	0/10	0/10	4/10	5/10*	3/10	6/10*	8/10***	10/10***
grade 1	0	0	0	0	3	3	3	5	1	2
grade 2	0	0	0	0	1	2	0	1	6	6
grade 3	0	0	0	0	0	0	0	0	1	2

[a] includes 5M + 5F for interim kill at 1-month

* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$ (Fisher Exact test)

F: female; M: male

2.6.7.10.B Carcinogenicity (Continued)

Report Title: 3-month repeated dose oral toxicity study in the Swiss mouse **Test Article:** etravirine

Noteworthy Findings (Continued):

Dose (mg base eq./kg/day)	0 (vehicle)		10		50		200		800	
No. of animals[a]	M:15	F:15	M:15	F:15	M:15	F:15	M:15	F:15	M:15	F:15
Histopathology (Week 13: terminal kill) (continued)										
- hepatocellular hypertrophy (centrilobular)	1/10	0/10	3/10	3/10	1/10	3/10	4/10	3/10	2/10	0/10
grade 1	1	0	3	3	1	3	2	3	0	0
grade 2	0	0	0	0	0	0	2	0	2	0
- hepatocellular hypertrophy (diffuse)	0/10	0/10	0/10	0/10	1/10	3/10	4/10	4/10	10/10***	10/10***
grade 1	0	0	0	0	1	3	4	4	8	5
grade 2	0	0	0	0	0	0	0	0	2	5
- inclusion bodies, eosinophilic, multifocal grade 1	0/10	0/10	1/10	0/10	0/10	0/10	2/10	0/10	6/10*	0/10
- single cell necrosis (multi) focal	1/10	2/10	2/10	3/10	4/10	5/10	6/10	5/10	9/10**	9/10**
grade 1	1	2	1	3	4	5	5	2	5	6
grade 2	0	0	1	0	0	0	1	3	3	3
grade 3	0	0	0	0	0	0	0	0	1	0

[a] includes 5M + 5F for interim kill at 1-month

** $p \leq 0.01$; *** $p \leq 0.001$ (Mann Whitney U test and Fisher Exact test)

F: female; M: male

2.6.7.10.C Carcinogenicity

Report Title: 13-week toxicity study with 1-month interim kill by oral route (dietary admixture) in mice **Test Article:** etravirine

Species/strain: Mouse/CD1
Age/weight at first dose: 8 weeks/18.3 - 36.6 g
Duration of dosing: 4 and 13 weeks
Duration of postdose: 0 days

Administration route/method: Oral/dietary admixture
Doses: 0 (diet), 0 (diet with hydroxypropyl-methylcellulose/microcrystalline cellulose), 450, 1620/800, 2320 and 200 mg/kg body weight/day as spray-dried TMC125 ([redacted] active to polymer ratio), dose correction factor was 3.5.
Test article batch: [redacted]
Vehicle/formulation: diet/hydroxypropyl-methylcellulose and microcrystalline cellulose

Testing facility: [redacted]
Study no.: TMC125-NC195
Location in CTD: 4.2.3.4.1
GLP compliance: Yes
Date start of study: [redacted] 20[redacted]

Special Features: Owing to marked adverse effects the animals given 2320 mg/kg/day were sacrificed after 7 weeks of treatment and the 1620 mg/kg/day dose was reduced to 800 mg/kg/day on Day 50. A new group, comprised of animals from the vehicle groups, was treated at 200 mg/kg/day from Week 9 to the end of Week 13

No Observed Adverse Effect Level: was not established

Toxicokinetics:

Dose (mg/kg/day)	0 (diet)		0 (+ HPMC/MCC)		450		1620/800[f]		2320[g]		200[h]	
No. of animals	M:0	F:0	F:18	F:18	M:36	F:36	M:36	F:36	M:36	F:36	M:6	F:6
Died or killed prematurely	-	-	0	0	1	0	3	0	7	0	0	0
$AUC_{0\text{-}24h}$ Day 7 (µg.h/mL)	-	-	-	-	3.20	4.50	9.76	12.3	18.9	16.8	-	-
$AUC_{0\text{-}24h}$ Day 29 (µg.h/mL)	-	-	-	-	2.95	2.59	8.79	7.98	11.5	7.89	-	-
$AUC_{0\text{-}24h}$ Day 90 (µg.h/mL)	-	-	-	-	1.98	2.59	4.54	3.47	10.2[a]	8.12[a]	2.35[b]	1.53[b]

[a] Day 49; [b] Day 34

Noteworthy Findings:

Dose (mg/kg/day)	0 (diet)		0 (+ HPMC/MCC)		450		1620/800[f]		2320[g]		200[h]	
No. of animals[a]	M:20[e]	F:20[e]	M:24[e]	F:24[e]	M:30	F:30	M:20[e]	F:20[e]	M:24[e]	F:24[e]	M:30	F:30
Mortality (treatment related)	0	0	0	0	1	0	6	0	11	0	0	0
Clinical observations - deteriorating clinical condition	0	0	0	0	0	0	3	1	5	3	0	0

[a] includes 10M + 10F for interim sacrifice at 1-month; [e] Week 1 - 8: 30M/30F, from Week 9: 24M/24F; [f] 1620 until Week 7, 800 from Week 8; [g] until Week 7; [h] from Week 9
F: female; HPMC: hydroxypropyl-methylcellulose; M: male; MCC: microcrystalline cellulose; - no treatment related changes

2.6.7.10.C Carcinogenicity (Continued)

Report Title: 13-week toxicity study with 1-month interim kill by oral route (dietary admixture) in mice **Test Article:** etravirine

Noteworthy Findings (Continued):

Dose (mg/kg/day)	0 (diet)		0 (+ HPMC/MCC)		450		1620/800[f]		2320[g]		200[h]	
No. of animals[a]	**M:20[e]**	**F:20[e]**	**M:24[e]**	**F:24[e]**	**M:30**	**F:30**	**M:20[e]**	**F:20[e]**	**M:24[e]**	**F:24[e]**	**M:30**	**F:30**
Body weight (g) (Week 4 + 13)	-	-	-	-	-	-	-	-	-	-	-	-
Body weight gain (g) (Week 1 – 4)[b]	3.7	2.5	0.62	1.20	0.78	1.08	0.92	1.16	0.43	1.44	-	-
Food consumption (g/week)	-	-	-		-	-	-	-	-	-	-	-
Ophthalmology	-	-	-	-	-	-	-	-	-	-	-	-
Hematology (Week 13)[b]												
- platelet (g/L)	1157	1144	1.04	0.87	1.10	1.02	1.29**	1.05	1.31[c]	1.09[c]	0.94[d]	1.08[d]
Serum chemistry (Week 13)[b]												
- ALP (U/Ll)	158	221	0.84	0.89	2.59**	1.15	1.99**	1.58	6.30[c]	2.38[c]**	2.10[d]*	1.02[d]
- AST (U/L)	172	308	1.02	0.54	1.27	1.16	1.60	1.18	2.11[c]	1.15[c]	1.48[d]	1.15[d]
- ALT (U/L)	57	51	0.67	0.78	1.47	4.04**	2.39**	4.16**	4.98[c]	6.51[c]**	1.51[d]	2.35[d]**
- triglyceride (mmol/L)	0.91	0.85	0.82	1.13	1.01	1.39	1.63*	1.41	2.38[c]*	2.18[c]**	1.04[d]	1.27[d]
Organ weights (Week 4)[b]												
- liver weight (%)	4.53	4.90	1.08	1.01	1.45**	1.45**	1.81**	1.83**	1.93**	1.82**	1.28[d]	1.35[d]
Organ weights (Week 13)[b]												
- liver weight (%)	4.41	4.96	1.01	1.00	1.46**	1.51**	1.79**	1.79**	2.31[c]	2.43[c]	1.31[d]	1.33[d]
Gross pathology (no. examined) (Week 13)	**10**	**10**	**14**	**14**	**20**	**20**	**20**	**20**	**20[c]**	**20[c]**	**10[d]**	**10[d]**
- liver, enlargement	0	0	0	0	0	2	4	5	9	6	0	0
- thorax, reddish content	0	0	0	0	0	0	3	0	6	0	0	0
Histopathology (no. examined) (Week 4)												
Heart	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	N/A	N/A
- pericarditis	0/10	0/10	0/10	0/10	0/10	0/10	2/10	0/10	6/10	0/10	N/A	N/A
grade 1	0	0	0	0	0	0	2	0	2	0	N/A	N/A
grade 2	0	0	0	0	0	0	0	0	4	0	N/A	N/A

[a] includes 10M + 10F for interim kill at 1-month; [b] for vehicle group means are shown. For treated groups, the multiples of vehicle/baseline are shown. Statistical significance based on actual data; [c] Week 7 of treatment; [d] 5 weeks of treatment; [e] Week 1 - 8: 30M/30F, from Week 9: 24M/24F; [f] 1620 until Week 7, 800 from Week 8; [g] until Week 7; [h] from Week 9

* $p \leq 0.05$; ** $p \leq 0.01$ (Hematology and serum chemistry: Dunnet test; organ weights: Dunn's test)

ALP: alkaline phosphatase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; F: female; HPMC: hydroxypropyl-methylcellulose; M: male; MCC: microcrystalline cellulose; - no treatment related changes

2.6.7.10.C Carcinogenicity (Continued)

Report Title: 13-week toxicity study with 1-month interim kill by oral route (dietary admixture) in mice **Test Article:** etravirine

Noteworthy Findings (Continued):

Dose (mg/kg/day)	0 (diet)		0 (+ HPMC/MCC)		450		1620/800[f]		2320[g]		200[h]	
No. of animals[a]	**M:20[e]**	**F:20[e]**	**M:24[e]**	**F:24[e]**	**M:30**	**F:30**	**M:30**	**F:30**	**M:30**	**F:30**	**M:10**	**F:10**
Histopathology (Week 4) (Continued)												
Heart	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	N/A	N/A
- degenerative/necrotic changes	0/10	0/10	0/10	0/10	0/10	0/10	4/10	0/10	7/10	0/10	N/A	N/A
grade 1	0	0	0	0	0	0	0	0	2	0	N/A	N/A
grade 2	0	0	0	0	0	0	0	0	2	0	N/A	N/A
grade 3	0	0	0	0	0	0	3	0	2	0	N/A	N/A
grade 4	0	0	0	0	0	0	1	0	1	0	N/A	N/A
- interstitial hemorrhage	0/10	0/10	0/10	0/10	0/10	0/10	4/10	0/10	6/10	0/10	N/A	N/A
grade 1	0	0	0	0	0	0	0	0	2	0	N/A	N/A
grade 2	0	0	0	0	0	0	2	0	2	0	N/A	N/A
grade 3	0	0	0	0	0	0	2	0	2	0	N/A	N/A
Liver	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	N/A	N/A
- hepatocellular hypertrophy	0/10	0/10	0/10	0/10	10/10	10/10	10/10	10/10	10/10	10/10	N/A	N/A
grade 1	0	0	0	0	1	0	0	0	0	0	N/A	N/A
grade 2	0	0	0	0	8	8	1	3	4	3	N/A	N/A
grade 3	0	0	0	0	1	2	9	7	5	7	N/A	N/A
grade 4	0	0	0	0	0	0	0	0	1	0	N/A	N/A
- degenerative/necrotic changes	0/10	0/10	0/10	0/10	0/10	2/10	1/10	1/10	1/10	5/10	N/A	N/A
grade 1	0	0	0	0	0	2	0	1	0	1	N/A	N/A
grade 2	0	0	0	0	0	0	1	0	1	2	N/A	N/A
grade 3	0	0	0	0	0	0	0	0	0	2	N/A	N/A
- focal coagulative necrosis	0/10	1/10	0/10	0/10	0/10	0/10	3/10	0/10	2/10	2/10	N/A	N/A
grade 1	0	0	0	0	0	0	0	0	0	0	N/A	N/A
grade 2	0	1	0	0	0	0	2	0	1	2	N/A	N/A
grade 3	0	0	0	0	0	0	1	0	1	0	N/A	N/A

[a] includes 10M + 10F for interim kill at 1-month; [e] Week 1 – 8: 30M/30F, from Week 9: 24M/24F; [f] 1620 until Week 7, 800 from Week 8; [g] until Week 7; [h] from Week 9

F: female; HPMC: hydroxypropyl-methylcellulose; M: male; MCC: microcrystalline cellulose

2.6.7.10.C Carcinogenicity (Continued)

Report Title: 13-week toxicity study with 1-month interim kill by oral route (dietary admixture) in mice **Test Article:** etravirine

Noteworthy Findings (Continued):

Dose (mg/kg/day)	0 (diet)		0 (+ HPMC/MCC)		450		1620/800[f]		2320[g]		200[h]	
No. of animals[a]	M:20[e]	F:20[e]	M:24[e]	F:24[e]	M:30	F:30	M:30	F:30	M:30	F:30	M:10	F:10
Histopathology (Week 13) (Continued)												
Heart	**10**	**10**	**14**	**14**	**20**	**20**	**20**	**20**	**20[c]**	**20[c]**	**10[d]**	**10[d]**
- pericarditis	0/10	0/10	0/14	0/14	1/20	0/20	4/20	0/20	9/20	0/20	0/10	0/10
grade 1	0	0	0	0	0	0	0	0	5	0	0	0
grade 2	0	0	0	0	1	0	4	0	3	0	0	0
grade 3	0	0	0	0	0	0	0	0	1	0	0	0
- degenerative/necrotic changes	0/10	0/10	0/14	0/14	1/20	0/20	4/20	0/20	11/20	0/20	0/10	0/10
grade 1	0	0	0	0	0	0	0	0	1	0	0	0
grade 2	0	0	0	0	0	0	0	0	2	0	0	0
grade 3	0	0	0	0	1	0	4	0	8	0	0	0
- interstitial hemorrhage	0/10	0/10	0/14	0/14	1/20	0/20	4/20	0/20	11/20	0/20	0/10	0/10
grade 1	0	0	0	0	0	0	1	0	1	0	0	0
grade 2	0	0	0	0	0	0	0	0	7	0	0	0
grade 3	0	0	0	0	1	0	3	0	3	0	0	0
Liver	**10**	**10**	**14**	**14**	**20**	**20**	**20**	**20**	**20[c]**	**20[c]**	**10[d]**	**10[d]**
- hepatocellular hypertrophy	0/10	0/10	0/14	0/14	12/20	10/20	20/20	20/20	20/20	20/20	0/10	0/10
grade 1	0	0	0	0	1	1	0	0	0	0	0	0
grade 2	0	0	0	0	5	5	2	7	6	6	0	0
grade 3	0	0	0	0	6	4	16	11	13	12	0	0
grade 4	0	0	0	0	0	0	2	2	1	2	0	0
- degenerative/necrotic changes	0/10	0/10	0/14	0/14	2/20	4/20	2/20	1/20	5/20	2/20	0/10	0/10
grade 1	0	0	0	0	1	3	1	0	2	2	0	0
grade 2	0	0	0	0	1	1	1	1	3	0	0	0
- focal coagulative necrosis	0/10	1/10	0/14	2/14	3/20	1/20	3/20	1/20	6/20	2/20	0/10	0/10
grade 1	0	1	0	1	2	1	1	0	1	1	0	0
grade 2	0	0	0	1	0	0	2	0	4	1	0	0
grade 3	0	0	0	0	1	0	0	1	1	0	0	0

[a] includes 10M + 10F for interim kill at 1-month; [c] week 7 of treatment; [d] 5 weeks of treatment; [e] Week 1 – 8: 30M/30F, from Week 9: 24M/24F; [f] 1620 until Week 7, 800 from Week 8; [g] until Week 7; [h] from Week 9

F: female; HPMC: hydroxypropyl-methylcellulose; M: male; MCC: microcrystalline cellulose

2.6.7.10.D Carcinogenicity

Report Title: 3-month repeated dose oral toxicity study with 1-month interim kill in the rat **Test Article:** etravirine

Species/strain: Rat/Sprague-Dawley
Age/weight at first dose: 6 weeks/137 - 223 g
Duration of dosing: 13 weeks
Duration of postdose: 0 days

Administration route/method: Oral/gavage
Doses: 0 (vehicle), 70, 200 and 600 mg base eq./kg body weight/day as TMC125 HBr
Test article batch: [redacted]
Vehicle/formulation: Polyethylene glycol 400

Testing facility: J&J PRD
Study no.: TMC125-NC140
Location in CTD: 4.2.3.4.1
GLP compliance: Yes
Date start of study: [redacted] 20[redacted]

No Observed Adverse Effect Level: was not established

Toxicokinetics:

Dose (mg base eq./kg/day)	0 (vehicle)		70		200		600	
No. of animals	M:0	F:0	M:6	F:6	M:6	F:6	M:6	F:6
Died or killed prematurely (unrelated to treatment)	0	0	0	0	1	0	0	0
$AUC_{0-\infty}$ Day 0 (µg.h/mL)	-	-	0.79	1.14	1.64	1.93	2.61	5.93
AUC_{0-24h} Day 30 (µg.h/mL)			0.53	1.05	0.99	2.01	1.47	6.85
AUC_{0-24h} Day 86 (µg.h/mL)	-	-	1.00	1.41	0.74	2.28	2.29	6.82

Noteworthy Findings:

Dose (mg base eq./kg/day)	0 (vehicle)		70		200		600	
No. of animals	M:20	F:20	M:20	F:20	M:20	F:20	M:20	F:20
Died or killed prematurely (unrelated to treatment)	0	1	1	0	0	0	0	0
Clinical observations	-	-	-	-	-	-	-	-
Body weight (g) (Week 13)[a]	501	285	1.01	1.01	1.06	0.98	0.92*	1.05
Body weight gain (g) (Week 13)[a]	305	136	1.01	1.02	1.09	0.97	0.87*	1.07
Food consumption (g/week) (Week 13)[a]	147	105	1.03	1.01	1.03	0.98	0.91*	1.07
Ophthalmoscopy	-	-	-	-	-	-	-	-
Hematology (Week 13)[a]								
- neutrophils (10^3/µL)	1.37	0.92	1.26	1.33	1.45**	1.52*	1.89	1.38
- monocytes (10^3/µL)	0.45	0.25	1.00	1.40*	1.11	1.48*	1.20	1.28
- eosinophils (10^3/µL)	0.20	0.13	0.85	1.08	1.05	0.85	0.60*	0.77
Serum Chemistry (Week 13)[a]								
- total protein (g/dL)	6.8	7.0	1.04	1.06*	1.09***	1.09***	1.09**	1.16***
- bilirubin (mg/dL)	0.12	0.12	0.92	1.00	0.75***	0.75***	0.58***	0.50***

[a] for vehicle group means are shown. For treated groups, the multiples of vehicle/baseline are shown. Statistical significance based on actual data.
* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$ (Mann Whitney U test)
F: female; M: male; - no treatment related changes

2.6.7.10.D Carcinogenicity (Continued)

Report Title: 3-month repeated dose oral toxicity study with 1-month interim kill in the rat **Test Article:** etravirine

Noteworthy Findings (Continued):

Dose (mg base eq./kg/day)	0 (vehicle)		70		200		600	
No. of animals	**M:20**	**F:20**	**M:20**	**F:20**	**M:20**	**F:20**	**M:20**	**F:20**
Serum Chemistry (continued)								
- albumin (g/dL)	4.6	5.1	1.00	1.02	1.02	1.02	0.99	1.10**
- glucose (mg/dL)	93	114	0.92*	0.82***	0.97	0.85*	0.99	0.88**
- urea nitrogen (mg/dL)	17.3	17.4	0.97	1.07	0.91	0.98	0.87*	0.89
Endocrinology (Week 13)[a]								
- TSH (ng/mL)	6.3	4.2	1.03	1.07	0.97	1.10	1.43	2.07*
- T4 (nmol/L)	77	46	0.77*	0.70**	0.64**	0.57**	0.40***	0.67*
- T3 (nmol/L)	0.85	0.89	0.87	0.94	0.74*	0.99	0.71**	1.11
Urinalysis (Week 13)								
- proteins (score)	1.00	0.00	1.33	0.40	1.10	0.30	1.20	1.40**
- occult blood (score)	0.80	0.89	0.89	0.30	1.20	0.60	2.00*	0.90
Organ weights (Week 13)[a]								
- adrenal (%)	0.012	0.026	1.01	0.97	1.03	1.02	1.25**	1.16
- liver (%)	2.64	2.68	1.07*	1.01	1.06*	1.05	1.15***	1.23***
Gross pathology (Week 13)								
Liver	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
- more pronounced lobulation	0/10	0/10	0/10	0/10	0/10	0/10	2/10	1/10
Histopathology (Week 4: interim kill)								
Adrenal gland	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
- swollen zona fasciculata cells, diffuse grade 1	0/10	0/10	0/10	1/10	0/10	0/10	0/10	4/10
Liver	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
- vacuolization (fatty-like), large grade 1	0/10	0/10	0/10	3/10	1/10	2/10	1/10	4/10
- vacuolization (fatty-like), small - perilobular	2/10	6/10	3/10	5/10	4/10	7/10	2/10	8/10
grade 1	1	5	2	4	4	5	2	6
grade 2	1	1	1	1	0	2	0	2

[a] for vehicle group means are shown. For treated groups, the multiples of vehicle/baseline are shown. Statistical significance based on actual data.
* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$ (Organ weight, urinalysis, endocrinology and serum chemistry: Mann Whitney U test; histopathology: Fisher Exact test)
F: female; M: male; - no treatment related changes; T3: triiodothyronine; T4: tetraiodothyronine/thyroxine; TSH: thyroid stimulating hormone

2.6.7.10.D Carcinogenicity (Continued)

Report Title: 3-month repeated dose oral toxicity study with 1-month interim kill in the rat **Test Article:** etravirine

Noteworthy Findings (Continued):

Dose (mg base eq./kg/day)	0 (vehicle)		70		200		600	
No. of animals	M:20	F:20	M:20	F:20	M:20	F:20	M:20	F:20
Histopathology (Week 4: interim kill) (continued)								
Thyroid gland								
- high follicular epithelium grade 1	2/10	0/10	2/10	1/10	7/10	4/9*	10/10***	7/9***
- small follicles	2/10	1/10	1/10	2/10	4/10	2/9	7/10	1/9
grade 1	2	1	1	2	4	1	6	1
grade 2	0	0	0	0	0	1	1	0
Histopathology (Week 13: terminal kill)								
Adrenal gland	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
- swollen zona fasciculata cells, diffuse grade 1	0/10	0/10	0/10	0/10	0/10	0/10	0/10	6/10*
Kidneys	**10**	**10**	**3**	**10**	**2**	**10**	**10**	**10**
- basophilic tubuli, (multi)focal grade 1	6/10	4/10	1/3	3/10	0/2	3/10	5/10	7/10
- hyaline cast(s)	0/10	2/10	0/3	2/10	0/2	2/10	0/10	4/10
grade 1	0	2	0	2	0	2	0	3
grade 2	0	0	0	0	0	0	0	1
Liver	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
- vacuolization (fatty-like), large	1/10	0/10	0/10	2/10	2/10	2/10	3/10	5/10*
grade 1	1	0	0	2	2	2	3	4
grade 2	0	0	0	0	0	0	0	1
- vacuolization (fatty-like), small - perilobular	1/10	3/10	0/10	4/10	0/10	8/10	3/10	6/10
grade 1	1	3	0	3	0	6	2	2
grade 2	0	0	0	1	0	2	1	4
- Hyperthrophy (centrilobular) grade 1	0/10	0/10	0/10	0/10	0/10	0/10	1/10	1/10
Thyroid gland	**10**	**10**	**10**	**10**	**10**	**9**	**10**	**10**
- high follicular epithelium	7/10	2/10	6/10	2/10	10/10	2/9	10/10	9/10**
grade 1	6	2	5	2	7	2	6	9
grade 2	1	0	1	0	3	0	4	0

* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$ (Fisher Exact test)
F: female; M: male

2.6.7.10.D Carcinogenicity (Continued)

Report Title: 3-month repeated dose oral toxicity study with 1-month interim kill in the rat **Test Article:** etravirine

Noteworthy Findings (Continued):

Dose (mg base eq./kg/day)	0 (vehicle)		70		200		600	
No. of animals	**M:20**	**F:20**	**M:20**	**F:20**	**M:20**	**F:20**	**M:20**	**F:20**
Histopathology (Week 13: terminal kill)								
- small follicles	3/10	1/10	4/10	1/10	6/10	4/9	10/10**	6/10
grade 1	3	1	3	1	6	4	3	1
grade 2	0	0	1	0	0	0	7	5

** $p \leq 0.01$ (Fisher Exact test)
F: female; M: male

2.6.7.10.E Carcinogenicity – Non-pivotal

Report Title: 13-week toxicity study with 1-month interim kill by oral route (dietary admixture) in rats **Test Article:** etravirine

Species/strain: Rat/Sprague-Dawley
Age/weight at first dose: 8 weeks/M = 246 - 365 g, F = 170 - 247 g
Duration of dosing: 4 weeks (interim kill) + 13 weeks
Duration of postdose: 0 days

Administration route/method: Oral/dietary admixture
Doses: 0 (diet), 0 (diet with hydroxypropyl-methylcellulose/microcrystalline cellulose), 330, 990 and 1300 mg/kg body weight/day as spray-dried TMC125 ([redacted] active to polymer ratio), dose correction factor was 3.5.
Test article batch: [redacted]
Vehicle/formulation: diet/hydroxypropyl-methylcellulose and microcrystalline cellulose

Testing facility: [redacted]
Study no.: TMC125-NC196
Location in CTD: 4.2.3.4.1
GLP compliance: Yes
Date start of study: [redacted] 20[redacted]

No Observed Adverse Effect Level: was not established

Toxicokinetics:

Dose (mg/kg/day)	0 (diet)		0 (+ HPMC/MCC)		330		990		1300	
No. of animals	M:0	F:0	M:3	F:3	M:6	F:6	M:6	F:6	M:6	F:6
Died or killed prematurely (unrelated to treatment)	0	0	0	0	0	0	0	2	0	0
$AUC_{0\text{-}24h}$ Day 7 (µg.h/mL)	-	-	-	-	0.91	2.37	1.51	4.73	2.14	7.12
$AUC_{0\text{-}24h}$ Day 31 (µg.h/mL)	-	-	-	-	1.07	3.02	1.35	4.87	2.07	4.09
$AUC_{0\text{-}24h}$ Day 84 (µg.h/mL)	-	-	-	-	1.21	2.95	2.09	6.73	2.96	5.34

Noteworthy Findings:

Dose (mg/kg/day)	0 (diet)		0 (+ HPMC/MCC)		330		990		1300	
No. of animals	M: 30	F: 30	M: 30	F: 30	M: 30	F: 30	M: 30	F: 30	M: 30	F: 30
Died or killed prematurely	0	0	0	0	0	0	0	0	0	0
Clinical observations	-	-	-	-	-	-	-	-	-	-
Body weight (g) (Week 13)[a]	550	306	0.98	0.93*	1.01	0.95	0.92*	0.96	0.93*	0.93
Body weight gain (g) (Difference from Vehicle + HPMC/MCC)	-	-	221	81	1.02	0.99	0.91	1.04	0.90	0.88
Food consumption (g/week)	-	-	-	-	-	-	-	-	-	-
Ophthalmoscopy	-	-	-	-	-	-	-	-	-	-

[a] for vehicle group means are shown. For treated groups, the multiples of controls are shown. Statistical significance versus controls based on actual data.
* $p \leq 0.05$ (Dunnet test)
F: female; HPMC: hydroxypropyl-methylcellulose; M: male; MCC: microcrystalline cellulose; - no treatment related changes

2.6.7.10.E Carcinogenicity – Non-pivotal (Continued)

Report Title: 13-week toxicity study with 1-month interim kill by oral route (dietary admixture) in rats **Test Article:** etravirine

Noteworthy Findings (Continued)

Dose (mg/kg/day)	0 (diet)		0 (+ HPMC/MCC)		330		990		1300	
No. of animals	**M: 30**	**F: 30**	**M: 30**	**F: 30**	**M: 30**	**F: 30**	**M: 30**	**F: 30**	**M: 30**	**F: 30**
Hematology (Week 13)[a]										
- PT (s)	15.0	15.8	1.01	0.95**	1.07*	0.91**	1.17**	0.87**	1.22**	0.88**
- APTT (s)	19.9	15.3	1.06	1.04	1.31**	1.05	1.50**	1.10**	1.61**	1.06
Serum chemistry	-	-	-	-	-	-	-	-	-	-
Urinalysis	-	-	-	-	-	-	-	-	-	-
Organ weights (Week 13)[a]										
- liver (%)	2.79	2.84	0.96	1.02	1.05	1.14**	1.12**	1.24**	1.12**	1.27**
- thyroid (%)	0.005	0.007	0.92	0.89	1.06	1.04	1.26**	1.16	1.30**	1.15
Gross pathology	-	-	-	-	-	-	-	-	-	-
Histopathology (Interim kill: Week 4)										
Thyroid glands (no. examined)	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
- morphological changes indicative of increased activity	9/10	7/10	10/10	9/10	10/10	9/10	10/10	9/10	10/10	10/10
grade 1	4	7	6	3	4	5	0	1	0	0
grade 2	4	0	3	5	5	4	6	4	5	5
grade 3	1	0	1	1	1	0	4	4	5	5
Histopathology (Terminal kill: Week 13)										
Thyroid glands (no. examined)	**20**	**20**	**20**	**20**	**20**	**20**	**20**	**20**	**20**	**20**
- morphological changes indicative of increased activity	19/20	15/20	19/20	15/20	19/20	20/20	20/20	20/20	20/20	20/20
grade 1	11	9	8	5	5	7	1	0	4	3
grade 2	6	5	9	6	12	11	12	11	7	5
grade 3	2	1	2	4	2	2	7	9	9	11
grade 4	0	0	0	0	0	0	0	0	0	1

[a] for vehicle group means are shown. For treated groups, the multiples of controls are shown. Statistical significance versus controls based on actual data.
* $p \leq 0.05$; ** $p \leq 0.01$ (Dunnett test, Dunn's test)
APTT: activated partial thromboplastin time; F: female; HPMC: hydroxypropyl-methylcellulose; M: male; MCC: microcrystalline cellulose; PT: prothrombin time; - no treatment related changes

2.6.7.10.F Carcinogenicity – Non-pivotal

Report Title: 1-month toxicity study by oral route (gavage) in CB6F1-nonTgrasH2 mice **Test Article:** etravirine

Species/strain: Mouse/CB6F1-nonTgrasH2
Age/weight at first dose: 10 weeks/20.3 - 33.3 g
Duration of dosing: 4 weeks
Duration of postdose: 0 days

Administration route/method: Oral/gavage
Doses: 0 (vehicle), 50, 200, 400 and 800 mg base eq./kg body weight/day as TMC125 HBr
Test article batch: [redacted]
Vehicle/formulation: Polyethylene glycol 400

Testing facility: [redacted]
Study no.: TMC125-NC197
Location in CTD: 4.2.3.4.3
GLP compliance: Yes
Date start of study: [redacted] 20[redacted]

No Observed Adverse Effect Level: 50 mg/kg/day in the absence of any relevant changes

Toxicokinetics:

Dose (mg base eq./kg/day)	0 (vehicle)		50		200		400		800	
No. of animals	M:9	F:9	M:13	F:13	M:13	F:13	M:13	F:13	M:13	F:13
Died or killed prematurely (unrelated to treatment)	0	0	0	1	0	1	1	1	1R	0
$AUC_{0-\infty}$ Day 1 (µg.h/mL)	-	-	2.41[a]	2.45	7.04	4.06	13.4	8.91	33.2	21.2
AUC_{0-24h} Day 28 (µg.h/mL)	-	-	1.15[a]	1.22	2.39	1.86	3.88	3.26	6.11	6.58

[a] AUC_{0-12h} reported

Noteworthy Findings:

Dose (mg base eq./kg/day)	0 (vehicle)		50		200		400		800	
No. of animals	M:10	F:10	M:10	F:10	M:10	F:10	M:10	F:10	M:10	F:10
Died or killed prematurely (unrelated to treatment)	0	0	0	0	0	0	0	0	0	1R
Clinical observations	-	-	-	-	-	-	-	-	-	-
Body weight (g)	-	-	-	-	-	-	-	-	-	-
Food consumption (g/week)	-	-	-	-	-	-	-	-	-	-
Hematology[a]										
- platelet count (G/L)	1264	1128	1.04	1.13	1.12*	1.15	1.06	1.21**	1.10	1.23**
- APTT (s)[b]	22.2	23.2	1.05	0.97	1.04	1.00	1.02	0.91	1.22**	1.04

[a] for vehicle group means are shown. For treated groups, the multiples of vehicle/baseline are shown. Statistical significance based on actual data; [b] APTT was examined in satellites, not in the main study animals
* $p \leq 0.05$; ** $p \leq 0.01$ (Dunnet test, Dunn's test)
APTT: activated partial thromboplastin time; F: female; M: male; R: replaced; - no treatment related changes

2.6.7.10.F Carcinogenicity – Non-pivotal (Continued)

Report Title: 1-month toxicity study by oral route (gavage) in CB6F1-nonTgrasH2 mice **Test Article:** etravirine

Noteworthy Findings (Continued):

Dose (mg base eq./kg/day)	0 (vehicle)		50		200		400		800	
No. of main animals	M:10	F:10	M:10	F:10	M:10	F:10	M:10	F:10	M:10	F:10
Serum chemistry[a]										
- potassium (mmol/L)	4.76	5.11	1.06	0.92	1.02	0.90	0.93	0.85*	0.94	0.86
- ALT (IU/L)	65	48	0.63	2.52	1.62	2.00	0.74	5.27*	1.02	5.85*
- cholesterol (mmol/dL)	2.1	1.6	1.19	1.13	1.05	1.13	1.10	1.19	1.10	1.25*
- triglyceride (mmol/dL)	0.56	0.48	0.95	1.10	0.88	1.04	1.11	1.17	1.95**	1.38
Organ weights[a]										
- liver (%)	4.52	4.68	1.10	1.10	1.18**	1.25**	1.23**	1.26**	1.62**	1.52**
Gross pathology	-	-	-	-	-	-	-	-	-	-
Histopathology										
Adrenal glands (no. examined)	**9**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**8**	**10**
- cortical cell hypertrophy	0/9	0/10	0/10	0/10	4/10	4/10	3/10	4/10	8/8	10/10
grade 1	0	0	0	0	2	1	0	1	0	0
grade 2	0	0	0	0	2	3	3	3	4	5
grade 3	0	0	0	0	0	0	0	0	4	5
Liver (no. examined)	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
- hepatocellular hypertrophy	0/10	0/10	0/10	0/10	10/10	10/10	10/10	10/10	10/10	10/10
grade 1	0	0	0	0	1	5	1	4	0	0
grade 2	0	0	0	0	8	5	7	5	1	2
grade 3	0	0	0	0	1	0	2	1	8	8
grade 4	0	0	0	0	0	0	0	0	1	0
- vacuolated hepatocytes	0/10	0/10	0/10	0/10	8/10	6/10	5/10	6/10	10/10	10/10
grade 1	0	0	0	0	2	5	1	4	0	0
grade 2	0	0	0	0	6	1	4	1	7	8
grade 3	0	0	0	0	0	0	0	1	2	2
grade 4	0	0	0	0	0	0	0	0	1	0

[a] for vehicle group means are shown. For treated groups, the multiples of vehicle/baseline are shown. Statistical significance based on actual data.
* $p \leq 0.05$; ** $p \leq 0.01$ (Dunnet test and Dunn's test)
ALT: alanine aminotransferase; F: female; M: male; - no treatment related changes

2.6.7.10.F Carcinogenicity – Non-pivotal (Continued)

Report Title: 1-month toxicity study by oral route (gavage) in CB6F1-nonTgrasH2 mice **Test Article:** etravirine

Noteworthy Findings (Continued):

Dose (mg base eq./kg/day)	0 (vehicle)		50		200		400		800	
No. of main animals	M:10	F:10	M:10	F:10	M:10	F:10	M:10	F:10	M:10	F:10
Liver (continued, no. examined)	10	10	10	10	10	10	10	10	10	10
- hepatocellular degeneration/necrosis	0/10	0/10	0/10	0/10	0/10	1/10	0/10	2/10	0/10	4/10
grade 1	0	0	0	0	0	1	0	2	0	2
grade 2	0	0	0	0	0	0	0	0	0	1
grade 3	0	0	0	0	0	0	0	0	0	1

F: female; M: male

2.6.7.11. Reproductive and Developmental Toxicity: Non-pivotal studies

Test article: etravirine

Species/Strain Sex/No. Per Group	Route/Method of Administration (Vehicle/ Formulation)	Duration of Dosing	Doses (mg/kg/day)	Batch no.	NOAEL[a] (mg/kg)	Noteworthy Findings	Study No./ Location in CTD
Rat/Wistar 6 pregnant F/group	Oral/gavage (PEG400)	Day 6 to 16 of gestation	Vehicle, 250, 500, 1000 as TMC125 HBr	[redacted]	1000	One death unrelated to treatment (1000: 1/6 F). No effect on the evaluated maternal or fetal parameters.	TMC125-NC123/ 4.2.3.5.2
Rabbit/New Zealand white 2 F/group	Oral/gavage (TPGS + HPMC)	5 days	210, 500, as TMC125K-G23, 750 as TMC125 HBr for suspension	[redacted] (suspension), [redacted] (powder for suspension)	750	No consistent adverse findings. Clinical signs in one high dosed female. TK data collected.	TMC125-NC127/ 4.2.3.5.2
Rabbit/New Zealand white 4 F/group	Oral/gavage (TPGS + HPMC)	5 days	Vehicle, 750, 1125, 1500 (750 b.i.d.) as TMC125 HBr	TMC125[redacted]	1125/750 b.i.d.	One death unrelated to treatment (vehicle: 1/4 F). No adverse findings. TK data collected.	TMC125-NC178/ 4.2.3.5.2
Rabbit/New Zealand white 5 pregnant F/group	Oral/gavage (HPMC + MCC)	Day 6 to 18 of gestation	Vehicle, 125, 250, 525 as spray-dried TMC125 ([redacted])	[redacted]	525 (maternal) 250 (fetal)	No relevant effect on the evaluated maternal parameters. Increased number pre- and post-implantation losses in one female dosed at 525 mg/kg.. TK data collected.	TMC125-NC235/ 4.2.3.5.2
Rat/Sprague-Dawley 12 pregnant F/group (6 main; 6 satellites/group)	Oral/gavage (HPMC)	Day 7 of gestation to Day 7 postpartum (TK satellites from Day 7 to 17 of gestation)	Vehicle, 125, 250, 500 as spray-dried TMC125 ([redacted])	[redacted]	500	Four deaths unrelated to treatment (125: 1/12 F, 250: 3/12F). No effect on the evaluated maternal or fetal parameters. TK data collected.	TMC125-NC236[a]/ 4.2.3.5.3

[a] GLP compliant

b.i.d.: twice daily; F: female; HPMC: hydroxypropyl-methylcellulose; MCC: microcrystalline cellulose; NOAEL: no observed effect level; PEG: polyethylene glycol; TK: toxicokinetic; TPGS: alpha-tocopheryl polyethylene glycol succinate

2.6.7.12.A Reproductive and Developmental Toxicity – Fertility and Early Embryonic Development to Implantation

Report Title: TMC125K (HBr): Fertility and early embryonic toxicity study by oral gavage in Wistar rats **Test Article:** etravirine

Species/strain: Rat/Wistar
Age/weight at first dose: 11 - 14 weeks/mean 398g M, 262 g F
Duration of dosing: M: 4 weeks before mating until necropsy; F: 2 weeks prior to mating up to Gestation Day (GD) 8
Duration of postdose: 0 days
Day of mating: GD0
Day of c-section: GD21

Administration route/method: Oral/gavage
Doses: 0, 127, 253 and 506 mg base eq./kg body weight/day as TMC125 HBr
Test article batch: [redacted]
Vehicle/formulation: Polyethylene glycol 400

Testing facility: [redacted]
Study no.: TMC125-NC125
Location in CTD: 4.2.3.5.1
GLP compliance: Yes
Date start of study: [redacted] 20[redacted]

Special features: none
No Observed Adverse Effect Level: 506 mg/kg/day in the absence of any effects

Toxicokinetics: Not determined

Noteworthy Findings:

Daily dose (mg base eq./kg)	0 (vehicle)		127		253		506	
No. of animals	M:24	F:24	M:24	F:24	M:24	F:24	M:24	F:24
Died or killed prematurely (unrelated to treatment)	1	0	1	0	2	0	1	1
Clinical observations	-	-	-	-	-	-	-	-
Body weight (g)	-	-	-	-	-	-	-	-
Food consumption (g/week)	-	-	-	-	-	-	-	-
Estrus cycle	-	-	-	-	-	-	-	-
Gross pathology	-	-	-	-	-	-	-	-

F: female; GD: gestation day; M: male; - no noteworthy findings

2.6.7.12.A Reproductive and Developmental Toxicity – Fertility and Early Embryonic Development to Implantation (Continued)

Report Title: TMC125K (HBr): Fertility and early embryonic toxicity study by oral gavage in Wistar rats **Test Article:** etravirine

Noteworthy Findings (Continued):

Daily dose (mg base eq./kg)	0 (vehicle)		127		253		506	
No. of animals	M:24	F:24	M:24	F:24	M:24	F:24	M:24	F:24
No. paired	24		24		24		23	
Mated in first period	22		20		23		23	
Mated in second period	1		2		0		0	
No mating	1		2		1		0	
Mean no. days prior to first mating	2.3		2.2		2.7		2.3	
No. pregnant females		22		19		20		22
No. aborted or with total resorption of litter		0		0		0		0
No. females with live fetuses		22		19		20		22
Mean no. corpora lutea		20		20		20		19
Mean no. implantations sites		17		17		17		17
Pre-implantation loss (% of corpora lutea)		11		13		17*		12
Post-implantation loss (% of implantation sites)		3		4		2		4
Embryonic/fetal deaths (% of implantation sites)		3		4		2		3
Embryonic/fetal resorptions (% of implantation sites)		3		4		2		3
Mean no. live conceptuses		17		17		16		16
No. dead conceptuses (% of the fetuses)		0		0		0		1

* $p \leq 0.05$ (Fisher Exact test)
F: female; M: male

2.6.7.12.B Reproductive and Developmental Toxicity – Fertility and Early Embryonic Development to Implantation

Report Title: Oral fertility study of TMC125 in the male and female rat

Test Article: etravirine

Species/strain: Rat/Sprague-Dawley
Age/weight at first dose: 10 - 12 weeks/199 - 236 g
Duration of dosing: M: 4 weeks before mating until necropsy; **F:** 2 weeks prior to mating up to GD7
Duration of post-dose: 0 days
Day of mating: GD0
Day of c-section: GD14

Administration route/method: Oral/gavage
Doses:0, 125, 250 and 500 mg/kg body weight/day as spray-dried TMC125 ([redacted] active to polymer ratio), dose correction factor was 4.5
Test article batch: [redacted]
Vehicle/formulation: hydroxypropyl-methylcellulose + microcrystalline cellulose in demineralized water

Testing facility: J&J PRD
Study no.: TMC125-NC337
Location in CTD: [redacted]
GLP compliance: Yes
Date start of study: [redacted] 20[redacted]

Special features: none
No Observed Adverse Effect Level: > 500 mg/kg/day in the absence of any relevant changes

Toxicokinetics:

Daily dose (mg/kg)	0 (vehicle)		125		250		500	
No. of animals	M:3	F:3	M:3	F:3	M:3	F:3	M:3	F:3
Died or killed prematurely (accidental)	0	0	0	0	0	0	0	0
Died or killed prematurely (unrelated to treatment)	0	0	0	0	0	0	0	0
$AUC_{0-\infty}$ Day 0			2.72	7.79	5.48	9.77	10.7	18.1
AUC_{0-24h} Day 14			NA	5.45	NA	5.02	NA	9.09
AUC_{0-24h} Day 28			1.53	NA	2.77	NA	4.54	NA

Noteworthy Findings:

Daily dose (mg/kg)	0 (vehicle)		125		250		500	
No. of animals	M:24	F:24	M:24	F:24	M:24	F:24	M:24	F:24
Died or killed prematurely (unrelated to treatment)	3	0	0	0	0	0	1	0
Clinical observations	-	-	-	-	-	-	-	-
Body weight gain (g)	-	-	-	-	-	-	-	-
Food consumption (g/week)	-	-	-	-	-	-	-	-
Estrus cycle	NA	-	NA	-	NA	-	NA	-
Gross pathology	-	-	-	-	-	-	-	-

F: female; GD: gestation day; M: male; NA: not applicable; - no noteworthy findings

2.6.7.12.B Reproductive and Developmental Toxicity – Fertility and Early Embryonic Development to Implantation (Continued)

Report Title: Oral fertility study of TMC125 in the male and female rat **Test Article:** etravirine

Noteworthy Findings (Continued):

Daily dose (mg/kg)	0 (vehicle)		125		250		500	
No. of animals	M:24	F:24	M:24	F:24	M:24	F:24	M:24	F:24
No. paired	23		24		24		23	
Mated	23		24		24		23	
No mating	0		0		0		0	
Mean no. days prior to first mating	2		2		2		3	
No. pregnant females		22		24		24		23
No. aborted or with total resorption of litter		0		0		0		0
No. females with live fetuses		22		24		24		23
Mean no. corpora lutea		17.0		17.5		16.0**		16.3*
Mean no. implantations sites		15.9		15.6		15.1		14.0*
Pre-implantation loss (% of corpora lutea)		7.07		9.55		5.24		13.42
Postimplantation loss (% of implantation sites)		5.76		7.71		6.09		9.64
Embryonic/fetal deaths (% of implantation sites)		ND		ND		ND		ND
Embryonic/fetal resorptions (mean)		0.91		1.17		0.88		0.91
Mean no. live conceptuses		15.0		14.4		14.3		13.1*
No. dead conceptuses (% of the fetuses)		ND		ND		ND		ND

* $p < 0.05$; ** $p < 0.01$ (Mann-Whitney U test)
F: female; M: male; ND: not determined

2.6.7.13.A Reproductive and Developmental Toxicity – Effects on Embryo-Fetal Development

Report Title: TMC125K (HBr): Embryo-foetal developmental toxicity study by oral gavage in female Wistar rats **Test Article:** etravirine

Species/strain: Rat/Wistar
Age/weight at pairing: about 10 weeks/207 - 302 g
Duration of dosing: Gestation Days (GD) 6-16
Day of mating: GD0
Day of c-section: GD21

Administration route/method: Oral/gavage
Doses: 0, 250, 500 and 1000 mg base eq./kg body weight/day, as TMC125 HBr
Test article batch: [redacted]
Vehicle/formulation: Polyethylene glycol 400

Testing facility: [redacted]
Study no.: TMC125-NC123
Location in CTD: 4.2.3.5.2
GLP compliance: Yes
Date start of study: [redacted] 20[redacted]

Special features: None
No Observed Adverse Effect Level: The maternal and embryo-fetal NOAEL was 1000 mg/kg based on the absence of any effect. TMC125 is considered not to be teratogenic in rats

Toxicokinetics:

Dose (mg base eq./kg/day)	0 (vehicle)	250	500	1000
No. of animals	F:2	F:6	F:4	F:6
Died or killed prematurely (unrelated to treatment)	0	0	1	1
$AUC_{0\text{-last}}$ GD16 (µg.h/mL)	NR	3.26	7.94	8.27

Noteworthy Findings

Daily dose (mg base eq./kg)	0 (vehicle)	250	500	1000
No. of animals	F:24	F:20	F:22	F:20
Maternal data:				
Died or killed prematurely (unrelated to treatment)	0	0	3	0
Clinical observations	-	-	-	-
Body weight (g)	-	-	-	-
Food consumption (g)	-	-	-	-
Gross pathology	-	-	-	-
No. mated	24	20	22	20
No. pregnant females	23	19	17	19
No. aborted or with total resorption of litter	0	1	0	1

F: female; GD: gestation day; NR: not reported; - no noteworthy findings

2.6.7.13.A Reproductive and Developmental Toxicity – Effects on Embryo-Fetal Development (Continued)

Report Title: TMC125K (HBr): Embryo-foetal developmental toxicity study by oral gavage in female Wistar rats **Test Article:** etravirine

Noteworthy Findings (Continued)

Daily dose (mg base eq./kg) **No. of animals**	**0 (vehicle)** **F:24**	**250** **F:20**	**500** **F:22**	**1000** **F:20**
No. females with live fetuses	23	18	17	18
Mean no. corpora lutea	17	16	17	18*
Mean no. implantations	15	14	15	16
Pre-implantation loss (% of corpora lutea)	11	11	12	13
Post-implantation loss (% of implantation sites)	5	7	4	7
Mean no. live conceptuses	14	13	14	15
Litter data:				
No. litters evaluated	23	18	17	18
No. live fetuses	322	254	240	276
Mean male fetuses (%)	52	51	54	52
Mean fetal bodyweight (g)	5.6	5.5	5.6	5.5
Fetal abnormalities				
Soft tissue examination	-	-	-	-
Skeletal evaluation - ossification	-	-	-	-
Skeletal evaluation - morphological				
- anomalous thoracic vertebral centrum (% fetal incidence)	33	33	30	47
- anomalous (wavy/thickened/bent) ribs (% fetal incidence)	10	15	12	23

* $p \leq 0.05$ (Steel test)
F: female; - no noteworthy findings

2.6.7.13.B Reproductive and Developmental Toxicity – Effects on Embryo-Fetal Development

Report Title: TMC125K (HBr): Embryo-foetal developmental toxicity study by oral gavage in female albino NZW rabbits

Test Article: etravirine

Species/strain: Rabbit/New Zealand white
Age at first hormone injection/weight at first dose: about 16 weeks/3 to 4 kg
Duration of dosing: GD6 - 18
Day of mating: GD0
Day of c-section: GD28

Administration route/method: Oral/gavage
Doses: 0, 50, 200 and 750 mg base eq./kg body weight/day, as TMC125 HBr
Test article batch: [redacted] and [redacted]
Vehicle/formulation: alpha-tocopheryl polyethylene glycol succinate + hydroxypropyl-methylcellulose

Testing facility: [redacted]
Study no.: TMC125-NC124
Location in CTD: 4.2.3.5.2
GLP compliance: Yes
Date start of study: [redacted] 20[redacted]

Special features: None
No Observed Adverse Effect Level: The maternal and embryo-fetal NOAEL was 750 mg/kg based on the absence of any effect. TMC125 is considered not to be teratogenic in rabbits

Toxicokinetics:

Dose (mg base eq./kg/day)	0 (vehicle)	50	200	750
No. of animals	F:6	F:6	F:6	F:6
Died or killed prematurely (accidental)	0	0	0	0
$AUC_{0\text{-}24h}$ GD18 (µg.h/mL)	NR	1.29	2.24	3.76

Noteworthy Findings

Daily dose (mg base eq./kg)	0 (vehicle)	50	200	750
No. of animals	F:25	F:22	F:22	F:29
Maternal Data:				
Died or killed prematurely (unrelated to treatment)	1	0	0	2
Clinical observations	-	-	-	-
Body weight (g)	-	-	-	-
Food consumption (g)	-	-	-	-
Gross pathology	-	-	-	-
No. inseminated	25	22	22	29
No. pregnant females	19	18	19	22
No. aborted or with total resorption of litter	1	1	1	4

F: female; GD: gestation day; NR: not reported; - no noteworthy findings

2.6.7.13.B Reproductive and Developmental Toxicity – Effects on Embryo-Fetal Development (Continued)

Report Title: TMC125K (HBr): Embryo-foetal developmental toxicity study by oral gavage in female albino NZW rabbits **Test Article:** etravirine

Noteworthy Findings (Continued)

Daily dose (mg base eq./kg)	**0 (vehicle)**	**50**	**200**	**750**
No. of animals	**F:25**	**F:22**	**F:22**	**F:29**
No. females with live fetuses	18	17	18	16
Mean no. corpora lutea	9	10	9	9
Mean no. implantations	7	8	7	8
Pre-implantation loss (% of corpora lutea)	21	17	23	16
Mean no. resorptions (% implantation sites)	4	7	4	3
Post-implantation loss (% of implantation sites)	4	7	4	3
Mean no. live fetuses	7	7	7	7
Litter data:				
No. litters evaluated	18	17	18	16
No. live fetuses	117	124	125	117
Mean male fetuses (% of fetuses)	52	53	38*	45
Mean fetal body weight (g)	36.4	35.3	33.9	36.7
Fetal abnormalities				
Soft tissue examination	-	-	-	-
Skeletal evaluation - ossification	-	-	-	-
Skeletal evaluation - morphological	-	-	-	-

* $p \leq 0.05$ (Fisher's exact test)
F: female; - no noteworthy findings

2.6.7.13.C Reproductive and Developmental Toxicity – Effects on Embryo-Fetal Development

Report Title: Oral (gavage) developmental toxicity study in the rabbit

Test Article: etravirine

Species/strain: Rabbit/New Zealand white
Age/weight at pairing: mature time-mated/3 to 4 kg
Duration of dosing: GD6 - 19
Day of mating: GD0
Day of c-section: GD28

Administration route/method: Oral/gavage
Doses: 0, 125, 250 and 375 mg/kg body weight/day, as spray-dried TMC125 ([redacted] active to polymer ratio), dose correction factor was 4.5
Test article batch: [redacted]
Vehicle/formulation: hydroxypropyl-methylcellulose + microcrystalline cellulose in purified water

Testing facility: [redacted]
Study no.: TMC125-NC150
Location in CTD: 4.2.3.5.2
GLP compliance: Yes
Date start of study: [redacted] 20[redacted]

Special features: None
No Observed Adverse Effect Level: The maternal NOAEL was 125 mg/kg and the embryo-fetal NOAEL was 375 mg/kg based on the absence of any relevant effects. TMC125 is considered not to be teratogenic in rabbits

Toxicokinetics:

Dose (mg/kg/day) No. of animals	0 (vehicle)[a] F:3	125 F:3	250 F:3	375 F:3
Died or killed prematurely (accidental)	0	0	0	0
AUC_{0-24h} GD6 (µg.h/mL)	-	7.58	11.6	12.3
AUC_{0-24h} GD18 (µg.h/mL)	-	6.09	5.27	9.67

[a] TMC125 plasma concentrations above the lower limit of quantitation (10 ng/mL) were found in 3 out of 42 control samples (0.007 – 0.075 µg/mL)

Noteworthy Findings

Daily dose (mg/kg) No. of animals	0 (vehicle) F:20	125 F:20	250 F:20	375 F:20
Maternal data:				
Died or killed prematurely (accidental)	0	0	0	0
Clinical observations	-	-	-	-
Body weight gain GD6 - 9 (g)	-21	-23	-96***	-93***
Food consumption GD6 - 8 (g)	93	96	66*	63*
Gross pathology	-	-	-	-
No. pregnant females	20	17	17	20
No. aborted or with total resorption of litter	1	0	0	1

* $p \leq 0.05$; *** $p \leq 0.001$ (Analysis of variance and William's test)
F: female; GD: gestation day; - no noteworthy findings

2.6.7.13.C Reproductive and Developmental Toxicity – Effects on Embryo-Fetal Development (Continued)

Report Title: Oral (gavage) developmental toxicity study in the rabbit **Test Article:** etravirine

Noteworthy Findings (Continued)

Daily dose (mg/kg)	0 (vehicle)	125	250	375
No. of animals	F:20	F:20	F:20	F:20
No. females with live fetuses	19	17	17	19
Mean no. corpora lutea	12	11	11	10*
Mean no. implantations	10	9	10	9
Pre-implantation loss (% of corpora lutea)	13.7	15.8	10.3	8.4
Number of early embryo/fetal deaths	13	10	17	7
Number of late embryo/fetal deaths	35	22	16	15
Number of dead fetuses	0	1	0	0
Post-implantation loss (% of implantation sites)	23	23	17	13
Mean no. live fetuses/female	8	7	8	8
Mean % of implantations	77	77	83	87
Litter data:				
No. litters evaluated	19	17	17	19
No. live fetuses	143	117	132	149
Mean male fetuses (%)	45	48	48	50
Mean fetal body weight (g)	32.6	36.6	34.6	34.2
Fetal abnormalities				
Soft tissue examination	-	-	-	-
Skeletal evaluation - ossification	-	-	-	-
Skeletal evaluation - morphological	-	-	-	-

* $p \leq 0.05$ (Analysis of variance and William's test)
F: female; - no noteworthy findings

2.6.7.14.A Reproductive and Developmental Toxicity – Effects on Pre- and Postnatal Development, Including Maternal Function

Report Title: TMC125: Pre- and post-natal developmental toxicity study in rats

Test Article: etravirine

Species/strain: Rat/Sprague-Dawley
Initial Age/weight 56 - 70 days time-mated/180 - 250 g
Duration of dosing: TK: GD7 - 17; main study: GD7 - LD21
Day of mating: GD1

Administration route/method: Oral/gavage
Doses: 0, 125, 250 and 500 mg/kg body weight/day, as spray-dried TMC125 ([redacted] active to polymer ratio), dose correction factor is 4.5.
Test article batch: [redacted]
Vehicle/formulation: hydroxypropyl-methylcellulose + microcrystalline cellulose in purified water

Testing facility: [redacted]
Study no.: TMC125-NC145
Location in CTD: 4.2.3.5.3
GLP compliance: Yes
Date start of study: [redacted] 20[redacted]

Special features:
No Observed Adverse Effect Level: 500 mg/kg/day

Toxicokinetics:

Dose (mg/kg/day)	0 (vehicle)	125	250	500
F0 Females: No. of Animals	F:6	F:6	F:6	F:6
Died or killed prematurely (accidental)	0	0	0	0
$AUC_{0-\infty}$ GD7 (µg.h/mL)	-	6.15	9.42	12.1
AUC_{0-24h} GD17 (µg.h/mL)	-	5.29	7.60	3.63

Noteworthy Findings

Dose (mg/kg/day)	0 (vehicle)	125	250	500
F0 Females: No. of Animals	F: 28	F: 28	F: 28	F: 28
No. pregnant	22	22	20	23
No. died or sacrificed moribund	0	0	0	0
No. with difficult parturition	2	0	0	1
No. with total litter loss	0	1	1	0
Clinical observations	-	-	-	-
Necropsy observations	-	-	-	-
Gestation body weight	-	-	-	-
Lactation body weight	-	-	-	-
Gestation food consumption	-	-	-	-
Lactation food consumption	-	-	-	-
Mean duration of gestation (days)	21.5	21.7	22.0**	21.9*
Mean no. implantations	12.6	13.0	13.1	12.1
Mean % post-implantation loss	6.7	9.8	8.0	6.2

* $p \leq 0.05$; ** $p \leq 0.01$ (Analysis of variance)
GD: gestation day; LD: lactation day; TK: toxicokinetics; - no noteworthy findings

2.6.7.14.A Reproductive and Developmental Toxicity – Effects on Pre- and Postnatal Development, Including Maternal Function (Continued)

Report Title: TMC125: Pre- and Post-natal Developmental Toxicity Study in Rats **Test Article:** etravirine

Noteworthy Findings (Continued)

Dose (mg/kg/day)	0 (vehicle)	125	250	500
F1 litters (preweaning to Day 5)				
Proportion of pups born live	98	99	98	99
Mean no. pups/litter	11.5	11.6	11.8	11.3
Postnatal survival to Day 5 (%)	95	97	96	98
Proportion of male pups pup sex ratios (% male)	-	-	-	-
Pup clinical signs	-	-	-	-
Pup necropsy observations	-	-	-	-
Pup body weights at Day 1 (g) - males	6.1	6.7*	6.6*	7.0**
Pup body weights at Day 1(g) - females	5.7	6.3*	6.2*	6.5**
Pup body weights at Day 22 (g) - males	57.5	59.3	61.4	61.4
Pup body weights at Day 22(g) - females	55.1	57.4	58.2	58.9
% male pups with ears open on Day 3	25	49	54*	70**
% female pups with ears open on Day 3	22	55*	57*	72**
% male pups - successful righting reflex Day 4	89	93	98*	92
% female pups - successful righting reflex Day 4	82	85	93	87
F1 animals (postweaning selected Day 22)				
Clinical observations	-	-	-	-
Necropsy observations	-	-	-	-
Body weight at Day 92 (g) - males	454.5	462.5	478.0	479.3
Body weight at Day 71 (g) - femaless	226.7	234.4	242.9	238.0
Eye opening (mean day) - males	15.5	15.2	15.1	14.9**
Eye opening (mean day) - females	15.4	15.1	15.1	14.9*
Pupillary reflex	-	-	-	-
Starle response	-	-	-	-
Preputial separation (Day of age)	-	-	-	-

* $p \leq 0.05$; ** $p \leq 0.01$ (Eyes open: Fisher Exact test; ears open: analysis of variance)
- no noteworthy findings

2.6.7.14.A Reproductive and Developmental Toxicity – Effects on Pre- and Postnatal Development, Including Maternal Function (Continued)

Report Title: TMC125: Pre- and Post-natal Developmental Toxicity Study in Rats **Test Article:** etravirine

Noteworthy Findings (Continued)

Dose (mg/kg/day)	0 (vehicle)	125	250	500
Tail flick	-	-	-	-
Pup development and learning postweaning	-	-	-	-
Motor activity	-	-	-	-
Reproductive performance				
Precoital interval (days)	2.8	2.8	3.2	3.3
Mated	20/20	20/20	20/20	20/20
No. of pregnant females	19	19	20	19
Mean no. corpora lutea	16.1	15.8	16.7	16.4
Mean no. implantations	14.8	14.9	16.2*	15.2
Mean % pre-implantation loss	7.3	5.1	2.8*	7.6
Mean % post-implantation loss	6.2	5.9	3.3	3.7
Mean no. live embryos per female	13.9	14.0	15.7**	14.6

Statistically significant from Controls: * $p \leq 0.05$; ** $p \leq 0.01$ (Mean no. implantations: analysis of variance; mean % pre-implantation loss: Fisher Exact test; mean no. live embryos: analysis of variance)

2.6.7.15 Studies in Juvenile Animals

Test article: etravirine

No juvenile studies have been performed.

2.6.7.16 Local Tolerance

Test Article: etravirine

Type of Study	In vitro System or Species/Strain	Route/Method of Administration	Gender and no. per Group	Dose (Batch) (Reference/ Controls)	Noteworthy Findings	Study No./ Location in CTD
In Vitro Tests						
Bovine corneal opacity-permeability eye irritation assay	In vitro	NA	NA	20% w/w TMC125 HBr in saline (positive control: imidazole) ([redacted])	Very strong increase in opacity and small increase in permeability. TMC125 HBr considered a "very severe" eye irritant	TMC125-NC159/ 4.2.3.6
Bovine corneal opacity-permeability eye irritation assay	In vitro	NA	NA	20% w/w TMC125 base in saline (positive control: imidazole) ([redacted])	Small increase in opacity and no increase in permeability. TMC125 base considered a "mild" eye irritant	TMC125-NC298/ 4.2.3.6
Phototoxicity - Balb/c mouse fibroblast assay	In vitro	NA	NA	0 (vehicle), 0.002 to 6.42 mg/L TMC125 HBr in DPBS/DMSO ([redacted]) (positive control: Chlorpromazine) In presence and absence of UVA light	TMC125 HBr was not phototoxic up to the highest concentration tested (6.42 mg/L)	TMC125-NC245/ 4.2.3.6
In Vivo Tests						
Skin sensitization - Magnusson and Kligman method	Guinea pig/ Hartley	Intradermal injection (Day 1). Cutaneous application (Day 8). Challenge on Day 22.	5M + 5F (control) 10M + 10F (treated)	FCA 50% v/v in saline, 0.1% TMC125 base (w/w) in corn oil, 0.1% TMC125 base (w/w) in FCA/saline Cutaneous route: 50% w/w ([redacted])	Grade 1 erythema in 2/20 at 24 h only. No delayed contact hypersensitivity. Classified as "nonsensitizing" to the skin.	CIT20822TSG[a]/ 4.2.3.6
Primary skin irritation	Rabbit/New Zealand white	Gauze patch applied to intact clipped skin for 4 hours	1M + 2F	0.5 g as 1% TMC125 base solution ([redacted])	No skin reactions, staining of skin or corrosive effects were observed. Classified as "nonirritant" to the skin.	TMC125-NC160[a]/ 4.2.3.6

[a] GLP compliant

DMSO: dimethyl sulfoxide; DPBS: Dulbecco's phosphate buffered saline; F: female; FCA: Freund's complete adjuvant; M: male; NA: not applicable; UVA: ultraviolet A

2.6.7.16 Local Tolerance (Continued)

Test Article: etravirine

Type of Study	In vitro System or Species/Strain	Route/Method of Administration	Gender and no. per Group	Dose (Batch) (Reference/ Controls)	Noteworthy Findings	Study No./ Location in CTD
In Vivo Tests (Continued)						
Mouse skin sensitization - local lymph node assay	Mouse/ CBA/J	Dermal application	4F	0 (vehicle), 2.5%, 5%, 10%, 25%, 50% w/v TMC125 base in DMF ([redacted])	Negative response. TMC125 base considered unlikely to have the potential to cause skin sensitization.	TMC125-NC324[a]/ 4.2.3.6

[a] GLP compliant
DMF: dimethyl formamide; F: female

2.6.7.17.A Other Toxicity Studies - Immunotoxicity

Test Article: etravirine

Species/Strain No. and Gender/Group	Route/Method of Administration (Vehicle/ Formulation)	Duration of Dosing	Batch no.	Doses (mg base equivalent/kg/day)	Noteworthy Findings	Study No./ Location in CTD
Rat/Sprague-Dawley 8M + 8F (main) 6M + 6F (satellite, only in treated groups)	Oral/gavage (PEG400)	4 Weeks	[redacted]	0 (vehicle), 70, 200, 600 as TMC125 HBr	No relevant effects on immune function (IgM response) at doses up to 600 mg/kg/day. NOAEL – 600 mg/kg/day, based on absence of hematological and histopathological changes. Body weight gain generally lower in high dose males and in females from 200 mg/kg/day.	TMC125-NC180[a]/ 4.2.3.7.2

[a] GLP compliant

F: female; M: male; NOAEL: no observed adverse effect level; PEG400: polyethylene glycol 400

2.6.7.17.B Other Toxicity Studies - Mechanistic

Test Article: etravirine

Species/Strain No. and Gender/Group	Route/Method of Administration (Vehicle/ Formulation)	Duration of Dosing	Batch no.	Doses (mg base equivalent/kg/day)	Noteworthy Findings	Study No./ Location in CTD
Mouse/CD1 20M + 20F (main) 8M + 8F (satellite)	Oral/dietary admixture (diet)	6 Weeks	[redacted]	0 (untreated diet), 0 (untreated diet + Vitamin K1 sc), 2320, 2320 (+ Vitamin K1 sc) as spray-dried TMC125 ([redacted] active to polymer ratio)	TMC125 alone: 1M showed hemorrhagic cardiomyopathy. No hemorrhages in any other organ. ↑ APTT + PT and ↓ vitamin K dependent factors II and VII. These effects were more pronounced in males. TMC125 with Vitamin K: normalization of coagulation times and clotting factors II and VII. Reduction of hepatic hemorrhagic necrosis.	TMC125-NC240/ 4.2.3.7.3
Mouse/CD1 20M + 20F (main) 8M + 8F (satellite)	Oral/gavage (PEG400)	4 Weeks	[redacted]	0 (vehicle), 0 (vehicle + Vitamin K1 sc), 1000, 1000 (+ Vitamin K1 sc) as TMC125 HBr	No animals with hemorrhagic cardiomyopathy. No hemorrhages in any other organ. TMC125 alone: ↑ APTT + PT and ↓ vitamin K dependent factors II and VII. These effects were more pronounced in males. No effects on factor II and VII in females. TMC125 with Vitamin K: normalization of coagulation times and clotting factors II and VII. Gavage administration of TMC125 HBr had lower impact on clotting times compared with dietary admixture.	TMC125-NC241/ 4.2.3.7.3

APTT: activated partial thromboplastin time; F: female; M: male; PEG400: polyethylene glycol 400; PT: prothrombin time; sc: subcutaneous; ↑: increased; ↓: decreased

2.6.7.17.C Other Toxicity Studies – Drug Substance Impurities

Test Article: etravirine

Species/Strain No. and Gender/Group	Route/Method of Administration (Vehicle/ Formulation)	Duration of Dosing	Batch no.	Doses (mg/kg/day)	Noteworthy Findings	Study No./ Location in CTD
R230187 (Impurity)						
S. typhimurium TA98, TA100, TA102, TA1535, TA1537 +/- S9	In vitro (DMSO)	---	TMC125 [redacted]	TMC125 base spiked with 2% impurity 0 (vehicle), 1.95 to 1000 µg/mL Precipitation at 250 µg/mL and above	No evidence of mutagenic properties.	TMC125-NC322[a]/ 4.2.3.7.6
Dog/Beagle 3M+3F/group	Oral/Gavage (PEG400)	13 Weeks	TMC125 HBr [redacted]	TMC125 HBr spiked with 2% impurity 0 (vehicle), 10, 20	No clinical signs or mortality. NOAEL is 20 (TMC125)/0.4 ([redacted]) mg/kg/day.	TMC125-NC246[a]/ 4.2.3.7.6
L5178Y mouse lymphoma cells (TK locus) +/- S9	In vitro (DMSO)	---	TMC125 [redacted]	TMC125 spiked with 2% impurity 0 (vehicle), 0.5 to 100 µg/mL Precipitation at 75 µg/mL and above	No evidence of mutagenic (gene mutations and/or chromosome aberrations) properties.	TMC125-NC248[a]/ 4.2.3.7.6
R405793 (Impurity)						
Dog/Beagle 3M+3F/group	Oral/Gavage (PEG400)	13 Weeks	TMC125 HBr [redacted]	TMC125 HBr spiked with 2% impurity 0 (vehicle), 10, 20	No clinical signs or mortality. NOAEL is 20 (TMC125)/0.4 ([redacted]) mg/kg/day.	TMC125-NC246[a]/ 4.2.3.7.6
S. typhimurium TA98, TA100, TA102, TA1535, TA1537 +/- S9	In vitro (DMSO)	---	TMC125 [redacted]	TMC125 base spiked with 2% impurity 0 (vehicle), 15.63, 31.25, 62.5, 125, 250, 500, 1000 µg/plate Precipitation at 250 µg/plate and above	No evidence of mutagenic properties.	TMC125-NC247[a]/ 4.2.3.7.6
L5178Y mouse lymphoma cells (TK locus) +/- S9	In vitro (DMSO)	---	TMC125 [redacted]	TMC125 spiked with 2% impurity 0 (vehicle), 1 to 50 µg/mL. Precipitation at 6 µg/mL and above	No evidence of mutagenic (gene mutations and/or chromosome aberrations) properties.	TMC125-NC323[a]/ 4.2.3.7.6

[a] GLP compliant

DMSO: dimethyl sulfoxide; F: female; M: male; NOAEL: no observed adverse effect level; PEG400: polyethylene glycol 400; TK: thymidine kinase

2.6.7.17.C Other Toxicity Studies – Drug Substance Impurities (Continued)

Test Article: etravirine

Species/Strain No. and Gender/Group	Route/Method of Administration (Vehicle/ Formulation)	Duration of Dosing	Batch no.	Doses (mg/kg/day)	Noteworthy Findings	Study No./ Location in CTD
R405792 (Impurity)						
S. typhimurium TA98, TA100, TA102, TA1535, TA1537 +/- S9	In vitro (DMSO)	---	[redacted] impurity of TMC125 Batch – [redacted]	0 (vehicle), 4.88, 9.77, 19.53, 39.06, 78.13, 156.25, 312.5 µg/plate Precipitation at 19.53 µg/plate and above	No evidence of mutagenic properties.	TMC125-NC226[a]/ 4.2.3.7.6

[a] GLP compliant
DMSO: dimethyl sulfoxide

2.6.7.17.D Other Toxicity Studies – Impurities

Test Article: etravirine

Species/Strain No. and Gender/Group	Route/Method of Administration (Vehicle/ Formulation)	Duration of Dosing	Batch no.	Doses (mg/kg/day)	Noteworthy Findings	Study No./ Location in CTD
Residual Intermediate Products						
T002615						
S. typhimurium TA98, TA100, TA1535, TA1537 *E. coli* WP2uvrA +/- S9	In vitro (DMSO)	---	[redacted]	0 (vehicle), 33, 100, 333, 1000, 2500, 5000 µg/plate	No evidence of mutagenic properties.	TMC125-NC381[a]/ 4.2.3.7.7
Chinese hamster V79 cells +/- S9	In vitro (THF)	---	[redacted]	0 (vehicle), 0.6, 1.3, 2.5, 5, 10, 20, 40 µg/mL Precipitation at 10 µg/mL and above	No evidence of clastogenic properties (induced chromosomal aberrations).	TMC125-NC385[a]/ 4.2.3.7.7
Human lymphocytes +/- S9	In vitro	---	[redacted]	0 (vehicle), 14.69, 29.38, 58.75, 117.5, 235, 470, 940, 1880, 3760 µg/mL Precipitation at 470 µg/mL and above	No evidence of clastogenic properties with up to 24 h exposure in absence or presence of S9.	TMC125-NC445/ 4.2.3.7.7

[a] GLP compliant
DMSO: dimethyl sulfoxide; THF: tetra hydrofurane

2.6.7.17.D Other Toxicity Studies – Impurities (Continued)

Test Article: etravirine

Species/Strain No. and Gender/Group	Route/Method of Administration (Vehicle/ Formulation)	Duration of Dosing	Batch no.	Doses (mg/kg/day)	Noteworthy Findings	Study No./ Location in CTD
T002327						
S. typhimurium TA98, TA100, TA1535, TA1537 *E. coli* WP2uvrA +/- S9	In vitro (DMSO)	---	[redacted]	0 (vehicle), 3, 10, 33, 100, 333, 1000, 2500, 5000 µg/plate Precipitation at 100 µg/plate and above	No evidence of mutagenic properties	TMC125-NC263[a]/ 4.2.3.7.7
Human lymphocytes +/-S9	In vitro	---	[redacted]	0 (vehicle), 1.74, 3.48, 6.97, 13.93, 20.89, 27.85, 41.77, 55.69 µg/mL Precipitation at 55.69 µg/mL and above	No evidence of clastogenic properties with up to 24 h exposure (up to 27.85 µg/mL) in the absence of S9.	TMC125-NC339/ 4.2.3.7.7
Chinese hamster V79 cells +/- S9	In vitro (DMSO)	---	[redacted]	0 (vehicle), 1.6, 3.1, 6.3, 12.5, 25.0, 50.0, 100.0 µg/mL Precipitation at 25 µg/mL and above	Reported as clastogenic (induced chromosomal aberrations) at only one concentration (12.5 µg/mL) in the absence of metabolic activation and as non-clastogenic in the presence of metabolic activation at 25 µg/mL.	TMC125-NC264[a]/ 4.2.3.7.7
Mouse/NMRI 6M – 6F/group micronucleus assay	Oral/gavage (corn oil)	Single dose	[redacted]	0 (vehicle), 500, 1000, 2000 (24 h interval) and 2000 (48 h interval)	No evidence of mutagenic properties. T002327 did not induce micronuclei in bone marrow cells of the mouse at 2000 mg/kg. TK determined: At 2000 mg/kg Cmax = 0.058 (M) and 0.074 (F) ng/mL, AUC_{0-7h} = 0.22 (M) and 0.19 (F) µg.h/mL.	TMC125-NC325[a]/ TMC125-NC357[a] 4.2.3.7.7

[a] GLP compliant
DMSO: dimethyl sulfoxide; F: female; M: male; TK: toxicokinetics

2.6.7.17.E Other Toxicity Studies – Batch Qualification

Test Article: etravirine

Species/Strain No. and Gender/Group	Route/Method of Administration (Vehicle/ Formulation)	Duration of Dosing	Batch no.	Doses (mg base eq./kg/day)	Noteworthy Findings	Study No./ Location in CTD
S. typhimurium TA98, TA100, TA102, TA1535, TA1537 +/- S9	In vitro (DMSO)	---	[redacted]	0 (vehicle), 0.61, 1.22, 2.44, 4.88, 9.77, 19.53, 39.06, 78.13, 156.25, 312.5, 625, 1250, 2500, 5000 µg/plate as TMC125 HBr Precipitation at 156.25 and above	No evidence of mutagenic properties.	TMC125-NC163[a]/ 4.2.3.7.7
S. typhimurium TA98, TA100, TA102, TA1535, TA1537 +/- S9	In vitro (DMSO)	---	[redacted]	0 (vehicle), 10, 25, 50, 100, 250, 500, 1000 µg/plate Precipitation at all concentrations Stressed capsule content of clinical trial formulation of TMC125	No evidence of mutagenic properties.	TMC125-NC211[a]/ 4.2.3.7.7
Dog/Beagle 3M + 3F/group	Oral/gavage (PEG400)	2 Weeks	[redacted]	0 (vehicle), 20 ([redacted]), 240 ([redacted]), 20 ([redacted]) 240 ([redacted]) as TMC125 HBr	No relevant difference in toxicological profile between the batches of TMC125.	TMC125-NC175[a]/ 4.2.3.7.7

[a] GLP compliant
DMSO: dimethyl sulfoxide; F: female; M: male; M6: M60261002; PEG400: polyethylene glycol 400