MODULE 2.4 NONCLINICAL OVERVIEW

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LIST OF ABBREVIATIONS

AE	adverse event
ALP	alkaline phosphatase
ALT	alanine aminotransferase
APTT	activated partial thromboplastin time
ARV	antiretroviral
AST	aspartate aminotransferase
AUC	area under the concentration vs. time curve
C_{max}	maximum concentration
CYP	cytochrome P450
ECG	electrocardiogram
F1	first generation
GLP	good laboratory practice
HBr	hydrogen bromide
HEK	human embryonic kidney
HERG	human-ether-à-go-go-gene
hERG.T.HEK293	human-ether-à-go-go-gene stably transfected human embryonic kidney cells
HIV	human immunodeficiency virus
HLM	human liver microsomes
HPMC	hydroxypropyl methylcellulose
ICH	international conference on harmonization
INR	international normalized ratio
K_i	inhibition constant
LC-MS/MS	liquid chromatography with tandem mass spectrometry
NNRTI	non-nucleoside reverse transcriptase inhibitor
NOAEL	no observed adverse effect level
PEG400	polyethylene glycol 400
P-gp	p-glycoprotein
PT	prothrombin time
QWBA	quantitative whole-body autoradiography
T3	triiodothyronine
T4	tetraiodothyronine/thyroxine
TSH	thyroid stimulating hormone
TPGS	alpha-tocopheryl polyethylene glycol succinate
UDP-GT	uridine diphosphate-glucuronosyltransferase

1 OVERVIEW OF THE NONCLINICAL TESTING STRATEGY

TMC125 (etravirine) is a new non-nucleoside reverse transcriptase inhibitor (NNRTI) belonging to the substituted diarylpyrimidine derivatives, which was selected for its high potency against wild-type and drug-resistant human immunodeficiency virus (HIV)-1. TMC125 is in development for the treatment of HIV-1 infected, antiretroviral (ARV) experienced adult patients, in combination with other ARV medicinal products. The recommended clinical dose of TMC125 for treatment of experienced HIV-1 infected patients is 200 mg (formulation A*) given orally twice daily. At this dose level in HIV-infected patients, the maximum TMC125 plasma concentration (C_{max}) was 0.45 µg/mL, and the area under the concentration-time curve (AUC_{0-24h}) was 7.4 µg.h/mL (Module 5.3.1.2/TMC125-C228). Throughout this overview, safety margins are presented based on comparison of plasma levels in animals with the above human exposure levels.

This nonclinical overview presents a summary of the nonclinical testing strategy and the information gathered. This overview also includes a critical assessment of the relevance and implications of the nonclinical test findings for human safety of TMC125 in the above indication and treatment dose. Whenever appropriate, reference is made to clinical trial findings to provide a clinical perspective in relation to the nonclinical findings.

The nonclinical safety evaluation program of TMC125 included safety pharmacology, pharmacokinetics, metabolism and toxicology studies conforming to relevant international regulatory guidelines. All nonclinical safety studies were performed orally. Owing to poor solubility and despite considerable effort, no suitable intravenous formulation has been produced that would permit exposures greater than the oral exposure. TMC125 was originally tested in its base form in a limited number of studies to support initial clinical trials. As part of the program to improve the pharmaceutical formulation it was found that the hydrogen bromide (HBr) salt of the compound (TMC125 HBr) showed superior oral bioavailability and exposure in nonclinical studies. 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 mice and rats than in dogs. Further improvement in exposure was also achieved with development of the spray-dried TMC125 formulation, which became available at a later stage of drug development. The spray-dried formulation was developed for the production of an oral tablet for clinical use. In the spray-dried form, TMC125 is amorphorized by spray-drying technology using hydroxypropyl methylcellulose (HPMC) as a stabilizing polymer and microcrystalline cellulose as an inert carrier (various active to polymer ratios were used). To assess the 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, a fertility study in rat and an embryo-fetal development study in rabbit. The modified Irwin test and the pre- and postnatal developmental toxicity study in the rat were directly performed with spray-dried TMC125. The dose levels used in all studies were expressed as the base equivalent and the appropriate correction factor was applied when the salt and the spray-dried forms were used. TMC125 in HBr and spray-dried forms was also tested orally by dietary admixture in rodents. This route, however, resulted in no further gain in exposure relative to oral gavage and therefore it was not considered further.

The nonclinical toxicology program was consistent with the best scientific principles and international guidelines. Pivotal studies have been conducted according to Good Laboratory

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Practice (GLP) standards[1,2]. The design and the conduct of several major nonclinical studies, including repeated dose toxicity, genotoxicity and carcinogenicity studies, were discussed with various Health Authorities worldwide (for an overview see Module 2.2) and concurrence was obtained. The animal species used in the various studies were from recognized sources and are standard models for nonclinical safety testing.

The nonclinical safety evaluation program consisted of:

Safety Pharmacology – The objective of the safety pharmacology program was to assess safety on vital organ systems. The potential effects of TMC125 on cardiovascular, central nervous and respiratory systems were investigated in a series of in vitro secondary pharmacodynamic studies and in vitro and in vivo safety pharmacology studies. The studies conducted are listed in Overview Table 2.6.3.1 and individual study details are given in Tabulated Summaries 2.6.3.3 and 2.6.3.4.

Pharmacokinetics and Metabolism – The objectives of the drug metabolism and pharmacokinetics program were to characterize the pharmacokinetics of TMC125 in various species, measure exposure after single and repeated administration, characterize the distribution in the body, identify metabolic pathways and metabolites formed, determine the rates and routes of elimination, predict the potential for drug-drug interactions and where appropriate, to examine the inter-relationships between dose, exposure and effects observed. Several in vitro and in vivo studies were performed. In vivo studies were conducted in CD1, NMRI and CB6F1-nonTgrasH2-transgenic mice, pigmented Lister-Hooded, Wistar and Sprague-Dawley rats, SPF albino or New Zealand white rabbits and Beagle dogs. With the exception of pigmented rats, all species and strains were the same as those used in the nonclinical pharmacology and toxicology studies. Radiolabeled TMC125 was used in plasma protein binding, tissue distribution and metabolism and elimination studies. The studies conducted are listed in Overview Table 2.6.5.1.

Toxicology – The objective of the toxicology program was to investigate TMC125 toxicity and dose response in relevant nonclinical species, to assess target organs of toxicity and to establish the potential of TMC125 to produce genotoxic effects and developmental toxicity. **Single dose** oral (gavage) and subcutaneous studies were conducted in mice and rats, with TMC125 base and TMC125 HBr. An escalating single dose oral study in dogs was conducted with spray-dried TMC125 as tablets or in aqueous suspension. **Repeated dose** studies evaluated the effects of TMC125 HBr in 2-week and 3-month studies in mice and these served as a dose range finder for the carcinogenicity study. Spray-dried TMC125, administered by dietary admixture, was also evaluated in a 3-month study in mice. In Wistar rats TMC125 base was used in a 2-week study but subsequently TMC125 HBr was used in 1-, 3- and 6-month studies. An additional 3-month study with TMC125 HBr was performed in Sprague-Dawley rats in preparation for the carcinogenicity study. Also in Sprague-Dawley rats, spray-dried TMC125, administered by dietary admixture, was evaluated in a 3-month study. In dogs TMC125 base was evaluated by oral (gavage) administration in a 2-week and a 1-month study but subsequently TMC125 HBr was used in 3-, 6- and 12-month studies. When the spray-dried form became available it was tested in dogs in 1- and 6-month studies. In the oral gavage studies polyethylene glycol (PEG400) was mainly used as a vehicle for TMC125 base and TMC125 HBr, while water was used for spray-dried TMC125. In all studies, control animals were administered the vehicle and toxicokinetic parameters evaluated (using satellite animals in the mouse and rat studies). **Genotoxicity** tests included in vitro gene mutation assays (Ames test and mouse lymphoma test), in vitro chromosomal aberration test (human lymphocytes) and an in vivo chromosomal

aberration test (mouse bone marrow micronucleus test). **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. **Reproduction and development** effects of TMC125 HBr were evaluated in a fertility and early embryonic development study in rats and in embryo-fetal development studies in rats and rabbits. 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. Also a pre- and postnatal development study in rats was performed with this form. **Immunotoxicity** has been evaluated in rats. Several **drug substance impurities** of TMC125 were investigated and batch qualification studies were conducted, as necessary. In all pivotal repeated dose toxicity studies plasma levels of TMC125 have been determined. Given the nature of the target organ toxicity in animals and the plasma levels in rats compared to man, investigation of the reversibility of the changes seen was limited to the 12-month dog study. The toxicity studies conducted are listed in Overview Table 2.6.7.1.

To facilitate the review of the nonclinical overview, the document has been structured as follows: for each of the relevant disciplines (safety pharmacology, pharmacokinetics and toxicology), a logical overview of the studies undertaken and their corresponding results is presented. A critical assessment of the key findings is also included. Specific cross-disciplinary issues and proposals for the inclusion of nonclinical items in the product labeling are discussed throughout the text, as appropriate, and summarized at the end of this document. The nonclinical overview does not include consideration of primary pharmacology, as these are dealt with separately in the virology section (Module 2.7.2, Virology Summary).

2 PHARMACOLOGY

2.1 PRIMARY PHARMACODYNAMICS

For the primary pharmacodynamics of TMC125, please refer to Module 2.7.2 Virology Summary.

2.2 SECONDARY PHARMACODYNAMICS

TMC125 was investigated in a series of in vitro secondary pharmacodynamic studies (Module 2.6.2 Pharmacology Written Summary). The studies conducted are listed in Overview Table 2.6.3.1 and individual study details are given in Tabulated Summaries 2.6.3.3.

The interaction of TMC125 base with a wide range of receptors was studied at concentrations up to 10 μM (4.4 μg/mL) (Module 2.6.2, Section 3.1). TMC125 only weakly interacted with glycine-1 transporter binding sites (inhibition constant (K_i) was 5.4 μM). No interaction of the compound with receptors could be observed in any other assays performed.

At concentrations of 1 and 10 μM TMC125 was found to have inhibitory effects on nicotinergic nerve-smooth muscle function, but was devoid of muscarinic effects (Module 2.6.2, Section 3.2).

2.3 SAFETY PHARMACOLOGY

TMC125 was investigated in a series of in vitro and in vivo safety pharmacology studies (Module 2.6.2 Pharmacology Written Summary). The studies conducted are listed in Overview Table 2.6.3.1 and individual study details are given in Tabulated Summary 2.6.3.4.

2.3.1 Cardiovascular Safety

Cardiovascular safety was evaluated in several in vitro and in vivo tests and these demonstrated that TMC125 has no relevant effects on cardiac electrophysiology or on cardio-hemodynamic parameters.

In vitro, TMC125 base at concentrations up to 10 μM (4.4 μg/mL) in dimethyl sulfoxide had no relevant effect on membrane potassium current in human-ether-à-go-go-gene (hERG) – stably transfected human embryonic kidney (HEK) cells (hERG.T.HEK293) (Module 2.6.2, Section 4.1.1). At concentrations of up to 1 μM (0.44 μg/mL) TMC125, in acidified ethanol, demonstrated no relevant effects on the electrophysiological parameters in guinea pig isolated papillary muscles (Module 2.6.2, Section 4.1.2).

In Beagle dogs, TMC125 base formulated in PEG400 had no relevant effect on electrocardiogram (ECG) and cardio-hemodynamic parameters following a single oral (gavage) dose of 10 or 40 mg/kg (Module 2.6.2, Section 4.2.1). The median plasma levels at 10 mg/kg were 0.05, 0.19 and 0.23 μg/mL, and at 40 mg/kg were 0.16, 0.30 and 0.23 μg/mL, at 30, 60 and 240 minutes after administration, respectively.

Spray-dried TMC125 improved the oral bioavailability and systemic exposure in the Beagle dog and a further in vivo cardiovascular study was performed (Module 2.6.2, Section 4.2.2). TMC125 had no relevant effects on ECG, cardio-hemodynamic or respiratory parameters following a single oral (gavage) dose of 100 or 200 mg/kg spray-dried TMC125 (1:3:0.5 active

to polymer ratio) as an aqueous suspension. An oral dose of 400 mg/kg (given as 200 mg/kg twice daily with 1-hour interval) resulted in slight reversible increases in heart rate and blood pressure during a period of restlessness and vomiting, approximately 1 hour after the second dose administration. The mean C_{max} values of TMC125 were 4.9, 4.8 and 3.3 µg/mL and the corresponding AUC_{0-24h} values were 76, 75 and 50 µg.h/mL, for 100, 200 and 400 mg/kg (200 mg/kg twice daily), respectively.

In addition, during repeated dose toxicology studies in dogs with TMC125 base or TMC125 HBr, of up to 12 months, at dose levels of up to 240 mg/kg/day and exposures (AUC_{0-24h}) of up to 40 µg.h/mL, no treatment-related effects on heart rate or ECG morphology were seen (Module 2.6.6, Section 3.2.). Also no cardiovascular effects were observed in the 6-month repeated dose study in dogs with spray-dried TMC125 (1:3:0.5 active to polymer ratio) at dose levels up to 500 mg/kg/day and exposures (AUC_{0-24h}) of up to 62 µg.h/mL day at the end of the study (Module 2.6.6, Section 3.2.7).

2.3.2 Neurobehavior and Motor Activity

Neurobehavior and motor activity, together with gastro-intestinal transit time and respiration rate, were assessed before treatment and over 3 hours after single oral administration of 80 mg/kg TMC125 base in PEG400 vehicle to Wistar rats. No relevant effects were observed (Module 2.6.2, Section 4.2.3).

Neurobehavior was further assessed in Sprague-Dawley rats with spray-dried TMC125 in a modified Irwin's test (Module 2.6.2, Section 4.2.3). Neurofunctional integrity was examined at doses up to 500 mg/kg with spray-dried TMC125 (1:3:0.5 active to polymer ratio) as an aqueous suspension. At the high dose only, there was a slight delay in righting reflex in 3 out of 5 animals but behavioral and autonomic functions were unaffected and there were no other relevant clinical effects or concerns for delayed neurotoxicity. The mean C_{max} values were 1.0, 1.2 and 1.7 µg/mL and the corresponding $AUC_{0-\infty}$ values were 4.7, 7.4 and 10 µg.h/mL for rats given 200, 350 and 500 mg/kg, respectively.

2.3.3 Pulmonary Safety

Pulmonary safety was determined following oral administration of TMC125 in base or spray-dried form and there were no effects on respiration in rats at a dose level of 80 mg/kg (Module 2.6.2, Section 4.2.3) or on respiratory rate and arterial blood oxygen in dogs at dose levels of up to 400 mg/kg (given as 200 mg/kg twice daily with 1-hour interval) (Module 2.6.2, Section 4.2.2). No toxicokinetic data was measured in the rat study and the exposure data in the dog are described above (cardiovascular safety).

2.4 ASSESSMENT OF SECONDARY PHARMACODYNAMICS AND SAFETY PHARMACOLOGY

The secondary pharmacodynamics and safety pharmacology program was compliant with international guidance for the core battery of studies and appropriate maximum practical doses were used. An additional cardiovascular study was conducted as spray-dried TMC125 improved the oral bioavailability and systemic exposure in the dog. There were no relevant effects of TMC125 on in vitro cardiovascular electrophysiological parameters at concentrations up to 10 µM (4.4 µg/mL). Due to lack of plasma protein in the incubation medium, TMC125

concentrations used in vitro correspond to approximately 1000-fold free maximum plasma level anticipated in HIV-1 infected subjects at the recommended therapeutic dose of 200 mg tablet twice daily (mean C_{max} 0.45 µg/mL, plasma protein binding 99.9%).

No relevant treatment-related effects were observed on ECG, cardio-hemodynamic or respiratory parameters in dogs after the administration of oral doses up to 400 mg/kg. 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 (Module 5.3.1.2/TMC125-C228) and values obtained with spray-dried TMC125 in the dog were about 10-fold higher than these.

The results of the thorough QTc trial in healthy volunteers using clinically relevant doses of TMC125 (200 mg twice daily and 400 mg once daily) were interpreted as negative (Module 5.3.4.1/TMC125-C178). When evaluating the 2-sided 90% confidence interval, the upper bounds of the time-matched mean changes in QTcF values versus placebo never exceeded the 10 ms boundary for the TMC125 treatment groups. Moxifloxacin (400 mg once daily) fulfilled the criterion of positive control according to the E14 ICH Guideline[3]. The clinical Phase IIb and Phase III trials also revealed no clinically relevant changes for vital signs or ECG parameters.

No relevant effects on neurobehavioral, motor activity or other body functions were seen in rats following oral administration of up to 80 or 500 mg/kg TMC125 base or spray-dried TMC125. Dosing at 500 mg/kg led to a slight delay in righting reflex but behavioral and autonomic functions were unaffected and there were no other relevant effects. At 350 mg/kg, where no clinical effects were seen in rats, C_{max} level (1.2 µg/mL) was approximately 3-fold higher than maximum plasma levels (C_{max} 0.45 µg/mL) observed in patients.

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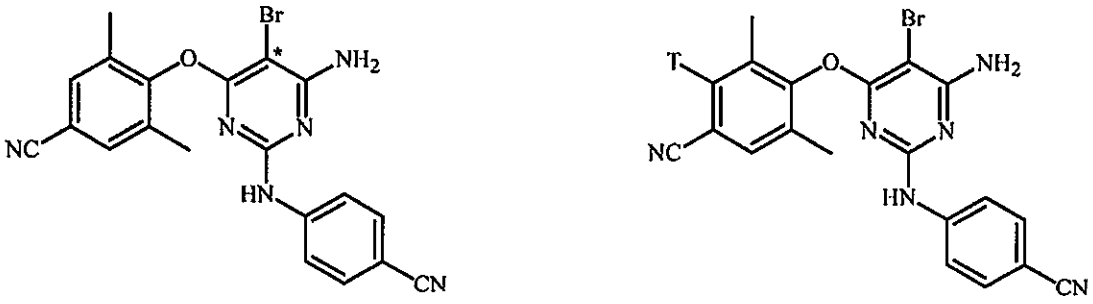
3 PHARMACOKINETICS

The pharmacokinetics of TMC125 was investigated in a series of in vitro and in vivo studies and is described in detail in the Pharmacokinetics Written Summary (Module 2.6.4). In vivo studies were conducted in CD1, NMRI and CB6F1-nonTgrasH2-transgenic mice, pigmented Lister-Hooded, Wistar and Sprague-Dawley rats, SPF albino or New Zealand white rabbits and Beagle dogs. With the exception of pigmented rats, all species and strains were the same as those used in the nonclinical pharmacology and toxicology studies. The studies conducted are listed in Overview Table 2.6.5.1.

3.1 METHOD OF ANALYSIS

TMC125 and/or its metabolites were determined in various in vitro media and biological samples (plasma, urine, bile and feces). Several studies were conducted with radiolabeled TMC125, which was labeled with ^{14}C or ^{3}H atom as outlined in Figure 1 (Module 2.6.4, Section 2.1 and 2.2).

Figure 1: Structural Formula of ^{14}C-TMC125 (Left) and ^{3}H-TMC125 (Right)



* : ^{14}C-label; T : ^{3}H-label

The tissue distribution of ^{14}C-TMC125 was studied by quantitative whole-body autoradiography (QWBA). The total radioactivity in biological samples was measured by liquid scintillation counting. The metabolites were identified by co-chromatography with authentic substances using different high performance liquid chromatography methods and detection systems such as radiodetection and tandem mass spectrometry (LC-MS/MS).

An LC-MS/MS method was validated for the determination of TMC125 in mouse, rat, rabbit and dog heparin and ethylenediamine tetra-acetic acid plasma. Tissue samples were analyzed with nonvalidated qualified research methods based on the validated plasma methods. (Module 2.6.4, Section 2.3).

The stability of TMC125 was assessed in the biological matrices at different conditions. TMC125 was sufficiently stable under the conditions tested which allowed the use of the developed assays under normal laboratory testing conditions (Module 2.6.4, Section 2.3.2).

Analysis of pharmacokinetic and toxicokinetic data from nonclinical studies was performed using non-compartmental methods contained in commercially available pharmacokinetic software (Win Nonlin®).

3.2 ABSORPTION

The transepithelial permeability of TMC125 was low to intermediate in Caco-2 cells. Passive transcellular diffusion is proposed as the predominant mechanism for TMC125 intestinal absorption. TMC125 was not a substrate of P-glycoprotein (P-gp) or other efflux transporter. TMC125 showed P-gp inhibitory properties with an apparent 50% inhibitory concentration of 24.2 μM (10.5 μg/mL). Inhibition of transepithelial permeation of P-gp substrates by TMC125, therefore, cannot be excluded. However, for most drugs it is unlikely that a possible effect on intestinal absorption will be clinically significant (Module 2.6.4, Section 3.1.1).

Absorption and plasma kinetic studies of TMC125 were conducted in CD1 and CB6F1-nonTgrasH2- transgenic mice, Wistar, pigmented Lister-Hooded and Sprague-Dawley rats, SPF albino and New Zealand white rabbits and Beagle dogs. The studies were performed after single and repeated oral administration either as specific pharmacokinetic studies or as toxicokinetic evaluations of toxicology studies. A summary of plasma pharmacokinetic parameters for TMC125 in laboratory animals following single and repeated oral administration is presented in Table 1.

In all species, including humans, TMC125 oral absorption was limited due to poor solubility and intestinal permeability. In rats, the fraction of the dose absorbed was low (< 11%). In dogs, the absolute oral bioavailability was estimated at 8-12% for TMC125 HBr and at 33-34% for spray-dried TMC125. Many studies were performed in order to examine whether a specific formulation or route of administration could improve absorption. Initial preclinical studies were performed with TMC125 base in PEG400. Subsequently, studies were performed with TMC125 HBr (in PEG400) or spray-dried TMC125 (in water), which showed higher exposure relative to TMC125 base to a varying extent in different species. In mice and to a more limited extent in rats, exposure to TMC125 was increased with the HBr salt relative to the base (by up to 6.3-fold and 1.6-fold, respectively). However, there was no relevant difference in exposure between the 2 forms in dogs. Later experiments in rats, rabbits and dogs, indicated that exposure further increased (by up to 4-fold, 21-fold and 5-fold, respectively) after single administration of spray-dried TMC125 relative to the HBr salt. This difference in exposure, however, between the 2 forms was less pronounced following repeated administration due to the enzymatic auto-induction. In healthy volunteers and in HIV-1 infected subjects, the relative oral bioavailability of TMC125 when spray-dried was significantly improved relative to previous formulations (Module 2.7.1, Section 3.2).
Most nonclinical studies were performed after once daily dosing; however, twice daily dosing was investigated using TMC125 HBr in rabbits and dogs and spray-dried TMC125 in dogs. Overall, the value of a twice daily regimen to improve exposure was limited with TMC125 HBr. In an attempt to further increase the exposure, TMC125 was also tested via the dietary admixture in rodents using HBr and spray-dried forms. No improvement in exposure (AUC) 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 effect of food was studied with TMC125 base and spray-dried TMC125 in dogs and the exposure to TMC125 increased by up to 2-fold in the presence of food. A similar finding was noticed in humans. When administered fasting, TMC125 exposure was decreased by approximately 50% compared to administration following a standard breakfast.

Following oral gavage administration, peak plasma concentrations (C_{max}) were generally reached within 4 hours in all species, including humans. The elimination from plasma was rapid in

animals with terminal half-life of approximately 5, 6 and 15 hours in mice, rats and dogs, respectively. In humans, the terminal half-life was longer (30-40 hours). In animals, across the dose range studied, plasma levels of TMC125 increased less than dose proportionally in mice, rats, rabbits and dogs after single oral administration, especially at the high dose levels due to poor solubility. There were no major gender differences in pharmacokinetics in mice and dogs, whereas exposure was higher in female rats compared to male rats. In humans, no major difference was noticed between males and females (Module 2.7.2, Section 3.8.1.2). In all animal species, the systemic exposure decreased after repeated dosing, especially at doses where high exposure values (exceeding the human exposure) were achieved in the first day of dosing. This effect was more pronounced with spray-dried TMC125 (although it was also seen with TMC125 HBr at high dose levels), and was caused by the induction of the liver enzymes involved in the metabolism of TMC125. No relevant decrease in exposure after repeated oral administration was observed in humans (Module 2.7.2, Section 2.5).

Table 1: Summary of Plasma Pharmacokinetic Parameters for TMC125 in Animals Following Single or Repeated Oral Administration of TMC125

Species	TMC125 formulation	Sampling period	Sex/n	Dose (mg/kg/day)	C_{max} (µg/mL)	t_{max} (h)	AUC[a] (µg.h/mL)	$t_{1/2}$ (h)
Mice	HBr salt in PEG400	Day 1	M/45	10	0.34	1	1.6[b]	4.9
				50	0.60	4	4.0[b]	8.6
				200	1.7	4	9.7	3.6
				800	5.2	4	43	2.6
			F/45	10	0.23	2	1.3[b]	4.5
				50	0.67	4	4.1[b]	4.4
				200	1.3	4	9.4	3.0
				800	4.6	4	41	2.6
		Day 91	M/45	10	0.24	1	1.4	3.4
				50	0.56	1	3.1	4.7
				200	0.41	1	3.9	5.3
				800	1.5	1	7.8	4.5
			F/45	10	0.22	2	1.1[b]	9.0
				50	0.37	1	1.7[b]	5.6
				200	0.50	2	4.6	5.2
				800	1.5	4	10	6.0
	Spray-dried/ Dietary	Day 7	M/36	450	0.23	3:00[c]	3.2	NA
			M/24	1620	0.50	19:00[c]	9.8	NA
			M/29	2320	1.3	19:00[c]	19	NA
			F/36	450	0.35	3:00[c]	4.5	NA
				1620	0.71	19:00[c]	12	NA
				2320	1.2	7:00[c]	17	NA
		Day 34	M/6	200	0.20	3:00[c]	2.4	NA
			F/6	200	0.10	3:00[c]	1.5	NA
		Day 49	M/5	2320	0.98	3:00[c]	10	NA
			F/36	2320	0.61	3:00[c]	8.1	NA
		Day 90	M/36	450	0.13	19:00[c]	2.0	NA
			M/9	1620/800[e]	0.35	7:00[c]	4.5	NA
			F/36	450	0.20	19:00[c]	2.6	NA
				1620/800[e]	0.23	15:00[c]	3.5	NA
Rat	Spray-dried as an aqueous suspension	Day 1	M/3	125	0.57	1	2.7	2.2
				250	0.93	2	5.5	3.4
				500	1.6	3	11	3.3
			F/3	125	1.4	1	7.8	3.4
				250	1.7	1	9.8	3.8
				500	2.1	4	18	2.7
		Day 15	F/3	125	1.1	1	5.5	5.3
				250	1.2	1	5.0	4.3
				500	1.4	2	9.1	5.2
		Day 29	M/3	125	0.38	1	1.5	1.6
				250	0.57	1	2.8	9.4
				500	0.76	3	4.5	3.5
	HBr salt in PEG400	Day 1	M/9	70	0.02	4	0.13[b]	15[d]
				200	0.04	4	0.63	8.2[d]
				600	0.33	4	2.0	4.5[d]
			F/9	70	0.05	4	0.27[b]	4.7[d]
				200	0.07	4	0.92	5.2
				600	0.24	4	2.0	6.7[d]
		Week 26	M/9	70	0.06	1	0.21[b]	2.5
				200	0.05	1	0.56	9.1
				600	0.12	8	1.7	50[d]
			F/9	70	0.06	1	0.66	7.1
				200	0.14	4	1.3	NC
				600	0.28	4	3.6	20[d]

a: $AUC_{0-\infty}$ after single dose and AUC_{0-24h} after repeated dose; b: AUC_{0-8h}; c: o'clock time; d: not accurately determined; e: dose was reduced due to poor tolerability; F: female; M: male; NA: not applicable; NC: not calculated

Table 1: Summary of Plasma Pharmacokinetic Parameters for TMC125 in Animals Following Single or Repeated Oral Administration of TMC125 (Continued)

Species	TMC125 formulation	Sampling period	Sex/n	Dose (mg/kg/day)	C_{max} (µg/mL)	t_{max} (h)	AUC[a] (µg.h/mL)	$t_{1/2}$ (h)
Rat (pf)	Spray-dried as an aqueous suspension	Day 1 (GD7)	F/6	125	1.0	4	6.2	3.7
				250	1.3	4	9.4	2.7
				500	1.7	4	12	3.1
		Day 11 (GD17)	F/6	125	0.65	4	5.3	4.2
				250	0.85	2	7.6	5.0
				500	0.64	1	3.6	2.2
		Day 1 (GD7)	F/6	125	0.89	2	6.6	3.2
				250	1.1	4	10	3.2
				500	1.6	8	24	6.1
		Day 11 (GD17)	F/6	125	1.0	2	7.2	4.0
				250	1.1	4	8.6	4.5
				500	0.99	4	13	5.9
Rabbit (pf)	Spray-dried as an aqueous suspension	Day 1 (GD6)	F/3	125	0.51	8	7.6	8.2
				250	0.63	8	12	NC
				375	0.70	2	12	NC
		Day 13 (GD18)	F/3	125	0.38	4	6.1	NC
				250	0.41	1	5.3	NC
				375	0.54	1	9.7	NC
Dog	Spray-dried as an aqueous suspension	Day 1	M/3	160 (80 b.i.d)	3.3[b]	1[b]		
					5.9[c]	1[c]	74	10
				500 (250 b.i.d)	2.5[b]	1.7[b]		
					5.3[c]	0.7[c]	73[d]	13
			F/3	160 (80 b.i.d)	2.9[b]	1[b]		
					6.0[c]	1[c]	63	8.3
				500 (250 b.i.d)	2.6[b]	1[b]		
					6.7[c]	1.7[c]	70[d]	13
		Day 181	M/3	160 (80 b.i.d)	2.2[b]	1[b]		
					3.9[c]	1[c]	55	16
				500 (250 b.i.d)	3.0[b]	1.7[b]		
					4.5[c]	2.8[c]	62	14
			F/3	160 (80 b.i.d)	2.8[b]	1[b]		
					4.0[c]	1.7[c]	45	13
				500 (250 b.i.d)	3.1[b]	1[b]		
					5.8[c]	1[c]	62	9.5
	HBr salt in PEG400	Day 1	M/4	30	0.90	3.8	13	10
			M/7	80	2.0	3.9	23[d]	24
			M/7	240	2.8	3.4	35[d]	10
			F/4	30	0.66	5.5	10	10
			F/7	80	1.6	2.6	18[d]	21
			F/7	240	2.3	3.7	27	31
		Day 366	M/4	30	0.96	3.0	14	22
			M/4	80	2.7	2.0	34	21
			M/7	240	2.2	4.0	36	22
			F/4	30	0.81	1.8	13	15
			F/4	80	1.7	4.0	19	13
			F/7	240	1.8	4.4	23	17

a: $AUC_{0-\infty}$ after single dose and AUC_{0-24h} after repeated dose; b: after the first dosing; c: after the second dosing; d: AUC_{0-24h}; F: female; GD: day of gestation; M: male; NC: not calculated; b.i.d: twice daily; pf: pregnant female

3.3 PLASMA PROTEIN BINDING AND BLOOD PARTITIONING

The plasma protein binding of TMC125 was studied, in vitro, by equilibrium dialysis in mice, rats, rabbits, dogs and healthy male subjects (Module 2.6.4, Section 4.2).

TMC125 was extensively bound to plasma proteins and this was species and concentration independent. In all species, plasma protein binding values ranged between 99.8% and 99.9%. TMC125 was extensively bound to both human albumin (99.60% at a physiological concentration of 4.3%) and α_1-acid glycoprotein (97.66% to 99.02% at physiological concentrations of 0.10% to 0.20%). When TMC125 was tested at a concentration of 0.1 or 1.0 µg/mL, the blood to plasma concentration ratio of TMC125 ranged from 0.66 to 1.4. The fraction of TMC125 distributed in red blood cells was limited (< 30%) in mice, dogs and humans. In rats and rabbits, TMC125 distributed approximately equally between plasma proteins and red blood cells.

3.4 TISSUE DISTRIBUTION

The tissue distribution of TMC125 in rats has been studied using QWBA following single oral administration of ^{14}C-labeled TMC125 (about 70 or 250 mg/kg). Studies were performed in pigmented male Lister-Hooded rats and in pregnant and nonpregnant Sprague-Dawley rats. Distribution to placenta and fetuses was examined in the latter study. Tissue distribution was investigated at several time points up to 336 hours in male pigmented Lister-Hooded rats, at 1, 2, 8 and 24 hours in female Sprague-Dawley rats and at 2 and 24 hours in pregnant female Sprague-Dawley rats (Module 2.6.4, Section 4.1.2).

In rats, tissue distribution of ^{14}C-TMC125 and its metabolites after a single oral dose was rapid and extensive. The highest concentrations of radioactivity were measured in the gastrointestinal tract (the mucosa of stomach, small intestine and cecum), liver and adrenal gland. There was no evidence of undue retention or accumulation and there were no indications of extensive or irreversible binding of TMC125 or its metabolites to melanin. In pregnant rats, there was distribution of ^{14}C-TMC125 to the placenta and the fetus and the total radioactivity levels in these tissues were about twice the levels observed in maternal blood.

3.5 METABOLISM

The metabolism of TMC125 following a single oral administration was quantitatively limited in rodents, dogs and humans. TMC125 was metabolized by Phase I and Phase II reactions and the most important metabolic pathways were methyl hydroxylation, glucuronidation of the resulting hydroxymethyl metabolites, and aromatic hydroxylation. In human, no unique metabolites were observed (Figure 2). In the plasma of animals and humans, unchanged TMC125 was more abundant than any metabolite (Table 2).

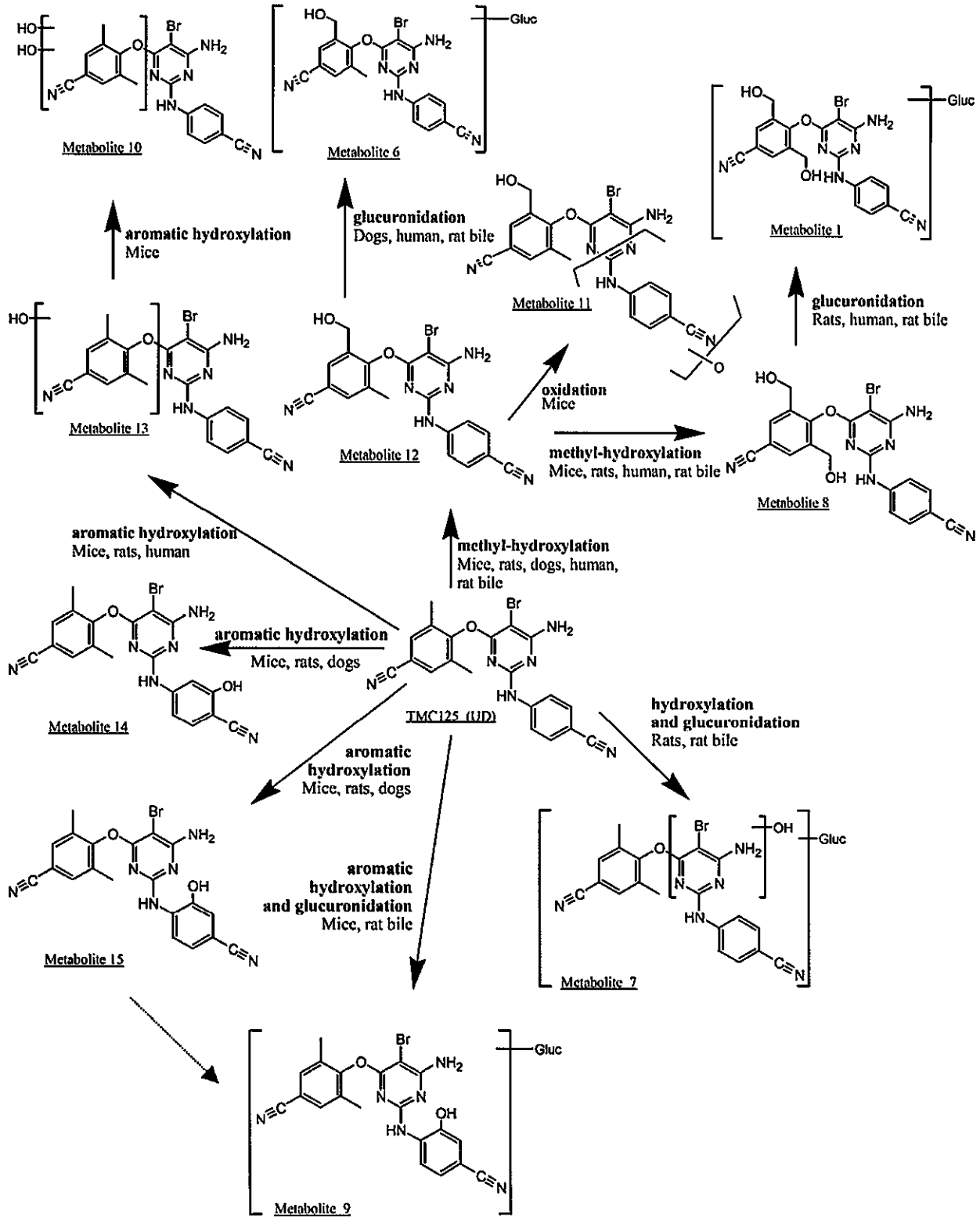
Table 2: Percentage of Administered ^{14}C-TMC125 Dose Metabolized per Major Pathways in the Mouse, Rat, Dog and Human

Metabolic pathway	Mouse		Rat		Dog	Human
	Male	Female	Male	Female	Male	Male
Methyl hydroxylation (M8, M11[a], M12)	8.0	6.5	11.3	6.8	9.7	3.8-9.0
Aromatic hydroxylation (M10, M13, M14, M15)[b]	4.3	4.3	< 1.3	< 1.3	5.3	0-0.2
Hydroxylation + glucuronidation (M1, M6, M7[c], M9[c])	1.1	1.2	< 0.03	0.04	< 0.7	0.2-0.8
Unchanged TMC125	57.5	58.0	85.1	87.4	81.9	81.2-86.4
Total Percentage	**70.9**	**70.0**	**97.7**	**95.5**	**97.6**	**85.2-96.2**

[a] the location of the second oxidation site is not known; [b] at either the dimethylbenzonitrile (M10, M13) or the (cyanophenyl)amine (M14, M15) moiety; [c] the location of the oxidation in the glucuronidated metabolites M7 and M9 is not known.

In vitro, TMC125 metabolism was studied in mice, rats, rabbits, dogs and humans. There was an extensive metabolism of TMC125 in the plated hepatocyte cultures of all animal species, while metabolism occurred to a significantly lower extent in human plated hepatocytes. Metabolism was also generally much less extensive in liver subcellular fractions compared to hepatocyte preparations, which may be due to more limited phase II metabolism. Metabolic pathways proposed on the basis of in vitro studies were consistent with those observed in vivo. (Module 2.6.4, Section 5). The antiviral activities of some metabolites (M8 and M12) were tested on a panel of wild-type and mutant HIV-1 virus strains. When compared to TMC125, the 50% effective concentration values for the wild-type HIV-1 were similar for M12 but were about 200-fold higher (less active) for M8. Antiviral activity on mutant virus strains was clearly lower for M12 as compared to TMC125. M8 did not show any activity on the mutant virus strain tested (Module 2.7.2, Section 3.3.1.2).

Figure 2: In Vivo Metabolic Pathways of TMC125 in Animals and Humans



Ex vivo induction studies in rodents showed that TMC125 was an inducer of cytochrome P450 (CYP) 3A and CYP2B isoenzymes and, to a lesser extent, of cytosolic glutathione-*S*-transferase activity. Additionally, TMC125 induced uridine diphosphate-glucuronosyltransferase (UDP-GT) activity in mice and to a lesser extent in rats. Ex vivo induction studies in dogs showed that TMC125 had an inducing effect on CYP3A and possibly also on CYP2B and other CYP subfamily isoenzymes, however, to a much lesser extent than in rodents. UDP-GT was not induced in dogs (Module 2.6.4, Section 5.5.1). The relevance of these data for drug-drug interaction potential is questionable but they can explain the liver and the thyroid toxicity (cellular hypertrophy) that was observed in rodents following chronic treatment (Module 2.6.6, Sections 3.1 and 5.1).

In vitro and in vivo, CYP was involved in the oxidative metabolism of TMC125. In human liver microsomes (HLM), CYP3A4 enzyme, and to a lesser extent also the CYP2C enzyme, played a major role in the biotransformation of TMC125. In line with this finding, in vivo drug-drug interaction studies in humans showed that well known inducers of CYP3A4 such as efavirenz and rifabutin decreased the exposure of TMC125 (Module 2.7.2, Section 3.9.3). TMC125 inhibited CYP2C9 in HLM with a K_i value of 0.58 µM (0.25 µg/mL). Given this relatively low value, the CYP2C9 inhibition was considered to be clinically relevant. A cocktail probe study on the interaction potential of TMC125 in humans indicated that TMC125 is a mild inhibitor of CYP2C9 and also an inhibitor of CYP2C19 (Module 2.7.2, Section 2.8.1.1). In human hepatocytes, TMC125 was also an inducer of CYP3A4 mRNA expression level as well as activity. Consistent with this finding, a mild induction of CYP3A4 by TMC125 was seen in vivo in the cocktail probe study and also in some drug-drug interaction studies with substrates of CYP3A4 (Module 2.7.2, Section 2.8.1.1).

Other clinical drug-drug interaction studies are described in detail in the Clinical Pharmacology Summary (Module 2.7.2, Section 3.9.3).

3.6 EXCRETION

The routes and extent of TMC125 excretion were studied after a single oral administration of ^{14}C-TMC125 in CD1 mice (200 mg/kg), Sprague-Dawley rats (70 mg/kg), dogs (20 mg/kg) and humans (800 mg). In all animal species, the predominant route of ^{14}C-TMC125 excretion was via feces and amounted to 84%, 100%, 101% and 94% of the administered dose in mice, rats, dogs and humans, respectively. Urinary excretion was less than 0.6% in mice, rats and dogs but was higher in humans i.e 1.2%. The majority of the radioactivity was excreted during the first 24 hours after administration. Unchanged TMC125 was mainly excreted in feces and was about 60%, 85%, 82% and 86% of the administered dose in mice, rats, dogs and humans, respectively. In all species including humans, no unchanged compound was present in urine. Therefore, the renal clearance of TMC125 is negligible. In plasma, unchanged compound accounted for the largest fraction of the radioactivity in all the species incuding humans (Module 2.6.4, Section 6.1). TMC125 was also excreted in the bile in rats (11% of radioactive dose in the first 24 hours). The amount of unchanged TMC125 excreted in the bile was very limited (about 0.1%).

3.7 COMPARISON OF EXPOSURE IN ANIMALS AND MAN

The C_{max} and AUC values of TMC125 after repeated oral administration in various animal species used in the toxicology studies are summarized in Table 1. In addition, exposure ratios for C_{max} and AUC values in comparison with humans are presented in Table 3.

The recommended clinical dose of TMC125 for treatment-experienced HIV-infected patients is 200 mg twice daily (formulation A*). At this dose level, the mean C_{max} was 0.45 µg/mL and the mean $AUC_{0\text{-}24h}$ was 7.4 µg.h/mL in HIV-infected patients after 8 days of treatment (Module 5.3.1.2/TMC125-C228). After comparison of these values with the exposures achieved in animal species after repeated administration, the highest C_{max} ratio (animal/human) was around 3 in mice and female rats, 2 in male rats and 13 in dogs. The highest AUC ratio (animal/human) was 8 in dogs and was around 1 in mice, female rats and rabbits but was less than 1 in male rats. In general, the ratio was influenced by the physical form of the drug substance and the duration of drug administration due to enzymatic auto-induction, which reduced exposure upon repeated administration in animals.

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Table 3: TMC125 Exposure in Animals Relative to Human after Oral Administration

Species	TMC125 formulation	Period (day or week)	Sex/n	Dose (mg/kg/day)	C_{max} (µg/mL)	AUC_{0-24h} (µg.h/mL)	C_{max} Ratio	AUC ratio
Mice	HBr salt in PEG400	91	M/45	10	0.24	1.4	0.53	0.19
				50	0.56	3.1	1.2	0.42
				200	0.41	3.9	0.92	0.53
				800	1.5	7.8	3.3	1.1
			F/45	10	0.22	1.1[a]	0.49	0.15
				50	0.37	1.7[a]	0.81	0.23
				200	0.50	4.6	1.1	0.62
				800	1.5	10	3.3	1.4
	Spray-dried/ dietary	34	M/6	200	0.20	2.4	0.44	0.32
			F/6	200	0.10	1.5	0.22	0.21
		49	M/5	2320	0.98	10	2.2	1.4
			F/36	2320	0.61	8.1	1.4	1.1
		90	M/36	450	0.13	2.0	0.28	0.27
			M/9	1620/800[c]	0.35	4.5	0.77	0.61
			F/36	450	0.20	2.6	0.43	0.35
				1620/800[c]	0.23	3.5	0.50	0.47
Rat	Spray-dried as an aqueous suspension	15	F/3	125	1.1	5.5	2.5	0.74
				250	1.2	5.0	2.7	0.68
				500	1.4	9.1	3.2	1.2
		29	M/3	125	0.38	1.5	0.84	0.21
				250	0.57	2.8	1.3	0.37
				500	0.76	4.5	1.7	0.61
	HBr salt in PEG400	Week 26	M/9	70	0.06	0.21[a]	0.14	0.03
				200	0.05	0.56	0.11	0.08
				600	0.12	1.7	0.27	0.23
			F/9	70	0.06	0.66	0.12	0.09
				200	0.14	1.3	0.31	0.17
				600	0.28	3.6	0.61	0.48
Rat (pf)	Spray-dried as an aqueous suspension	11 (GD17)	F/6	125	0.65	5.3	1.4	0.71
				250	0.85	7.6	1.9	1.0
				500	0.64	3.6	1.4	0.49
		11 (GD17)	F/6	125	1.0	7.2	2.3	0.98
				250	1.1	8.6	2.4	1.2
				500	0.99	13	2.2	1.7
Rabbit (pf)	Spray-dried as an aqueous suspension	13 (GD18)	F/3	125	0.38	6.1	0.8	0.82
				250	0.41	5.3	0.9	0.71
				375	0.54	9.7	1.2	1.3
Dog	Spray-dried as an aqueous suspension	181	M/3	160 (80 b.i.d)	3.9[b]	55	8.5	7.4
				500 (250 b.i.d)	4.5[b]	62	10	8.4
			F/3	160 (80 b.i.d)	4.0[b]	45	8.8	6.0
				500 (250 b.i.d)	5.8[b]	62	13	8.3
	HBr salt in PEG400	366	M/4	30	0.96	14	2.1	1.8
			M/4	80	2.7	34	6.0	4.6
			M/7	240	2.2	36	4.8	4.8
			F/4	30	0.81	12	1.8	1.6
			F/4	80	1.7	19	3.8	2.6
			F/7	240	1.8	23	3.9	3.1

a: AUC_{0-8h} ; b: after the second dosing; c: dose was reduced due to poor tolerability F: female; M: male; pf: pregnant female; b.i..d : twice daily; GD: day of gestation; At 200 mg twice daily (formulation A*), the mean C_{max} was 0.45 µg/mL and the AUC_{0-24h} was 7.4 µg.h/mL in HIV-infected patients after 8 days of treatment. The dose levels underlined are the NOAEL.

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4 TOXICOLOGY

The toxicology of TMC125 was investigated in a series of in vitro and in vivo studies and is described in detail in the Toxicology Written Summary (Module 2.6.6). All pivotal studies and some pilot studies were GLP compliant. All studies used recognized sources of suitable rodent and non-rodent animals in sufficient numbers to meet the objectives of the various studies. The studies conducted are listed in Overview Table 2.6.7.1.

4.1 SINGLE DOSE TOXICITY STUDIES

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.

In **mice**, there were no relevant effects following oral administration of TMC125 base or TMC125 HBr in PEG400 vehicle at doses up to 1000 mg/kg (Module 2.6.6, Section 2.1.1 and 2.1.2). Similarly, there was no effect of TMC125 base in PEG400 vehicle administered by subcutaneous injection to mice at doses of up to 320 mg/kg (Module 2.6.6, Section 2.1.3).

In **rats**, there were no relevant effects following oral administration of TMC125 base or TMC125 HBr in PEG400 vehicle at doses up to 1000 mg/kg (Module 2.6.6, Section 2.2.1 and 2.2.2). Similarly, there was no effect of TMC125 base in PEG400 vehicle administered by subcutaneous injection to rats at doses of up to 320 mg/kg, other than slight skin irritation/necrosis at the site of injection in 1 female given the highest dose (Module 2.6.6, Section 2.2.3).

In **dogs**, the single dose oral toxicity was determined using spray-dried TMC125 (1:3:0.5 active to polymer ratio) formulated 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 conditions. No relevant toxicity findings were observed. The highest exposure was seen in the 350 mg/kg/day fed animals where mean C_{max} and $AUC_{0\text{-}24h}$ values on Day 5 were 11 and 7.6 µg/mL and 135 and 92 µg.h/mL in males and females, respectively (Module 2.6.6, Section 2.3.1).

4.2 REPEATED DOSE TOXICITY STUDIES

Repeated dose studies evaluated the effects of TMC125 HBr formulated in PEG400 in studies of 2 weeks and 3 months in mice and these served as a dose range finder for the carcinogenicity study. Spray-dried TMC125, administered by dietary admixture, was also evaluated in a 3-month study in mice. In Wistar rats, TMC125 base in PEG400 vehicle was used in a 2-week study but subsequently TMC125 HBr formulated in PEG400 was used in 1-, 3- and 6-month studies. An additional 3-month study with TMC125 HBr in PEG400 vehicle was performed in Sprague-Dawley rats in preparation for the carcinogenicity study. Also in Sprague-Dawley rats, spray-dried TMC125, administered by dietary admixture, was evaluated in a 3-month study.

In dogs, TMC125 base in PEG400 vehicle was evaluated by oral (gavage) administration in a 2-week and a 1-month study but subsequently TMC125 HBr formulated in PEG400 was used in

3-, 6- and 12-month studies. When the spray-dried form became available it was tested as an aqueous suspension in dogs in 1- and 6-month studies.

In all studies, control animals were administered the vehicle, and toxicokinetic parameters were evaluated (using satellite animals in the rodent studies).

4.2.1 Mice

In CD1 mice, the oral (gavage) toxicity of TMC125 HBr in PEG400 vehicle was evaluated in a 2-week study up to 1200 mg/kg/day and in a 3-month study up to 800 mg/kg/day (Module 2.6.6, Section 5.1.1).
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. The main target organ in these studies was the liver. Dose-related increases in transaminases, alkaline phosphatase (ALP), triglyceride and cholesterol were seen. Histopathological changes were mainly confirmed as hepatocellular hypertrophy and necrosis. Owing to limited liver changes at the lowest dose level a no observed adverse effect level (NOAEL) could not be established. Systemic exposures ($AUC_{0\text{-}24h}$) observed at the highest dose in the 3-month study were 7.8 and 10 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 (Module 2.6.6, Section 5.1.1). The cause of mortality was attributed to a disturbance in blood coagulation leading to cardiomyopathy, hemothorax and bleeding. These findings were further investigated in 2 mechanistic studies (1 with TMC125 HBr in PEG400 vehicle 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 (Module 2.6.6, Section 8.2). Cardiac lesions and hemorrhages were observed in male mice only and were associated with dietary dosing since no such effects were seen after gavage dosing. No mortality, hemorrhages or cardiac changes were observed in females, although systemic exposure was comparable between both sexes. Changes in the liver, similar to those seen in the gavage study, were also noted. The NOAEL was not determined in this study due to the hemorrhagic cardiomyopathy and liver changes. The highest systemic exposures ($AUC_{0\text{-}24h}$) observed in mice in the dietary study were 10 and 8.1 µg.h/mL in males and females, respectively.

4.2.2 Rats

In rats, the oral (gavage) toxicity of TMC125 in PEG400 vehicle was evaluated in Wistar rats in a 2-week study, at doses of up to 320 mg/kg/day using TMC125 base (Module 2.6.6, Section 3.1.1) and in studies up to 6 months and 600 mg/kg/day using TMC125 HBr in PEG400 vehicle (Module 2.6.6, Section 3.1.2, 3.1.3 and 3.1.4). As dose range finding studies for a carcinogenicity study in Sprague-Dawley rats, a 3-month gavage study with TMC125 HBr in PEG400 vehicle was conducted, as well as a 3-month dietary study with spray-dried TMC125 (Module 2.6.6, Section 5.1.2).
There were no deaths associated with TMC125 in any study, but there were a limited number of accidental gavage 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 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. Owing to limited liver changes at the lowest dose level (330 mg/kg/day) a NOAEL could not be established in the 3-month dietary study. A prolongation of coagulation times was seen in the dietary study but this was not associated with bleedings or cardiac changes. Systemic exposures ($AUC_{0\text{-}24h}$) observed at 330 mg/kg/day were 1.2 and 3.0 µg.h/mL in males and females, respectively. At the maximum feasible dose, exposure values obtained with dietary administration of spray-dried TMC125 (2.1 - 6.7 µg.h/mL at 990 mg/kg/day) were similar to those obtained with gavage administration of TMC125 HBr (2.3 - 6.8 µg.h/mL at 600 mg/kg/day).

4.2.3 Dogs

The oral (gavage) toxicity of TMC125 base formulated in PEG400 was evaluated in Beagle dogs in 2-week and 1-month studies at dose levels of up to 160 mg/kg/day (Module 2.6.6, Section 3.2.2 and 3.2.3), and using TMC125 HBr in PEG400 vehicle, in studies of up to 12 months and 240 mg/kg/day (Module 2.6.6, Section 3.2.5, 3.2.6 and 3.2.8). 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 in the 12-month study was 240 mg/kg/day corresponding to an exposure ($AUC_{0\text{-}24h}$) of 36 and 23 µg.h/mL in males and females, respectively. Additional 4-day, 1- and 6-month dog studies with spray-dried TMC125 as an aqueous suspension were conducted with dose levels of up to 500 mg/kg/day given over 2 equal doses separated by 5 hours (Module 2.6.6, Section 3.2.1, 3.2.4 and 3.2.7). Vomiting was noted in a dose-related frequency and severity. The highest dose level (500 mg/kg/day) was associated with decreases in body weight and food consumption. Also at this dose level, increases in total bilirubin, ALP and alanine aminotransferase (ALT) were noted in male animals. At dose levels of 160 mg/kg/day and above, hepatic changes (microgranuloma) were observed, while gall bladder changes (inspissated bile) were seen at 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 ($AUC_{0\text{-}24h}$) of 62 µg.h/mL in males and females after 6 months of dosing at 500 mg/kg/day.

4.3 GENOTOXICITY

Genotoxicity tests, in vitro and in vivo, (Module 2.6.6, Section 4) have shown TMC125 to be free of genotoxic potential, with and without liver metabolic activation system. These tests included in vitro gene mutation assays (Ames test and mouse lymphoma test), an in vitro chromosomal aberration test (human lymphocytes) and an in vivo chromosomal aberration test (mouse bone marrow micronucleus test; up to a single dose of 2000 mg/kg). In the latter test, mean C_{max} and $AUC_{0\text{-}\infty}$ values were 7.9 µg/mL and 111 µg.h/mL, respectively.

4.4 CARCINOGENICITY

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, formulated in PEG400, given by oral gavage, or spray-dried TMC125 administered via the diet (Module 2.6.6, Section 5.1.1 and 5.1.2). Gavage administration using TMC125 HBr was chosen for the carcinogenicity studies as spray-dried TMC125 in the diet did not increase the exposure and was not tolerated in male mice. The use of transgenic RasH2 mice has been explored in a dose range finding study (Module 2.6.6, Section 5.3.1), 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[4].

4.5 REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Reproduction and development effects of TMC125 HBr were evaluated in a fertility and early embryonic development study in rats and in embryo-fetal development studies in rats and rabbits. 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. Also a pre- and postnatal development study in rats was performed with this form.

4.5.1 Fertility and Early Embryonic Development

A first fertility and early embryonic development study in rats was performed with TMC125 HBr in PEG400 vehicle (Module 2.6.6, Section 6.1.1). 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 as an aqueous suspension up to a dose level of 500 mg/kg/day (Module 2.6.6, Section 6.1.2). No relevant effects were observed in either study and the NOAEL was considered to be 506 or 500 mg/kg/day. Exposure ($AUC_{0\text{-}24h}$) in the study using the spray-dried form was 4.5 and 9.09 µg.h/mL in males and females, respectively.

4.5.2 Embryo-fetal Development

In the rat embryo-fetal development study, following a dose range finding study, TMC125 HBr in PEG400 vehicle was administered by oral gavage at dose levels of up to 1000 mg/kg to pregnant Wistar rats during the period of fetal organogenesis (days 6 to 16 post-coitum inclusive) (Module 2.6.6, Section 6.2.1). There was no maternal toxicity and no effects on embryo-fetal development at any of the doses administered. The fetal NOAEL was considered to be 1000 mg/kg/day and TMC125 is considered not to be teratogenic in the rat. Maternal exposure ($AUC_{0\text{-}24h}$) at this dose level amounted to 8.3 µg.h/mL.

In an embryo-fetal developmental toxicity study TMC125 HBr formulated in alpha-tocopheryl polyethylene glycol succinate (TPGS)/HPMC was dosed up to 750 mg/kg/day to pregnant New Zealand white rabbits during the period of fetal organogenesis (gestation days 6 to 18) (Module 2.6.6, Section 6.2.2). There was no maternal toxicity and no effects on embryo-fetal development at any of the doses administered. However, exposure ($AUC_{0\text{-}24h}$) at the end of treatment at the highest dose level was only 3.8 µg.h/mL and, therefore, an additional study was conducted using spray-dried TMC125 as an aqueous suspension at doses up to 375 mg/kg/day (Module 2.6.6, Section 6.2.2). There was an initial decrease in body weight and food consumption of dams at doses of 250 and 375 mg/kg/day, but no effects were seen on embryo-fetal development at any of the doses administered. The maternal NOAEL was 125 mg/kg/day ($AUC_{0\text{-}24h}$ 6.1 µg.h/mL)

and the fetal NOAEL was considered to be 375 mg/kg/day ($AUC_{0\text{-}24h}$ 9.7 µg.h/mL). TMC125 is considered not to be teratogenic in rabbits.

4.5.3 Pre- and Postnatal Development

A pre- and postnatal development study was conducted in rats with spray dried TMC125 as an aqueous suspension. Following a dose-range finding study, spray-dried TMC125 was administered by oral gavage, at dose levels of up to 500 mg/kg/day to females from Day 7 of gestation to Day 21 of lactation and the first generation (F1) was allowed to mature untreated (Module 2.6.6, Section 6.3.1 and 6.3.2). No adverse effects were observed in the study. 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. The maternal NOAEL was considered to be 500 mg/kg/day. The highest maternal exposure ($AUC_{0\text{-}24h}$) achieved at the end of treatment was 7.6 µg.h/mL.

4.6 LOCAL TOLERANCE

Both TMC125 base and TMC125 HBr were evaluated in a range of local tolerance studies (Module 2.6.6, Section 7.1.1 and 7.1.2). Skin sensitization and irritation were evaluated using in vivo models, whereas eye irritation and phototoxicity were evaluated using in vitro models. TMC125 was classified as "non sensitizing" in an in vivo guinea pig skin sensitization study and in a mouse local lymph node assay. TMC125 was also classified as "non irritant" in an in vivo rabbit skin irritation study. In an in vitro eye irritation assay, TMC125 in base form was considered to be a "mild" eye irritant, whereas in HBr-salt form, it was considered to be a "very severe" eye irritant. No effects were observed in an in vitro phototoxicity study.

4.7 OTHER TOXICITY STUDIES

4.7.1 Immunotoxicity

Immunotoxicity has been evaluated in rats. TMC125 HBr formulated in PEG400 was administered at doses of up to 600 mg/kg/day (Module 2.6.6, Section 8.1.1). There were no relevant effects of treatment with TMC125 and the immune response, as measured by IgM production, was not affected by treatment. Exposure ($AUC_{0\text{-}24h}$) at the highest dose was 1.5 and 5.4 µg.h/mL, in males and females respectively at the end of the study.

4.7.2 Mechanistic Toxicity Studies

Two mechanistic toxicity studies were conducted to address the hemorrhagic cardiomyopathy finding that was observed with spray-dried TMC125 in male mice in the 3-month dietary study. The literature describes identical cases of hemorrhagic cardiomyopathy and hemothorax caused by a coagulopathy in vitamin K-deficient mice which was more specific to male than female mice[5,6]. One mechanistic study was performed with spray-dried TMC125 (1:2:0.5 active to polymer ratio) given via the diet at a dose level of 2320 mg/kg/day, with and without vitamin K_1 injection, for 6 weeks. Unlike the 3-month study, only one male mouse, given spray-dried TMC125 without vitamin K_1, developed hemorrhagic cardiomyopathy and had to be sacrificed. This animal had marked prolongation in coagulation time, decreased clotting factors, hemothorax and elevated cardiac troponin. Compared to the vehicle group without vitamin K_1,

prothrombin time (PT) was prolonged by 3.9-fold and activated partial thromboplastin time (APTT) was >100 sec in this animal. Furthermore, all clotting factors investigated were markedly decreased: II (-90%), VII (-65%), VIII (-73%) and XI (–93%). 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, however, were not associated with bleedings and 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. No cardiac lesions nor increases in cardiac troponin were observed in any animal in the study other than the one animal that died of hemorrhagic cardiomyopathy. In line with literature data, this study demonstrated that heart lesions could occur in association with very pronounced effects on coagulation parameters in mice. The study also confirmed that TMC125 related changes in coagulation times and clotting factors are mediated via a mechanism involving vitamin K.
The second mechanistic study was performed with TMC125 HBr in PEG400 vehicle given via oral gavage at a dose level of 1000 mg/kg/day, with and without vitamin K_1 injection, for 4 weeks. 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 mostly (clotting times: increase up to 31% in APTT and 10% in PT) or totally absent (vitamin K-dependent factors) in females dosed with TMC125 without vitamin K_1. Hemorrhagic cardiomyopathy was not seen in any animal in this study and this correlated with an absence of changes in cardiac troponin. This study also demonstrated that the effect of TMC125 on clotting parameters in mice was mediated via a mechanism involving vitamin K. The effects on coagulation parameters seen with gavage dosing were less pronounced relative to those reported after dietary dosing.
The reports of the mechanistic studies are described in the repeated dose toxicity summary (Module 2.6.6, Section 8.2.1 and 8.2.2).

4.7.3 Drug Substance Impurities and Intermediates

Several drug substance impurities and intermediates were investigated (Module 2.6.6, Sections 8.3, 8.4 and 8.5). The impurity A* and impurity B* were qualified above their specified levels and they were considered non-genotoxic in an Ames test and in a chromosomal aberration test and also non-toxic in a repeated dose (3 months) toxicity study. The impurity C* was tested in an Ames test and considered non-mutagenic.

Genotoxicity studies were conducted on 2 intermediate products, intermediate A* and intermediate B*. Intermediate A* did not induce mutagenicity in bacteria, nor clastogenicity in human lymphocytes or Chinese hamster V79 cells. Therefore, intermediate A* is considered to be a non-genotoxic intermediate. 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 the negative in vitro clastogenicity results obtained with peripheral human lymphocytes at higher concentrations and the negative in vivo micronucleus test. Based on the weight of evidence analysis including all available data intermediate B* is not considered as a genotoxic intermediate.

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4.8 TOXICOLOGY EVALUATION

4.8.1 Mortality

No TMC125-related mortality was noted in the different single dose studies performed by subcutaneous injection. Lethality related to TMC125 either as TMC125 base, TMC125 HBr or the spray-dried form could not be demonstrated following single or repeated oral (gavage) administration in mouse, rat and dog. In some occasions animals were found dead or were killed prematurely due to poor clinical condition. In most cases, this was confirmed to be associated with the dosing procedures and mainly caused by the irritant and viscous nature of the vehicle (PEG400) used.

In the 3-month dietary study in mice, unexpected high mortality was noted in male animals only from a dose of 450 mg/kg/day and at exposure ($AUC_{0\text{-}24h}$ 2.0 µg.h/mL) below that seen in HIV-infected patients. The cause of death was hemorrhagic cardiomyopathy, often associated with hemothorax and hemorrhages in several organs. In the same study, no mortality, hemorrhages or cardiac changes were observed in females, although systemic exposure was comparable between both sexes. The underlying mechanism for the cardiac lesions was explained by severe disturbance in the coagulation system. The results of the mechanistic studies performed demonstrated that the effect of TMC125 on coagulation times and clotting factors was mediated via a mechanism involving vitamin K. 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 mortality seen in male mice and its underlying pathology are considered as being gender and species specific and, therefore, of no relevance to humans.

4.8.2 Safety Margin

The safety margin for TMC125, expressed on the basis of AUC, is close to or less than 1 in rodents but up to about 5 in dogs at the recommended clinical dose regimen (200 mg twice daily; formulation A*) in treatment-experienced HIV-1 infected subjects (C_{max} value of 0.45 µg/mL and an $AUC_{0\text{-}24h}$ of 7.4 µg.h/mL; Module 5.3.1.2/TMC125-C228). However, it is not uncommon for ARV drugs to have no or low margins of safety. TMC125 was originally tested in its base form in a limited number of studies. Exposure was improved by the use of 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 and mice than in dogs. Some further improvement in exposure was achieved with the development of the spray-dried form. To assess the nonclinical safety of TMC125 at the highest possible exposure, some studies were repeated with spray-dried TMC125. These studies included: 1- and 6-month repeated dose studies in dogs, a fertility study in rats and an embryo-fetal development study in rabbits. The modified Irwin test and the pre- and postnatal developmental toxicity study in the rat were directly performed with spray-dried TMC125. Toxicokinetic data indicate that the absorption of TMC125 is limited and dependent on the TMC125 form used after oral administration in all species used in the nonclinical safety evaluation program. Saturation of absorption, due to poor solubility and permeability across the intestine, was frequently noted over the dose ranges tested. 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 animal species, mainly with TMC125 HBr

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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, was discontinued as it did not increase exposure versus gavage administration with the HBr salt and was poorly tolerated in male mice.

4.8.3 Assessment of Target Organ Toxicity

The key target organ/systems identified in the nonclinical studies were the liver (in mouse, rat and dog), the thyroid (in rat) and the coagulation system (in mouse and rat). The heart (hemorrhagic cardiomyopathy) was identified as a species/gender specific target organ in male mice secondary to changes in the coagulation system.

4.8.3.1 LIVER

Liver changes were observed in mice, 2 strains of rats and dogs but were most pronounced in mice. In mice, in addition to the increased liver weight, most apparent with spray-dried TMC125 (up to 2.4-fold), the macroscopic 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 demonstrated accumulation of lipid globules in the hepatocytes as compared to that in control mice. Liver changes were observed in mice at the lowest tested dose in the 3-month study (10 mg/kg/day) with TMC125 HBr and at systemic exposures below those reported in HIV-infected patients ($AUC_{0\text{-last}}$ of 1.4 and 1.1 µg.h/mL in male and female animals, respectively). Ex-vivo metabolic study following 3-month administration of TMC125 HBr in mice showed a dose-related induction (up to 11-fold) in the microsomal activities of CYP3A and CYP2B. This strong enzymatic induction may have contributed to the liver changes seen in mice.

In rats, liver weight was increased slightly (up to 27%) in the 3- and 6-month studies with TMC125 HBr at the highest tested dose level only (600 mg/kg/day, $AUC_{0\text{-}24h}$ 1.7 to 4.5 µg.h/mL), and in the 3-month study with spray-dried TMC125 from a dose of 330 mg/kg ($AUC_{0\text{-}24h}$ 1.2 to 3.0 µg.h/mL). In 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, liver changes were limited to basophilic stippling (Wistar) or fatty-like vacuolization (Sprague-Dawley). Ex-vivo metabolic study following 3-month administration of TMC125 HBr in rats showed induction of microsomal CYP3A, CYP2B and other CYP subfamily forms. Based on the above, the 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[7].

In the dog, in the 1-month and 6-month repeated dose toxicity study with spray-dried TMC125, different liver changes were observed (microgranuloma and minor inflammatory changes, and

inspissated bile in gall bladder). In the 1-month study with spray-dried TMC125 in dogs, these liver changes were observed in 2 high dose animals with associated increases in serum bilirubin and ALP. 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. Increases in total bilirubin, ALP and ALT were also noted at 500 mg/kg/day but only in male animals. The dose level of 160 mg/kg/day corresponds to an $AUC_{0\text{-}24h}$ of 55 and 45 µg.h/mL in males and females, respectively, while values after 500 mg/kg/day were 62 µg.h/mL in males and females. No liver changes were observed in dogs in the 12-month study with TMC125 HBr at doses up to 240 mg/kg/day and exposures ($AUC_{0\text{-}24h}$) of 36 and 23 µg.h/mL in males and females, respectively. The occurrence of these changes after dosing with spray-dried TMC125 in the dog is therefore likely to be 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 at the recommended therapeutic dose. Ex-vivo metabolic study following 1-month administration of spray-dried TMC125 in dogs showed an induction in the microsomal activities of CYP3A and CYP2B. This enzymatic induction may have contributed to the liver changes seen in dogs.

Monitoring of transaminases and other hepatobiliary parameters was performed in all clinical trials conducted. No signs of drug-induced liver toxicity were observed. In the pooled Phase III trials (Module 5.3.5.1/TMC125-C206 and TMC125-C216), the incidence of liver-related adverse events (AEs) was comparable to placebo (5.3% in the TMC125 group versus 5.1% in the placebo group). The most common liver-related AEs in subjects that initiated treatment with TMC125 were related to increases in liver enzymes (3.5% versus 3.0% in the placebo group). Most AEs were mild to moderate in severity; 2.5% of subjects in each treatment group had grade 3 or 4 liver-related AEs. Four (0.7%) subjects in each treatment group discontinued due to liver-related AEs. The laboratory evaluation of hepatic parameters for the TMC125 treatment group showed that mean changes (i.e. decreases) from baseline for aspartate aminotransferase (AST) and ALT were small, not different from the placebo group and considered not clinically relevant. Most individual liver abnormalities in subjects who started treatment with TMC125 were grade 1 or 2 (i.e. mild or moderate) in severity; their incidence was generally similar to placebo. The incidence of grade 3 or 4 increases in ALT and AST was low (2.5% versus 1.7% in the placebo group). Data from other clinical trials (Phase I and II) in both healthy volunteers and HIV-1 infected subjects did not reveal any hepatic safety signals or findings substantially different from that in the pooled Phase III dataset. Taken together, these results showed that liver abnormalities in subjects receiving TMC125 are generally non-severe, and that their frequency is low and not different from that observed in subjects receiving placebo.

4.8.3.2 THYROID

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 ($AUC_{0\text{-}24h}$ 0.74 and 2.3 µg.h/mL in males and females, respectively). Measurement of triiodothyronine (T3), thyroxine (T4) and thyroid stimulating hormone (TSH) in the 3-month gavage study with TMC125 HBr in rats demonstrated decreases in T3 and T4 and a compensatory rise in TSH. In the same study, induction of microsomal thyroxine UDP-GT activity was reported ex-vivo. These changes are considered to be rodent-specific and pose no safety risk to humans[8]. In one 3-month study with TMC125 HBr in the Wistar rat, these findings were associated with a slight increase in severity

of hypertrophy and vacuolation of the TSH-producing cells in the pars anterior of the pituitary gland in high dose (600 mg/kg/day) males only ($AUC_{0\text{-}24h}$ 1.7 µg.h/mL). No thyroid or pituitary changes have been observed in studies in mice or dogs. The thyroid and pituitary changes seen in rats are considered to be rodent-specific and related to adaptive response to enhanced thyroid hormone elimination and therefore considered of no relevance to humans.

In several clinical trials in HIV-1 infected subjects and healthy volunteers, TSH, T3 and T4 were assessed at several time points including at baseline, during the treatment and/or follow-up periods. From the amassed clinical data to date, there has been no trend or signal of thyroid dysfunction. Thyroid-related AEs and laboratory abnormalities were infrequent in the clinical trials with TMC125. In the pooled Phase III trials, hypothyroidism was reported in one (0.2%) subject who initiated treatment with TMC125 compared to 2 (0.3%) subjects in the placebo group (Module 5.3.5.1/TMC125-C206 and TMC125-C216). Hyperthyroidism was reported in 2 (0.3%) TMC125-treated subjects. The laboratory evaluation showed no consistent changes over time for the thyroid parameters in subjects who initiated treatment with TMC125. The incidence of individual laboratory abnormalities was generally similar in subjects who initiated treatment with TMC125 compared to placebo. Both increases and decreases were observed and their incidence was low. Increased TSH, T4 and T3 levels were observed in 4.9% (versus 5.7% in the placebo group), 1.0% (versus 0.7% in the placebo group) and 6.6% of subjects (versus 8.6% in the placebo group) who initiated treatment with TMC125, respectively. The incidence of decreased TSH, T4 and T3 levels was similar to placebo: decreased TSH was observed in 2.1% of subjects who initiated treatment with TMC125 (versus 0.8% in the placebo group), decreased T4 in 3.1% (versus 1.7% in the placebo group) and decreased T3 in 1.7% (versus 2.4% in the placebo group). Data from other clinical trials (Phase I and II) in both healthy volunteers and HIV-1 infected subjects did not reveal any thyroid-related safety signals or findings substantially different from that in the pooled Phase III dataset. In conclusion, the clinical data show that TMC125 has no specific effect on thyroid function in humans and that the frequency of thyroid abnormalities is low and not different from placebo.

4.8.3.3 COAGULATION SYSTEM/HEART

Changes in the coagulation system were observed in mice and rats. In rats, increases in coagulation times with TMC125 were only observed during a 3-month dietary study and not in any gavage study, demonstrating a higher impact of this dosing route on the coagulation system. In the dietary study where animals were treated at 330, 990 and 1300 mg/kg/day, an increase in PT (up to 22%) was seen in all treated males. However, all treated females showed a reduction in PT (up to 13%). APTT was also increased (up to 61%) in all treated males but only females treated at 990 mg/kg/day showed a marginally elevated APTT (10%). These changes were not associated with micro- or macroscopic hemorrhages or with cardiac lesions. TMC125 exposure was similar in male and female rats and was lower than the exposure observed in patients at the recommended clinical dose.

In mice, changes in the coagulation parameters were observed in oral gavage and dietary studies and in the dietary study only these changes led to hemorrhagic cardiomyopathy and hemothorax in male mice. In the 3-month dietary study with spray-dried TMC125, an unexpected high mortality in male mice from a dose of 450 mg/kg/day ($AUC_{0\text{-}24h}$ 2.0 µg.h/mL) was noted. 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. The literature described identical cases of hemorrhagic cardiomyopathy and hemothorax caused by a coagulopathy in vitamin K-deficient mice[5,6]. The disorder affects primarily the heart muscle by producing myofiber degeneration, necrosis, and hemorrhage. In all these cases males were much more sensitive to coagulopathy and hemorrhagic cardiomyopathy than females. It has been suggested that females are less susceptible to vitamin K deficiencies since they are able to produce the active form of vitamin K more rapidly and at a more effective concentrations based on estrogen-induced epoxidase activity[9]. At the same time, enteric vitamin K absorption is more efficient in females[10]. To examine if a vitamin K mediated mechanism is involved in the heart injury findings seen in male mice treated with TMC125 by the dietary route, two mechanistic studies, one by gavage (4 weeks at 1000 mg/kg/day) and the other by dietary administration (6 weeks at 2320 mg/kg/day) were conducted. Changes in the coagulation parameters were observed in both studies, but they were more pronounced after the dietary study relative to the gavage study, in spite of similarities in liver and plasma exposure. In the dietary mechanistic study, increases in PT (99%) and APTT (68%) were noted in males at 2320 mg/kg/day. In females, similar changes were noted but were less pronounced: PT (34%) and APTT (51%). Decreases in the vitamin K-dependent clotting factors II and VII were also seen, more pronouncedly in males than in females. In the mechanistic study performed via gavage (at 1000 mg/kg/day), increases in PT (72%) and APTT (56%) were noted in males, while again in females the changes were less pronounced: PT (10%) and APTT (31%). Decreases in the vitamin K dependent clotting factors II and VII were noted in males only. In both studies, the changes seen in the coagulation parameters were counteracted by daily administration of Vitamin K_1. The studies demonstrated that the effect of TMC125 on clotting times and clotting factors in mice is mediated via a vitamin K pathway. Unlike the 3-month dietary study, only one animal (male) developed hemorrhagic cardiomyopathy in the dietary mechanistic study. This animal showed a marked prolongation in clotting times (3.9-fold for PT; >100 sec. for APTT) and a pronounced reduction of clotting factors: II (-90%), VII (-65%), VIII (-73%) and XI (-93%). In this animal, elevated levels of cardiac troponin I were detected in serum (4.73 ng/mL). Troponin levels, however, were below the limit of detection in all other TMC125 treated animals in both mechanistic studies indicating no cardiac damage. 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.

Overall, the effects of TMC125 on coagulation times in rodents 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. 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 and no cardiac changes were noted in female mice or in rats or dogs of either sex. Based on the above, the findings observed in male mice are considered to be of no relevance to humans.

Coagulation parameters (PTT, PT, international normalized ratio (INR)) were monitored at regular intervals in clinical trials, and bleeding events were included as events of interest to allow for detection of any signal of coagulation disturbance. No evidence of alteration of coagulation or of bleeding disorders has been observed. In the pooled Phase III clinical trials, changes over time from baseline for PTT, PT and INR did not reveal any evidence for a clinically relevant effect with TMC125 treatment (Module 5.3.5.1/TMC125-C206 and TMC125-C216). Evaluation

of individual abnormalities for subjects who initiated treatment with TMC125 showed that most PTT, PT and INR abnormalities were grade 1 or 2 in severity and that their incidence was similar to placebo. The incidence of grade 3 or 4 abnormalities in PTT (1.2% versus 1.7% in the placebo group), PT (0.3% versus 0.5% in the placebo group) and INR (0.7% in each treatment group) was low. Bleeding AEs were infrequent and the incidence was similar in subjects who initiated treatment with TMC125 as in the placebo group (3.8% vs. 4.6%). There was no pattern of specific bleeding events. Grade 3 or 4 bleeding events were not reported in the TMC125 group and none of the subjects discontinued the trials due to a bleeding event. Data from other clinical trials (Phase I and II) in both healthy volunteers and HIV-1 infected subjects did not reveal any coagulation-related safety signals or findings substantially different from that in the pooled Phase III dataset. Therefore, from the current data, there is no evidence in clinical trials that TMC125 is associated with an increased frequency of coagulation abnormalities.

4.8.4 Assessment of Genotoxicity and Carcinogenicity

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). Carcinogenicity studies in mice and rats are currently being performed. The information available to date does not suggest potential carcinogenic risk based on a genotoxic mechanism.

4.8.5 Assessment of Reproduction and Development Toxicity

Reproduction and developmental toxicity studies demonstrated that TMC125 did not affect reproductive parameters.

Fertility and early embryonic development studies performed in the rat with TMC125 HBr and spray-dried TMC125, revealed no effect on mating or fertility in male and female animals treated at TMC125 dose levels up to 500 mg/kg/day and at exposures (C_{max}, AUC) equivalent to those in humans at the recommended therapeutic dose. There was also no evidence of teratogenicity in embryo-fetal development toxicity studies conducted in rats (up to 1000 mg/kg/day) and rabbits (up to 375 mg/kg/day). In both species, the maternal exposure at the end of the treatment was equivalent to human exposure at the recommended therapeutic dose. In the rabbit, however, the exposure levels exceeded the clinical exposure at the beginning of dosing i.e. during the stages of the organogenesis but levels subsequently declined due to auto-induction. The safety of TMC125 has not been assessed in pregnant women in well controlled clinical trials. Therefore, women of child bearing potential should only use TMC125 if the potential benefit justifies the potential risk.

In rats, TMC125 had also no effect on offspring development during lactation or postweaning when the dams were dosed up to 500 mg/kg/day at exposures equivalent to those observed in the clinic. In rats, no studies have been conducted to assess directly the excretion of TMC125 into the milk. In a tissue distribution study, some radioactivity was detected in the mammary glands in rats, which indicates the possibility of TMC125 excretion via milk. In humans, it is not known if TMC125 is excreted in milk. Because of the potential for HIV transmission and adverse events in nursing infants, mothers should be instructed not to breastfeed if they are receiving TMC125.

5 INTEGRATED OVERVIEW AND CONCLUSIONS

The secondary pharmacodynamics and safety pharmacology program revealed no relevant effects of TMC125 on in vitro and in vivo cardiovascular electrophysiological parameters respiratory parameters, neurobehavior, motor activity or any other body functions.

Pharmacokinetic data revealed low to intermediate transepithelial intestinal permeability of TMC125. Passive transcellular diffusion was proposed as a mechanism for TMC125 absorption. However, the solubility, and in the case of suspensions and solid dosage forms, possibly also dissolution seem to limit the rate and extent of absorption, resulting in saturation of absorption and exposure at high dose levels. Many studies were performed to investigate whether a specific formulation or route of administration could improve absorption. Any potential improvements in absorption after single administration were somewhat negated after repeated administration due to the induction of liver enzymes. It was considered that the maximum feasible exposures were obtained in the nonclinical program of studies. Following oral (gavage) administration peak plasma concentrations were generally reached within 4 hours in all species. Tissue distribution and the elimination from plasma was rapid. The human profile of metabolites was reflected in the species tested and the animal pharmacokinetic data in general reflected the clinical data, albeit at lower levels in some species. In all species, the contribution of metabolic elimination to the overall disposition of TMC125 was quantitatively limited as in the plasma and feces of animals and humans unchanged TMC125 was more abundant than any metabolite. TMC125 was predominantly excreted unchanged via the feces in all species. Urinary elimination of TMC125 was negligible.

In single dose and repeated dose toxicity studies, up to 3 months in mice, 6 months in rats and 12 months in dogs, there were only limited effects of treatment with TMC125. The key target organ/systems identified in the nonclinical studies were the liver (in mouse, rat and dog), the thyroid (in rat) and the coagulation system (mouse and rat). A species/gender specific effect on the heart (hemorrhagic cardiomyopathy) occurred in male mice, only in the dietary studies. In rats, the changes seen in liver and thyroid (cellular hypertrophy with increase in organ weight) were consistent with the liver enzyme inducing property of TMC125 and are considered to reflect an adaptive response to liver enzyme induction after administration of a xenobiotic, rather than a direct adverse effect of TMC125. In the mice, however, liver changes were more pronounced and included elevation of serum transaminases and histopathological changes such as hepatocellular hypertrophy and necrosis. The liver changes seen in rodents occurred at exposures below those anticipated in the clinic at the recommended therapeutic dose. In the dog, repeated dosing with spray-dried TMC125 resulted also in liver changes (increases in transaminases, microgranuloma and minor inflammatory changes, and inspissated bile in gall bladder). However, these were observed at exposures at least 8-fold higher than the anticipated human exposure. In rodents, changes in the coagulation parameters were also noted, more pronouncedly in mice relative to rats. In mice and following dietary administration only, disturbances in the coagulation parameters led to mortality, hemorrhagic cardiomyopathy and hemothorax in male animals only. These effects are considered to be of no relevance to human as this was demonstrated to be an effect of TMC125 on coagulation times and clotting factors mediated by a mechanism involving vitamin K to which male mice are particularly susceptible.

Genotoxicity tests, in vitro and in vivo, have shown TMC125 to be free of genotoxic potential. Rat and mouse carcinogenicity studies are ongoing but the information available to date does not

suggest potential carcinogenic risk based on a genotoxic mechanism. Reproduction and developmental toxicity studies demonstrated that TMC125 is not teratogenic and did not affect reproductive parameters. TMC125 was shown not to be immunotoxic.

The recommended clinical dose regimen of TMC125 for treatment-experienced HIV-1 infected subjects is 200 mg twice daily (twice daily formulation A*). At this dose, the mean C_{max} value was 0.45 µg/mL and the mean $AUC_{0\text{-}24h}$ was 7.4 µg.h/mL (Module 5.3.1.2/TMC125-C228). The safety margin for TMC125, expressed on the basis of AUC, was close to or less than 1 in rodents but up to about 5-fold in dogs. However, it is not uncommon for ARV drugs to have no or low margins of safety. In general, exposure was influenced by the physical form of the drug substance and the duration of drug administration due to enzymatic auto-induction, which reduced exposure upon repeated administration. The use of TMC125 HBr rather than TMC125 base improved exposure 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. Some further improvement in exposure was achieved with the development of the spray-dried form and to assess the nonclinical safety of TMC125 at the highest possible exposure, some studies were repeated with spray-dried TMC125.

The nonclinical program is considered adequate to support the use of TMC125 in the stated indication with a number of statements in the product label reflecting findings and/or limitations of nonclinical studies.

＊：新薬承認情報提供時に置き換え

6 NONCLINICAL FINDINGS TO CONSIDER FOR THE PRODUCT LABEL

With the exception of the ongoing carcinogenicity studies, all nonclinical studies required to support the chronic use of drugs in non-life threatening conditions have been performed as part of the safety assessment of this novel HIV NNRTI. These include: complete safety pharmacology and pharmacokinetic evaluation, chronic toxicity studies, genotoxicity, reproductive assessment of fertility, embryonic and pre- and postnatal development and immunotoxicity. However, based on the data generated and discussed above, there are several points to consider for inclusion in the appropriate sections of the product label, these include:

- TMC125 is a substrate of CYP3A4 and CYP2C family and as a result potent inducers or inhibitors of these iso-enzymes may alter plasma concentrations of TMC125 and may alter its therapeutic effect or adverse events. TMC125 is also an inhibitor of CYP2C9 and an inducer of CYP3A and drug-drug interaction can occur for therapeutic agents predominantly eliminated by these CYP450 iso-enzymes.
- TMC125 is a P-gp inhibitor (not a substrate) in vitro. Therefore, drug-drug interactions via this pathway cannot be ruled out.
- TMC125 is approximately 99.9% bound to plasma proteins, primarily to albumin (99.6%) and α1-acid glycoprotein (97.66%-99.02%) in vitro.
- The predominate route of TMC125 elimination is via the feces. Urinary elimination is limited (<1.2% of the radioactive dose in humans). Therefore, the impact of renal impairment on TMC125 clearance is expected to be minimal.
- Carcinogenicity data are not yet available and the lack of information on potential carcinogenic risk should be reflected in the product label. However, it should be noted that there was no evidence of mutagenicity in vitro or in vivo.
- There were no effects on reproductive functions and development, and there is no evidence of teratogenicity in rats or rabbits.
- There were indirect indications of TMC125 milk excretion in rats. It is not yet known if TMC125 is excreted in human milk and whether there is potential for adverse events in nursing infants.

7 LIST OF LITERATURE REFERENCES

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