5.3 PRESYSTEMIC METABOLISM (GI/HEPATIC FIRST-PASS EFFECTS)

Across species, the first pass effect on TMC278 is limited and appears to be higher in mice and in rats than in dogs.

5.4 IN VITRO METABOLISM, INCLUDING P450 STUDIES

5.4.1 In Vitro Metabolic Pathways

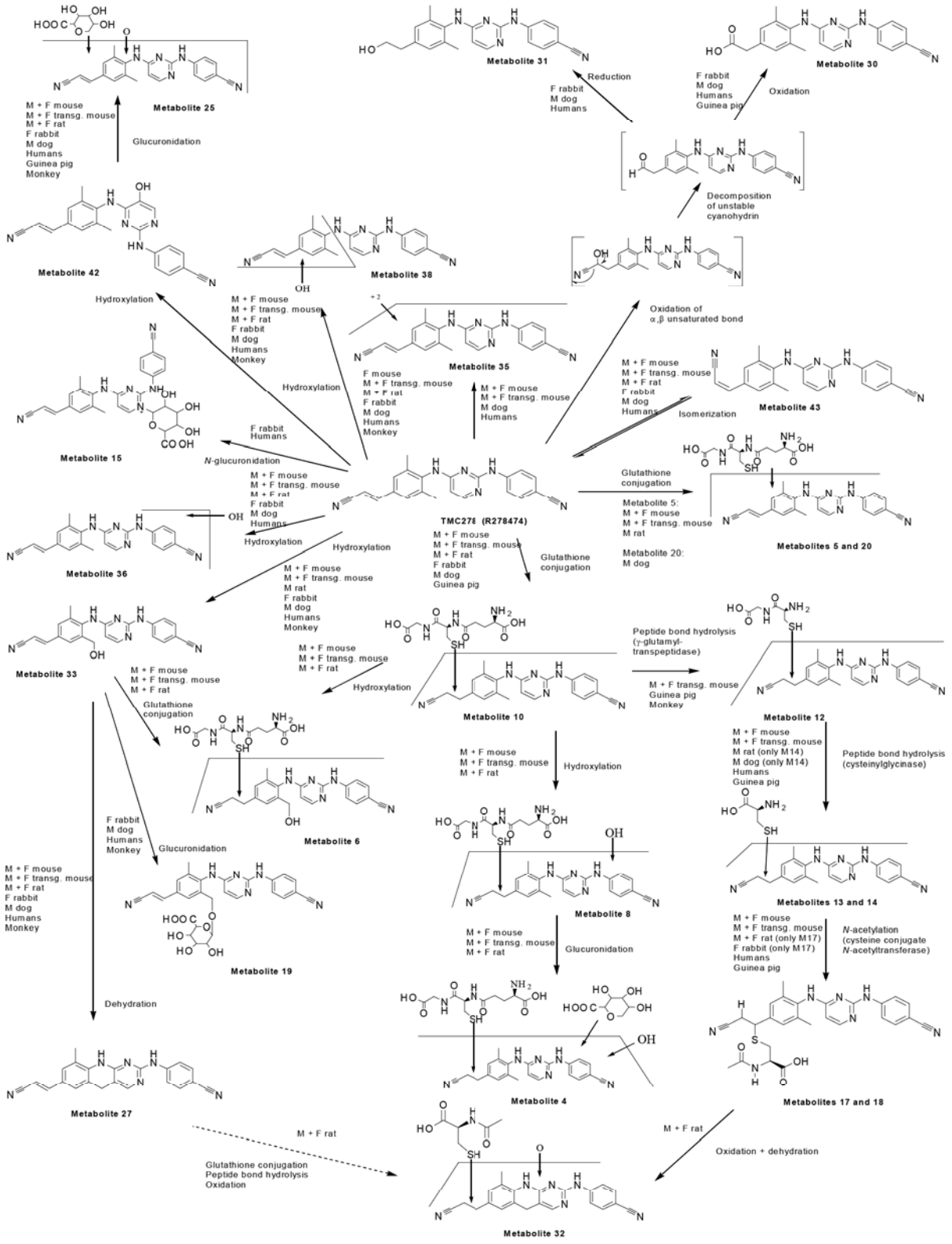
The in vitro metabolism of ^{14}C-TMC278 was studied in hepatocytes (suspensions and primary cultures) and liver subcellular fractions (microsomes and 12,000 x g supernatant fractions) of male and female Swiss albino mice, male and female black agouti ras H2 microinjected mice, male and female Sprague Dawley rats, female New Zealand white rabbits, male beagle dogs and man[66] (Tabulated Summary 2.6.5.10A). In addition, in vitro metabolism was also studied in hepatocyte primary cultures and 12,000 x g liver supernatant fractions from female Dunking Hartley Guinea pigs and female or male cynomolgus monkeys[67] (Tabulated Summary 2.6.5.10B). TMC278 (5 µM) was incubated in the above systems at 37°C for various time periods. Incubates were analyzed for metabolites by radio-HPLC. Co-chromatography, enzyme hydrolysis, LC-MS/MS and NMR techniques were used for the identification of metabolites.

In each species, a large number of metabolites was detected (see Figure 8). Overall, TMC278 was metabolized via different metabolic pathways including aromatic and aliphatic hydroxylation, glutathione conjugation, N-glucuronidation, nitrile release followed by reduction/oxidation, and isomerization. Aromatic hydroxylation at the pyrimidinyl moiety (M42) subsequently followed by glucuronidation (M25) was an important metabolic pathway in all the species, and it was the most important in vitro biotransformation route in human, dog and rabbit. Aliphatic hydroxylation at one of the methyl groups of the cyanoethenyl-2,6-dimethylphenyl moiety (M33) subsequently followed by dehydration to form a tricyclic metabolite (M27), proved to be an important metabolic pathway in human, monkey and rabbit, but was less important in the other animal species. The combination of aliphatic hydroxylation with glutathione conjugation (M6) occurred in the mouse strains and in male and female rats, but not in the other species. Aliphatic hydroxylation in combination with glucuronidation (M19), on the contrary, was observed in rabbit, dog, monkey and human, but not in mouse, rat and Guinea pig. Glutathione conjugation subsequently followed by conversions leading to mercapturic acid metabolites (M17 and M18) was a main metabolic route in mouse, rat and Guinea pig. In the other species, the mercapturic acid biosynthesis route proved to be a minor pathway, and not all intermediary metabolites were detected. Hydroxylation of the glutathione conjugate (M8), subsequently followed by glucuronidation was also solely observed in mouse and rat. The release of the nitrile group followed by reduction/oxidation, resulting in the formation of an alcohol metabolite (M31) and a carboxylic acid metabolite (M30), was a minor metabolic pathway in rabbit, Guinea pig, dog and human, and could not be detected in mouse, rat and monkey. N-glucuronidation at the pyrimidinyl moiety of TMC278 (M15) was an important biotransformation pathway in rabbit and could also be detected in human but not in the other species.

All identified TMC278 metabolites that were detected in human in vitro systems were also detected in at least one animal species.

In a previous in vitro metabolism study with cold TMC278[68], glutathione conjugation of TMC278 was identified as the most important metabolic pathway in man and rodents. This was not confirmed in the in vitro study with ^{14}C-TMC278 and in vivo where hydroxylation was the most important metabolic pathway in man.

Figure 8: In Vitro Metabolic Pathways of TMC278 in the Liver of Swiss Albino Mouse, Sprague Dawley Rat, Guinea Pig, Rabbit, Dog, Monkey, Man and Black Agouti ras H2 Mouse.



5.4.2 Isozymes Involved in the Metabolism of TMC278 in Human Liver

5.4.2.1 CYP450 Isozymes Involved in TMC278 Metabolism

The in vitro metabolism of ^{14}C-TMC278 was studied in human liver microsomes (HLM) in the presence of a nicotinamide adenine dinucleotide phosphate (NADPH)-generating system[69] (Tabulated Summary 2.6.5.10C). The CYP reaction phenotyping of TMC278 metabolism was performed by different approaches including effect of CYP diagnostic inhibitors on TMC278 metabolism, metabolism in expressed CYP systems (E.coli cells and Supersomes®) and correlation analysis of metabolism rate in a panel of 10 batches of characterized HLMs. Incubations were conducted at various TMC278 concentrations (0.5-50 µM) for 15 minutes with a protein concentration of 0.25 mg/mL. In a preceding nonradiolabeled pilot metabolism study, the identification of CYP isoenzymes was based on inhibitor and metabolism experiments with heterologous expression systems[70].

In the radiolabeled study, one primary TMC278 metabolite, M42, and 4 minor metabolites i.e. M33, M27 and the co-eluting metabolites M35 and M36 were formed. The apparent Michaelis-Menten constant K_m and V_{max} values for the metabolism of TMC278 in HLMs were 4.17 µM and 381 pmol/mg/min, respectively. The use of different CYP diagnostic inhibitors showed that TMC278 metabolism was markedly inhibited by the different CYP3A diagnostic inhibitors. Formation of M33 was moderately inhibited with the CYP2C8/9/10 inhibitor sulphaphenazole. Metabolism experiments in expressed CYP P450 *E.coli* and Supersomes® systems clearly indicated the involvement of CYP3A isoforms and to some extent of the CYP1A2 isoform. Correlation analysis showed involvement of CYP3A and CYP2C19 in the formation of several metabolites, though for CYP2C19 this was not confirmed in the other phenotyping experiments. CYP1A2 might also play a role in the formation of M33. During some of the experiments in this study, the recovery of the total radioactivity was around 70% in the presence of NADPH and cofactor. Addition of glutathione to the incubation mixture resulted in a 50% decrease of bound radioactivity. This suggests that glutathione was able to scavenge hypothetical reactive intermediates. Comparison of the metabolic profile of TMC278 after incubation in HLMs in the absence and presence of glutathione confirmed the formation of several glutathione conjugates. In conclusion, overall TMC278 metabolism as well as formation of all its metabolites were mainly catalyzed by CYP3A4. Additionally, it was observed that formation of certain metabolites could also be catalyzed to a lesser extent by CYP2C19, CYP1A2 and CYP2C8/9/10.

In the nonradiolabeled metabolism study, CYP3A4 was clearly involved in the metabolism of TMC278 based on both inhibition and metabolism data in heterologous expression systems. Metabolism experiments with heterologous expression systems also indicated the possible involvement of CYP1A1, CYP1B1, CYP2C18 and CYP3A5 in the metabolism of TMC278.

In an earlier study, using cold compound, a K_m value of 4.94 µM and a V_{max} value of 0.84 nmol/mg/min was calculated. Based on these kinetic parameters, a human in vivo intrinsic clearance of 1410 L/h and a hepatic clearance of 0.042 L/h/kg were predicted[71].

5.4.2.2 GST Isoforms Involved in TMC278 Metabolism

The identification of the glutathione S-transferase (GST) isoforms (alpha, mu and pi) involved in the metabolism of ^{14}C-TMC278 was studied in vitro using heterologous expressed GST[72]. ^{14}C-TMC278 was tested at 5 and 200 µM using reduced glutathione (GSH, 1 mM) as

co-substrate. In addition, incubations were performed in the absence of GST to estimate the amount of non-enzymatic conjugation.

Conjugation with glutathione was more dependent on the mu than the pi isoform of GST, although both isoforms were involved.

5.5 ENZYME INDUCTION AND INHIBITION

5.5.1 In Vitro Study Measuring CYP Activity and CYP mRNA Induction in Human Hepatocytes

The potential of TMC278 to induce CYP450 activities was determined in primary cultures from cryopreserved human hepatocytes originating from 3 different donors and compared to the data obtained with the positive controls omeprazole, rifampicin and ethanol[73] (Tabulated Summary 2.6.5.12D). Cells were treated for 2 consecutive days either with vehicle (dimethyl sulfoxide (DMSO)), with TMC278 (2.5, 10 and 25 µM) or with the CYP inducers, i.e. omeprazole (CYP1A2), rifampicin (CYP2B6/2C19/3A4) or ethanol (CYP2E1). Induction of CYP activities (CYP1A2, CYP2B6, CYP2C19, CYP2E1 and CYP3A4) was assessed at the end of the 48-hour treatment period, using corresponding probe substrates (phenacetin (CYP1A2), S-mephenytoin (CYP2B6 and CYP2C19), chlorzoxazone (CYP2E1) and testosterone (CYP3A4)). LC-MS/MS was used to measure the products of the probe substrates in order to determine the CYP activity of the hepatocytes. In addition, induction of CYP activities was also determined by measurement of mRNA expression levels by TaqMan real-time reverse transcription-polymerase chain reaction.

Most of the batches responded well to the treatment of positive inducers in all assays, except for the CYP2E1 assay. However, the inhibition control (positive control + 25 µM TMC278) revealed that TMC278 seemed to mask the induction of all investigated CYPs. Based on the observed fold-changes of mRNA expression and fold-induction of CYP activities, it can be concluded that TMC278 might be a very weak inducer of CYP1A2 (6-fold less than omeprazole) and CYP2B6 (4.5-fold less than rifampicin) in human hepatocytes. In addition, the results indicate that TMC278 appears to be a moderate inducer of CYP2C19 (1.4-fold less than rifampicin) and CYP3A4 (2-fold less than rifampicin) in human hepatocytes. No conclusion could be drawn for CYP2E1.

5.5.2 In Vitro Study Measuring GST Activity Induction in Human Hepatocytes

The potential of TMC278 to induce GST was evaluated in one batch of primary human hepatocytes in the presence of 3 concentrations of TMC278 (1, 10, and 30 µM), incubated for 3 consecutive days[74].

TMC278 had a low or no effect on GST activity or GST-alpha and GST-mu immunoreactive protein levels when compared with enzyme rates or levels observed in hepatocytes treated with the vehicle control DMSO. However, the positive controls (phenobarbital, rifampin or 2,3,7,8-tetrachlorodibenzo-p-dioxin) did not result in induction of GST in human hepatocytes and therefore no conclusions can be drawn on the inducing properties of TMC278 on the GST activity and expression from this study.

5.5.3 Ex-vivo Studies Measuring Enzyme Activities in Mouse, Rat and Dog Liver

TMC278 in aqueous HPMC (0.5%) was administered for 3 months to male and female CD-1 mice at doses of 20, 80 and 320 mg/kg/day[75] (Tabulated Summary 2.6.5.12A). TMC278 base in PEG400/CA (10%) was administered to male and female Sprague Dawley rats at doses of 40, 120 and 400 mg/kg/day and to male and female beagle dogs at doses of 5, 10 and 40 mg/kg/day for 6 months[76,77] (Tabulated Summaries 2.6.5.12B and 2.6.5.12C).
To examine the effect of TMC278 on some hepatic enzyme activities, microsomal fractions of livers from the above mentioned TMC278 treated animals were assayed for protein and total CYP content, and for the activities of 7-ethoxyresorufin O-deethylase, 7-pentoxyresorufin O-dependentylase, 4-nitrophenol hydroxylase, testosterone 6β-hydroxylase and lauric acid 12-hydroxylase. These enzyme activities are well known markers for the induction of CYP1A, CYP2B, CYP2E, CYP3A and CYP4A forms, respectively. Microsomes were also assayed for lauric acid 11-hydroxylase activity, which is largely catalyzed by CYP2E1 and for thyroxine uridine diphosphate-glucuronosyltransferase (UDP-GT) activity. Additionally, liver cytosolic fractions were assayed for protein content and GST activity towards 1-chloro-2, 4 dinitrobenzene as a substrate. In addition, in rats, the effect of TMC278 on some hepatic enzyme activities were also examined in liver samples from a 2-week study (TMC278 base in PEG400/CA (10%) at 40, 120 and 400 mg/kg/day)[78]. In this study, the liver microsomes were assayed for protein and for 7-ethoxyresorufin *O*-deethylase (CYP1A1, CYP1A2), 7-pentoxyresorufin-dealkylase (CYP2B), aniline hydroxylase (CYP2E1), *N*-ethyl morphine *N*-demethylase (CYP3A1, CYP3A2), lauric acid hydroxylase (CYP4A1) and thyroxine (T4) glucuronosyltransferase activities.

In mice, TMC278 was an inducer of the CYP4A forms in both male and female animals (up to 25- and 20-fold, respectively) (see Table 11). Some induction was also seen with the CYP3A forms (up to 1.7-fold in both males and females). TMC278 treatment induced UDP-GT activity in male and female mice (up to 2.1- and 2.3-fold, respectively) and decreased GST activity in male mice to 44% at 320 mg/kg/day.

In rats, TMC278 was an inducer of CYP4A forms in male rats (4.7-fold) whereas in female rats TMC278 was an inducer of CYP3A forms (6-fold) and possibly also of CYP2B and CYP4A forms (see Table 11). TMC278 treatment had some effect on UDP-GT activity in male rats (induction of 1.3 fold only at high dose level) and on GST activity in female rats (induction of 1.5-fold). In the 2-week study, the results were similar.

In dogs, treatment with TMC278 did not result in any induction of CYP1A1, CYP2B, CYP2E, and CYP4A, UDP-GT or GST activity. TMC278 produced some decrease in microsomal CYP3A-dependent testosterone 6β-hydroxylase activity but this effect was confined to the two highest dose levels and was not dose-dependent (see Table 11).

Table 11: Percentage of Testosterone 6β-hydroxylase, Lauric Acid 12-hydroxylase and UDP-GT Activities Relative to Control Values in Hepatic Microsomal Fractions of CD-1 Mouse, Sprague Dawley Rat and Beagle Dog After Repeated Administration of TMC278 or TMC278 Base

Species	Dose (mg/kg/day)	Testosterone 6β-hydroxylase (CYP3A)		Lauric acid 12-hydroxylase (CYP4A)		Thyroxine UDP glucuronosyltransferase (UDP-GT)	
		Male	Female	Male	Female	Male	Female
Mouse[75] NC192	20	111	153**	147*	126	108	138*
	80	156***	174***	525***	521***	150**	164***
	320	174***	175***	2499***	1966***	210***	229***
Rat[76] NC193	40	95	120	140	75	65**	127
	120	125	300***	262**	93	77*	98
	400	120	600***	466***	127*	125*	134
Dog[77] NC140	5	85		100		82	
	10	57**		102		75	
	40	74*		113		68	

* p<0.05; ** p<0.01; *** p<0.001

5.5.4 In Vitro Inhibition of Human CYP450 Enzymes by TMC278

TMC278 was tested for its inhibitory effect on the metabolism of various human CYP450 probe substrates to gain information about the possibility of clinically relevant interactions with other drugs[79,80]. Incubations with P450 probe substrates, selective towards CYP1A2, CYP2A6, CYP2C8/9/10, CYP2C19, CYP2D6, CYP2E1, CYP3A4, CYP4A and CYP3A4/5 were performed in HLM in the absence and presence of TMC278 at 8 different concentrations ranging between 0.03 and 400 µM (Table 12).

Table 12: Interaction of TMC278 With Human CYP450 in Vitro

Substrate	CYP involved	IC_{50}-value (µM)
Phenacetin	CYP1A2	34.0
Coumarin	CYP2A6	>100 (15.7)[a]
Tolbutamide	CYP2C8/9/10	3.99
Dextromethorphan	CYP2D6	3.88
Bufuralol	CYP2D6	12.0
Testosterone	CYP3A4	6.29
Cyclosporin A	CYP3A4	16.8
Midazolam	CYP3A**4**/5 CYP3A4/**5**	4.20 18.3
Lauric acid	CYP4A CYP2E1	>100 (15.9) [a] 9.79

[a] % inhibition at 100 µM

Under the conditions of this experiment, TMC278 was a potent inhibitor of CYP2C19 and CYP2E1. CYP2C19 activity was blocked 70% at a concentration of 0.06 µM (0.02 µg/mL) and 86% of CYP2E1 activity was inhibited at a concentration of 0.03 µM (0.01 µg/mL). However, an in vitro study with cultured hepatocytes (see Section 5.5.1) indicated a moderate induction of CYP2C19 by TMC278. In addition, an in vivo drug-drug interaction trial with omeprazole (Clinical Study TMC278-TiDP6-C114 (Module 2.7.2/Clinical Pharmacology Summary/Section 2.8.3.9)) indicated a weak induction of CYP2C19 by TMC278. For CYP2E1, there were some discrepancies in this in vitro study: TMC278 seemed to be a strong inhibitor of CYP2E1 with chlorzoxazone as a substrate and not with lauric acid as a substrate. However, in the in vitro drug-drug interaction study (see Section 7) and also in the in vivo clinical study (Clinical Study C139 (Module 2.7.2/Clinical Pharmacology Summary/Section 2.8.3.12)), no interaction was observed between TMC278 and chlorzoxazone. Therefore, as they were not confirmed by subsequent studies, the inhibition of CYP2C19 and CYP2E1 by TMC278 are considered not relevant. For the other CYPs, taking into account a mean C_{max}-value of about 0.13 µg/mL for TMC278 in human, inhibition in vivo is unlikely.

Inhibition of CYP2C8-mediated paclitaxel 6α-hydroxylation and CYP2C9-mediated S-warfarin-7-hydroxylation by TMC278 (0.1 - 300 or 200 µM, respectively) was also investigated in HLMs[80]. TMC278 is an inhibitor of CYP2C8 and CYP2C9 with a K_i of 10 and 1.7 µM, respectively. Taking into account a mean C_{max}-value of about 0.13 µg/mL for TMC278 in human, inhibition of CYP2C8 and CYP2C9 by TMC278 is not expected ($C_{max}/K_i<0.1$).

5.5.5 Effect of TMC278 on Adrenal Gland

The effect of TMC278 on cortisol biosynthesis in dog adrenal cortex cell-free extracts was determined[81] (see also Module 2.6.6/Section 8.3.3).

TMC278 at a nominal concentration of 75 µM (27.75 µg/mL) caused 39% inhibition of the metabolism of pregnenolone compared to control. A concentration-dependent increase in

progesterone and 17α-hydroxyprogesterone concentrations was noted concomitant with decreases of 11-deoxycorticosterone, 11-deoxycortisol, and corticosterone concentrations.

6 EXCRETION

6.1 ROUTES AND EXTENT OF EXCRETION

The excretion of TMC278 was studied after single oral administration of ^{14}C-TMC278 base in male and female CD-1 mice at 20 and 320 mg/kg[59], in Sprague Dawley rats at 40 mg/kg[60,61] and in male beagle dogs at 5 mg/kg[63] (Tabulated Summaries 2.6.5.9A, 2.6.5.9B, 2.6.5.9C and 2.6.5.9D). Healthy male subjects were dosed orally with 150 mg ^{14}C-TMC278 base[64] (Tabulated Summary 2.6.5.9E). Urine and feces samples were collected up to 96 hours after dosing in rodents, up to 168 hours after dosing in dogs and humans (only urine) and up to 336 hours in humans (only feces). TR was measured by scintillation counting.

In rodents, the TR was rapidly excreted with 90% to 94% (at 20 mg/kg) and 69% to 74% (at 320 mg/kg) of the radioactive dose eliminated in mice and 79% to 84% eliminated in rats within the first 24 hours after dosing. In dogs, excretion was relatively slow with 54% of the radioactive dose eliminated within the first 24 hours. In mice, rats and dogs, the predominant route of excretion of ^{14}C-TMC278 was via the feces. The majority of the TR was eliminated in feces as unchanged TMC278 in mice (33-34% at 320 mg/kg), in rats (43-47%) and in dogs (43%) at 48 hours after dosing. Only, in mice at 20 mg/kg, one metabolite M42 was the most abundant in feces. Renal excretion was very limited (0.45 to 4.2% of the radioactivity dose) in all animal species and the amount of unchanged TMC278 in urine was negligible. The excretion was virtually complete at 96 hours after dosing in rodents and at 168 hours after dosing in dogs (Table 13). In a biliary excretion study in male Sprague Dawley rats, the amount of radioactivity excreted in bile within 24 hours after dosing was rather low, only 18% and 25% of the administered radioactivity, in restrained and nonrestrained animals, respectively. The amount of unchanged TMC278 excreted in bile during this time period was negligible (about 0.2%). The biliary excretion study demonstrated that the major part of unchanged TMC278 excreted in feces in rats had not been absorbed.

The excretion of TMC278 in humans was similar to that seen in the nonclinical species. 85% of the dose was excreted in feces and excretion was virtually complete at 336 hours after dosing. Unchanged TMC278 represented on average 26% of the administered dose in feces. In humans, the amount of TR recovered in urine was somewhat higher (6.1% of the administered dose over the 7-day period) than in animals. Unchanged TMC278 in urine was negligible.

Table 13: Urinary and Fecal Excretion of the Radioactivity Following a Single Oral Dose of ^{14}C-TMC278 base in Mouse and Rat at 96 Hours After Dosing and in Dog and Human at 168 Hours After Dosing

% of administered dose	Mouse[59]				Rat[60]		Dog[63]	Human[64]
	20 mg/kg		320 mg/kg		40 mg/kg		5 mg/kg	150 mg
	Male	Female	Male	Female	Male	Female	Male	Male
Urine	3.51	4.19	1.84	3.62	0.45	1.77	1.73	6.13
Feces	87.8	87.1	95.8	88.8	93.3	92.6	94.7	85.1
Cage washings	3.61	3.79	1.18	3.37	0.12	0.68	0.38	-
Total Recovered	**94.9**	**95.1**	**98.9**	**95.8**	**93.9**	**95.1**	**96.8**	**91.2**[a]

[a] Expressed as percent of the administered dose in the 0-168h urine and 0-336h feces

6.2 EXCRETION IN MILK

No studies have been conducted to assess directly the excretion of TMC278 into milk. In the QWBA study in pregnant Sprague Dawley rats (see Section 4.1.1), some radioactivity was seen in the mammary glands (tissue/blood $AUC_{0\text{-}8h}$ ratio = 3), which indicates the potential for excretion of TMC278-related radioactivity via the milk.

In a dose range finding study for a pre- and postnatal developmental study[32] it was found that pups were exposed to TMC278 through the milk of the dams dosed with TMC278 (40, 120 and 400 mg/kg/day). On Day 7 of lactation, exposure ($AUC_{0\text{-}24h}$) in pups was 0.62 and 0.74 µg.h/mL at 40 mg/kg, 0.94 and 0.91 µg.h/mL at 120 mg/kg and 1.9 and 1.8 µg.h/mL at 400 mg/kg in males and females, respectively. Exposure in pups dosed through milk on Day 7 of lactation was approximately 20- to 35-fold lower than in pups directly dosed by oral gavage on Day 25 of age (see also Section 3.1.3.2).

7 PHARMACOKINETIC DRUG INTERACTIONS

The in vitro interaction of TMC278 with the metabolism of sertraline (substrate of multiple CYPs, Monoamine oxidase and UDP-GT), paroxetine (CYP2D6), clarithromycin (CYP3A4), sildenafil (CYP3A4), omeprazole (CYP2C19 and CYP3A4), chlorzoxazone (CYP2E1), 17α-ethinylestradiol (phase II metabolism), S-mephenytoin (CYP2C19) and norethindrone (different isoenzymes) was investigated in a pooled batch of human liver microsomes and the same was done for abacavir (alcohol dehydrogenase) in a pooled batch of human liver cytosol[82] (Tabulated Summary 2.6.5.15).

TMC278 seemed to have a significant inhibitory effect (IC_{50} < 5 µM) on the metabolism of clarithromycin, sildenafil, S-mephenytoin and norethindrone and a moderate effect (5 µM < IC_{50} < 10 µM) on sertraline, paroxetine and 17α-ethinylestradiol. Omeprazole metabolism was only poorly inhibited by TMC278, displaying an IC_{50}-value of 12 µM. TMC278 has under these conditions no measurable effect on the metabolism of abacavir or chlorzoxazone, as metabolite formation of the latter compounds was not inhibited (IC_{50} > 30 µM).

These in vitro data indicate a possible effect of TMC278 on the in vivo metabolism of clarithromycin, sildenafil, S-mephenytoin, and norethindrone and also albeit somewhat less likely, with sertraline, paroxetine and 17α-ethinylestradiol. No inhibition is expected for omeprazole, abacavir, and chlorzoxazone.

In the Nonclinical Overview (Module 2.4/Nonclinical Overview) these results are compared to the clinical data.

8 OTHER PHARMACOKINETIC STUDIES

There are no additional studies to report under this heading.

9 DISCUSSION AND CONCLUSIONS

The objectives of the ADME program were to determine the pharmacokinetics of TMC278 in various animal species, to measure the exposure after oral administration, to characterize the distribution to the different organs and tissues within the body and the rates and routes of elimination, to identify the metabolites and to predict the drug-drug interaction potential. Throughout this section, references are made to human data (where available), which are helpful in providing a clinical perspective in relation to the results obtained in nonclinical studies.

Human colon carcinoma-derived Caco-2 cells revealed that TMC278 can be classified as a compound with an intermediate permeability. Passive transcellular diffusion was proposed as a mechanism for TMC278 absorption. However, the solubility, and in case of suspension and solid dosage forms, possibly also dissolution seem to limit the rate and extent of absorption. After oral administration of TMC278 base, the absolute oral bioavailability of TMC278 was 32%, 54%, 31% and 24% in rats, rabbits, dogs and monkeys, respectively. With CA in the formulation, the absolute bioavailability in dogs was increased by a factor of 2.6 and was 80% at 5 mg/kg whereas at 80 mg/kg, there was no effect of CA on exposure. In rats, the bioavailability remained the same at 40 mg/kg with or without citric acid and increased with citric acid at 400 mg/kg.

After oral gavage administration of TMC278 (2 forms), peak plasma concentrations were generally reached rapidly followed by a decline at lower dose levels whereas at higher dose levels the plasma profiles showed a plateau until at least 8 hours in all species. Across the dose range studied, plasma concentrations of TMC278 increased dose-proportionally or more often less than dose-proportionally, due to poor solubility. At very high dose levels, no further increase in exposure was seen. There were no major gender differences in pharmacokinetics in mice at low dose levels and dogs whereas in mice at high dose levels and rats, exposures in females were higher than in males (up to 2-fold in mice and 2- to 4-fold in rats). In rats, the gender effect was less pronounced after administration of HCl salt particularly at Day 1 in the carcinogenicity study. In mice, exposure after repeated administration was comparable or slightly lower than that at Day 1 in males and females (only at 20 mg/kg/day). In females, at higher dose levels (60 to 320 mg/kg/day) exposure after repeated administration was higher (up to 2.2-fold) than at Day 1. In rats, after repeated administration of the base, systemic exposure increased slightly (up to 1.6-fold) in comparison to Day 1 in females while in males particularly at high dose levels, exposure decreased slightly (up to 40%). After repeated administration of the HCl salt, the decrease in exposure seen in males was more pronounced (up to 76%) compared to base, particularly in the carcinogenicity study, while no further decrease was observed between Week 27 and Week 39. In female rats no clear tendency for time-dependent pharmacokinetics was seen. In rabbits, the exposures obtained after repeated administration were similar to those obtained at Day 1. In dogs, after repeated administration (2 forms), exposure increased after repeated administration as compared to Day 1 mainly due to the long elimination half-life ($t_{1/2}$ = 31 hours) of TMC278. In monkey, after repeated administration of the HCl salt, exposure had a tendency to increase but due to the high inter-individual variability it is difficult to reach firm conclusions. In the species (rats and dogs) where the base and the HCl salt were administered, the exposure was comparable at low dose in rats. At high dose in rats, exposure after administration of the base was higher (1.7- to 2.7-fold) than that after administration of the HCl salt. In dogs no clear difference was noted. However, inter-individual variability was high especially after administration of the HCl salt.

In rats, tissue distribution of ^{14}C-TMC278 and its metabolites after single dose was rapid and extensive. The highest concentrations of radioactivity were measured in the liver, adrenal gland brown fat and kidney. There was no evidence of undue retention and there were no indications of irreversible binding of TMC278 and its metabolites to melanin. In pregnant rats, there was distribution of ^{14}C-TMC278 to the placenta and the fetus.TR exposure values in the placenta and in whole fetus were 0.94 and 0.64-fold those of maternal blood, respectively.

TMC278 was extensively bound to plasma proteins and this was independent of the concentration and species. In the various animal species and man, plasma protein binding ranged from 99.08% to 99.97%. TMC278 was extensively bound to human albumin and much lower to α_1-acid glycoprotein. The distribution of TMC278 to red blood cells is limited in all species.

Some differences were seen in clearance across species. In rats, blood clearance of TMC278 is moderate whereas in rabbits, dogs and monkeys it is low compared to the hepatic blood flow. Large differences in elimination half-life of TMC278 were observed between the rat (4.4 hours), rabbit (12 hours), dog (31 hours) and monkey (7.1 hours). Vd_{ss} is larger in rats, dogs and monkeys and very low in rabbits.

TMC278 is metabolized by Phase I and Phase II pathways including aromatic and aliphatic hydroxylation, glutathione conjugation, N-glucuronidation and nitrile split-off followed by reduction/oxidation, whether or not in combination with secondary pathways like glucuronidation, dehydration and catabolism of the glutathione conjugate. In mice, oxidation of TMC278 and to a lesser extent glutathione conjugation were the predominant pathways. In rats the glutathione conjugation pathway is the predominant pathway whereas in dog and man, oxidation of TMC278 is the predominant one. No unique human metabolites were observed. In plasma of animals and human, unchanged TMC278 was more abundant than any metabolite.

In rodents dosed with ^{14}C-TMC278, TR was rapidly excreted whereas in dogs, excretion was relatively slow. In all animal species and human, the predominant route of excretion was via feces (> 85%) and generally, the majority of the TR eliminated was unchanged TMC278. Renal excretion of TR was very limited (0.45% to 6.1% of the dose) in all animal species and human and the amount of unchanged TMC278 in urine was negligible. In rats, biliary excretion was limited (18%-25% of the dose) and the amount of unchanged TMC278 in bile is negligible. In rats, there was indication that TMC278 was excreted in milk.

In vitro, the CYP3A4 isoenzyme plays a major role in the biotransformation of TMC278. TMC278 might be a very weak inducer of CYP1A2 and CYP2B6 and a moderate inducer of CYP2C19 and CYP3A4. TMC278 is an inhibitor of CYP2C8 and CYP2C9 in vitro whereas no inhibition is expected in vivo. However, not confirmed by subsequent studies, inhibition of CYP2E1 and CYP2C19 by TMC278 are considered not relevant.

TMC278 was shown to have P-gp inhibitor properties with an apparent IC_{50} value of 9.2 µM (3.4 µg/mL). Inhibition of transepithelial permeation of P-gp substrates cannot be excluded, but a possible effect is unlikely to be clinically relevant. Pg-p inhibitors are not expected to play a role in modulating the intestinal absorption of TMC278, given the limited role of efflux transporters in the epithelial permeability of TMC278.

Ex vivo induction studies in rodents showed that TMC278 is an inducer of CYP3A-family (up to 1.7-fold in mice and up to 6-fold in rats) and CYP4A-family (up to 25-fold in mice and up to 4.7-fold in rats). Additionally, TMC278 induced UDP-GT activity in mice (up to 2.3-fold) and to

a lesser extent in rats (up to 1.3-fold only at high dose in males). In dogs, treatment with TMC278 did not result in any enzyme induction.

The recommended dose of TMC278 in HIV-infected treatment-naïve patients is 25 mg q.d. At this dose level, mean C_{max} (Week 4-8) was 0.13 µg/mL and mean AUC_{0-24h} (Week 48; population PK) was 2.4 µg.h/mL (Clinical Study TMC278-TiDP6-C209 and TMC278-TiDP6-C215 (Module 2.7.2/Summary of Clinical Pharmacology Studies/Section 2.6.3). These values were compared with those obtained at the highest doses tested in animal species.
At the end of administration, the C_{max} and AUC ratios (animal/human) ranged from 1.9 to 2.4 in monkeys, 25 to 42 in dogs, 97 to 115 in pregnant rabbits, 7.5 to 48 in male rats, 35 to 123 in female rats, 63 to 100 in pregnant rats, 21 to 70 in juvenile rats, and 210 to 446 in mice.

10 TABLES AND FIGURES

In-text tables and figures are included at appropriate locations throughout the summary within the text. This excludes the Tabulated Summaries, which are located in Module 2.6.5.

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2.6.5.1 Pharmacokinetics: Overview

Test Article: rilpivirine

Type of Study	Species/ Strain	Route/Method of Administration (Vehicle/Formulation)	GLP Compliance	Testing Facility	Study/Report Number	Location in CTD
Absorption						
Drug Permeability and Transport	Caco-2 cells	In vitro	No	J&J PRD	R278474-JRF FK4155	4.2.2.2
Drug Permeability and Transport	Caco-2 cells	In vitro	No	J&J PRD	TMC278-NC104	4.2.2.2
Absorption, Single Dose	Mouse/ CD-1	Oral/Gavage (base in PEG400/CA (10%))	Yes	J&J PRD	TMC278-FK4259	4.2.3.3.2
Absorption, Single Dose	Rat/ Sprague Dawley	Intravenous (base in PEG400/sterile water (25%) Oral/Gavage (base in PEG400 or PEG400/CA (10%))	No	J&J PRD	TMC278-FK4195	4.2.2.2
Absorption, Single Dose	Rat/ Sprague Dawley	Oral/Gavage (base in PEG400; HCl and fumarate salt in Tween 20/HPMC/water)	No	J&J PRD	TMC278-NC106	4.2.2.2
Absorption, Single Dose	Rat/ Sprague Dawley	Oral/Gavage (base in PEG400)	No	J&J PRD	TMC278-FK4278	4.2.3.1
Absorption, Single Dose	Rabbit/ New Zealand white	Intravenous (base in PEG400/sterile water (25%))	No	J&J PRD	TMC278-FK4293	4.2.2.2
Absorption, Single Dose	Dog/ beagle	Intravenous (base in PEG400/sterile water (25%)) Oral/Gavage (base in PEG400 or PEG400/CA (10%))	No	J&J PRD	TMC278-FK4231	4.2.2.2
Absorption, Single Dose	Dog/ beagle	Oral/Gavage (base in PEG400 or PEG400/CA (10%))	No	J&J PRD	TMC278-FK4102	4.2.3.1
Absorption, Single Dose	Dog/ beagle	Oral/Gavage (base in PEG400/CA (10%))	No	J&J PRD	TMC278-NC163	4.2.2.2
Absorption, Single Dose	Monkey/ Cynomolgus	Intravenous (base in PEG400/sterile water (25%)) Oral/Gavage (base in PEG400/CA (10%))	No	J&J PRD	TMC278-DM02403	4.2.2.2
Absorption, Single Dose	Monkey/ Cynomolgus	Oral/Gavage (HCl salt in HPMC (1%) with Tween 20 (0.5%))	No	[redacted]	TMC278-NC326	4.2.2.2
Absorption, Repeat Dose (2 weeks)	Mouse/ CD-1	Oral/Gavage (HCl salt in HPMC (0.5%)) Oral/Diet (HCl salt)	No	J&J PRD	TMC278-NC118	4.2.3.2
Absorption, Repeat Dose (1 month)	Mouse/ CB6F1-nonTgrasH2	Oral/Gavage (HCl salt in HPMC (0.5%))	Yes	[redacted]	TMC278-NC121	4.2.3.2
Absorption, Repeat Dose (3 months)	Mouse/ CD-1	Oral/Gavage (HCl salt in HPMC (0.5%))	Yes	J&J PRD	TMC278-NC119	4.2.3.2

CA: citric acid; HPMC: hydroxypropyl-methylcellulose; PEG400: polyethylene glycol 400

Approved, issued date: [redacted]

2.6.5.1 Pharmacokinetics: Overview (Continued)

Type of Study	Species/ Strain	Route/Method of Administration (Vehicle/Formulation)	GLP Compliance	Testing Facility	Study/Report Number	Location in CTD
Absorption, Repeat Dose (2 years)	Mouse/ CD-1	Oral/Gavage (HCl salt in HPMC (0.5%))	Yes	[redacted]	TMC278-NC120	4.2.3.4.1
Absorption, Repeat Dose (5 days)	Rat/ Sprague Dawley	Oral/Gavage (base in PEG400)	No	J&J PRD	TMC278-FK4103	4.2.3.2
Absorption, Repeat Dose (2 weeks)	Rat/ Sprague Dawley	Oral/Gavage (base in PEG400/CA (10%))	Yes	J&J PRD	TMC278-FK4243	4.2.3.2
Absorption, Repeat Dose (2 weeks)	Rat/ Sprague Dawley	Oral/Gavage (HCl salt in HPMC (0.5%)) Oral/Diet (base and HCl salt)	No	J&J PRD	TMC278-NC136	4.2.3.2
Absorption, Repeat Dose (2 weeks)	Rat/ Sprague Dawley	Oral/Gavage (HCl salt in HPMC (0.5%))	No	J&J PRD	TMC278-NC177	4.2.3.2
Absorption, Repeat Dose (1 month)	Rat/ Sprague Dawley	Oral/Gavage (base in PEG400/CA (10%))	Yes	[redacted]	TMC278-TOX5692	4.2.3.2
Absorption, Repeat Dose (1 month)	Rat/ Sprague Dawley	Oral/Gavage (base in PEG400/CA (10%), HCl salt in HPMC (0.5%))	Yes	J&J PRD	TMC278-NC117	4.2.3.2
Absorption, Repeat Dose (6 months)	Rat/ Sprague Dawley	Oral/Gavage (base in PEG400/CA (10%))	Yes	J&J PRD	TMC278-NC101	4.2.3.2
Absorption, Repeat Dose (2 years)	Rat/ Sprague Dawley	Oral/Gavage (HCl salt in HPMC (0.5%))	Yes	[redacted]	TMC278-NC123	4.2.3.4.1
Absorption, Repeat Dose (2 weeks)	Juvenile rat/ Sprague Dawley	Oral/Gavage (HCl salt in HPMC (0.5%))	Yes	[redacted]	TMC278-NC168	4.2.3.5.3
Absorption, Repeat Dose (5 days)	Rabbit/ New Zealand white	Oral/Gavage (base in HPMC (0.5%))	No	J&J PRD	TMC278-NC126	4.2.3.2
Absorption, Dose Escalating (5 days)	Dog/ beagle	Oral/Gavage (base in PEG400/CA (10%))	No	J&J PRD	TMC278-FK4102	4.2.3.1
Absorption, Repeat Dose (7 days)	Dog/ beagle	Oral/Gavage (base in PEG400/CA (10%))	Yes	J&J PRD	TMC278-FK4244	4.2.3.2
Absorption, Repeat Dose (1 month)	Dog/ beagle	Oral/Gavage (base in PEG400/CA (10%))	Yes	J&J PRD	TMC278-TOX5650	4.2.3.2
Absorption, Repeat Dose (1 month)	Dog/ beagle	Oral/Gavage (base in PEG400/CA (10%), HCl salt in HPMC (0.5%))	Yes	J&J PRD	TMC278-NC116	4.2.3.2
Absorption, Repeat Dose (6 months)	Dog/ beagle	Oral/Gavage (base in PEG400/CA (10%))	Yes	J&J PRD	TMC278-NC115	4.2.3.2
Absorption, Repeat Dose (12 months)	Dog/ beagle	Oral/Gavage (base in PEG400/CA (10%))	Yes	[redacted]	TMC278-NC107	4.2.3.2

CA: citric acid; HPMC: hydroxypropyl-methylcellulose; PEG400: polyethylene glycol 400

Approved, issued date: [redacted]

2.6.5.1 Pharmacokinetics: Overview (Continued)

Type of Study	Species/ Strain	Route/Method of Administration (Vehicle/Formulation)	GLP Compliance	Testing Facility	Study/Report Number	Location in CTD
Absorption, Repeat Dose (7 days)	Monkey/ Cynomolgus	Oral/Gavage (HCl salt in HPMC (0.5%))	No	[redacted]	TMC278-NC249	4.2.2.2
Absorption, Repeat Dose (14 days)	Monkey/ Cynomolgus	Oral/Gavage (HCl salt in HPMC (1%) with Tween 20 (0.5%))	No	[redacted]	TMC278-NC326	4.2.2.2
Absorption, Repeat Dose (8 weeks)	Monkey/ Cynomolgus	Oral/Gavage (HCl salt in HPMC (1%) with Tween 20 (0.5%))	No	[redacted]	TMC278-NC248	4.2.3.2
Absorption, Repeat Dose (gestation day 6 to 16)	Pregnant rat/ Sprague Dawley	Oral/Gavage (base in PEG400/CA (10%))	Yes	J&J PRD	TMC278-NC105	4.2.3.5.2
Absorption, Repeat Dose (gestation day 6 to 19)	Pregnant rabbit/ New Zealand white	Oral/Gavage (base in HPMC (0.5%))	No	J&J PRD	TMC278-NC128	4.2.3.5.2
Absorption, Repeat Dose (gestation day 6 to 19)	Pregnant rabbit/ New Zealand white	Oral/Gavage (base in HPMC (0.5%))	Yes	J&J PRD	TMC278-NC130	4.2.3.5.2
Distribution						
Tissue Distribution (Single dose)	Rat/ pigmented Long Evans	Oral/Gavage (^{14}C-TMC278 in PEG400/CA (10%)	No	J&J PRD	TMC278-NC108	4.2.2.3
	Pregnant rat/ Sprague Dawley	Oral/Gavage (^{14}C-TMC278 in PEG400/CA (10%)	No	J&J PRD	TMC278-NC109	4.2.2.3
	Rat/ Sprague Dawley	Intravenous (base in PEG400/sterile water (25%) Oral/Gavage (base in PEG400 or PEG400/CA (10%))	No	J&J PRD	TMC278-FK4195	4.2.2.2
Tissue Distribution (Repeat dose)	Dog/ beagle	Oral/Gavage (base in PEG400/CA (10%))	Yes	J&J PRD	TMC278-NC115	4.2.3.2
	Dog/ beagle	Oral/Gavage (base in PEG400/CA (10%))	Yes	J&J PRD	TMC278-TOX5650	4.2.3.2
Protein Binding Blood Distribution	Mouse, rat, dog, human	In vitro	No	J&J PRD	TMC278-FK4217	4.2.2.3
	Mouse, rat, rabbit, dog, human	In vitro (^{3}H-TMC278)	No	J&J PRD	TMC278-NC112	4.2.2.3
	Guinea pig, monkey	In vitro (^{14}C-TMC278)	No	J&J PRD	TMC278-NC332	4.2.2.3

CA: citric acid; HPMC: hydroxypropyl-methylcellulose; PEG400: polyethylene glycol 400

Approved, issued date: [redacted]

2.6.5.1 Pharmacokinetics: Overview (Continued)

Type of Study	Species/ Strain	Route/Method of Administration (Vehicle/Formulation)	GLP Compliance	Testing Facility	Study/Report Number	Location in CTD
Metabolism						
Metabolism Excretion (Single dose)	Mouse/ CD-1	Oral/Gavage (^{14}C-TMC278 in PEG400/CA (10%)	No	J&J PRD	TMC278-NC190	4.2.2.4
	Rat/ Sprague Dawley	Oral/Gavage (^{14}C-TMC278 in PEG400/CA (10%)	No	J&J PRD	TMC278-NC113	4.2.2.4
	Dog/ beagle	Oral/Capsule (^{14}C-TMC278 in PEG400/CA (10%)	No	J&J PRD	TMC278-NC114	4.2.2.4
	Human	Oral/Gavage (^{14}C-TMC278 in PEG400)	No	J&J PRD	TMC278-NC157	4.2.2.4
Metabolism Excretion in Bile (Single dose)	Rat/ Sprague Dawley	Oral/Gavage (^{14}C-TMC278 in PEG400/CA (10%)	No	J&J PRD	TMC278-NC145	4.2.2.4
Metabolism (Repeat dose)	Rat/ Sprague Dawley	Oral/Gavage (HCl salt in HPMC (0.5%))	No	J&J PRD	TMC278-NC290	4.2.2.4
Metabolism (Single and/or repeat dose)	Mouse, rat, rabbit, dog, human	Plasma samples from different studies	No	J&J PRD	TMC278-NC155	4.2.2.4
Metabolism	Mouse, rat, rabbit, dog, human	In vitro: hepatocytes, subcellular liver fractions	No	J&J PRD	TMC278-FK4152	4.2.2.4
	Mouse, rat, rabbit, dog, human	In vitro: hepatocytes, subcellular liver fractions (^{14}C-TMC278)	No	J&J PRD	TMC278-NC102	4.2.2.4
	Guinea pig, monkey	In vitro: hepatocytes, subcellular liver fractions (^{14}C-TMC278)	No	J&J PRD	TMC278-NC333	4.2.2.4
Metabolism	Human	In vitro: human liver microsomes, *E. coli* expressed CYP isoforms	No	J&J PRD	TMC278-FK4151	4.2.2.4
Metabolism	Human	In vitro: human liver microsomes, *E. coli* expressed CYP isoforms, supersomes	No	J&J PRD	TMC278-NC141	4.2.2.4
Metabolism	Human	In vitro: heterologous expressed GST isoforms	No	J&J PRD	TMC278-FK4789	4.2.2.4
Induction	Human	In vitro: cryopreserved human hepatocytes (CYP activity and mRNA level)	No	J&J PRD	TMC278-NC186	4.2.2.4
Induction	Human	In vitro: human hepatocytes (GST activity)	No	J&J PRD	TMC278-FK4824	4.2.2.4
Metabolism Induction/Inhibition (3 months)	Mouse/ CD-1	Ex vivo: hepatic microsomes	Yes	[redacted]	TMC278-NC192	4.2.2.4

CA: citric acid; CYP: cytochrome P450; GST: glutathione S-transferase; HPMC: hydroxypropyl-methylcellulose; PEG400: polyethylene glycol 400

Approved, issued date: [redacted]

2.6.5.1 Pharmacokinetics: Overview (Continued)

Type of Study	Species/ Strain	Route/Method of Administration (Vehicle/Formulation)	GLP Compliance	Testing Facility	Study/Report Number	Location in CTD
Metabolism Induction/Inhibition (6 months)	Rat/ Sprague Dawley	Ex vivo: hepatic microsomes	Yes	[redacted]	TMC278-NC193	4.2.2.4
Metabolism Induction/Inhibition (6 months)	Dog/ beagle	Ex vivo: hepatic microsomes	Yes	[redacted]	TMC278-NC140	4.2.2.4
Induction/Inhibition (2 weeks)	Rat/ Sprague Dawley	Ex vivo: hepatic microsomes	No	J&J PRD	TMC278-FK4247	4.2.2.4
Inhibition	Human	In vitro: human liver microsomes	No	J&J PRD	TMC278-FK4123	4.2.2.4
Inhibition	Human	In vitro: human liver microsomes	No	J&J PRD	TMC278-NC283	4.2.2.4
Effect Adrenal Gland	Dog	In vitro: adrenal cortex cell-free extracts	No	J&J PRD	TMC278-FK4790	4.2.2.4
Excretion						
Excretion (Single dose) Metabolism	Mouse/ CD-1	Oral/Gavage (^{14}C-TMC278 in PEG400/CA (10%)	No	J&J PRD	TMC278-NC190	4.2.2.4
	Rat/ Sprague Dawley	Oral/Gavage (^{14}C-TMC278 in PEG400/CA (10%)	No	J&J PRD	TMC278-NC113	4.2.2.4
	Dog/ beagle	Oral/Capsule (^{14}C-TMC278 in PEG400/CA (10%)	No	J&J PRD	TMC278-NC114	4.2.2.4
	Human	Oral/Gavage (^{14}C-TMC278 in PEG400)	No	J&J PRD	TMC278-NC157	4.2.2.4
Excretion in Bile (Single dose) Metabolism	Rat/ Sprague Dawley	Oral/Gavage (^{14}C-TMC278 in PEG400/CA (10%)	No	J&J PRD	TMC278-NC145	4.2.2.4
Drug-drug Interaction						
Drug-drug Interactions	Human	In vitro: human liver microsomes	No	J&J PRD	TMC278-NC194	4.2.2.6

CA: citric acid; PEG400: polyethylene glycol 400

2.6.5.3A Pharmacokinetics: In Vitro Absorption

Test Article: rilpivirine

Location in CTD	**4.2.2.2**	
Study No.	**TMC278-NC104**	
Type of Study	Transepithelial permeation of TMC278 across Caco-2 cell monolayers	
Method: Caco-2 cells were maintained for 21-23 days on cell culture inserts. ^{14}C-TMC278 (3 - 300 µM) was added to the apical or basolateral side of the monolayers and transport was measured for 15, 45 and 90 minutes. ^{14}C-alniditan, ^{3}H-levocabastine and ^{3}H-theophylline at a final concentration of 20 µM were included as control compounds for low, medium and high permeability, respectively. The possible inhibition of human P-glycoprotein (or other efflux transporters) was assessed after incubation of ^{3}H-taxol (75.8 nM) in absence or presence of TMC278 (1 - 100 µM). Measurement of bidirectional transport in the presence of 100 µM verapamil was used as a positive control.		
Condition, Compound	P_{app} (10^{-6} cm/s) ± SD (n=4)	
	Apical to Basolateral	Basolateral to Apical
3 µM TMC278	11.1 ± 2.0	22.8 ± 3.3
10 µM TMC278	12.5 ± 2.2	19.0 ± 3.9
30 µM TMC278	13.4 ± 1.1	26.5 ± 5.7
100 µM TMC278	12.8 ± 1.8	16.1 ± 2.3
300 µM TMC278	9.8 ± 0.8	15.1 ± 3.7
30 µM TMC278 + 100 µM verapamil	11.8 ± 1.6	27.1 ± 4.8
20 µM alniditan	0.6 ± 0.3	0.7 ± 0.0
20 µM levocabastine	20.2 ± 4.2	24.8 ± 0.9
20 µM theophylline	28.4 ± 2.4	31.4 ± 5.6
Additional Information Apical to Basolateral (absorptive) and Basolateral to Apical (secretory) P_{app} values were calculated from the slopes of 15 - 45 - 90 min transport-time profiles. All incubations in this experiment were conducted in the presence of Hank's Balanced Salt Solution + 10% Fetal Calf Serum, at pH 6.5 (+ 25 mM MES) in the apical compartment and at pH 7.4 in the basolateral compartment. The average mannitol permeability values were below 0.8 x 10^{-6} cm/s. TMC278 has P-glycoprotein inhibitory properties with an apparent IC_{50} value of 9.2 µM (3.4 µg/mL).		

IC_{50}: concentration resulting in 50% of maximum inhibition; MES: 2-(N-morpholino)ethanesulfonic acid; P_{app}: apparent permeability coefficient; SD: standard deviation

Approved, issued date: [redacted]

2.6.5.3B Pharmacokinetics: Absorption after a Single Oral Dose in Mice

Test Article: rilpivirine

Location in CTD	**4.2.3.3.2**					
Study No.	**TMC278-FK4259** [a]					
Species	**Mouse (CD-1)**					
Feeding Condition	**Not fasted**					
Vehicle/Formulation	**TMC278 base in PEG400/CA (10%)**					
Route	**Oral (gavage)**					
Gender (M/F)/Number of Animals	M/6	F/6	M/6	F/6	M/10	F/10
Dose (mg/kg)	100		400		1600	
Concentration (mg/mL)	5		20		80	
Sample (whole blood, plasma, serum, etc.)	plasma		plasma		plasma	
Analyte	TMC278		TMC278		TMC278	
Assay	LC-MS/MS		LC-MS/MS		LC-MS/MS	
Pharmacokinetic Parameters						
C_{1h} (µg/mL)	39	33	59	68	60	58
Estimated AUC (µg.h/mL)	158	130	262	258	307	287
(Time for calculation –h)	(0-6)	(0-6)	(0-6)	(0-6)	(0-6)	(0-6)
Additional Information						
The sampling times were 1 and 6 h after dose administration.						

[a] the toxicology study number is TOX5538

CA: citric acid; LC-MS/MS: liquid chromatography with tandem mass spectrometry; PEG400: polyethylene glycol 400

2.6.5.3C Pharmacokinetics: Absorption after a Single Dose in Rats

Test Article: rilpivirine

Location in CTD	**4.2.2.2**				
Study No.	**TMC278-FK4195**				
Species	**Rats (Sprague Dawley)**				
Feeding Condition	-				
Vehicle/Formulation	**TMC278 base in PEG400/sterile water (25%)**	**TMC278 base in PEG400**	**TMC278 base in PEG400/CA (10%)**		
Route	**Intravenous (slow bolus injection)**	**Oral (gavage)**	**Oral (gavage)**		
Gender (M/F)/Number of Animals	M/18	M/15	M/15	M/15	M/15
Dose (mg/kg)	4	40	40	160	400
Concentration (mg/mL)	2	4	4	16	40
Sample (whole blood, plasma, serum, etc.)	plasma	plasma	plasma	plasma	plasma
Analyte	TMC278	TMC278	TMC278	TMC278	TMC278
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Pharmacokinetic Parameters					
C_{max} (µg/mL)	5.3 [a]	1.3	1.7	3.3	6.6
t_{max} (h)	NA	1.0	1.0	8.0	8.0
AUC (µg.h/mL)	3.1	9.8	12	48	64
(Time for calculation –h)	(0-∞)	(0-∞)	(0-∞)	(0-∞)	(0-∞)
$t_{1/2}$ (h)	4.4	2.8	4.6	5.7	3.2
(Time for calculation –h)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)
Bioavailability (Fabs %)	NA	32	39	39	21
Clearance (L/h/kg)	1.3	NA	NA	NA	NA
Vd_{ss} (L/kg)	4.1	NA	NA	NA	NA

Additional Information

The sampling times were 7 min (iv only), 20 min, 1, 3, 8 and 24 hours after dose administration.

Tissue samples from the adrenal gland, brain, liver and muscle were collected as well in this study. Maximum tissue concentrations were observed within 20 to 60 min after administration. Tissue levels declined in parallel with plasma concentrations. Tissue to plasma concentration (AUC_{0-24h}) ratios were 3.4, 2.7, 0.49 and 0.45 for liver, adrenal gland, brain and muscle, respectively.

[a] C_0: extrapolated value at 0 h.

CA: citric acid; LC-MS/MS: liquid chromatography with tandem mass spectrometry; NA: not applicable; PEG400: polyethylene glycol 400; Vd_{ss}: volume of distribution at steady state

2.6.5.3D Pharmacokinetics: Absorption after a Single Oral Dose in Rats

Test Article: rilpivirine

Location in CTD	**4.2.2.2**		
Study No.	**TMC278-NC106**		
Species	**Rats (Sprague Dawley)**		
Feeding Condition	**Not fasted**		
Vehicle/Formulation	**TMC278 base in PEG400**	**TMC278.HCl in Tween20/HPMC/water**	**TMC278 fumarate in Tween20/HPMC/water**
Route	**Oral (gavage)**	**Oral (gavage)**	**Oral (gavage)**
Gender (M/F)/Number of Animals	M/6	M/6	M/6
Dose (mg base eq./kg)	40	40	40
Concentration (mg base eq./mL)	4	4	4
Sample (whole blood, plasma, serum, etc.)	plasma	plasma	plasma
Analyte	TMC278	TMC278	TMC278
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS
Pharmacokinetic Parameters			
C_{max} (µg/mL)	1.9	0.77	1.7
t_{max} (h)	1.0	6.0	4.0
AUC (µg.h/mL)	10	7.4	11
(Time for calculation –h)	(0-∞)	(0-∞)	(0-∞)
$t_{1/2}$ (h)	6.0	2.0	3.3
(Time for calculation –h)	(8-24)	(8-24)	(8-24)
Additional Information			
The sampling times were 0.5, 1, 2, 4, 6, 8, 12 and 24 hours after dose administration.			

HPMC: hydroxypropyl methyl cellulose; LC-MS/MS: liquid chromatography with tandem mass spectrometry; PEG400: polyethylene glycol 400

2.6.5.3E Pharmacokinetics: Absorption after a Single Oral Dose in Rats

Test Article: rilpivirine

Location in CTD	**4.2.3.1**	
Study No.	**TMC278-FK4278** [a]	
Species	**Rats (Sprague Dawley)**	
Feeding Condition	**Fasted**	
Vehicle/Formulation	**TMC278 base in PEG400/CA (10%)**	
Route	**Oral (gavage)**	
Gender (M/F)/Number of Animals	M/4	F/4
Dose (mg/kg)	800	
Concentration (mg/mL)	80	
Sample (whole blood, plasma, serum, etc.)	plasma	
Analyte	TMC278	
Assay	LC-MS/MS	
Mean Pharmacokinetic Parameters		
C_{max} (µg/mL)	8.0	18
t_{max} (h)	8.0	8.0
AUC (µg.h/mL)	86	233
(Time for calculation –h)	(0-∞)	(0-∞)
$t_{1/2}$ (h)	5.1	6.3
(Time for calculation –h)	(8-24)	(8-24)
Additional Information		
The sampling times were 0.3, 1, 3, 8 and 24 h after dose administration.		

[a] the toxicology study number is Exp5559

CA: citric acid; LC-MS/MS: liquid chromatography with tandem mass spectrometry; PEG400: polyethylene glycol 400

2.6.5.3F Pharmacokinetics: Absorption after a Single Intravenous Dose in Rabbits

Test Article: rilpivirine

Location in CTD	**4.2.2.2**		
Study No.	**TMC278–FK4293**		
Species	**Rabbits (New Zealand white)**		
Feeding Condition	**Not fasted**		
Vehicle/Formulation	**TMC278 base in PEG400/sterile water (25%)**		
Route	**Intravenous (slow bolus injection)**		
Gender (M/F)/Number of Animals	F/3	F/1	F/2
Dose (mg/kg)	1.25[a]	2.9[b]	4
Concentration (mg/mL)		2	
Sample (whole blood, plasma, serum, etc.)		plasma	
Analyte		TMC278	
Assay		LC-MS/MS	
Mean Pharmacokinetic Parameters			
C_0 (µg/mL)[c]	8.5	12	28
AUC (µg.h/mL)	44	104	151
(Time for calculation –h)	(0-∞)	(0-∞)	(0-∞)
$t_{1/2}$ (h)	12	21	21
(Time for calculation –h)	(24-48)	(48-72)	(48-72)
Clearance (L/h/kg)	0.030	0.028	0.027
Vd_{ss} (L/kg)	0.32	0.56	0.45
Additional Information: The sampling times were 0 (predose), 0.13, 0.25, 0.5, 1, 3, 8, 24, 48 and 72 h (2.9 and 4 mg/kg dose levels only) after dose administration.			

[a] Since dosing of the rabbits with PEG400 formulation at 4 mg/kg, in general, went difficult, an extra 3 animals were dosed at a much lower concentration of 1.25 mg/kg; [b] Originally 3 animals should receive 4 mg/kg; because one animal was difficult to handle it only received 2.9 mg/kg; [c] C_0: extrapolated concentrations at 0 h.
LC-MS/MS: liquid chromatography with tandem mass spectrometry; PEG400: polyethylene glycol 400; Vd_{ss}: volume of distribution at steady state

2.6.5.3G Pharmacokinetics: Absorption after a Single Oral and Intravenous Dose in Dogs

Test Article: rilpivirine

Location in CTD	**4.2.2.2**		
Study No.	**TMC278-FK4231**		
Species	**Dog (beagle)**		
Feeding Condition	-		
Vehicle/Formulation	**TMC278 base in PEG400/sterile water (25%)**	**TMC278 base in PEG400**	**TMC278 base in PEG400/CA (10%)**
Route	**Intravenous (slow bolus injection)**	**Oral (gavage)**	**Oral (gavage)**
Gender (M/F)/Number of Animals	M/2	M/2	M/2
Dose (mg/kg)	1.25	5	5
Sample (whole blood, plasma, serum, etc.)	plasma	plasma	plasma
Analyte	TMC278	TMC278	TMC278
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS
Pharmacokinetic Parameters			
C_{max} (µg/mL)	NA	0.34	1.2
t_{max} (h)	NA	4.0	3.0
AUC (µg.h/mL)	8.7	11	28
(Time for calculation –h)	(0-∞)	(0-∞)	(0-∞)
$t_{1/2}$ (h)	31	39	18
(Time for calculation –h)	(8 or 32-72)	(32-72)	(8-72 or 48-72)
Bioavailability (%)		31	80
Clearance (L/h/kg)	0.14	-	-
Vd_{ss} (L/kg)	5.2	-	-

Additional Information

The sampling times were 0, 0.13 (IV only), 0.25 (IV only), 0.5, 1, 2, 4, 6, 8, 24, 32, 48 and 72 h after dose administration.

Other formulations were tested (capsules containing PEG400/CA, CA/cromophor RH40, CA/HPC E5/vitamin E TPGS and CA/HP-β-CD/vitamin E TPGS). Bioavailability after administration of these formulations was <61% except for capsule containing CA/HPC E5/vitamin E TPGS.

CA: citric acid; HPC: hydroxypropylcellulose; HP-β-CD: hydroxypropyl-β-cyclodextrine; LC-MS/MS: liquid chromatography with tandem mass spectrometry; NA: not applicable; PEG400: polyethylene glycol 400; TPGS: d-alpha-tocopheryl polyethylene glycol 1000 succinate; Vd_{ss}: volume of distribution at steady state

2.6.5.3H Pharmacokinetics: Absorption after a Single Oral and IV Dose in Monkeys

Test Article: rilpivirine

Location in CTD	**4.2.2.2**	
Study No.	**TMC278-DM02403**	
Species	**Monkey (cynomolgus)**	
Feeding Condition	**Not fasted**	**Fasted**
Vehicle/Formulation	**TMC278 base in PEG400/sterile water (25%)**	**TMC278 base in PEG400/CA (10%)**
Route	Intravenous (bolus)	Oral (gavage)
Gender (M/F)/Number of Animals	M/4	M/4
Dose (mg/kg)	1.25	5
Concentration (mg/mL)	2	5
Sample (whole blood, plasma, serum, etc.)	plasma	plasma
Analyte	TMC278	TMC278
Assay	LC-MS/MS	LC-MS/MS
Pharmacokinetic Parameters		
C_{max} or C_0 of infusion (µg/mL) [a]	0.50	0.10
t_{max} (h)	-	9.3
AUC (µg.h/mL)	1.4	1.3
(Time for calculation –h)	(0-∞)	(0-∞)
$t_{1/2}$ (h)	7.1	8.4
(Time for calculation –h)	NS	NS
Cl (L/h/kg)	0.93	-
Vd_{ss} (L/kg)	4.2	-
Bioavailability (%)	-	24
Additional Information		
The sampling times were 0, 0.13, 0.25, 0.5, 1, 3, 7, 24, 48 and 72 h after intravenous dosing, and 0, 0.5, 1, 2, 4, 7, 24, 48 and 72 h after oral dose administration. The same animals were used for intravenous and oral administrations after a 2-week washout period.		

[a] C_0: extrapolated concentrations at 0 h.

CA: citric acid; LC-MS/MS: liquid chromatography with tandem mass spectrometry; NS: not specified; - : not applicable