SECTION 2.6 NONCLINICAL SUMMARY

Section 2.6.4 Pharmacokinetics Written Summary

EMTRICITABINE/ RILPIVIRINE/ TENOFOVIR DISOPROXIL FUMARATE FIXED-DOSE COMBINATION

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GLOSSARY OF ABBREVIATIONS AND DEFINITION OF TERMS

ADME	absorption, distribution, metabolism, and excretion
amu	atomic mass unit
BID	twice daily
CA	citric acid
CSF	cerebrospinal fluid
CYP450	cytochrome P450
DMSO	dimethyl sulfoxide
EDTA	ethylenediamine tetra-acetic acid
FMOs	FAD-containing monooxygenases
FTC	emtricitabine
GD	gestation day
GFR	glomerular filtration rate
GI	gastrointestinal
GLP	Good Laboratory Practices
GST	glutathione S-transferase
HCl	hydrochloride
HLM	human liver microsomes
hOAT1/3	human organic anion transporter type 1/3
HPLC	high performance liquid chromatography
HPMC	hydroxypropyl methylcellulose
IC_{50}	inhibitory concentration of 50%
IV	intravenous
LC-ARC	liquid chromatography-accurate radioisotope counting
LC/MS	HPLC-mass spectrometry
LC/MS/MS	HPLC-tandem mass spectrometry
LLOQ	lower limit of quantification
LSC	liquid scintillation counting
MDCK	Madin-Darby canine kidney
MRM	multiple reaction monitoring
MRP1/2/4	multidrug resistance protein type 1/2/4
MS/MS	Tandem mass spectrometry
NADPH	nicotinamide adenine dinucleotide phosphate
NMR	nuclear magnetic resonance
NNRTI	nonnucleoside reverse transcriptase inhibitor
NRTI	nucleoside reverse transcriptase inhibitor

NtRTI	nucleotide reverse transcriptase inhibitor
PBMC	peripheral blood mononuclear cell
PBS	phosphate buffered saline
PEG400	polyethylene glycol 400
P-gp, MDR1	P-glycoprotein
PI	protease inhibitors
PO	by mouth
QC	quality control
QWBA	quantitative whole-body autoradiography
RBCs	red blood cells
RLG	radioluminography
RPV, TMC278	rilpivirine (27.5 mg rilpivirine hydrochloride is equivalent to 25 mg rilpivirine)
SD	standard deviation
SIV	simian immunodeficiency virus
SPE	solid phase extraction
TDF	tenofovir DF
TFV	tenofovir
UDP-GT	uridine diphosphate-glucuronosyltransferase
ULOQ	upper limit of quantification
US	United States
UV	ultraviolet

PHARMACOKINETIC ABBREVIATIONS

λ_z	Terminal elimination rate constant, estimated by linear regression of the apparent terminal elimination phase of the serum, plasma, or peripheral mononuclear blood cells (PMBCs) concentration of drug versus time curve
AUC_{inf}	The area under the serum, plasma, or PMBC concentration versus time curve extrapolated to infinite time, calculated as $AUC_{0-last} + (C_{last}/\lambda_z)$
AUC_{x-xx}	Partial area under the serum, plasma, or PMBC concentration versus time curve from time "x" to time "xx"
AUC_{ss}	The area under the concentration versus time curve extrapolated to time at steady state
CL	The systemic clearance of the drug after intravenous administration
CL/F	The apparent oral clearance after administration of the drug: $CL/F = Dose/AUC_{inf}$, where "Dose" is the dose of the drug
Cl_p	Total plasma clearance
Cl_b	Total blood clearance
$C_{b/}\ C_p$	Concentration blood/concentration plasma
C_{last}	The last observed quantifiable concentration of the drug in serum, plasma, or PBMCs
C_{max}	The maximum observed concentration of drug in serum, plasma, or PMBC
F	The estimated oral bioavailability of the drug (%), calculated as $100 \bullet (AUC_{oral} \bullet Dose_{iv})/(AUC_{iv.} \bullet Dose_{oral})$
F_{abs}	Absolute oral bioavailability
$t_{½}$	An estimate of the terminal elimination half-life of the drug in serum, plasma, or PBMCs, calculated by dividing the natural log of 2 by the terminal elimination rate constant (λ_z)
T_{last}	The time (observed time point) of C_{last}
T_{max}	The time (observed time point) of C_{max}
V_{ss}	The apparent steady-state volume of distribution of the drug
V_{ss}/F	The apparent steady-state volume of distribution of the drug after oral administration
V_z	The volume of distribution of the drug after intravenous administration
V_z/F	The apparent volume of distribution of the drug

2.6. NONCLINICAL SUMMARY

2.6.4. PHARMACOKINETICS WRITTEN SUMMARY

2.6.4.1. Brief Summary

This dossier is being submitted in support of a marketing authorization application (MAA) for a fixed-dose combination (FDC) film-coated tablet that contains the active substances emtricitabine (FTC), rilpivirine (RPV, which is also referred to as TMC278 throughout this document), and tenofovir disoproxil fumarate (tenofovir DF, TDF). Emtricitabine and TDF are antiretroviral agents developed by Gilead Sciences that have been approved for the treatment of human immunodeficiency virus type 1 (HIV-1) infection as the stand-alone agents Emtriva® (Commission Decision granted on [redacted]) and Viread® (Commission Decision granted on [redacted]), and in a FDC product Truvada® (emtricitabine/tenofovir DF [FTC/TDF]; Commission Decision granted on [redacted]). Emtricitabine, a nucleoside reverse transcriptase inhibitor (NRTI), and TDF, a nucleotide reverse transcriptase inhibitor (NtRTI), are listed as preferred agents in United States (US) and international treatment guidelines {15207}, {12716}, {14065}. Emtricitabine and TDF are also approved for the treatment of HIV-1 infection in combination with efavirenz (EFV), a nonnucleoside reverse transcriptase inhibitor (NNRTI). This FDC product is Atripla® (efavirenz/emtricitabine/tenofovir DF, [EFV/FTC/TDF]; Commission Decision granted on [redacted]). These products are currently approved in the US, the European Community, and other countries worldwide for use in adults. In some regions, Emtriva and Viread are approved for use in adolescents; Emtriva may be administered to children as young as 4 months of age. Rilpivirine, an NNRTI, is an investigational agent that is being submitted for approval by Tibotec BVBA.

Gilead Sciences has coformulated FTC and TDF, the standard of care NRTI backbone, with RPV into a FDC tablet. This FDC tablet is referred to as emtricitabine/rilpivirine/tenofovir disoproxil fumarate (FTC/RPV/TDF; dose strength 200/25/300 mg, respectively) throughout this document. Each FTC/RPV/TDF FDC tablet contains FTC, RPV, and TDF at the same dosages as recommended for the individual components, i.e., 200 mg of FTC, 25 mg RPV (27.5 mg rilpivirine hydrochloride is equivalent to 25 mg RPV), and 300 mg of tenofovir disoproxil fumarate (equivalent to 245 mg tenofovir disoproxil or 136 mg of tenofovir [TFV]). The FTC/RPV/TDF FDC tablet has demonstrated bioequivalence to each of the individual dosage forms (FTC, TDF, and RPV). It is proposed that the FTC/RPV/TDF FDC tablet be indicated for the treatment of HIV-1 infection in adults and taken orally once daily with a meal.

In accordance with the advice received from the European Medicines Evaluation Agency (EMA) at the EMA Strategy meeting held on [redacted] (see meeting minutes provided in Module 1.2, Annex 5.14), the MAAs for RPV as a single agent and for the FTC/RPV/TDF FDC tablet are being submitted in parallel by Tibotec BVBA and Gilead Sciences, respectively.

Comprehensive programs of nonclinical studies with FTC, RPV, and TDF have been conducted. Within this Module 2.6.4 and in the pharmacokinetics Tabulated Summary 2.6.5, RPV is referred to as TMC278 in the text and tables describing the nonclinical studies of RPV alone.

As the RPV component is a new chemical entity, this FDC MAA dossier contains full data on this new component while providing the key data on the Truvada (i.e., FTC, TDF, and FTC/TDF) components. All Truvada studies considered to support the FDC are included to ensure that this is a 'stand-alone dossier.' This is in agreement with the feedback received at the presubmission meeting with the EMA on [redacted] 20[redacted] and with the meeting with the Rapporteur/Co-Rapporteur on [redacted] 20[redacted] (see Module 1.2.5.14, final minutes). To assist the reviewer, a listing of all the FTC, TDF, FTC/TDF, and EFV/FTC/TDF nonclinical reports is provided in Module 2.4, Section 2.4.7.

Information from all nonclinical studies with FTC, RPV, and TDF should be considered in the context of the substantial clinical experience with FTC and TDF within antiretroviral combination therapy for the treatment of HIV-1 infection, the Phase 2 and Phase 3 clinical experience with RPV, and with RPV administered in combination with Truvada (FTC/TDF).

Emtricitabine

Preclinical studies to characterize the absorption, distribution, metabolism, and excretion (ADME) of FTC have been performed in mice, rats, rabbits, and primates. The pharmacokinetic studies are listed in the overview table (Tabulated Summary 2.6.5.1), and study details are given in the individual study overview tables in Module 2.6.5.

In mice, rats, and cynomolgus monkeys, FTC was rapidly and extensively absorbed with oral bioavailability ranging from 58% to 97%. In general, there was no difference in pharmacokinetics following single and multiple dosing. Systemic exposure to FTC (C_{max} and AUC) increased approximately proportionally with dose and was similar between males and females. With chronic dosing, somewhat higher exposures were observed in the mouse and rat studies when compared to short term dosing; however, there was no evidence of accumulation in the monkey studies.

Emtricitabine is widely distributed throughout the body, with a volume of distribution similar to that of total body water. After oral administration, the highest concentrations of FTC were found in the kidneys, gut, and liver well exceed those in plasma, while concentrations in CNS tissues were less than 10% of those in plasma. Emtricitabine is almost completely eliminated within 72 hours following dosing with no evidence of tissue accumulation. Emtricitabine does not undergo extensive first-pass or systemic metabolism, and is eliminated primarily by renal excretion of unchanged drug. Metabolism is similar in humans and monkeys, and includes oxidation of the thiol moiety (Phase 1 metabolism) to form the 3'-sulfoxide diastereomers and conjugation with glucuronic acid (Phase 2 metabolism) to form the 2'-O-glucuronide. The major metabolite, a 3'-sulfoxide accounting for 2% to11% of the dose and several minor metabolites (generally all < 2% of dose) are also eliminated primarily in the urine. Importantly, FTC does not form 5-fluorouracil. The total body clearance of FTC

exceeds the glomerular filtration rate, suggesting the drug is actively secreted by renal tubules into the urine. Emtricitabine was readily transferred across the placenta.

Rilpivirine

TMC278 has been examined in both in vitro and in vivo test systems. In vivo studies were conducted in CD-1 and CB6F1-nonTgrasH2-transgenic mice; pigmented Long Evans and Sprague Dawley rats; New Zealand white rabbits; beagle dogs; and cynomolgus monkeys. With the exception of pigmented rats, all species and strains were the same as those used in the nonclinical pharmacology and toxicology studies. Results from a mass-balance trial evaluating the metabolism and excretion of TMC278 in humans, as well as exposure to TMC278 in patients, are included to provide comparison with results obtained from studies in animal species and to support overall conclusions about the exposure, metabolism, and elimination of TMC278.

The pharmacokinetic studies are listed in the overview table (Tabulated Summary 2.6.5.1), and study details are given in the individual study overview tables in Module 2.6.5. The drug substance, TMC278, has been used in 2 forms in the nonclinical studies, in base form and as the hydrochloride (HCl) salt form. The HCl salt form was used in a limited number of preclinical studies and in the clinical tablet formulations. Throughout this document, these forms are referred to as "TMC278 base" and "TMC278," respectively. The dose levels used in all studies are expressed as base equivalent, and the appropriate correction factor was used when the HCl salt was administered. The form determined in the in vitro media or in in vivo compartments is referred to as "TMC278."

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 the 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.

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 dogs and in mice at low dose levels whereas in rats and in mice at high dose levels, exposures in females were higher than in males (up to 2-fold in mice and 4-fold in rats). In mice, exposure after repeated administration of TMC278 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, the exposure increased compared to Day 1 mainly due to the long half-life ($t_{1/2}$ = 31 hours) of TMC278. In monkeys, after repeated administration of TMC278, exposure had a tendency to increase but due to the high inter-individual variability it is difficult to reach firm conclusions.

In rats, tissue distribution of [^{14}C]TMC278 and its metabolites after a 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. Total radioactivity exposure values in the placenta and in whole fetus were 0.94- and 0.64-fold those of maternal blood, respectively.

TMC278 is extensively bound to plasma proteins and this is independent of the concentration and species. In the various animal species and human, plasma protein binding ranged from 99.08% to 99.97%. TMC278 is extensively bound to human albumin and to a much lesser extent 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 $t_{1/2}$ of TMC278 were observed between the rat (4.4 hours), rabbit (12 hours), dog (31 hours), and monkey (7.1 hours). The volume of distribution at steady-state was 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 such as 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 was the predominant pathway, whereas in dog and man, oxidation of TMC278 was 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, total radioactivity 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 total radioactivity eliminated was unchanged TMC278. Renal excretion of total radioactivity 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% to 25% of the

dose) and the amount of unchanged TMC278 in bile was negligible. In rats, there was an indication that TMC278 was excreted in milk.

In vitro, the cytochrome P450 (CYP450) 3A4 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. TMC278 was shown to have P-glycoprotein (P-gp, MDR1) inhibitor properties with an apparent inhibitory concentration of 50% (IC_{50}) value of 9.2 µM (3.4 µg/mL). Inhibition of transepithelial permeation of P-gp substrates cannot be excluded, but any effect is unlikely to be clinically relevant. P-glycoprotein 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 the 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 uridine diphosphate-glucuronosyltransferase (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-naive patients is 25 mg once daily. At this dose level, mean C_{max} (Weeks 4–8) was 0.13 µg/mL and mean $AUC_{0–24h}$ (Week 48; population PK) was 2.4 µg·h/mL (Clinical Studies TMC278-TiDP6-C209 and TMC278-TiDP6-C215 (Module 2.7.2, Sections 2.7.2.2.2.6.4 and 2.7.2.2.2.6.5). 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.

Tenofovir DF

The ADME of tenofovir (TFV) and TDF have been investigated in mice, rats, rabbits, woodchucks, dogs, and monkeys. Following oral administration of TDF in these species, maximum TFV plasma concentrations were reached within 0.25 to 1 hour and declined in a biphasic manner. The observed terminal $t_{1/2}$ values were approximately 7, 9, and 60 hours in rats, monkeys, and dogs, respectively. Due to the long terminal $t_{1/2}$ observed in dogs, a substantial degree of accumulation was observed upon daily repeat dosing in this species. The oral bioavailability of TDF was greatest in dogs and monkeys (30% to 40%) and least in rodents (10% to 20%). The prodrug moiety was efficiently cleaved in all species. The pharmacokinetic studies are listed in the overview table (Tabulated Summary 2.6.5.1), and study details are given in the individual study overview tables in Module 2.6.5.

No circulating metabolites of TFV, other than the monoester observed at early time points in rats, were detected. This was consistent with the lack of metabolism of TFV in intestinal and

liver preparations. Little or no inhibition of CYP P450 isozymes was observed in human hepatic microsomes; little or no induction was observed in livers from rats given a high dose of TDF. Extensive tissue distribution, suggested by the plasma pharmacokinetics of TFV, was confirmed in studies with [^{14}C]labeled TFV in dogs. Major sites of tissue uptake included the liver and kidney. Placental transfer of TFV appeared to be significant in monkeys.

Tenofovir was excreted unchanged in the urine of all animal species tested and renal excretion was identified as the primary route of elimination by a combination of glomerular filtration and tubular secretion. In vitro transport studies indicate that the active tubular secretion of TFV in humans is mediated by human organic anion transporter type 1 (hOAT1) and multi-drug resistance protein type 4 (MRP4) acting in series as the major uptake and efflux transporters in proximal tubules, respectively. Human organic anion transporter type 3 (hOAT3) may play a secondary role in the tubular uptake of TFV. Neither P-gp nor multidrug resistance protein type 1 or 2 (MRP1 and MRP2) appear to be involved in the tubular efflux of TFV. As the primary transporter handling the tubular uptake of TFV, hOAT1 has been assessed for its potential role in drug interactions between TFV and other renally secreted therapeutics including antibiotics, anti-inflammatory agents, and other antivirals (including protease inhibitors [PIs]). Under physiologically relevant conditions, a number of renally excreted drugs showed no effect in vitro on the hOAT1-mediated transport of TFV. Similarly, PIs did not exhibit any effect on the in vitro active cellular elimination of TFV mediated by the MRP4 efflux pump indicating that PIs are unlikely to exert any substantial effect on the accumulation of TFV in renal proximal tubules.

In vitro studies have shown that the intestinal absorption of TDF, the oral prodrug of TFV, can be slightly affected by a combination of P-gp mediated efflux transport and esterase degradation in gastrointestinal (GI) tissue. Further studies in human intestinal S9 fractions, the human colon carcinoma cell line Caco-2, and Madin-Darby canine kidney (MDCKII) cells stably transfected with the human gene that encodes P-gp have suggested that the relative ability of PIs to inhibit esterase activity and inhibit or induce intestinal P-gp may account for the modest changes in plasma TFV levels when TDF is coadministered in humans with some PIs.

Emtricitabine/Tenofovir DF

Because the pharmacokinetic properties and safety profiles of the individual agents in preclinical animal models is well established and raises no concern for pharmacokinetic interactions, no nonclinical pharmacokinetic studies have been performed with the combination of FTC and TDF.

Emtricitabine/Rilpivirine/Tenofovir DF

Based on the data supporting the individual components and the FTC/TDF combination, adverse pharmacokinetic interactions that would negatively affect pharmacological efficacy are not anticipated. This assumption is based on the discrete routes of absorption and elimination demonstrated for each compound and the differences in physicochemical

properties between the compounds which influence drug distribution. Single-dose pharmacokinetic studies in dogs demonstrate that comparable exposures for each component can be achieved through co-formulation in a bilayer tablet relative to the clinical formulations administered separately. More compelling support for this assumption may be derived from the human clinical data.

2.6.4.2. Methods of Analysis

2.6.4.2.1. Emtricitabine

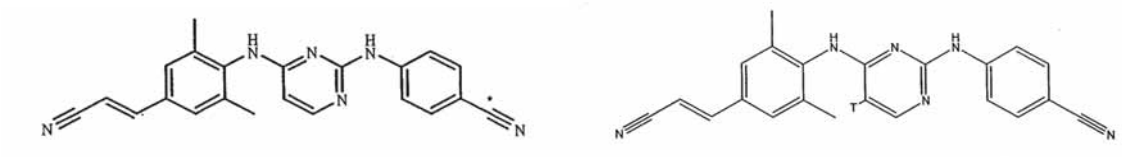
Analytical methods used to quantify FTC in mouse, rat, and monkey plasma from the early preclinical ADME studies were reverse-phase high performance liquid chromatography (HPLC) with ultraviolet (UV) detection at 280 nm. The lower limit of quantification (LLOQ) was 0.063 to 0.125 µg/mL {4251}. Additional mouse, rat, rabbit, and monkey ADME and toxicokinetic studies used HPLC-mass spectrometry (LC/MS)-based assays for the quantitation of FTC in plasma and urine. Initially, a method employing selected ion monitoring was developed (Report 97/001.01 [TPI# 1010]) which was subsequently improved by incorporation of full-scan tandem mass spectrometry MS/MS (Reports TPI #6447 v5, TPI #7582 v2, and TPI #6159 v1). Methods were cross-validated, and the LLOQ was generally in the range of 0.100 to 0.200 µg/mL.

2.6.4.2.2. Rilpivirine

Radio-Labeled TMC278

A large number of studies discussed in this summary were conducted with radiolabeled TMC278. Two radiolabeled (^{14}C and ^{3}H) TMC278 compounds were used but most of the studies were performed with ^{14}C. The ^{14}C atom was on the nitrile carbon of the benzonitrile of the TMC278 molecule (Figure 1). The original material had a radiochemical purity of 98.2% and a specific activity of 2.03 GBq/mmol. The ^{3}H atom was in the pyrimidine moiety of TMC278 molecule. The radiochemical purity was 99.7% and the specific activity was 8.07 TBq/mmol (Figure 1), and was used in one study to investigate plasma protein binding and distribution in blood.

Figure 1. Structural Formula of [^{14}C]TMC278 (Left) and [^{3}H]TMC278 (Right)



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

The metabolic stability of the ^{14}C label of TMC278 was investigated following a single oral dose of [^{14}C]TMC278 at 10 mg/kg in Sprague Dawley rats (Report TMC-FK4686). The

recovery of [^{14}C]O^2 from the expired air collected for 25 hours after administration was negligible. This indicates that the ^{14}C label is metabolically stable.

Radiochemical Methods

The detailed description of radiochemical methods used in ADME studies is given in the various study reports. The following techniques were used:

- Tissue distribution of total radioactivity was studied by quantitative whole-body autoradiography (QWBA) in male pigmented rats and pregnant female Sprague Dawley rats (see Section 2.6.4.4.2.5). The concentration of radioactivity in the different tissues was determined by radioluminography, whereas the concentration of radioactivity in the eye and in biological fluids (blood and plasma) was determined by liquid scintillation counting (LSC).
- Total radioactivity in biological samples was measured by LSC, using appropriate scintillation cocktails. Aliquots of biological fluids were counted directly (plasma, urine, and bile) following extraction or combustion (blood and feces residues).

In metabolism and/or mass balance studies, unchanged compound and/or its major metabolites were determined in various biological samples (plasma, urine, bile, and feces). Mass balance was based on the recovery of radioactivity from various samples or pools of samples. In in vitro and in vivo studies with [^{14}C]TMC278, metabolite profiles were determined by radio-HPLC. Metabolite identification was done by a combination of liquid chromatography with tandem mass spectrometry (LC-MS/MS) and co-chromatography with synthesized metabolites (see Sections 2.6.4.5.1.2 and 2.6.4.5.2.2).

Bioanalytical Methods

Bioanalytical methods were developed to support the TMC278 toxicokinetic and pharmacokinetic program. Methods were all based on the same detection technique, i.e., tandem mass spectrometry. The performance of most of these assays was characterized by validation processes, in line with the internal procedures and bioanalytical guidelines {15698}, {15551}, (Bioanalytical Method Validation, Guidance for Industry. US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research, Center for Veterinary Medicine May 2001). The validation data for each assay are described in detail in the respective method validation reports.

An LC-MS/MS method was validated for the determination of TMC278 in mouse (Report TMC278-FK4240), rat (Report TMC278-FK4170), rabbit (Report TMC278-BA1014), dog (Report TMC278-FK4169), and monkey (Report TMC278-NC273) ethylenediamine tetra-acetic acid (EDTA) plasma, and dog (Report TMC278-FK4169) heparin plasma. Tissue samples were analyzed with non-validated qualified research methods based on the validated plasma methods. The validation data for LC-MS/MS methods (heparin and EDTA plasma) are summarized below and details are outlined in the respective method validation reports.

In-study validation was conducted for nonclinical GLP studies. These validation data are appended to the individual preclinical study reports.

LC-MS/MS Methods

The LC-MS/MS method for mouse, rat, rabbit, and dog plasma consisted of a solid-phase extraction (SPE) followed by reversed-phase HPLC coupled to tandem mass spectrometry.

The selectivity of the LC-MS/MS assay towards endogenous compounds was proven in 6 different batches of non-pooled blank EDTA or heparin plasma.

The inter-batch accuracy and inter-batch precision were calculated by comparing the theoretical concentration with the mean measured concentration for sets of QC samples at 4 concentrations (LLOQ, Low, Medium, and High). The accuracy was within the criteria of 80% to 120% at the LLOQ QC level and 85% to 115% at the other levels. The inter-batch precision of the LLOQ QC was ≤ 20% and ≤ 15% at the other levels. Detailed information on the accuracy and precision can be found in the method validation reports.

Stability

The stability of TMC278 was assessed in the stock solution solvent (methanol) and in biological matrices (both heparin and EDTA blood and plasma at several temperatures). The test article was found not to be stable in daylight. When TMC278 is exposed to daylight, the drug is transformed to the Z-isomeric form. Therefore, each assay was carried out under yellow-light conditions and samples were protected from light.

TMC278 was stable in methanol for at least 6 months after storage in a freezer (–18°C), for 1 month in a refrigerator (4°C ± 2°C), and for 3 days at room temperature (yellow-light conditions) (Report TMC278-FK4170).

2.6.4.2.3. Tenofovir Disoproxil Fumarate

Two assays have been used to determine plasma TFV concentrations. The first is a reverse phase ion-pair HPLC method with fluorescence derivatization (Reports P1278-00028, P1278-00001, P1278-00017, 97-TOX-4331-08, OLI-RE748-9901-DNS-1, and P4331-00008). This assay was validated in plasma for mice, rats, rabbits, dogs, and cynomolgus monkeys with a limit of quantitation of 25 ng/mL, and was used in earlier studies. The method proved adequate to demonstrate exposure for toxicokinetic studies but lacked the sensitivity required to appropriately characterize the pharmacokinetics and oral bioavailability of TFV. A more sensitive assay has been developed and key pharmacokinetic studies were repeated in order to obtain definitive data. This second assay utilizes HPLC-mass spectroscopy (LC/MS/MS). The method was validated in plasma for mice, rats, dogs, and rhesus monkeys with a limit of quantitation (LOQ) of 3, 3, 3, and 1 ng/mL, respectively (Reports P1278-00028, 002092/OFH, and 003296/OTN). The LC/MS/MS assay was also validated for the determination of TFV in rat milk with an LOQ of 10 ng/mL (Report 003105/OUI). Stability studies have demonstrated that TFV is highly stable in all

matrices from all species tested to date. In addition, a HPLC method has been validated for the determination of TDF in dosing solutions (Report P4331-00009).

2.6.4.3. Absorption

2.6.4.3.1. In Vitro Absorption Studies for Rilpivirine

Human colon carcinoma-derived Caco-2 cells were used as an in vitro model to investigate the transepithelial transport characteristics of [^{14}C]TMC278 in both the absorptive (apical to basolateral) and the secretory (basolateral to apical) directions at initial donor concentrations between 3 and 300 µM (1.1 and 111 µg/mL). In addition, in order to evaluate the role of P-gp in TMC278 transport, TMC278 at 30 µM (11 µg/mL) was incubated in the presence of the P-gp inhibitor verapamil (100 µM). Possible inhibition of P-gp mediated transport of taxol (75.8 nM) across Caco-2 cells was also investigated in the presence of increasing concentrations of TMC278 (1-100 µM; 0.37-37 µg/mL) (Tabulated Summary 2.6.5.3.F, TMC278-NC104).

TMC278 showed apparent permeability coefficients ranging from 10×10^{-6} to 13×10^{-6} cm/s and 15×10^{-6} to 27×10^{-6} cm/s for absorptive and secretory transport, respectively. Comparison of these permeation rates with those of the reference compounds alniditan (low permeability), levocabastine (medium permeability), and theophylline (high permeability) indicated that TMC278 can be classified as a compound with an intermediate permeability.

The permeability of TMC278 across the monolayers was concentration-independent between 3 and 300 µM (1.1 and 111 µg/mL). TMC278 permeation at steady state across the monolayers was slightly polarized with efflux ratio (secretory/absorptive permeation) values of 1.5 to 2. Coincubation with the P-gp inhibitor verapamil did not affect TMC278 transport. These results indicate that TMC278 permeation occurs predominantly by passive transcellular diffusion and that TMC278 is not a substrate of P-gp.

Bidirectional transport experiments with the P-gp substrate taxol demonstrated that TMC278 has P-gp inhibitory properties with an apparent IC_{50} value of 9.2 µM (3.4 µg/mL).

Based on the above, it is concluded that TMC278 will exhibit sufficient membrane permeability to obtain adequate intestinal absorption, provided solubility and dissolution are not rate limiting. Inhibition of transepithelial permeability of P-gp substrates by TMC278 can not be excluded, but for most drugs a possible effect is unlikely to be clinically significant at the intestinal absorption level.

The results of the present study are consistent with previously reported results using unlabeled TMC278 in Caco-2 monolayers (Report TMC278-TiDP6-JRF [FK4155]).

2.6.4.3.2. In Vitro Absorption Studies for Tenofovir DF

Human colon carcinoma-derived Caco-2 cells were used as an in vitro model to investigate the transepithelial transport characteristics of tenofvoir DF. A concentration-dependent

increase in forward (apical to basolateral) permability was noted as TDF concentration increased from 1 to 1000 μM. Based on the clinical dose and solubility of TDF it is likely that luminal concentrations are sufficient to maximize permeability and drive absorption in vivo. A lack of direct interaction between P-gp and TFV has been established. Further in vitro studies have shown that the intestinal absorption of the oral prodrug of TFV (TDF) is limited by a combination of P-gp-mediated efflux transport and esterase degradation {5939}. Further studies in human intestinal S9 fractions, the human colon carcinoma cell line Caco-2, and Madin-Darby canine kidney (MDCKII) cells stably transfected with the human gene that encodes P-gp have suggested that the relative ability of PIs to inhibit esterase activity and inhibit or induce intestinal P-gp may account for the modest changes in plasma TFV levels when TDF is coadministered with some PIs (Tabulated Summary 2.6.5.14.J, AD-104-2010) {11255}.

2.6.4.3.3. Single-Dose Studies for Emtricitabine, Rilpivirine, and Tenofovir DF

2.6.4.3.3.1. Emtricitabine: Mouse, Oral/Intravenous

Two groups of male CD-1 mice were administered a single 10-mg/kg dose of a solution of FTC by either the oral (PO) or intravenous (IV) route (Tabulated Summary 2.6.5.3.A, TEIN/93/0003). After IV administration, plasma clearance was bi-exponential, with $t_{½}\alpha$ equal to 4 minutes and $t_{½}\beta$ equal to 23 minutes. The volume of distribution at steady state was 0.89 L/kg. After oral administration, absorption was rapid with T_{max} at 25.4 minutes and C_{max} at 9.8 μM (2.4 μg/mL). The absolute bioavailability was 96%.

Two groups of male CD-1 mice were given a single dose of 100 mg/kg FTC by either the PO or IV routes (Tabulated Summary 2.6.5.3.B, TEIN/93/0004). Plasma clearance was described by a tri-exponential equation, with a $t_{1/2}\alpha$ of 1.7 minutes, a $t_{1/2}\beta$ of 15.5 minutes, and a $t_{1/2}\gamma$ of 82 minutes. After oral administration, absorption was rapid, with the oral T_{max} at 24.5 minutes and the C_{max} at 89 μM (22 μg/mL). The absolute bioavailability was 79%.

Following IV administration of FTC to male mice at a dose of 600 mg/kg, plasma concentrations of FTC declined in an apparent tri-phasic manner with a terminal $t_{1/2}$ of approximately 4 hours (Tabulated Summary 2.6.5.3.C, IUW00101). Systemic clearance was 1.3 L/h/kg, which is nominally similar to the glomerular filtration rate (GFR) in mice. Apparent volume of distribution was approximately 1 L/kg indicating that FTC is widely distributed to tissues. The disposition kinetics of FTC in male mice observed in this study were similar to those reported previously. In the same study, following oral administration at a dose of 600 mg/kg, plasma concentrations of FTC attained maximum mean levels of 139 μg/mL at 40 minutes. Absorption of FTC appeared to be both rapid and extensive; the mean absorption time was 2 hours and the extent of systemic availability (bioavailability) was 63%.

2.6.4.3.3.2. Emtricitabine: Monkey, Oral/Intravenous

Eight male cynomolgus monkeys were administered single 10-mg/kg or 80-mg/kg doses of FTC (4 monkeys at each dose) by IV infusion and nasogastric gavage in a crossover design

study (Tabulated Summary 2.6.5.3.D, TEZZ/93/0019). Plasma concentrations were determined by an HPLC method. Dose-independent kinetics were observed over the concentration range used in this study. The terminal $t_{1/2}$ was 1.0 ± 0.2 hours. The mean C_{max} values after oral dosing were 14.1 ± 2.0 and 11.1 ± 3.4 µM (3.48 and 27.4 µg/mL, respectively) for 10 mg/kg and 80 mg/kg, respectively. The corresponding T_{max} values were 1.3 ± 0.5 and 2.3 ± 0.3 hours. The AUCs after IV and oral administration increased linearly with the dose. The bioavailability ranged from 44% to 69% (overall mean 60%) and did not differ significantly between dose levels.

Following single IV administration of FTC at a dose of 80 mg/kg to male cynomolgus monkeys, plasma concentrations of FTC declined in an apparent tri-phasic manner with a terminal $t_{1/2}$ of approximately 1 hour (Tabulated Summary 2.6.5.3.E, IUW00301). Following single oral administration at 80 mg/kg, plasma concentrations of FTC attained maximum levels of approximately 40 µg/mL at 1 hour. Absorption of FTC appeared to be both rapid and extensive; the mean absorption time was less than 1 hour, on average, and the extent of systemic availability (bioavailability) was virtually complete (97%). Plasma FTC concentrations declined in parallel with those following IV administration.

2.6.4.3.3.3. Rilpivirine: Mouse, Oral

TMC278 base formulated in polyethylene glycol 400 (PEG400)/10% citric acid (CA) was administered once, by oral gavage, at 100, 400, and 1600 mg/kg to male and female CD-1 mice (Tabulated Summary 2.6.5.3.G, TMC278-FK4259).

Due to the limited sampling points (1 and 6 hours), only an estimation of the AUC was possible. At 1600 mg/kg, estimated AUC_{0-6h} values were 307 and 287 µg·h/mL in males and females, respectively.

2.6.4.3.3.4. Rilpivirine: Rat, Oral/IV

TMC278 base was administered to male Sprague Dawley rats by oral gavage at 40 mg/kg formulated in PEG400 and at 40, 160, 400, and 800 mg/kg (males and females) formulated in PEG400/citric acid (CA) (10%) (Tabulated Summaries 2.6.5.3.H and 2.6.5.3.J, TMC278-FK4195 and TMC278-FK4278, respectively). TMC278 base formulated in PEG400/sterile water was administered intravenously to male Sprague Dawley rats at 4 mg/kg (Tabulated Summary 2.6.5.3.H, TMC278-FK4195). In addition, TMC278 was administered to male Sprague Dawley rats by oral gavage at 40 mg/kg as the base in PEG400 and as the HCl salt in Tween20/hydroxypropyl methylcellulose (HPMC)/water (Tabulated Summary 2.6.5.3.I, TMC278-NC106).

The absorption of TMC278 in PEG400 or PEG400/CA was rapid at the low dose (40 mg/kg) with C_{max} reached at 1 hour after dose administration. At higher doses, the plasma profiles showed a plateau and C_{max} values were only reached at 8 hours. $AUC_{0-\infty}$ values at 800 mg/kg were 86 and 233 µg·h/mL in males and females, respectively. AUC values increased dose-proportionally from 40 to 160 mg/kg and less than dose-proportionally from 160 to

800 mg/kg. At 800 mg/kg, exposure values (C_{max} and AUC) were up to 2.7-fold higher in females than in males.

The $t_{1/2}$ of TMC278 after IV administration was moderate (4.4 hours). The Vd_{ss} was 4.1 L/kg, which is approximately 6 times the total body water of rats (0.67 L/kg), indicating extensive distribution to the tissues. The total plasma clearance (Cl_p) was 1.3 L/h/kg, corresponding to a total blood clearance (Cl_b) of 1.9 L/h/kg (concentration blood/concentration plasma (C_b/C_{pl}) = 0.7 [Tabulated Summary 2.6.5.6.B, TMC278-NC112]), which is moderate compared to the hepatic blood flow of rats (3.3 L/h/kg). The absolute oral bioavailability (F_{abs}) was 32% and 39% after an oral dose of 40 mg/kg in the absence and presence of CA, respectively (compared to an IV dose of 4 mg/kg).

TMC278 in Tween20/HPMC/water gave a 26% lower exposure than the base in PEG400/CA, at an oral dose of 40 mg/kg.

2.6.4.3.3.5. Rilpivirine: Rabbit, IV

TMC278 base formulated in PEG400/water (25%) was administered intravenously to female New Zealand white rabbits at 1.25 (n=3), 2.9 (n=1), and 4 mg/kg (n=2) (Tabulated Summary 2.6.5.3.K, TMC278-FK4293).

After IV administration, AUC values increased dose-proportionally from 1.25 mg/kg to 4 mg/kg. Plasma levels declined slowly with a mean $t_{1/2}$ of 12 hours at 1.25 mg/kg and about 21 hours at the higher dose levels. The Vd_{ss} ranged between 0.32 and 0.56 L/kg, which is low compared to the total body water of rabbits (0.72 L/kg) indicating limited distribution. Total Cl_p was on average 0.03 L/h/kg, corresponding to a total Cl_b of 0.049 L/h/kg (C_b/C_{pl} = 0.61 [Tabulated Summary 2.6.5.6.B, TMC278-NC112]), which is very low compared to a hepatic blood flow of 4.3 L/h/kg.

2.6.4.3.3.6. Rilpivirine: Dog, Oral/IV

TMC278 base was administered to male beagle dogs by oral gavage at 5 mg/kg formulated in either PEG400 or PEG400/CA (10%) and intravenously at 1.25 mg/kg formulated in PEG400/water (25%) (Tabulated Summary 2.6.5.3.L, TMC278-FK4231). TMC278 base formulated in either PEG400 (40 and 80 mg/kg) or PEG400/CA (80 mg/kg) was also administered to male and female beagle dogs by oral gavage in a tolerance study (Tabulated Summary 2.6.5.4.Q, TMC278-FK4102). In addition, TMC278 base formulated in PEG400/CA was administered by oral gavage to male beagle dogs at 5 mg/kg under fed and fasted conditions to investigate the food effect on the pharmacokinetics of TMC278 (Report TMC278-NC163 [FK5141]).

The plasma profiles of TMC278 in PEG400 or PEG400/CA showed a plateau after an initial rapid absorption until at least 7 to 8 hours postdose. At 40 mg/kg in PEG400, $AUC_{0-\infty}$ values were 41 and 52 µg.h/mL in the male and female dog, respectively. Between 40 and 80 mg/kg in PEG400, no further increase in exposure was seen. Mean C_{max} and AUC values increased

substantially at 5 mg/kg with the addition of CA to the formulation (~ 3-fold). At 80 mg/kg, only C_{max} increased with addition of CA while AUC values were comparable.

TMC278 was slowly eliminated following IV administration with a $t_{1/2}$ of 31 hours. The Vd_{ss} was 5.2 L/kg, which is about 9 times the total body water of dogs (0.604 L/kg), indicating extensive distribution to tissues. Total Cl_p was on average 0.14 L/h/kg, corresponding to a total Cl_b of 0.20 L/h/kg (C_b/C_{pl} = 0.69 [Tabulated Summary 2.6.5.6.B, TMC278-NC112]), which is very low compared to a hepatic blood flow of 1.9 L/h/kg.

The F_{abs} was 31% and 80% after an oral dose of 5 mg/kg in the absence and presence of CA, respectively (as compared to an IV dose of 1.25 mg/kg).

The effect of food was tested in dogs at 5 mg/kg of TMC278 formulated in PEG400/CA. Under fed conditions, the absorption rate of TMC278 was slower but the extent of absorption was similar, leading to a lower C_{max} without any change in AUC.

2.6.4.3.3.7. Rilpivirine: Monkey, Oral

TMC278 base was administered to male cynomolgus monkeys by oral gavage at 5 mg/kg formulated in PEG400/CA (10%) and intravenously at 1.25 mg/kg formulated in PEG400/water (25%) (Tabulated Summary 2.6.5.3.M, TMC278-DM02403). TMC278 formulated in aqueous HPMC (1%)/Tween 20 (0.5%) was administered to female cynomolgus monkeys at 250 mg/kg twice daily (BID; dosing interval of 8 hours) and at 500 mg/kg once daily (Tabulated Summary 2.6.5.4.X, TMC278-NC326).

After once-daily administration, the inter-individual variability was high particularly for C_{max} (CV = 71%). C_{max} occurred between 2 and 24 hours after oral administration. The $t_{1/2}$ after IV administration was 7.1 hours. The Vd_{ss} was 4.2 L/kg, which is about 6 times the total body water of monkeys (0.692 L/kg), indicating that TMC278 is widely distributed to tissues. Total Cl_p was on average 0.93 L/h/kg, corresponding to a total Cl_b of 0.98 L/h/kg (C_b/C_{pl} = 0.95 [Tabulated Summary 2.6.5.6.C, TMC278-NC332]), which is low compared to a hepatic blood flow of 2.62 L/h/kg. At 5 mg/kg, $AUC_{0-\infty}$ was 1.3 µg.h/mL.

The F_{abs} was 24% after an oral dose of 5 mg/kg (compared to an IV dose of 1.25 mg/kg).

A higher exposure (AUC) to TMC278 was observed after a BID dose of 250 mg/kg of TMC278 compared to a single dose of 500 mg/kg. However, this was not confirmed in the subsequent repeat-dose study. At 250 mg/kg BID, the $AUC_{0-\infty}$ value was 10.1 µg·h/mL.

2.6.4.3.3.8. Tenofovir DF: Mouse, Oral

Concentrations of TFV were determined in plasma samples following oral gavage of TDF in albino mice at doses of 100, 300, and 1000 mg/kg (Tabulated Summary 2.6.5.4.Z, M990203-PK). Tenofovir DF was rapidly absorbed and converted to TFV with T_{max} values that ranged from 0.083 to 0.5 hours and C_{max} values of 3.38, 5.89, and 35.1 µg/mL for the 100-, 300-, and 1000-mg/kg dose groups, respectively. Overall, TFV plasma C_{max} appeared

to increase in a dose-proportional manner. Due to limited sampling, only C_{max} and T_{max} pharmacokinetic parameters could be accurately estimated following the Day 1 dose.

Levels of TFV were determined in mouse plasma samples obtained during an in vivo micronucleus assay (Tabulated Summary 2.6.5.3.N**,** 97-TOX-4331-008-PK)**.** Plasma samples (5 mice/dose/time point) were obtained at 1, 24, and 48 hours following dosing. At 1 hour after dosing, plasma TFV concentrations were dose proportional. Mean plasma TFV concentrations at the 500, 1000, and 2000 mg/kg doses were 3.73, 8.13, and 28.18 µg/mL, respectively. At 24 hours, mean plasma TFV levels remained dose proportional. Plasma TFV levels were 0.68, 1.21, and 2.06 µg/mL at the 500-, 1000-, and 2000-mg/kg dose levels, respectively. At 48 hours, mean plasma levels were no longer dose proportional. Plasma TFV levels were 0.045, 0.152, and 0.921 µg/mL at the 500-, 1000-, and 2000-mg/kg dose levels, respectively. The limited plasma data precluded determination of plasma $t_{1/2}$ and AUC. However, from the plasma concentration data it was apparent that greatest exposure (> 85% of total) to TFV occurred during the initial 24-hour period.

2.6.4.3.3.9. Tenofovir DF: Rat, Oral

Tenofovir pharmacokinetics in the Sprague-Dawley rat were evaluated following single-dose oral administration of TDF at doses ranging from 20 to 1000 mg/kg and are summarized in Tabulated Summaries 2.6.5.4.BB, 2.6.5.4.CC, 2.6.5.4.DD, and 2.6.5.4.EE (96-TOX-4331-003-PK, R2000036-PK, 97-TOX-4331-002-PK, and R990204**,** respectively). Maximum plasma TFV concentrations (C_{max}) were reached within 0.25 to 1 hour following administration. The C_{max} of TFV in rats appeared to be nonlinear with dose, with C_{max} values increasing in a less than dose proportional manner. The $AUC_{(0–\infty)}$ values appeared to be linear with dose, although the longer terminal $t_{1/2}$ and corresponding large values for % AUC extrapolated at higher doses weakens these conclusions. Combined, these data could indicate slower absorption at higher doses of TDF in rats. No apparent gender-related differences were observed in the pharmacokinetics of TFV at any dose, although the data were limited and highly variable. Oral bioavailability values for TFV from TDF in these studies ranged from 10% to 28%.

2.6.4.3.3.10. Tenofovir DF: Woodchuck, Oral/Intravenous

The oral bioavailability of TDF was determined in a crossover design study where animals (3/sex/group) received a single IV dose of 2.5, 7.5, or 12.5 mg/kg followed by oral administration of 5.0, 15, or 25 mg/kg after a 1-week washout period (Tabulated Summary 2.6.5.3.O, W20000108). C_{max} and $AUC_{(0-\infty)}$ increased with dose; however, oral bioavailability was noted to be reduced at the highest dose. Mean oral bioavailability for the 5.0, 15, and 25 mg/kg groups was 20.6 ± 12.9%, 32.4 ± 16.2%, and 10.0 ± 4.16%, respectively.

2.6.4.3.3.11. Tenofovir DF: Dog, Oral/Intravenous

In a single-dose 3-way crossover design study, TFV plasma pharmacokinetics were evaluated in adult male beagle dogs following: (1) single-dose IV bolus administration of TDF at doses

of 1 and 10 mg/kg; (2) single-dose oral administration of 5 and 30 mg/kg TDF in the fed state; and (3) single-dose oral administration of 5 and 30 mg/kg TDF in the fasted state (Tabulated Summary 2.6.5.3.P**,** D2000076). Absorption following oral administration was rapid, with T_{max} values of 0.5 to 2 hours and 0.5 to 4 hours for the fed and fasted groups, respectively. Mean C_{max} values of 0.490 ± 0.211 and 4.69 ± 2.03 µg/mL were achieved in the fed 5- and 30-mg/kg TDF groups, respectively, and 0.401 ± 0.141 and 3.49 ± 1.88 µg/mL for the same doses in fasted animals. Mean $AUC_{(0-\infty)}$ values of 3.21 ± 0.905 and 25.8 ± 6.64 µg·h/mL were estimated for the fed 5- and 30-mg/kg TDF groups, respectively, and 3.07 ± 0.620 and 23.0 ± 4.76 µg·h/mL for the same doses in fasted animals. There was an apparent enhancement of absorption of TDF with food in some but not all animals and even though study samples were collected out to 120 hours postdose, the % AUC extrapolated in 6 out of 16 observations still exceeded 15%. The absolute oral bioavailability of TFV following oral TDF administration was calculated for each animal. Mean values of 31.8 ± 9.21% and 46.4 ± 13.0% were estimated in the fed 5- and 30-mg/kg TDF groups, respectively, and 30.2 ± 4.08% and 41.2 ± 8.28% for the same doses in fasted animals.

Additional single-dose oral pharmacokinetic studies have been conducted with TDF in beagle dogs at doses ranging from 3 to 210 mg/kg and are summarized in Tabulated Summaries 2.6.5.4.GG, 2.6.5.4.II, and 2.6.5.4.HH (96-TOX-4331-004-PK, 97-TOX-4331-001-PK, and 98-TOX-4331-003-PK, respectively). Maximum TFV concentrations were reached within 0.25 to 1 hour following oral administration. C_{max} values increased in direct proportion with dose at doses of 3 to 60 mg/kg. Bioavailability of TFV following oral TDF based on IV administration of TFV in dogs ranged from 16.4% to 27.5% for the 30- and 60-mg/kg groups.

2.6.4.3.3.12. Tenofovir DF: Monkey, Oral/Intravenous

Tenofovir pharmacokinetics were evaluated in rhesus monkeys (*macaca mulatta*) following oral administration of TDF at doses of 5.0, 50, and 250 mg/kg and IV administration at 5 and 30 mg/kg (3/sex/group) (Tabulated Summary 2.6.5.3.Q**,** P2000031). All doses were administered to animals in the fed state. In addition, the 250-mg/kg dose cohort was evaluated in the fasted state to assess the effect of food. Oral administration of TDF resulted in the rapid appearance of TFV in plasma with mean T_{max} values of 0.83 ± 0.41 hour (5 mg/kg), 1.0 ± 0.55 hour (50 mg/kg), and 1.1 ± 0.56 hour (250 mg/kg) indicating rapid absorption and cleavage of the prodrug to TFV. Mean C_{max} values of 0.113 ± 0.042, 1.15 ± 0.676, and 1.68 ± 1.05 µg/mL were achieved in the 3 dose groups, respectively. Mean values for $AUC_{(0-\infty)}$ were 0.725 ± 0.125, 6.38 ± 1.74, and 14.8 ± 7.81 µg·h/mL. A comparison of C_{max} and AUC values suggested nonlinear pharmacokinetics across these doses. There was a trend towards decreased extent of absorption with increasing dose, that reached statistical significance between the 5- and 250-mg/kg dose groups ($p = 0.0053$). No differences in pharmacokinetic parameters between gender were observed, nor was there a significant food effect observed in monkeys administered a 250-mg/kg oral dose of TDF.

Overall, the results of this study indicated that TDF was well absorbed in this species. Mean oral bioavailability for the 5-, 50-, and 250-mg/kg TDF dose groups was 32.4 ± 7.90%, 23.7 ± 7.82%, and 17.0 ± 5.66% of the administered TFV dose, respectively.

2.6.4.3.4. Repeat Dose Studies for Emtricitabine, Rilpivirine, and Tenofovir DF

2.6.4.3.4.1. Emtricitabine: Mouse, Oral

Toxicokinetic data have been generated from number of short- to long-term studies done in mice following 14-day to 6-month dosing of FTC doses ranging from 0 to 3000 mg/kg/day. The systemic exposure of FTC over steady state increased proportionally with dose administered (Tabulated Summary 2.6.5.1, IUW00701, TOX 599, TOX 022, TOX 628).

A 2-year oral oncogenicity study on FTC was performed in CD-1 mice (n = 60/sex/group main study animals and 40/sex/group satellite toxicokinetic animals) in accordance with GLP guidelines (Tabulated Summary 2.6.5.4.A**,** TOX-109). Emtricitabine was administered once daily by oral gavage at doses of 0, 80, 250, and 750 mg/kg/day. On Weeks 2 and 26, blood samples were collected from 3 mice/sex/group for pharmacokinetic evaluation. On Week 104, terminal plasma samples were also taken 1-hour postdose from 10 male and 10 female mice in the control and treatment groups of the main study. Emtricitabine was rapidly absorbed following all doses with peak plasma concentrations occurring 0.5 to 1.0-hour postdose. C_{max} and $AUC_{0–24}$ results are summarized in (Tabulated Summary 2.6.5.4.A**,** TOX-109). $AUC_{0–24}$ and C_{max} increased proportionally with dose over the range of 80 to 750 mg/kg/day. In general, the exposures ($AUC_{0–24}$) in male mice were similar to those in female mice at all doses. $AUC_{0–24}$ and C_{max} values were higher on Week 26 compared to Week 2. At Week 104, FTC concentrations were below the LOQ in most control animals. The average exposure in mice in this oncogenicity study ranged from 2.6- (at 80 mg/kg/day) to 26.3-fold greater (at 750 mg/kg/day) than in humans receiving 200 mg/day.

2.6.4.3.4.2. Emtricitabine: Rat, Oral

Toxicokinetic data from a subchronic study in rats with FTC doses showed linear relationship between systemic exposure and daily dose of FTC from 120 to 3000 mg/kg (Tabulated Summary 2.6.5.1, TOX 097).

A 2-year oral oncogenicity study on FTC was performed in CD rats (n = 60/sex/group main study animals and 20/sex/group satellite toxicokinetic animals) in accordance with GLP guidelines (Tabulated Summary 2.6.5.4.B, TOX-108). Emtricitabine was administered once daily by oral gavage at doses of 0, 60, 200, and 600 mg/kg/day. On Weeks 2 and 26, 6 blood samples were collected from 3 rats/sex/group. On Week 104, terminal plasma samples were also taken 1 hour postdose from 10 male and 10 female rats in the control and all treatment groups of the main study. Emtricitabine was rapidly absorbed following all doses with peak plasma concentrations occurring at 0.5 hours postdose. C_{max} and $AUC_{0–24}$ results are summarized in Tabulated Summary 2.6.5.4.B (TOX-108), $AUC_{0\text{-}24}$ and C_{max} increased with dose over the range of 60 to 600 mg/kg/day. In general, exposure ($AUC_{0–24}$) in male rats was

similar to those in female rats. $AUC_{0–24}$ and C_{max} values were higher on Week 26 compared to Week 2. The average exposure, based on Week 2 and Week 26 $AUC_{0–24}$ values, in rats in this oncogenicity study ranged from 3.9- (at 60 mg/kg/day) to 31.3-fold greater (at 600 mg/kg/day) than in humans receiving 200 mg/day (Tabulated Summary 2.6.5.4.B, TOX-108).

2.6.4.3.4.3. Emtricitabine: Monkey, Oral

A 1-month toxicology study of FTC was conducted in cynomolgus monkeys at oral doses of 0, 80, 400, and 2000 mg/kg/day, given in 2 divided doses, 6 hours apart (Tabulated Summary 2.6.5.1, TOX 600). Plasma concentrations of FTC were measured in samples drawn predose and over the first 6 hours after the first dose on Days 3 and 27. Cerebrospinal fluid (CSF) and corresponding plasma samples were obtained for analysis 1 hour postdose on Day 28. There were no significant differences in drug levels in plasma and CSF between males and females. No significant differences in pharmacokinetic parameters were determined between Day 3 and Day 28. Mean C_{max} values increased with dose and the AUCs (0 to 6 hours) were proportional to the dose. The overall mean (combined male, female and dose day) C_{max} values were 13.9, 62.8, and 198 µg·h/mL for monkeys given 40, 200, and 1000 mg/kg/dose, respectively. The $AUC_{0–6hr}$ values were proportional to the dose. The overall $AUC_{0–6hr}$ were 13.9, 62.8, and 198 µg·h/mL for monkeys given 40, 200, and 1000 mg/kg/dose, respectively. The concentration of FTC in CSF 1 hour after dosing on Day 28 averaged 3.9 ± 0.7 percent of the corresponding plasma levels (Tabulated Summary 2.6.7.7.U, TOX 600).

A 3-month oral toxicity study was performed with FTC in cynomolgus monkeys (Tabulated Summary 2.6.5.1, TOX 627). The doses tested were 0, 40, 200, and 1000 mg/kg/day (n = 5/sex/group), given as 2 divided doses by nasogastric intubation, with approximately 6 hours between doses. On Days 3 and 87, plasma samples were taken prior to the first daily dose and at 1, 2, 3, 4, and 6 hours postdose for pharmacokinetic evaluation. Emtricitabine was rapidly and well absorbed following oral gavage administration, with peak plasma concentrations occurring between 0 and 2 hours. No significant differences were seen between results from males and females at any dose level. Also, there were no significant changes in pharmacokinetic parameters between dose Days 3 and 87. Maximum plasma levels of FTC increased with dose, but increased linearly only between the 20- and 100-mg/kg doses. The overall mean (combined male, female and dose day) C_{max} values were 5.63, 25.2, and 101 µg·h/mL for monkeys given 20, 100, and 500 mg/kg/dose, respectively. The AUC_{0-6hr} values were proportional to the dose. The overall AUCs were 13.3, 60.8, and 310 µg·h/mL for monkeys given 20, 100, and 500 mg/kg/dose, respectively. Although measurable amounts of FTC were found in predose plasma on both Days 3 and 87, the similar AUCs on both days suggest that there was no significant accumulation of FTC over the dosing period (Tabulated Summary 2.6.7.7.V, TOX 627).

A 1-year oral toxicity study was performed with FTC in cynomolgus monkeys (Tabulated Summary 2.6.5.1, TOX 032). The doses tested were 0, 50, 200, and 500 mg/kg/day (n = 8, 4, 4, 8/sex, respectively), given as 2 divided doses by nasogastric

intubation, with approximately 5 hours between doses. On the first day of dosing and during Weeks 13, 26, and 52 of the study, blood samples for toxicokinetic evaluations were collected from the first 4 animals/sex/dose group at predose and 0.5, 1, 2, 4, and 6 hours after the first dose, with the second daily dose withheld until after the final blood collection (at 6 hours). Emtricitabine was rapidly and well absorbed following oral gavage, with peak plasma concentrations occurring between 0.5 to 2 hours after dosing. Plasma FTC was eliminated with a terminal $t_{1/2}$ of 2 to 4 hours at all dose levels. The $t_{1/2}$ estimates did not change after multiple-dose administration, although the $t_{1/2}$ may have been underestimated due to the short sampling time period. There were no major differences in plasma FTC exposure between male and female monkeys. Slightly higher plasma exposures to FTC were achieved in Weeks 13, 26, and 52 as compared to Day 0 for each dose level, which is consistent with the expected $t_{1/2}$ estimates or could be due to day-to-day variability. Longer $t_{1/2}$ estimates were expected based on the higher FTC levels and the measurable predose drug levels after repeated dosing. In addition, plasma C_{max} $AUC_{(0–6\ hr)}$ and estimated steady-state $AUC_{(0–24\ hr)}$ (Weeks 13, 26, and 52) increased linearly with the dose administered over the range of 50 to 500 mg/kg/day in both male and female monkeys. Systemic exposure increased linearly with dose over the dose range of 50 to 500 mg/kg/day in both male and female monkeys (Tabulated Summary 2.6.7.7.W, TOX032).

2.6.4.3.4.4. Rilpivirine (RPV/TMC278): Mouse, Oral

TMC278 formulated in aqueous HPMC (0.5%) was administered to male and female CD-1 mice by oral gavage in a 3-month study (20, 80, and 320 mg/kg/day) and a carcinogenicity study (20, 60, and 160 mg/kg/day) (Tabulated Summaries 2.6.5.4.E and 2.6.5.4.F, TMC278-NC119 and TMC278-NC120, respectively). TMC278 was administered to male and female CD-1 mice by dietary admixture at 40 and 400 mg/kg/day and by oral gavage at 400 mg/kg/day formulated in aqueous HPMC (0.5%) in a 14-day study (Tabulated Summary 2.6.5.4.C, TMC278-NC118). In addition, TMC278 formulated in aqueous HPMC (0.5%) was administered to male and female CB6F1-nonTgrasH2 transgenic mice in a 1-month study (20, 80, and 320 mg/kg/day) (Tabulated Summary 2.6.5.4.D, TMC278-NC121).

In all these studies, after oral gavage administration of TMC278, peak plasma levels of TMC278 were generally reached between 0.5 and 2 hours after dosing, indicating rapid absorption. At 20 mg/kg/day TMC278, plasma concentrations declined rapidly after peak time. At higher dose levels (up to 320 mg/kg/day), the plasma profiles showed a plateau after an initial rapid absorption resulting in constant or even increasing plasma levels until at least 8 hours postdose (Figure 2). Plasma peak concentrations increased less than dose-proportionally. $AUC_{0–\infty}$ values increased dose-proportionally or slightly less than dose-proportionally in males and females at Day 1. After repeat-dose administration, $AUC_{0–24h}$ values increased less than dose-proportionally in males and dose-proportionally to more than dose-proportionally in females.

At low dose levels (20 mg/kg/day in females and up to 160 mg/kg/day in males), exposure after repeated administration was comparable to that at Day 1. 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 males, at 320 mg/kg/day, exposure after repeated administration was lower (up to 34% at Day 87) compared to Day 1 (Table 1).

Overall, exposure in females was similar to the exposure in males, except at 160 and 320 mg/kg/day after repeated administration, where exposure in females was higher than in males (up to 2-fold).

Exposure ($AUC_{0\text{-}24h}$) to TMC278 after dietary administration at 400 mg/kg/day was similar in females and somewhat higher (1.6-fold) in males when compared to oral gavage treatment.

After repeated administration of TMC278, exposure to TMC278 in CB6F1-nonTgrasH2 transgenic mice was similar to that observed in CD-1 mice for both C_{max} and AUC.

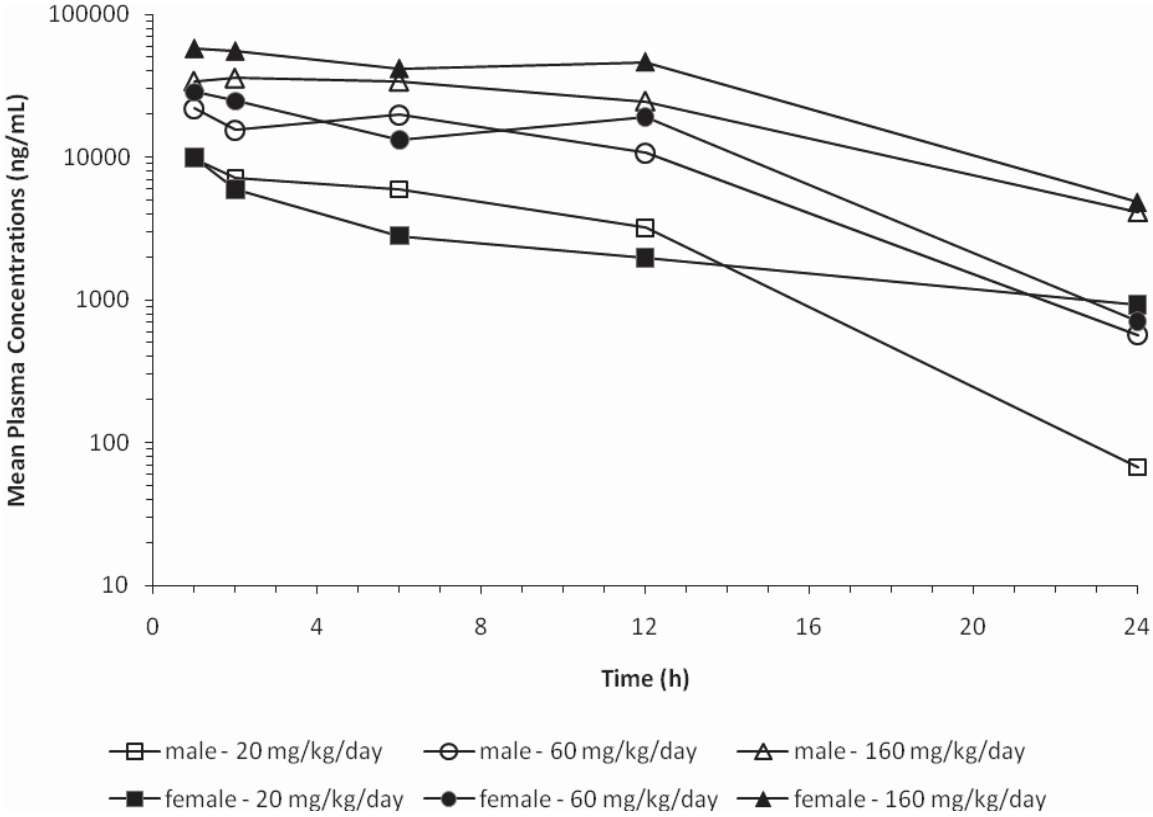
Table 1. Pharmacokinetic Parameters of TMC278 After Single or Repeated Administration by Oral Gavage of TMC278 in Mice

Study	Dose Level (mg/kg/day)	Sampling Time (Day)	C_{max} (µg/mL)		AUC[a] (µg·h/mL)	
			Male	Female	Male	Female
3-month study in CD-1 mice (TMC278-NC119)	20	1	14	13	71	59
		31	14	18	61[b]	74
		87	18	19	80	61
	80	1	28	32	236	250
		31	38	37	263	313
		87	34	42	210	313
	320	1	63	55	1010	707
		31	63	84	860	1170
		87	61	90	665	1360
24-month study in CD-1 mice (TMC278-NC120)	20	1	9.9	13	60	61
		Week 28	9.8	9.9	76	51
	60	1	23	24	239	182
		Week 28	22	29	230	278
	160	1	41	38	440	345
		Week 28	36	58	505	766

a $AUC_{0-\infty}$ after single dose and AUC_{0-24h} after repeated dose

b AUC_{0-8h}

Figure 2. Plasma Concentrations of TMC278 in CD-1 Mice After Repeated Administration of TMC278 by Oral Gavage Once Daily for 28 Weeks in the Carcinogenicity Study



2.6.4.3.4.5. Rilpivirine (RPV/TMC278): Rat, Oral

In a pilot study, TMC278 base formulated in PEG400 was administered by oral gavage to male rats in a 5-day toxicity study (40 and 400 mg/kg/day) (Tabulated Summary 2.6.5.4.G, TMC278-FK4103). TMC278 base formulated in PEG400/CA (10%) was administered by oral gavage to male and female rats in a 2-week toxicity study (40, 120, and 400 mg/kg/day), a 1-month toxicity study (10, 40, and 160 mg/kg/day), and a 6-month toxicity study (40, 120, and 400 mg/kg/day) (Tabulated Summaries 2.6.5.4.H, 2.6.5.4.K, and 2.6.5.4.M; TMC278-FK4243, TMC278-TOX5692, and TMC278-NC101, respectively). TMC278 formulated in aqueous HPMC (0.5%) was administered by oral gavage to male and female rats in a 2-week toxicity study (400, 1500, and 2000 mg/kg/day) (Tabulated Summary 2.6.5.4.J, TMC278-NC177) and a carcinogenicity study (40, 200, 500, and 1500 mg/kg/day) (Tabulated Summary 2.6.5.4.N, TMC278-NC123). In a 1-month bridging study, TMC278 base formulated in PEG400/CA (10%) and TMC278 formulated in aqueous HPMC (0.5%) were administered by oral gavage to male and female rats (10 and 400 mg/kg/day) (Tabulated Summary 2.6.5.4.L, TMC278-NC117). TMC278 (400 and 1200 mg/kg/day) and TMC278 base (400 mg/kg/day) were also administered by dietary admixture to male and female rats in a 2-week toxicity study. In the same study, TMC278

formulated in aqueous HPMC (0.5%) was administered by oral gavage at 400 mg/kg/day (Tabulated Summary 2.6.5.4.I, TMC278-NC136).

In addition, TMC278 base formulated in PEG400/CA (10%) was administered by oral gavage to pregnant rats in an embryo-fetal toxicity study from gestation day (GD) 6 to GD 16 (40, 120, and 400 mg/kg/day) (Tabulated Summary 2.6.5.7.C, TMC278-NC105). TMC278 formulated in aqueous HPMC (0.5%) was also administered to juvenile rats of 12-days-old by oral gavage for 14 days (Tabulated Summary 2.6.5.4.O, TMC278-NC168). Sprague Dawley rats were used in all the above-mentioned toxicity studies.

After oral administration of TMC278 base for 5 days using PEG400 (40 and 400 mg/kg/day), $AUC_{0-\infty}$ values at Day 1 were similar at 40 mg/kg and lower at 400 mg/kg (up to 70%) compared to PEG400/CA.

After oral gavage administration of TMC278 base formulated in PEG400/CA, C_{max} was reached within 4 hours after administration at low doses (up to 40 mg/kg/day), whereas at higher dose levels (up to 400 mg/kg/day) plasma profiles showed a plateau between 2 and 10 hours. Exposure, expressed as C_{max} and AUC, generally increased less than dose-proportionally at Day 1 and after repeated administration in both males and females (Figure 3). In females, C_{max} and AUC_{0-24h} values increased slightly (up to 1.6-fold) with repeated administration, while in males, particularly at high doses, exposure decreased slightly (up to 40%) (Table 2). In all studies, exposure in females appeared to be higher (2- to 4-fold) than in males at Day 1 and after repeated administration.

After oral gavage administration of TMC278 formulated in aqueous HPMC, C_{max} was reached between 2 and 8 hours. Exposure to TMC278, expressed as C_{max} and AUC, increased less than dose-proportionally at Day 1 and after repeated administration in both males and females up to 1500 mg/kg/day. Between 1500 and 2000 mg/kg/day no further increase in exposure was seen in males, whereas an increase (1.3-fold) was seen in females. In males, C_{max} and AUC_{0-24h} values decreased after repeated administration particularly in comparison to Day 1 in the carcinogenicity study where the decrease was between 67% and 76% at all doses. However, no further decrease in exposure (C_{max} and AUC) was seen between Week 27 and Week 39. In females, no clear tendency was observed across studies. For example, in the carcinogenicity study, exposure at Week 39 was similar to Day 1 at 200 and 1500 mg/kg/day and was slightly lower (up to 27%) at 40 and 500 mg/kg/day. C_{max} and AUC tended to be similar or higher (2-fold) in females than in males at Day 1, whereas after repeated dose, values in females were 1.3- to 5-fold higher.

In a 1-month bridging study comparing oral administration of TMC278 in aqueous HPMC with TMC278 base in PEG400/CA, AUC values after the base were comparable to those after the salt at the low dose (10 mg/kg/day). At 400 mg/kg/day, AUC_{0-24h} values at Day 24 after the base were 1.7- to 2.7-fold higher than those after the salt.

After repeated dietary administration, exposure to TMC278 at 400 mg/kg/day was slightly (1.3- to 1.6-fold) higher for TMC278 and similar for TMC278 base in both males and females compared to oral gavage treatment.

In pregnant rats, following daily oral administration of TMC278 base (PEG400/CA) at 40, 120, and 400 mg/kg/day from GD 6 to GD 16, exposures were similar to the exposures in nonpregnant females (see Table 2). Exposure, expressed as C_{max} and AUC, increased less than dose-proportionally and was approximately the same from GD 6 to GD 16.

In a dose range-finding pre- and postnatal development study in rats, TMC278 in aqueous HPMC was administered at 40, 120, and 400 mg/kg/day to dams from Day 6 of gestation to Day 7 of lactation. The same dose levels were also administered directly to the pups from Day 12 until Day 25 of age. On Day 7 of lactation, exposure ($AUC_{0–24h}$) in pups via milk 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/day in males and females, respectively. In pups, exposure to TMC278 via milk increased with the dose, but not dose-proportionally.
After repeated direct administration to pups for 14 days, exposure increased less than dose-proportionally. There was no difference in exposure between male and female pups in contrast to the adult rats (see Table 2).

Table 2. Pharmacokinetic Parameters of TMC278 After Single or Repeated Administration by Oral Gavage of TMC278 Base or TMC278 in Sprague Dawley Rats

Study	Formulation	Dose Level (mg/kg/day)	Sampling Time (Day)	C_{max} (µg/mL)		AUC[a] (µg·h/mL)	
				Male	Female	Male	Female
6-month study in rats (TMC278-NC101)	TMC278 base in PEG400/CA (10%)	40[b]	1	2.9	6.5	19	32
			175	1.7	6.6	12	50
		120[b]	1	6.4	8.5	53	83
			175	3.0	8.8	35	116
		400[b]	1	9.1	17	92	160
			175	6.2	16	73	244
Carcinogenicity study in rats (TMC278-NC123)	TMC278 in HPMC (0.5%)	40	1	1.6	2.2	19	17
			Week 39	0.82	2.1	6.3	14
		200	1	2.6	4.2	34	40[c]
			Week 39	1.3	4.7	8.2	41
		500	1	4.5	6.5	45	63
			Week 39	1.8	8.5	14	46
		1500	1	6.1	7.0	58	81
			Week 39	2.2	9.4	18	84
Embryo-fetal study in rats (TMC278-NC105)	TMC278 base in PEG400/CA (10%)	40	1 (GD6)	—	4.9	—	33
			11 (GD16)	—	5.6	—	37
		120	1 (GD6)	—	6.0	—	65
			11 (GD16)	—	7.2	—	63
		400	1 (GD6)	—	14	—	182
			11 (GD16)	—	13	—	152
Pre- and postnatal study in rats: administration to pups (TMC278-NC168)	TMC278 in HPMC (0.5%)	40[d]	Day 25 of age	2.6	5.8	12	18
		120[d]	Day 25 of age	3.7	3.6	34	28
		400[d]	Day 25 of age	9.1	7.3	50	53

a $AUC_{0-\infty}$ after single dose and $AUC_{0\text{-}24h}$ after repeated dose

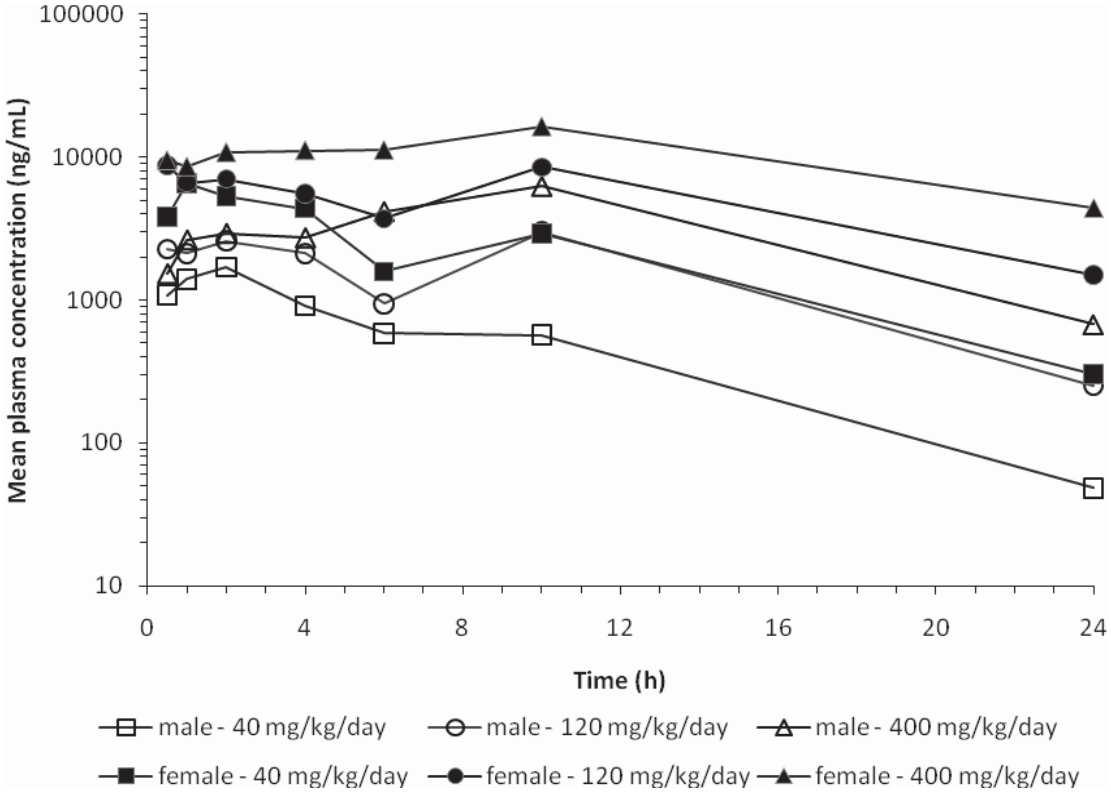
b total dosing volume of 10 mL/kg was changed after Day 83 and split in 2 administrations of 5 mL/kg each, with 1.5 hours between the 2 administrations

c AUC_{0-24h}

d Dose administered to pups from Day 12 to Day 25 of age.

CA: citric acid; GD: gestation day; PEG400: polyethylene glycol 400; HPMC: hydroxypropyl methyl cellulose.

Figure 3. Plasma Concentrations of TMC278 in Sprague Dawley Rats After Repeated Administration of TMC278 Base by Gavage Once Daily for 6 Months (Day 175)



2.6.4.3.4.6. Rilpivirine (RPV/TMC278): Rabbit, Oral

TMC278 base formulated in aqueous HPMC (0.5%) was administered by oral gavage to nonpregnant New Zealand white rabbits in a 5-day toxicity study (100, 300, and 1000 mg/kg/day) and to pregnant rabbits in a pilot (25, 75, and 150 mg/kg/day) and a main (5, 10, and 20 mg/kg/day) embryo-fetal developmental toxicity study from GD 6 to GD 19 (Tabulated Summaries 2.6.5.4.P, 2.6.5.7.D, and 2.6.5.7.E; TMC278-NC126, TMC278-NC128, and TMC278-NC130, respectively).

After oral gavage administration for 5 days of TMC278 base in nonpregnant rabbits and for 2 weeks in pregnant rabbits, C_{max} were reached between 7 and 19 hours after dosing. In the main embryo-fetal developmental study, mean C_{max} and $AUC_{0–24h}$ values at GD 19 were 15 µg/mL and 232 µg·h/mL at 20 mg/kg/day, respectively. C_{max} and AUC_{0-24h} values increased less than dose-proportionally in pregnant and nonpregnant rabbits from 5 to 1000 mg/kg/day. The exposure values observed following 2 weeks of repeated dosing were similar to those observed at Day 1.

2.6.4.3.4.7. Rilpivirine (RPV/TMC278): Dog, Oral

In a pilot study, TMC278 base formulated in PEG400/CA (10%) was administered by oral gavage to a male and a female beagle dog in a 5-day toxicity study (80 mg/kg/day) (Tabulated Summary 2.6.5.4.Q, TMC278-FK4102). TMC278 base formulated in PEG400/CA (10%) was administered by oral gavage to male beagle dogs in a 7-day toxicity study (20, 40, and 80 mg/kg/day) and at 5, 10, and 40 mg/kg/day to male and female beagle dogs in a 1-month toxicity study, a 6-month toxicity study, and a 12-month toxicity study (Tabulated Summaries 2.6.5.4.R, 2.6.5.4.S, 2.6.5.4.U and 2.6.5.4.V; TMC278-FK4244, TMC278-TOX5650, TMC278-NC115, TMC278-NC107, respectively). In addition, TMC278 base formulated in PEG400/CA (10%) and TMC278 formulated in aqueous HPMC (0.5%) were administered by oral gavage to male and female beagle dogs (5 and 40 mg/kg/day) in a 1-month bridging study (Tabulated Summary 2.6.5.4.T, TMC278-NC116).

In all studies with TMC278 base formulated in PEG400/CA, the rate of absorption was highly variable based on mean time to reach C_{max} (t_{max}) values ranging between 1.3 and 24 hours. The plasma concentration-time profile showed a plateau (see Figure 4). In general, exposure expressed as mean C_{max} and $AUC_{0\text{-}24h}$ increased less than dose-proportionally in both males and females (Table 3). No further increase in exposure was seen between 40 and 80 mg/kg/day. Exposure (AUC) increased after repeated administration as compared to Day 1 mainly due to the long terminal $t_{1/2}$ of TMC278. No clear difference in pharmacokinetics was seen between males and females.

In general, a high inter-study variability was seen particularly at 40 mg/kg/day after repeated administration.

In the 1-month bridging study no clear difference was noted between exposure (AUC) obtained after administration of TMC278 base and TMC278. However, inter-individual variability was high especially after administration of TMC278.

Table 3. Pharmacokinetic Parameters of TMC278 After Single or Repeated Administration by Oral Gavage of TMC278 Base in Beagle Dogs

Study	Formulation	Dose Level (mg/kg/day)	Sampling Time (Day)	C_{max} (µg/mL) (CV in %)		AUC_{0-24h} (µg·h/mL) (CV in %)	
				Male	Female	Male	Female
12-month study in dogs (TMC278-NC107)	TMC278 base in PEG400/CA (10%)	5	1	0.70 (12.9)	0.75 (7.1)	11 (14.5)	9.7 (16.5)
			363	1.1 (12.8)	1.5 (42.4)	17 (13.5)	19 (32.2)
		10	1	0.90 (23.1)	1.2 (37)	15 (24.8)	15[b] (31.1)
			363	1.3[a]	2.2 (13.7)	24[a]	36[c] (22.1)
		40	1	2.4 (39.7)	2.5 (31.3)	37 (36.3)	41 (27.6)
			363	4.1 (55.6)	5.5[d] (57.8)	65 (63.1)	61[d] (52.1)

a n=2

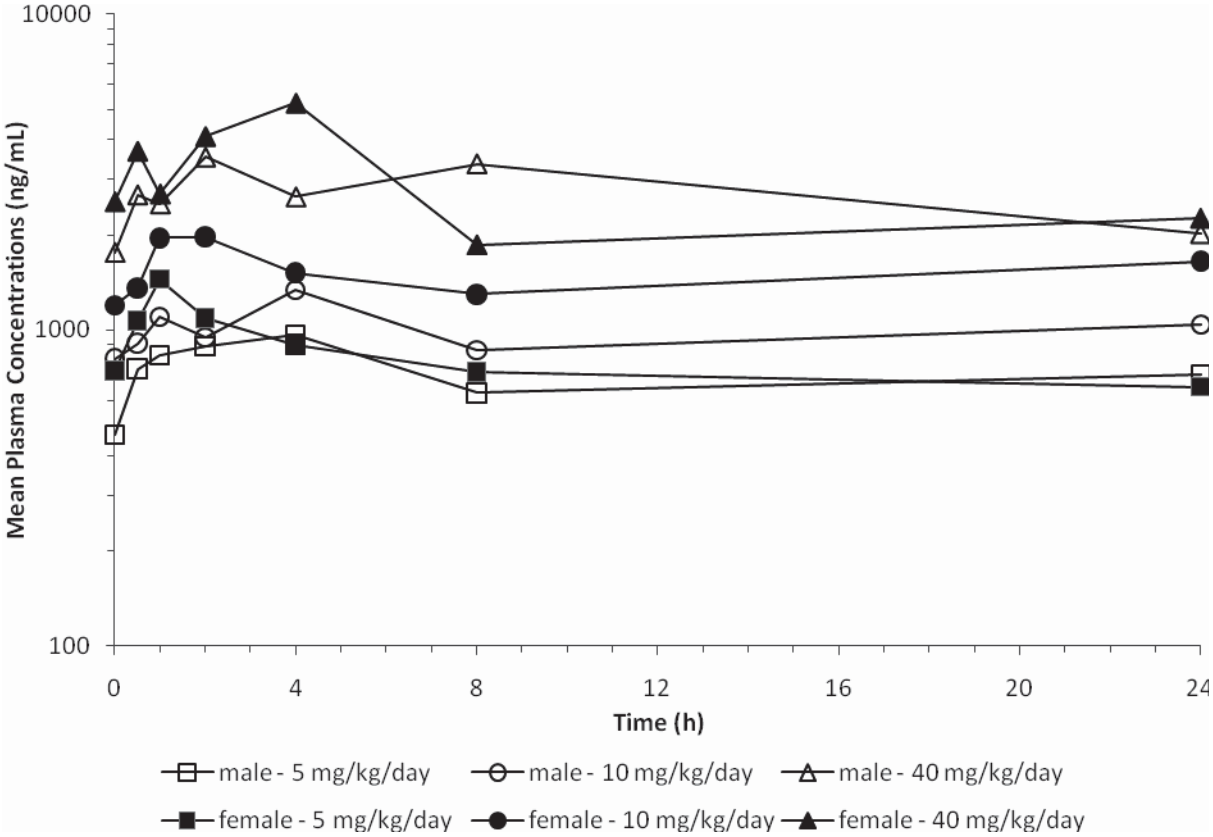
b n=5

c 1 animal was sampled at Day 335

d n=3

CV: coefficients of variation

Figure 4. Plasma Concentrations of TMC278 in Beagle Dogs After Repeated Administration of TMC278 Base by Gavage Once Daily for 12 Months (Day 363)



2.6.4.3.4.8. Rilpivirine: Monkey, Oral

TMC278 formulated in aqueous HPMC (0.5%) was administered by oral gavage to female cynomolgus monkeys at 10 and 100 mg/kg/day for 7 days (Tabulated Summary 2.6.5.4.W, TMC278-NC249). TMC278 formulated in aqueous HPMC (1%)/Tween 20 (0.5%) was administered by oral gavage to female cynomolgus monkeys at 100 mg/kg BID and at 250 mg/kg BID (dosing interval of 8 hours) for 2 weeks (Tabulated Summary 2.6.5.4.X, TMC278-NC326). TMC278 formulated in aqueous HPMC (1%)/Tween 20 (0.5%) was also administered to immature female monkeys at 100 mg/kg BID and at 250 mg/kg BID (dosing interval of 8 hours) for 55 days (Tabulated Summary 2.6.5.4.Y, TMC278-NC248).

In all studies inter-individual variability was high with a CV for C_{max} and AUC higher than 50%. The absorption rate was moderate based on t_{max} values ranging between 2.5 and 8 hours after the first dose. After BID dosing, C_{max} after the first dose (C_{max1}) and the second dose (C_{max2}) were comparable. After repeated dose administration, plasma concentration-time profiles became rather flat. A dose-proportional increase in C_{max} and AUC was observed after single and repeated dosing for 2 weeks. After repeated administration for 2 weeks,

exposure expressed as C_{max} and AUC seemed to increase by 2-fold compared to Day 1. In the 55-day study in immature monkeys, C_{max} and AUC increased less than dose-proportionally after repeated dose administration (Table 4).

Table 4. Pharmacokinetic Parameters of TMC278 After Single or Repeated Administration by Oral Gavage of TMC278 in Female Cynomolgus Monkeys

Study	Formulation	Dose Level (mg/kg/day)	Sampling Time (Day)	C_{max1} (µg/mL) (CV in %)	C_{max2} (µg/mL) (CV in %)	AUC_{0-24h} (µg·h/mL) (CV in %)
2-week study in monkeys (TMC278-TiDP38-NC326)	TMC278 in HPMC (1%)/Tween 20 (0.5%)	100 BID	1	0.094 (72)	0.19 (62)	2.8 (64)
			14	0.29 (72)	0.21 (50)	4.4 (58)
		250 BID	1	0.25 (63)	0.38 (61)	5.9 (65)
			14	0.50 (42)	0.56 (52)	10 (42)
55-day study in immature monkeys (TMC278-TiDP38-NC248)	TMC278 in HPMC (1%)/Tween 20 (0.5%)	100 BID	55	0.14 (52)	0.18 (56)	2.7 (52)
		250 BID	55	0.31 (82)	0.31 (71)	4.6 (57)

a C_{max1} and C_{max2}: C_{max} after the first and second dose administration, respectively; dosing interval of 8 hours

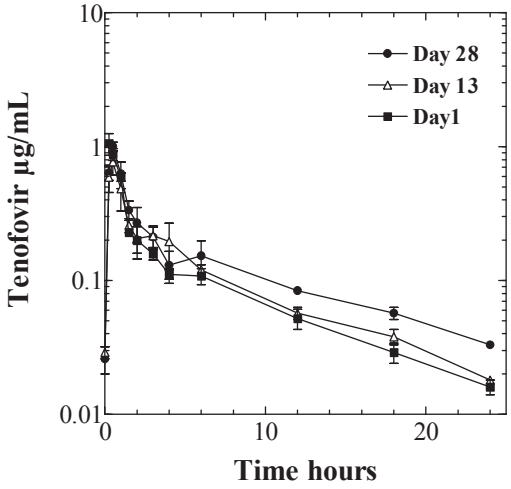
2.6.4.3.4.9. Tenofovir DF: Mouse, Oral

Concentrations of TFV were determined in plasma samples following daily oral gavage of TDF in albino mice for 13 weeks at doses of 100, 300, and 1000 mg/kg (Tabulated Summary 2.6.5.4.Z, M990203-PK). The dose level of the 1000-mg/kg/day dose was reduced to 600 mg/kg/day on Day 9 and for the remainder of the study due to problems attributed to the administration of the test suspension. Overall, TFV plasma C_{max} and $AUC_{(0-t)}$ appeared to increase in a dose proportional manner. C_{max} values on Day 91 were similar to values obtained on Day 1 indicating similar pharmacokinetics over time with no accumulation. In the mouse carcinogenicity study the AUC_{ss} (Day 180) was 46.7 µg·h/mL at 600 mg/kg/day, similar to the AUC_{inf} on Day 1 (Tabulated Summary 2.6.5.4.AA**,** M990205). Oral bioavailability of TFV from TDF has not been determined in this species (no IV TFV mouse data).

2.6.4.3.4.10. Tenofovir DF: Rat, Oral

Tenofovir pharmacokinetics in the Sprague Dawley rat were evaluated following repeat oral administration of TDF at doses ranging from 20 to 1000 mg/kg over 2 to 42 weeks (Tabulated Summaries 2.6.5.4.BB, 2.6.5.4.DD, 2.6.5.4.CC, and 2.6.5.4.EE; 96-TOX-4331-003-PK, 97-TOX-4331-002-PK, R20000036-PK, and R990204). In general, repeat-dose pharmacokinetics of TFV were similar to those after single doses (Figure 5). Maximum plasma TFV concentrations (C_{max}) were reached within 0.25 to 1 hour following TDF administration. The C_{max} of TFV in rats was nonlinear with dose, with C_{max} increasing in a less than dose proportional manner. $AUC_{(0-t)}$ appeared to be linear with dose, suggesting slower absorption at higher doses of TDF in rats. No apparent gender-related differences were observed in the pharmacokinetics of TFV at any dose, within the constraints imposed by the limited and highly variable data set.

Figure 5. **Mean Plasma Tenofovir Concentrations in Sprague-Dawley Rats Following Daily Oral Administration of Tenofovir DF 40 mg/kg (mean ± SD; n = 3 per time point, 18 animals per group)**
(R2000036-PK)

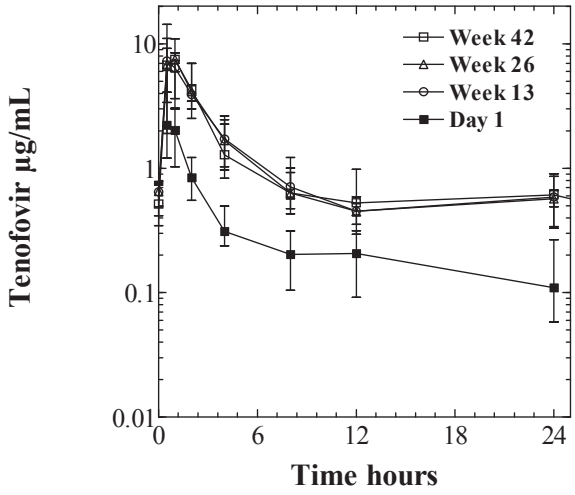


2.6.4.3.4.11. Tenofovir DF: Dog, Oral

Tenofovir plasma pharmacokinetics were evaluated in beagle dogs following daily oral administration of TDF at doses of 3 to 30 mg/kg over time periods of 4 to 42 weeks (Tabulated Summary 2.6.5.4.GG, 2.6.5.4.II, and 2.6.5.4.HH; 96-TOX-4331-004-PK, 97-TOX-4331-001-PK, and 98-TOX-4331-003-PK). Plasma concentrations versus time for the 30-mg/kg dose cohort on Day 1 and Weeks 13, 26, and 42 are shown in Figure 6. As with single-dose studies, the time to C_{max} was between 0.25 and 1.25 hours following administration. C_{max} values at the 3-mg/kg dose level were similar across time. For the

10- and 30-mg/kg dose cohorts, a 2- to 3-fold increase in both C_{max} and AUC occurred over time (generally reached by Day 28). Based on the single-dose dog data (97-DDM-4331-001), this increase in exposure is due to the long terminal $t_{1/2}$ (approximately 60 hours) in this species, rather than changes in absorption or clearance. AUC and C_{max} values remained constant from 28 days through 42 weeks of treatment. In general there were no apparent gender-related differences in absorption in this species upon multiple oral dosing of TDF.

Figure 6. **Median Plasma Tenofovir Concentrations in Dogs Following Daily Oral Administration of Tenofovir DF 30 mg/kg (median, min, max; n = 4 or 6 Males and Females/group)** (97-TOX-4331-001-PK)



2.6.4.3.4.12. Tenofovir DF /Tenofovir: Monkey, Oral/Subcutaneous

Plasma and peripheral blood mononuclear cell (PBMC) exposure to TFV following oral administration of TDF, or subcutaneous administration of TFV in monkeys was investigated (Tabulated Summary 2.6.5.4.JJ, P2000078-PK). Four groups of adult rhesus monkeys (3 per sex per group) were given daily doses of 30, 250, or 600 mg/kg/day oral TDF (Groups 2–4), or 30 mg/kg/day subcutaneous TFV (Group 5) for up to 56 days. All monkeys showed sustained exposure to TFV over the course of the study. Increasing oral doses of TDF led to increased, though not dose-proportional, C_{max} and AUC values.

The mean AUC observed at steady state for the 30 mg/kg oral TDF dose group was 4.05 ± 0.958 µg·h/mL, which was approximately 33% greater than the AUC observed in humans following 300 mg doses of TDF. Similar, although slightly lower, AUC values were observed in monkeys administered 600 mg/kg/day oral TDF (56.3 ± 22.1 µg·h/mL) as compared to 30 mg/kg/day subcutaneous TFV (62.3 µg·h/mL), when comparing values from animals without clinical signs of toxicity.

In general, PBMCs showed exposure to TFV for the 250- and 600-mg/kg/day oral TDF doses and the 30-mg/kg/day subcutaneous TFV dose. Variability in the data precluded any assessments of trends.

2.6.4.3.5. Emtricitabine/Tenofovir DF: Absorption

The absorption kinetics of FTC/TDF tablet have not been evaluated after single or multiple doses in any preclinical studies as there is no mechanistic reason to expect any interaction. In addition, clinical studies have documented that when these 2 products are administered together there is no change in absorption kinetics. Administration of FTC/TDF tablet resulted in plasma concentration-time profiles of FTC and TFV similar to those after concurrent administration of the 2 separate formulations.

2.6.4.3.6. Emtricitabine/Rilpivirine/Tenofovir DF: Absorption

Although formal studies of the absorption kinetics of FTC/RPV/TDF have not been conducted, a single dose comparison of the exposure of FTC/RPV/TDF after oral administration of a bilayer formulation and the individual clinical formulations in fasted dogs has demonstrated comparable systemic exposure to all agents (Tabulated Summary 2.6.5.3.S, AD-264-2023). Previously, similar results were obtained in single dose comparison of the exposure of FTC/RPV/TDF after oral administration of co-formulated formulations representative of those in the first human bioequivalence study and the individual clinical formulations in fasted dogs (Tabulated Summary 2.6.5.3.R, AD-264-2001).

2.6.4.4. Distribution

2.6.4.4.1. Plasma Protein Binding for Emtricitabine, Rilpivirine, and Tenofovir DF

2.6.4.4.1.1. Emtricitabine: Plasma Protein Binding

The binding of FTC to human, monkey, mouse, and rabbit plasma proteins was determined over the concentration range 0.020 to 200 µg/mL by equilibrium dialysis at 37ºC (Tabulated Summary 2.6.5.6.A, TBZZ/93/0025). The mean percentage bound for all species studied was ≤ 3.6%, with no indication of concentration dependency.

2.6.4.4.1.2. Rilpivirine: Plasma Protein Binding

The plasma protein binding of TMC278 was studied in vitro by equilibrium dialysis. Plasma samples from male and female CD-1 mice; male and female Sprague Dawley rats; female New Zealand white rabbits; male beagle dogs; and healthy male adult subjects were fortified with ^{3}H-TMC278 at concentrations ranging from 0.01 to 100 µg/mL (animals) and from 0.01 to 3.0 µg/mL (human) (Tabulated Summary 2.6.5.6.B, TMC278-NC112). The distribution of TMC278 to various compartments of blood and the binding of TMC278 to purified human serum albumin and α_1-acid glycoprotein were also studied (Tabulated Summary 2.6.5.6.B, TMC278-NC112). The distribution of [^{14}C]TMC278 in blood and the protein binding of [^{14}C]TMC278 in plasma were also investigated in samples from female guinea pigs at 2.5

and 8 µg/mL and from female monkeys at 2.5 and 5 µg/mL (Tabulated Summary 2.6.5.6.C, TMC278-NC332). In addition, the protein binding of unlabeled TMC278 was determined in plasma samples from male and female CD-1 mice; male and female Sprague Dawley rats; male beagle dogs; and healthy male subjects at a concentration of 0.03, 0.3 or 1 µg/mL (animals) and 0.01, 0.03, 0.1, 0.3, 1, or 3 µg/mL (human) (Report TMC278-FK4217).

TMC278 was extensively bound to plasma proteins in all species and the plasma protein binding was found to be concentration independent. Plasma protein-binding values ranged between 99.08% and 99.97%. TMC278 was extensively bound to human albumin (99.5% at a physiological concentration of 4.3% and irrespective of the TMC278 concentration) and to a much lesser extent to α_1-acid glycoprotein (48.8% at a physiological concentration of 0.07% and a TMC278 concentration of 1 µg/mL). The rank order of blood to plasma concentration ratio in all species was monkey > dog > rat > man > guinea pig > rabbit > mouse and ranged from 0.96 to 0.58. In all species, irrespective of the concentration, a very limited percentage of TMC278 distributed to the plasma water compartment and the values ranged from 0.06% to 0.5%. In guinea pig, male and female rats, male dog, monkey, and man, the percentage of TMC278 distributed to plasma proteins ranged from 61.8% to 94.1%, and the percentage of TMC278 distributed to blood cells ranged from 5.8% to 37.6%. In mouse and rabbit, TMC278 distributed almost completely to plasma proteins (see Table 5).

The protein binding results of unlabeled TMC278 were in line with the ones obtained with radiolabeled compound at the same range of concentrations. No data were obtained at 0.01 and 0.03 µg/mL due to the fact that concentrations were below the limit of quantification.

Table 5. Plasma Protein Binding and Blood Distribution of TMC278 at 1 µg/mL (Various Species) or 2.5 µg/mL (Guinea pig and Monkey)

	Mouse		Rat		Guinea Pig	Rabbit	Dog	Monkey	Human
	M	F	M	F	F	F	M	F	M
Plasma protein binding (%)	99.93	99.94	99.84	99.86	99.87	99.97	99.35	99.14	99.67
Free drug (%)	0.07	0.06	0.16	0.14	0.13	0.03	0.66	0.86	0.33
Blood to plasma ratio	0.60	0.58	0.67	0.67	0.64	0.61	0.68	0.96	0.66
Distribution to									
Plasma water (%)	0.07	0.07	0.15	0.14	0.11	0.03	0.50	0.53	0.20
Plasma proteins (%)	100	> 100	93.0	94.1	84.7	> 100	73.4	61.8	78.1
Blood cells (%)	< 0	< 0	6.8	5.8	15.2	< 0	26.2	37.6	21.7

2.6.4.4.1.3. Tenofovir DF: Plasma Protein Binding

The protein binding of TFV has been determined in human plasma and serum using centrifugal ultra-filtration (Tabulated Summary 2.6.5.6.D, P0504-00039.1). Five concentrations of TFV were prepared in duplicate in phosphate buffered saline (PBS), human

plasma, and human serum over the range of 0.01 to 25.01 μg/mL. The percent unbound for TFV was 99.3 ± 3.3% in human plasma, and 92.8 ± 3.6% in human serum. Tenofovir therefore showed very low protein binding in either human plasma (< 1.7%) or serum (< 7.2%).

2.6.4.4.2. Tissue Distribution Studies for Emtricitabine, Rilpivirine, and Tenofovir DF

2.6.4.4.2.1. Emtricitabine: Mouse, Oral/Intravenous

The pharmacokinetics and bioavailability of FTC after oral dosing were studied in mice dosed with 10, 100, and 600 mg of FTC per kg of body weight by the oral and IV routes. The clearance of FTC was rapid, via the kidney, and was independent of dose. Absolute bioavailability in mice after oral dosing was 96% at a dose of 10 mg/kg. Mice dosed orally with 120 mg of [6-^{3}H]FTC per kg excreted 67% ± 7% of the radiolabel in the 0 to 48 hour urine. Small amounts of the 3'-sulfoxide and glucuronide metabolites were observed in the urine, but 5-fluorouracil was not detected {4251}.

2.6.4.4.2.2. Emtricitabine: Rat, Oral

Twenty male Sprague Dawley nonpigmented rats (Group 1) and 6 male Long-Evans pigmented rats (Group 2) received a single 200-mg/kg oral dose containing approximately 135 μCi/kg of [^{14}C]FTC via gavage (Tabulated Summary 2.6.5.5.A, TOX092). The distribution of [^{14}C]FTC-derived radioactivity in the nonpigmented tissues of Long-Evans pigmented rats was similar to that of Sprague Dawley rats. For both groups, the absorption of [^{14}C]FTC following oral administration was rapid, and the radioactivity was widely distributed among all of the examined tissues. The clearance of [^{14}C]FTC drug-derived radioactivity from the plasma and tissues was also rapid with a $t_{1/2}$ of approximately 1 to 6 hours for blood and tissues and ≤ 3.42 hours for plasma.

The pharmacokinetic parameters for [^{14}C]FTC derived radioactivity in eyes and skin were not markedly different for nonpigmented and pigmented rats, indicating that [^{14}C]FTC-associated radioactivity does not bind appreciably to melanin.

The mean recovery of the administered radioactive dose over 6 days was 98.6%. Urine and feces accounted for 79.3% and 18.4% of the administered radioactivity, respectively. The excretion of radioactivity was rapid, with 95% of the administered radioactivity excreted in the urine and feces within 24 hours postdose.

In both urine and feces samples, the predominant radiochromatographic peak was associated with unchanged FTC. In urine, FTC represented a mean of 73.3% of the dose for the 0 to 72 hour samples. In feces, FTC represented a mean of 17.7% of the dose for the 0 to 48 hour samples. Metabolite peaks collectively accounted for < 5% of the dose in urine and < 0.3% of the dose in feces.

Based on data in albino rats, the whole body radiation dose in a 70-kg man following administration of a single 250-pCi dose of [^{14}C]FTC was estimated to be 1.47 mrem, which

is well below the annual cumulative whole-body radiation exposure limit of 5 rem for human isotope studies.

2.6.4.4.2.3. Emtricitabine: Rat, Oral/Intravenous

Male Sprague Dawley (CD) rats were administered FTC at 10 or 100 mg/kg orally or intravenously. Plasma clearance of 10 mg of FTC per kg of body weight was biexponential in rats, with a $t_{1/2}$ at alpha phase of 4.7 ± 1.1 min (mean ± standard deviation [SD]) and a $t_{1/2}$ at beta phase of 44 ± 8.8 min (n = 5). Emtricitabine in the brains of rats was initially less than 2% of the plasma concentration but increased to 6% by 2 hours postdose. Probenecid elevated levels of FTC in plasma as well as in brains but did not alter the brain-to-plasma ratio. The urinary and fecal recoveries of unchanged FTC after a 10 mg/kg IV dose were 87% ± 3% and 5% ± 1.6%, respectively. After a 10 mg/kg oral dose, respective urinary and fecal recoveries were 70% ± 2.5% and 25% ± 1.6%. Two sulfoxides of FTC were observed in the urine, accounting for 0.4% ± 0.03% and 2.7% ± 0.2% of the IV dose and 0.4% ± 0.06% and 2.5% ± 0.3% of the oral dose. Also observed were 5-fluorocytosine, representing 0.4% ± 0.06% of the IV dose and 0.4% ± 0.07% of the oral dose, and FTC glucuronide, representing 0.7% ± 0.2% of the oral dose and 0.4% ± 0.2% of the IV dose. Neither deaminated FTC nor 5-fluorouracil was observed in the urine (less than 0.2% of dose) {4570}.

2.6.4.4.2.4. Emtricitabine: Monkey, Oral

Cynomolgus monkeys were dosed with 10 and 80 mg/kg of FTC. The clearance of FTC was rapid, via the kidney, and was independent of dose. The total body clearance of 10 mg/kg FTC was 0.7 ± 0.1 L/h/kg, and the volume of distribution at steady state was 0.8 ± 0.02 L/kg. The terminal elimination $t_{1/2}$ was 1.0 ± 0.2 hours. The absolute bioavailability after oral dosing was 63% ± 4% at 10 mg/kg. Concentrations of FTC in the CSF were 4% ± 0.7% of the corresponding levels in plasma. Monkeys excreted 41% ± 6% of the radioactive dose in the 0 to 72 hour urine, 33% ± 10% in the feces, and 10% ± 7% in the cage wash. Unchanged FTC was 64% of the total radiolabeled drug recovered in the urine. The glucuronide was a minor urinary metabolite. 5-Fluorouracil was not detected (less than 0.02% of the dose) {4251}.

The tissue distribution of radioactivity after oral administration of [^{14}C]FTC in the cynomolgus monkey is described further in Section 2.6.4.5.2.1 (Tabulated Summary 2.6.5.8.B).

2.6.4.4.2.5. Rilpivirine: Rat, Oral

The tissue distribution of TMC278 and its metabolites was studied in male pigmented Long Evans rats and pregnant female Sprague Dawley rats by QWBA, following a single oral dose of [^{14}C]TMC278 base in PEG400/CA (10%) at 40 mg/kg (Tabulated Summaries 2.6.5.5.B and 2.6.5.5.C; TMC278-NC108 and TMC278-NC109, respectively). The tissue distribution of unchanged TMC278 was assessed in a limited set of tissues obtained in a pharmacokinetic

study in male Sprague Dawley rats given a single oral dose of 40 mg/kg (PEG400/CA (10%) (Tabulated Summary 2.6.5.3.H, TMC278-FK4195).

In pigmented rats, highest total radioactivity levels were observed at 4 hours postdose in nonpigmented and most pigmented tissues, indicating a rapid distribution of [^{14}C]TMC278 related radioactivity. Only in the pigmented parts of the eye and the uveal tract, the highest concentration of radioactivity was measured at 24 hours after dosing.

In most non-pigmented tissues, radioactivity levels were only quantifiable by radioluminography (RLG) until 4 hours after dosing. The highest concentration of radioactivity was measured in the liver and the exposure ($AUC_{0–4h}$) was 12-fold higher than the $AUC_{0–4h}$ observed in blood (Figure 8). In the adrenal gland, brown fat and kidney $AUC_{0–4h}$ values were about 4- to 5-fold those in blood. In pancreas and white fat, the $AUC_{0–4h}$ values were almost 3-fold that in blood. In spleen, based on C_{4h}, the radioactivity level was almost 3-fold that in blood. Tissue to blood $AUC_{0–4h}$ ratios in lung, heart, white skin, and thyroid were about 2. $AUC_{0–4h}$ values in prostate gland, bone marrow, muscle, testis and brain were similar or a bit lower than those in blood.

In pigmented tissues, the radioactivity decreased more slowly than in the other tissues and was still quantifiable by RLG 14 days postdose. Tissue to blood $AUC_{0–336h}$ ratios were 146 (uveal tract), 18 (brain meninges) and 15 (pigmented skin). Although levels in pigmented tissues at 14 days postdose still represented about 20% of corresponding peak levels, radioactivity levels decreased from 4 or 24 hours onwards. Therefore no undue retention of [^{14}C]TMC278 derived material is expected.

The QWBA study in non-pigmented pregnant rats showed a similar tissue distribution profile as the one observed in the male rat (Figure 7). In most tissues, highest radioactivity levels were observed at 4 hours postdose, indicating rapid distribution into the tissues. Only in uterine epithelium and to a lesser extent in adrenal gland, radioactivity concentrations declined more slowly than in blood from 8 to 24 hours.

Some specific reproductive tissues were evaluated. The AUC_{0-8h} values of uterine epithelium, mammary gland, and ovary were 4-, 3-, and 2-fold higher than the corresponding blood $AUC_{0–8h}$ values, respectively. The $AUC_{0–8h}$ values in uterus, placenta, and vagina were similar to slightly lower than those in blood. The $AUC_{0–8h}$ in whole fetus was 0.64-fold that in maternal blood, suggesting that the placenta presents a partial barrier for TMC278 and/or its metabolites.

In a pharmacokinetic study after administration of non-radiolabeled TMC278 base in male Sprague Dawley rats, plasma, and tissue samples from the adrenal glands, brain, liver, and muscle were collected. Maximum tissue concentrations were observed within 20 to 60 minutes after administration. Tissue levels declined in parallel with plasma concentrations. Highest tissue concentrations were observed in the liver and the adrenal gland reaching tissue to plasma $AUC_{0–24h}$ ratios of 3.4 and 2.7, respectively. In brain and muscle, the tissue to plasma ratios were 0.49 and 0.45, respectively. These results were

similar to the data obtained with radiolabeled material, showing high distribution to the liver and adrenal glands and lower distribution to the muscles and brain.

Figure 7. Tissue to Blood $AUC_{0–4h}$ Ratios of Total Radioactivity, as Determined by Radioluminography of Whole-Body Sections of Tissues in the Male Pigmented Long Evans Rats After a Single Oral Administration of ^{14}C-TMC278 base at 40 mg/kg

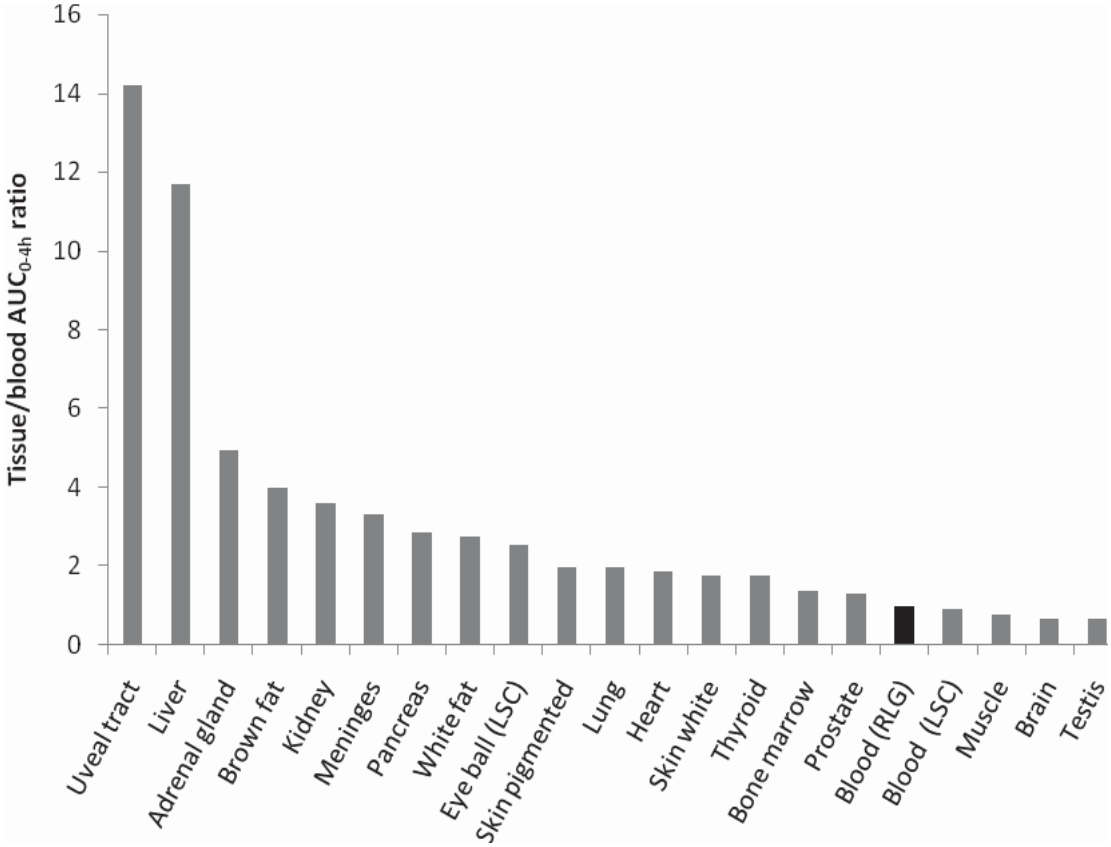
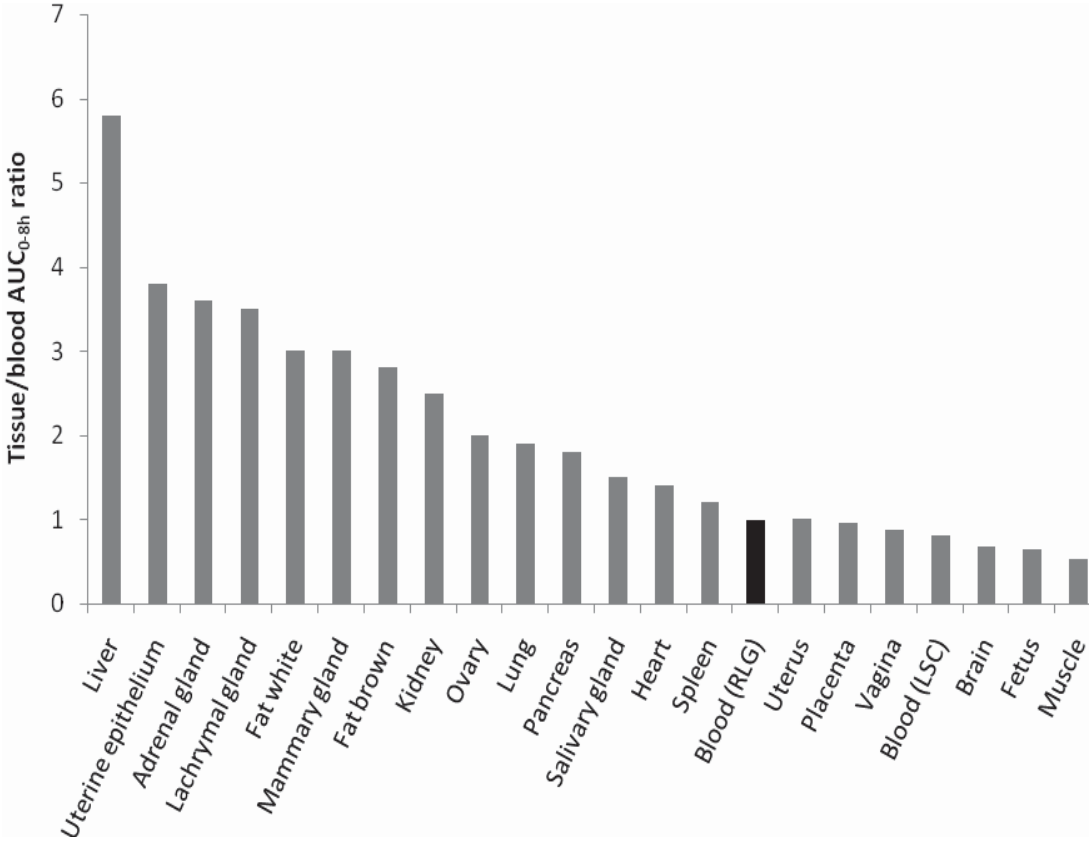


Figure 8. Tissue to Blood AUC_{0-8h} Ratios of Total Radioactivity, as Determined by Radioluminography of Whole-Body Sections of Tissues in the Pregnant Female Sprague Dawley Rats After a Single Oral Administration of ^{14}C-TMC278 base at 40 mg/kg



2.6.4.4.2.6. Rilpivirine: Dog, Oral

Tissue distribution assessments were conducted on some tissues collected in association with repeat dose administration in toxicology studies. Plasma and adrenal glands were obtained from dogs (3 animals/sex/group) following a 1-month toxicity study (Tabulated Summary 2.6.5.4.S, TMC278-TOX5650). Plasma, adrenal gland, and liver were also collected from dogs (3 animals/sex/group) following a 6-month study (Tabulated Summary 2.6.5.4.U, TMC278-NC115). In both studies, animals received TMC278 base (PEG400/CA (10%) oral doses of 5, 10, and 40 mg/kg/day. Plasma and tissues were collected at autopsy on Day 28/29 and Day 56 (recovery period) in the 1-month study or on Day 93 and Day 184/185 in the 6-month study.

In both studies, the adrenal gland concentrations were higher than the corresponding plasma concentrations with mean tissue to plasma concentration ratios ranging from 1.3 to 8.1. After 1 month of recovery, however, the adrenal gland levels as well as the plasma levels decreased below the limit of quantification. In the liver, TMC278 concentrations were also

higher than in plasma, with tissues to plasma concentration ratios ranging from 7.7 to 13. In the 6-month study, adrenal, and liver tissue to plasma ratios were similar between Day 93 and Day 184/185 of the study.

2.6.4.4.2.7. Tenofovir DF/Tenofovir: Rat, Oral/Intravenous

The tissue distribution of total radioactivity in male Sprague Dawley rats after single administration of [^{14}C]TFV was investigated using qualitative whole body autoradiography (Tabulated Summary 2.6.5.5.D, 95-DDM-1278-002). A single 10 mg/kg dose of [^{14}C]TFV was administered to 2 male rats, one intravenously and the other orally. At 24 hours following dose administration, the animals were sacrificed by carbon dioxide inhalation. Autoradiographic images were analyzed visually to determine drug distribution within selected tissues and organs. The extent of distribution of [^{14}C]TFV radio-equivalents was limited following both routes of administration at the 24-hour time point. Using the IV route distribution was more widespread, with the kidney, liver, urine, and large intestine containing the highest levels of radioactivity. The highest radioactivity concentrations 24 hours after oral administration of [^{14}C]TFV were found in the contents of the mid- to lower-gastrointestinal tract. Low levels of radioactivity were also found in the kidney, liver, and urine.

Male Sprague Dawley rats administered a single IV bolus dose of either 10 mg/kg or 50 mg/kg of TFV achieved maximum observed plasma concentrations of 22.0 and 162 µg/mL, respectively (Tabulated Summary 2.6.5.5.F, R2000075). Tenofovir plasma concentrations declined in a biphasic manner with apparent terminal half-lives of 4.02 and 5.41 hours (no substantive difference in $t_{½}$ between dose groups). Tenofovir plasma $AUC_{(0–\infty)}$ was 5.86 µg·h/mL following the 10-mg/kg dose and 53.7 µg·h/mL following the 50-mg/kg dose. Tenofovir distributed to a volume at steady state (V_{ss}) of 2210 and 1122 mL/kg following the administration of the 10- and 50-mg/kg doses, respectively. Plasma clearance of TFV was 1706 and 931 mL/h/kg for the 2 dose groups, respectively. Comparison of the pharmacokinetic parameters between the 2 doses, 10 and 50 mg/kg, suggests nonlinear pharmacokinetics with dose. The 5-fold increase in dose resulted in a 7.2-fold increase in C_{max} and 9.4-fold increase in AUC in association with apparent slower plasma clearance and reduced volume of distribution.

2.6.4.4.2.8. Tenofovir DF: Dog, Oral

The tissue distribution and recovery of radioactivity were examined in beagle dogs following oral administration of [^{14}C]TDF (Tabulated Summary 2.6.5.5.E, 97-DDM-4331-001). Three groups of dogs (2/sex/group) received a single dose of [^{14}C]TDF (10 mg/kg; 25 µCi/kg) by oral gavage. Animals were euthanized at 1, 6, and 24 hours after dosing. Tissues were removed and aliquots analyzed for total radioactivity by sample oxidation and scintillation counting. There was no significant effect of gender on tissue distribution. At 1-hour postdose, radioactivity was detected in all tissues except brain. The majority of the radioactivity was present in the contents of the GI tract, jejunum, and liver (> 66%). Concentrations were highest in bile, kidney, liver, and jejunum. By 6 hours postdose, radioactivity levels were significantly decreased in all tissues. At 24 hours postdose, the

highest concentrations of radioactivity were present in kidney, liver, and the intestinal contents. Total recovery of the dose at 24 hours was > 90% (24% to 44% in feces, 24% in urine, 15% to 20% in tissues, 4% to 24% GI contents, and 1% to 4% in cage wash).

2.6.4.4.2.9. Tenofovir DF: Dog, Intravenous

Male beagle dogs were administered a single IV 1- or 10-mg/kg dose of TFV and plasma concentrations were monitored over time for 120 hours postdose (Tabulated Summary 2.6.5.2.O, D2000076). Maximal plasma concentrations were 2.99 ± 0.410 and 31.0 ± 4.91 µg/mL following the 1- and 10-mg/kg doses, respectively, and declined in a biphasic manner. Tenofovir distributed to a V_{ss} of 3089 ± 275 and 2512 ± 275 mL/kg following the administration of the 1- and 10-mg/kg doses, again, suggesting extensive tissue distribution. Plasma clearance of TFV was 226 ± 29.3 and 244 ± 19.9 mL/h/kg for the 2 dose groups, respectively. Comparison of the pharmacokinetic parameters between the 2 doses, 1 and 10 mg/kg, suggests linear pharmacokinetics with dose. The apparent terminal $t_{1/2}$ for IV TFV was approximately 40 hours as compared to the 60-hour value observed following oral administration of TDF in this study. Interestingly, 2 of the dogs demonstrated detectable TFV plasma concentrations following the 2-week washout period. The incorporation of these values (312 hours postdose) into the data analysis resulted in terminal $t_{1/2}$ of 64 and 65 hours for the 2 animals.

2.6.4.4.2.10. Tenofovir: Monkey, Intravenous

Tenofovir pharmacokinetics were evaluated in rhesus monkeys following IV administration of TFV at doses of 5.0 and 30 mg/kg (3/sex/group) (Tabulated Summary 2.6.5.3.Q, P4331-00033, P2000031). Following IV administration, mean peak plasma concentrations of TFV were 13.8 ± 3.08 and 79.0 ± 12.6 µg/mL, respectively, and declined in a biphasic manner with terminal half-lives of 5.37 ± 1.35 and 8.79 ± 2.79 hours. Mean values for $AUC_{(0-\infty)}$ were 5.12 ± 1.15 and 38.4 ± 16.2 µg·h/mL. Both C_{max} and AUC demonstrated dose linearity. The apparent difference in half-lives between the dose groups can be ascribed to the availability of more quantifiable TFV concentrations at later time points that resulted from the higher dose and gave a more reliable assessment of the terminal elimination phase. No differences in pharmacokinetic parameters were observed between gender.

Naive rhesus monkeys (n = 4) administered a single IV dose of 10 mg/kg TFV (Tabulated Summary 2.6.5.5.G, T1278-00034) reached a mean plasma C_{max} of 27.7 µg/mL and AUC of 14.7 µg·h/mL. Plasma concentrations demonstrated biphasic elimination with a terminal $t_{1/2}$ of 10.6 hours and plasma clearance of 710 mL/h/kg.

2.6.4.4.2.11. Tenofovir: Monkey, Subcutaneous

Pharmacokinetic data were obtained from selected SIV-infected or noninfected juvenile/adult rhesus monkeys with bone lesions that were administered TFV (30 mg/kg/day, subcutaneous) daily for 14 months (Tabulated Summary 2.6.5.5.G, T1278-00034). The pharmacokinetics of TFV have also been examined in SIV-infected and non-infected rhesus monkeys that were

dose reduced from 30 mg/kg/day (subcutaneous) to 2.5, 5, or 10 mg/kg/day (subcutaneous), and maintained at these doses for 5 to 38 months. Additional data have been obtained from monkeys administered TFV subcutaneously at doses of 10 mg/kg/day for 27 to 30 months, as well as from animals dose escalated from 10 to 20 mg/kg/day and maintained at this dose from 3 to 5 months (Tabulated Summary 2.6.5.5.H, P2000117).

Following subcutaneous administration to monkeys on long-term therapy, concentrations of TFV in plasma generally reached a maximum at 30-minutes postdose and declined thereafter in a biphasic manner (similar to single dose subcutaneous data from naive monkeys (Tabulated Summary 2.6.5.7.I, P2000116). The steady-state AUC values in monkeys undergoing long-term TFV treatment (subcutaneous) at 30 mg/kg/day ranged from 97.9 to 240 µg·h/mL, while naive monkeys given a single subcutaneous dose of TFV at 30 mg/kg had a mean $AUC_{(0-\infty)}$ value of 62.6 µg·h/mL. The apparent clearance (CL/F) of TFV in monkeys on long-term TFV therapy was significantly less (1.5- to 3.9-fold lower) than in naive monkeys given a single subcutaneous dose of TFV. Monkeys whose doses were reduced to 2.5, 5, or 10 mg/kg/day (total of n = 5) continued to demonstrate reduced clearance compared to monkeys that had been started and maintained at 10 mg/kg/day (n = 3), or those started at 10 mg/kg and escalated to 20 mg/kg/day (n = 8). Apparent clearance values were 3.3- to 7.9-fold less in these monkeys (Tabulated Summary 2.6.5.5.H, P2000117). Monkeys in this study who were started at 10 mg/kg/day and either maintained at this dose, or dose escalated to 20 mg/kg/day of TFV, demonstrated plasma pharmacokinetics of TFV in keeping with the 2 healthy treatment naive monkeys (Tabulated Summary 2.6.5.7.I, P2000116). Both AUC and C_{max} were roughly dose proportional between the 10 and 20 mg/kg/day, and consistent with what might be predicted from the limited single-dose data.

Two studies have been reported on the penetration of TFV into CSF of SIV-infected rhesus monkeys. In a chronic administration study, monkeys were administered 30 mg/kg/day subcutaneously for 14 months (Tabulated Summary 2.6.5.5.G, T1278-00034). Cerebrospinal fluid was obtained from 5 monkeys prior to dosing and at 3 time points up to 100-minutes postdose. Penetration of TFV into CSF was minimal up to 100-minutes postdose. The highest concentrations of TFV in CSF were approximately 100-fold lower than corresponding plasma concentrations. In a single-dose study {2634}, a single 30-mg/kg dose of TFV was administered to SIV-infected and healthy monkeys. Tenofovir was not detected in the CSF from either the infected or control animals (n = 2/group).

The pharmacokinetics of TFV have been determined in infant rhesus monkeys following subcutaneous administration (Tabulated Summary 2.6.5.5.I, 96-DDM-1278-005). Tenofovir was formulated as an aqueous solution and was evaluated in infant rhesus monkeys in 4 age groups (newborn, 1, 3, and 12 months old) (n = 2 per group). Tenofovir was administered as a 30-mg/kg injection into the dorsal subcutis region. Plasma samples were obtained over the course of 24 hours and concentrations of TFV were determined by HPLC following fluorescence derivatization. The mean TFV C_{max} values in newborn, 1, 3, and 12-month-old monkeys were 51.8, 30.7, 34.6, and 18.8 µg/mL, respectively, with a T_{max} of 0.5 hour for all age groups. The corresponding plasma clearance (CL/F) of TFV was 0.18, 0.54, 0.41, and

1.02 L/h/kg, respectively, showing an increase in the clearance from birth through 1 year. These results suggest that, at an equivalent dose, younger monkeys received greater TFV exposure. The clearance of TFV correlated well with both weight and age of the infant monkeys. It is likely that newborn monkeys lack the anion transport system responsible for tubular secretion of TFV F222.

2.6.4.4.3. Studies in Pregnant or Nursing Animals for Emtricitabine, Rilpivirine, and Tenofovir DF

2.6.4.4.3.1. Emtricitabine: Pregnant Mice

Following oral administration of FTC to pregnant mouse dams, the exposure of murine fetuses to FTC was examined (Tabulated Summary 2.6.5.7.A, TOX103). Emtricitabine suspended in the vehicle, 0.5% methylcellulose (aqueous), was administered orally by gavage to 1 group of 8 bred Crl:CDR®-1(TCR) BR mice twice daily (approximately 6 hours between doses) from gestation Days 6 to 14 at a dose of 1000 mg/kg/day administered at a dose volume of 5 mL/kg/dose. On gestation Day 15, following approximately 10 days administration of 500 mg/kg of FTC twice daily, pregnant mice and their viable fetuses had measurable concentrations of FTC 1 hour following administration of the first 500 mg/kg dose. The mean (± SD) plasma concentration in the pregnant mice was 137.1 ± 28.0 µg/mL. The mean concentration of FTC in pooled fetal homogenate was 55.7 ± 10.4 µg/g. The mean fetal/maternal concentration ratio was 0.41 ± 0.04.

2.6.4.4.3.2. Emtricitabine: Pregnant Rabbits

In an oral GLP embryo-fetal toxicity study, pregnant New Zealand White rabbits (20/dose) were given FTC at 0, 100, 300, and 1,000 mg/kg/day in 0.5% methylcellulose as equal divided doses 6 hours apart on gestation Days 7 to 19. The does were necropsied on gestation Day 19 (Tabulated Summary 2.6.5.7.B, TOX038). Additional pregnant rabbits (5/dose) given the same doses on the same dose regimen were used to provide plasma for systemic exposure assessment on gestation Day 19. They were killed at 1 hour postdose on Day 20 to provide maternal/fetal blood samples to confirm fetal exposures.

Emtricitabine was rapidly absorbed in dams with C_{max} occurring generally within 1-hour postdose. Systemic exposure to FTC (AUC and C_{max}) increased linearly with dose from 100 to 1000 mg/kg/day in both dams and fetuses. On gestation Day 19, $AUC_{(0\text{-}24\ hr)}$ in dams was 87, 315, and 1258 µg·h/mL at 100, 300, and 1000 mg/kg/day, respectively. Plasma elimination $t_{1/2}$ was 3 to 4 hours at all dose levels. Fetal/maternal exposure ratios, as determined by analysis of umbilical cord blood, were around 0.4 to 0.5 at 1 hour after dosing (at T_{max}) for all dose levels. Emtricitabine was therefore readily transferred across the placenta (Tabulated Summary 2.6.5.7.B**,** TOX-038).

2.6.4.4.3.3. Rilpivirine: Pregnant Rats

The placental transfer of TMC278 was studied in pregnant Sprague Dawley rats, by QWBA, after a single oral dose (gavage) of [^{14}C]TMC278 base in PEG400/CA (10%) at 40 mg/kg

(Tabulated Summary 2.6.5.5.C, TMC278-NC109). The $AUC_{0–8h}$ values in the placenta and in whole fetuses were 0.95- and 0.64-fold the $AUC_{0–8h}$ value of maternal blood, respectively. This suggests that the placenta is only a partial barrier for TMC278 and its metabolites.

2.6.4.4.3.4. Tenofovir DF: Pregnant Rats

Tenofovir concentrations were determined in plasma and milk from pregnant female rats dosed with TDF by oral gavage for approximately 27 days at 0, 50, 150, 450, or 600 mg/kg/day (Tabulated Summary 2.6.5.7.F, R990202-PK). Blood samples were collected on Day 7 of presumed gestation (the first day of dosing) and Day 20 postpartum (the last day of dosing). Milk samples were collected at approximately 1-hour postdose on Day 11 postpartum.

Comparison of single-dose Day 7 pharmacokinetic data with steady-state Day 20 data revealed overall similar T_{max} and $t_{1/2}\lambda_z$ values. Results showed generally increasing values with dose for C_{max} and AUC and some apparent accumulation with multiple dosing, although there was high variability and limited data from the highest dose groups. Results for Day 11 median TFV concentrations from rat milk for the 50, 150, 450, and 600 mg/kg/day dose groups, respectively, showed 13.5%, 11.0%, 20.8%, and 23.5% of the TFV plasma concentration at similar times. This data is, however, limited by the comparison of data from different rats and the different numbers of observations between plasma and milk data.

All rats in dose groups that received TDF demonstrated exposure based on the measurable levels of TFV in plasma on Day 7 and Day 20. Both Day 7 and Day 20 postpartum pharmacokinetic rat plasma profiles demonstrated increased exposure of TFV with increasing oral doses of TDF. Day 11 postpartum data confirmed the presence of TFV in rat milk with the results suggesting that TFV was not concentrated in milk (Tabulated Summary 2.6.5.7.F, R990202-PK).

2.6.4.4.3.5. Tenofovir DF: Pregnant Rabbits

Concentrations of TFV were determined in plasma samples obtained during the course of a developmental toxicity study in rabbits (Tabulated Summary 2.6.5.7.G, 98-TOX-4331-005-PK)**.** Pregnant rabbits (n = 4 per group) received TDF at 30, 100, or 300 mg/kg/day by oral gavage, on Days 6 through 18 following gestation (DGs 6–18). Plasma samples were collected on DG 18. Concentrations of TFV in plasma on DG 18 reached mean peak values of 4.92 ± 1.34, 20.65 ± 4.09, and 47.62 ± 9.51 µg/mL at the 30, 100, and 300 mg/kg/day dose levels, respectively. The T_{max} in plasma was similar for each dose level; 0.75 hour at the 30 and 300 mg/kg/day dose levels and 0.5 hour at the 100 mg/kg/day dose level. There was a biphasic decline in plasma concentrations with time, with an apparent terminal $t_{1/2}$ of 8.32 ± 1.63, 10.12 ± 3.3.6, and 9.67 ± 3.23 at the 30, 100, and 300 mg/kg/day dose levels, respectively. Tenofovir C_{max} and $AUC_{(0-t)}$ appeared to increase in a dose-proportional manner following repeated dose administration of TDF and indicated linear pharmacokinetics following multiple daily administration to pregnant rabbits.

2.6.4.4.3.6. Tenofovir: Pregnant Monkeys

Placental transfer of TFV following subcutaneous administration to a pregnant rhesus monkey was determined in association with an infant rhesus monkey study (Tabulated Summary 2.6.5.7.H, 96-DDM-1278-005). One rhesus monkey received daily subcutaneous injection of 30 mg/kg/day TFV, beginning at GD 111. Maternal and fetal blood samples were drawn at Days 115, 127, 134, 140, and 151. Placental transfer of TFV appeared to be significant with a mean ± SD ratio of fetal/maternal serum concentrations of 0.17 ± 0.07 at approximately 30-minutes postdose.

2.6.4.4.3.7. Tenofovir: Lactating Monkeys

The pharmacokinetics of TFV were investigated in 2 healthy adult lactating rhesus monkeys which were administered a single 30 mg/kg subcutaneous dose of TFV (Tabulated Summary 2.6.5.7.I, P2000116). Following dosing, maximal serum TFV concentrations (C_{max}) of 18.3 and 30.2 µg /mL were observed in the 2 monkeys, respectively. Absorption was rapid, with T_{max} occurring at 0.5 hours. As observed in other species, elimination was biphasic, with an apparent half-life of 3.97 and 2.85 hours for the 2 animals, respectively. This was shorter than the approximately 9-hour terminal half-life observed in another study where TFV and TDF was given IV and orally, respectively, to male and female adult rhesus monkeys (Tabulated Summary 2.6.5.3.Q, P2000031) and may have resulted from the more limited period of sampling (24 vs. 48 hours) conducted in the present experiment.

Serum $AUC_{(0-\infty)}$ values in this study were 68.9 and 56.2 µg·h/mL for the 2 animals, respectively. In comparison to AUC values obtained following 30 mg/kg IV doses of TFV (Tabulated Summary 2.6.5.3.Q, P2000031), these data suggest essentially complete absorption of TFV after subcutaneous administration.

2.6.4.4.4. Emtricitabine/Tenofovir DF: Distribution

Preclinical studies to evaluate the distribution kinetics of the FTC/TDF combination have not been conducted as both TFV and FTC exhibit extremely low protein binding to plasma proteins of < 0.7% and < 4%, respectively. Interaction, in terms of changes in the distribution profile when the drugs are coadministered, seems unlikely. This conclusion has been confirmed in clinical trials when the actives are coadministered, and the apparent volumes of distribution for TFV and FTC are unaltered relative to the estimates when administered separately.

2.6.4.4.5. Emtricitabine/Rilpivirine/Tenofovir DF: Distribution

No formal study has been conducted to evaluate the distribution of these drugs when administered in combination. Based on the available data for each compound, the distribution profiles are unlikely to be altered in combination.

2.6.4.5. Metabolism

2.6.4.5.1. Metabolism In Vitro for Emtricitabine, Rilpivirine, and Tenofovir DF

2.6.4.5.1.1. Emtricitabine: Metabolism In Vitro

An in vitro metabolism study was performed to identify the potential human CYP450 isoenzyme(s) responsible for the metabolism of FTC using human liver microsomes and bactosomes containing cDNA-expressed human CYP P450 iosenzymes (Tabulated Summary 2.6.5.9.A, PDM-007). The results showed that FTC was relatively stable in the incubation medium. One minor metabolite (~1%) was detected in cDNA-expressed CYP 3A4 incubations only. It was not formed by any other isoenzyme investigated (CYP 1A2, 2A6, 2B6, 2D6, 2E1, 2C8, 2C9, or 2C19), indicating that CYP 3A4 was the sole CYP P450 isoenzyme responsible for the metabolism of FTC. Microsomal incubations in the presence and absence of selective inhibitors of various CYPs confirmed the low rate of FTC metabolism, and also suggested the possible involvement of FAD-containing monooxygenases (FMOs) in the metabolism of FTC.

In addition, studies have shown that FTC was not an inhibitor for human CYP1A2, 2A6, 2B6, 2C9, 2C19, 2D6, 2E1, and 3A4/5 (Tabulated Summary 2.6.5.9.B, PDM-006). Emtricitabine also did not show inhibition of 7-hydroxy-coumarin glucuronidation. Thus, it is unlikely that FTC would affect the metabolism of the coadministered medications.

2.6.4.5.1.2. Rilpivirine: Metabolism In Vitro

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 × 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 (Tabulated Summary 2.6.5.9.C, TMC278-NC102). In addition, in vitro metabolism was also studied in hepatocyte primary cultures and 12,000 × g liver supernatant fractions from female Dunking Hartley guinea pigs and female or male cynomolgus monkeys (Tabulated Summary 2.6.5.9.D, TMC278-NC333). TMC278 (5 µM) was incubated in the above systems at 37^{o}C for various time periods. Incubates were analyzed for metabolites by radio-HPLC. Co-chromatography, enzyme hydrolysis, LC-MS/MS, and nuclear magnetic resonance (NMR) techniques were used for the identification of metabolites.

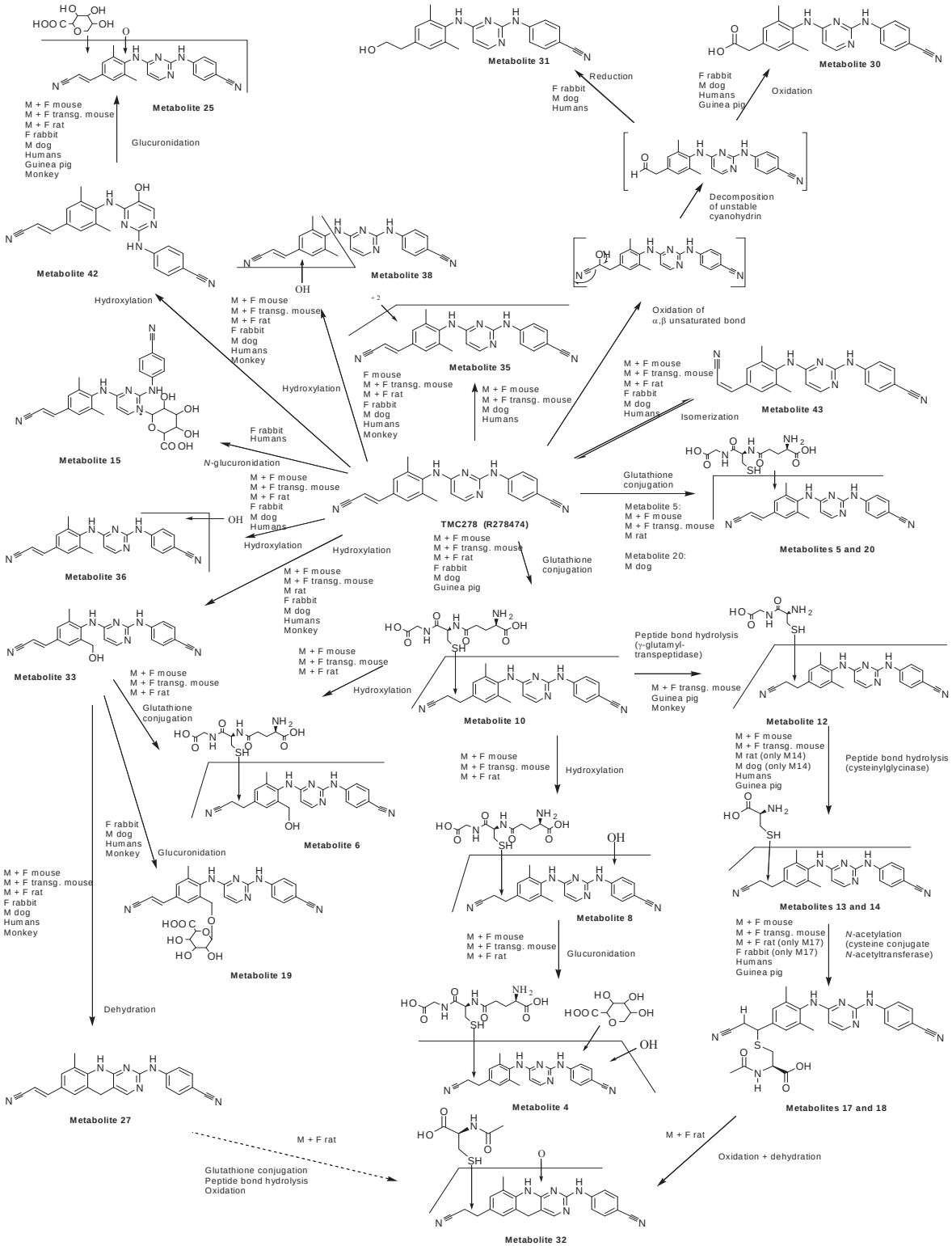
In each species, a large number of metabolites was detected (see Figure 9). Overall, TMC278 was metabolized via different metabolic pathways including aromatic and aliphatic hydroxylation; glutathione conjugation; N-glucuronidation; nitrile release followed by reduction/oxidation; and isomerization. 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 1 animal species.

In a previous in vitro metabolism study with cold TMC278 (Report TMC278-FK4152), 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 9. **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**



Isozymes Involved in the Metabolism of TMC278 in Human Liver

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 (Tabulated Summary 2.6.5.9.E, TMC278-NC141). 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 (Report TMC278-FK4151).

In the radiolabeled study, 1 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 non-radiolabeled 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 (Report TMC278-FK4288).

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 (Report TMC278-FK4789). [^{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 nonenzymatic conjugation.

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

Enzyme Induction and Inhibition

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 (Tabulated Summary 2.6.5.11D, TMC278-NC186). Cells were treated for 2 consecutive days either with vehicle (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.

In Vitro Study Measuring GST Activity Induction in Human Hepatocytes:

The potential of TMC278 to induce GST was evaluated in 1 batch of primary human hepatocytes in the presence of 3 concentrations of TMC278 (1, 10, and 30 µM), incubated for 3 consecutive days (Report TMC278-FK4824).

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.

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 (Tabulated Summary 2.6.5.11.A, TMC278-NC192). 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 (Tabulated Summaries 2.6.5.11.B and 2.6.5.11.C; TMC278-NC193 and TMC278-NC140, respectively).

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 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) (Report TMC278-FK4247). 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 6). 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 6). 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, 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 2 highest dose levels and was not dose-dependent (see Table 6).

Table 6. 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 (TMC278-NC192)	20	111	153**	147*	126	108	138*
	80	156***	174***	525***	521***	150**	164***
	320	174***	175***	2499***	1966***	210***	229***
Rat (TMC278-NC193)	40	95	120	140	75	65**	127
	120	125	300***	262**	93	77*	98
	400	120	600***	466***	127*	125*	134
Dog (TMC278-NC140)	5	85		100		82	
	10	57**		102		75	
	40	74*		113		68	

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

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 (Reports TMC278-FK4123 and TMC278-NC283). 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 7).

Table 7. 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	CYP3A4/5 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 2.6.4.5.1.2) 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, Section 2.7.2.2.2.8.13) 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 2.6.4.7.2) and also in the in vivo clinical study (Clinical Study C139 (Module 2.7.2, Section 2.7.2.2.2.8.17), no interaction was observed between TMC278 and chlorzoxazone. Therefore, as it was not confirmed by subsequent studies, the inhibition of CYP2C19 and CYP2E1 by TMC278 is 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 (Report TMC278-NC283). 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 ($C_{max}/K_i<0.1$).

Effect of TMC278 on Adrenal Gland:

The effect of TMC278 on cortisol biosynthesis in dog adrenal cortex cell-free extracts was determined (Report TMC278-FK4790) (see also Module 2.6.6, Section 2.6.6.8.3.1.2).

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.

2.6.4.5.1.3. Tenofovir DF/Tenofovir: Metabolism In Vitro

The in vitro metabolism of TDF was studied in Sprague-Dawley rat and beagle dog plasma and also in beagle dog liver and intestinal homogenates (Tabulated Summary 2.6.5.9.F, P4331-00003). An NADPH regenerating system was added to the liver homogenates. Tenofovir DF was added to each matrix and incubated at 37°C. Samples of each mixture were collected for analysis by HPLC with UV detection over time up to 60 minutes. Tenofovir DF was rapidly converted to the monoester in all test systems, especially rat plasma and dog intestinal homogenate ($t_{1/2}$ < 5 minutes). Estimated half-lives for the disappearance of TDF in dog plasma and dog liver homogenate were 21 and 53 minutes, respectively. No other metabolites were observed.

Similarly, TDF was converted to monoester and TFV in Caco-2 cell permeability experiments (P4331-00015.1). Tenofovir disoproxil, tenofovir soproxil, and TFV were detected inside the monolayer following 60 minutes incubation. Tenofovir soproxil was the major intracellular metabolite observed.

The in vitro metabolism of TFV was studied in dog plasma, in control and induced (Araclor™ 1254) rat liver microsomes, and also in dog liver and intestinal homogenates (Tabulated Summary 2.6.5.9.F, 96-DDM-1278-003). [^{14}C]TFV was incubated at 37°C with each matrix, and samples of each mixture were collected for analysis by HPLC with radiometric detection over time up to 60 minutes. Potential isomerization of TFV was determined using a chiral HPLC assay with radioactive flow detection. Radioactivity associated with the protein pellet was also determined by sample oxidation and liquid scintillation. The microsomal incubations were carried out with and without the addition of a NADPH regenerating system. No metabolites were detected in either rat microsomal preparation, with or without the addition of cofactors. In addition, TFV was recovered unchanged and less than 0.1% of the counts were associated with the protein pellet. There was no evidence of chiral inversion. Similarly, there was no apparent loss of TFV following incubation with either dog plasma, liver, or intestinal homogenates and no metabolites were detected.

The potential for TFV and TDF to inhibit CYP450 mediated drug metabolism was examined in vitro using human hepatic microsomes (Tabulated Summary 2.6.5.11.E, V990172-104). Probe substrates specific for the CYP450 isoforms were utilized to examine the effect of TFV and TDF on the activities of the distinct isoforms. The metabolism of the probe

substrates for CYP 3A4 (terfenadine), CYP 2D6 (dextromethorphan), CYP 2C9 (tolbutamide), CYP 2E1 (chlorzoxazone) and CYP 1A (7-ethoxycoumarin) was evaluated in the presence and absence of 100 mM TFV or TDF. Tenofovir did not inhibit the metabolism of any of the probe substrates. Tenofovir DF had no effect on the activity of any of the CYP450 isoforms, except for CYP 1A, where a small (6%), but significant reduction in the metabolism of 7-ethoxycoumarin was observed. Since the concentration of TFV and TDF used in the in vitro studies were 300-fold higher than the maximal plasma levels of these compounds observed in patients receiving an oral dose of TDF, these data indicate that TFV and TDF should have little or no potential to inhibit CYP-450 mediated metabolism in vivo.

2.6.4.5.2. Metabolism In Vivo for Emtricitabine, Rilpivirine, and Tenofovir DF

2.6.4.5.2.1. Emtricitabine: Metabolism In Vivo

Mice

The metabolism and elimination of orally administered [6-^{3}H]FTC was studied in male CD-1 mice (Tabulated Summary 2.6.5.8.A, TEIN/93/0015). Urine and feces were assayed by liquid scintillation counting and HPLC. Urinary recovery of radioactivity was 67% ± 7% of the dose. In the feces, 18% ± 3% of the dose was recovered, all as unchanged FTC. Total recovery of radioactivity excreted in urine and feces was 85% ± 4% of dose. In the urine, 64% ± 7% of the radioactivity was recovered as unchanged FTC in the 0- to 24-hour sample. Three metabolites of FTC were measurable in the urine. These metabolites were tentatively identified as: 3742W92 and 3743W92 (2 isomeric, 3'-sulfoxides of FTC, 1.7% ± 0.3% and 2.0% ± 0.4% of dose recovered, respectively); and 5-fluorocytosine (1.4% ± 0.1% of dose recovered). Traces of 5 other metabolites and a peak tentatively identified as tritiated water were also observed at levels of less than 1% of dose. 5-Fluorouracil was not observed (< 0.1% of dose).

Monkeys

An in vivo metabolism study was performed in cynomolgus monkeys (Tabulated Summary 2.6.5.8.B, TOX063). The purpose of the first phase of this study was to provide biological samples for the evaluation of the pharmacokinetics of radioactive FTC in plasma, and to obtain information on the rate and extent of excretion of radioactivity in urine and feces following a single oral dose of [^{14}C]FTC to male cynomolgus monkeys. The purpose of the second phase of this study was to provide samples for identification of metabolites and evaluation of the tissue distribution of FTC.

During Phase 1, 4 male cynomolgus monkeys received a single 200 mg/kg (10 mL/kg dose volume) oral dose containing 138 µCi of [^{14}C]FTC. Blood samples were collected for 120 hours postdose. After approximately 3 weeks, at the initiation of Phase 2, the same 4 male cynomolgus monkeys were dosed using the same regimen as Phase 1. Immediately following the 1-hour blood collection, the time of maximum concentration of radioactivity in plasma (T_{max}) as determined in Phase 1, the animals were anesthetized with sodium pentobarbital and the CSF was collected. After CSF collection, the animals were

exsanguinated under sodium pentobarbital and the aqueous humor, blood, and selected tissues were collected.

After the Phase-1 dose administration, the mean maximum concentration of radioactivity in blood (54.3 µg equivalents/g) and plasma (63.5 µg equivalents/g) was obtained at 1-hour postdose. The blood and plasma concentrations declined rapidly over time with [^{14}C]FTC-derived radioactivity only detectable through 48 hours postdose. A mean $t_{1/2}$ of 8.05 hours for the total radioactivity was estimated. The mean $AUC_{(0-48)}$ and $AUC_{(0-\infty)}$ values were 269 and 272 µg equivalents·h/g (0.0576 and 0.0583 µCi h/g), respectively.

The mean total recovery of radioactive dose in urine, feces, cage wash, and cage wipes over the 120-hour collection interval was 84.5%. Urine and feces accounted for a mean of 40.8% and 35.3% of the total radioactive dose, respectively, with 66.4% of the radioactivity excreted within 48 hours postdose. The cage wash and cage wipes accounted for an additional 8.31% of the total radioactive dose.

At 1 hour following Phase-2 dose administration, the animals were necropsied, and radioactivity was detected in all matrices examined. With the exception of the GI tract tissues (small intestine, large intestine, and stomach), the highest mean concentrations of radioactivity in the tissues were observed in the kidneys (596 µg equivalents/g) and liver (121 µg equivalents/g). The ratios for radioactivity concentrations found in the brain, CSF, bone, aqueous humor, and eyes as compared to blood were 0.028, 0.031, 0.039, 0.063, and 0.098, respectively, indicating distribution of radioactivity into these tissues (2.17 to 7.53 µg equivalents/g).

The metabolism and elimination in urine and feces of 80 mg/kg orally administered [6-^{3}H]FTC was studied in 4 female cynomolgus monkeys (Tabulated Summary 2.6.5.8.C, TEIN/93/0016). After 72 hours, 41% of the radioactivity in the dose was excreted into the urine. Most of the urinary excretion occurred in the first 8 hours postdose. Recovery of radioactivity in the feces averaged 33% of dose after 72 hours. Recovery of radioactivity in the cage wash averaged 10% of dose over the same time period. The overall recovery of dose was 84%. Radiochemical-HPLC analysis of urine and fecal samples showed that unchanged FTC was 64% of the radiolabel recovered in the urine and 98% of the radiolabel in feces. The major urinary metabolite constituted 11% of dose. This metabolite, designated M950, co-eluted with 1 isomer of authentic 524W913'-sulfoxide. Also present in the urine were 7 other radio-labeled compounds: none of these accounted for more than 1.7% of dose. Another potential urinary metabolite, 5-fluorouracil, was less than 0.02% of dose.

2.6.4.5.2.2. Rilpivirine: Metabolism In Vivo

Mice

Male and female CD-1 mice were dosed orally, by gavage, with a single dose of [^{14}C]TMC278 base in PEG400/CA (10%) at 20 or 320 mg/kg (Tabulated Summary 2.6.5.10.B, TMC278-NC190). Plasma samples were collected up to 24 hours after dosing and urine and feces samples up to 96 hours after dosing. Radioactivity

was determined by scintillation counting and samples were analyzed by radio-HPLC. Plasma concentrations of TMC278 were measured by LC-MS/MS and the metabolite profiling and identification were done by radio-HPLC and LC-MS/MS analyses.

After oral administration of [^{14}C]TMC278 base, 87% to 96% of the total radioactivity was eliminated in feces and 1.8% to 4.2% was excreted in urine. Unchanged TMC278 accounted for 7.9% to 8.8% and 33% to 34% of the administered dose in the overall pooled 0- to 48-hour feces at 20 and 320 mg/kg, respectively. Renal elimination was limited, only traces of unchanged TMC278 were excreted in urine (0.02% to 0.63% of the dose) at the 2 dose levels.

TMC278 was extensively metabolized in the mouse, as a large number of metabolites were detected (Figure 10). Metabolic pathways included oxidative pathways (aliphatic and aromatic hydroxylation) and glutathione conjugation, followed by secondary metabolism (metabolism of the glutathione conjugate, glucuronidation, dehydration).

In feces, by far the predominant metabolite fraction was composed of M41 (hydroxy metabolite of S-methyl conjugate of TMC278) and M42 (aromatic hydroxylation at the 5-position of the pyrimidinyl moiety), accounting for 18% to 26% of the 20 mg/kg dose and for 9% to 13% of the 320 mg/kg dose. In this fraction, M42 was the most abundant metabolite for both genders and at the 2 dose levels, estimated at about 14% and 17% of the 20 mg/kg dose, and at 5.9% and 8.0% of the 320 mg/kg dose in male and female mice, respectively. The cysteinyl conjugates (M13 co-eluted with M14) and the mercapturic acids (M17 co-eluted with M18) accounted for 6.2% to 9.2% of the dose in total (2 dose levels, both genders). M24 (hydroxylation on the cyanoethenyl moiety) co-eluted with M25 (oxidation followed by glucuronidation of TMC278) and accounted for less than 3.4% in total (2 dose levels, both genders). M30 (carboxylic acid metabolite on the cyanoethenyl moiety) accounted for about 1.6% to 3.1% of the 20-mg/kg dose and about 1.2% to 1.5% of the 320-mg/kg dose. Several minor metabolites in both male and female mice were present: M38 (hydroxylation of TMC278) and M43 (Z isomer of TMC278) that co-eluted with M45 (S methyl conjugate) accounted for less than 2.2%. M21 (hydroxylated methyl sulphonyl conjugate) and M33 (hydroxylation at the methyl group of TMC278) accounted for less than 1.4%. M27 (tricyclic metabolite) co-eluted with M28 (tricyclic metabolite of S-methyl conjugate of TMC278) and M29 (sulphoxidation of M45); and M47 (dimer) accounted for less than 0.8%. M35 and M46 (unknown structures) accounted for less than 0.7%.

In urine, M25 (oxidation in combination with glucuronidation) was the most abundant metabolite at both dose levels and accounted for 0.4% to 1.6% of the dose. M13, M14, M17, and M18 were more formed in female mice than in male mice and accounted for 1.2% and 1.1% of the dose in females (at 20 and 320 mg/kg, respectively) and for 0.54% and 0.27% of the dose in males (at 20 and 320 mg/kg, respectively). M42 was also excreted in urine (0.06% to 0.37% of the dose).

In plasma, unchanged TMC278 was by far the main circulating compound at both dose levels. At all time points, M33 (hydroxymethyl TMC278) was the most abundant plasma metabolite; it accounted for 1.5% to 6.6% of the plasma radioactivity. Other metabolites

(M13 co-eluted with M14, M27, M30, and M36) accounted for less than 1.3% of the sample radioactivity. Traces of several other metabolites (M17, M18, M31 [hydroxyl metabolite on the cyanoethenyl moiety], M42, and M43) were detected.

The metabolite profile of TMC278 was qualitatively comparable in male and female mice.

Rats

Single Dose Administration:

Male and female Sprague Dawley rats were dosed orally, by gavage, with a single dose of [^{14}C]TMC278 base in PEG400/CA (10%) at 40 mg/kg (Tabulated Summary 2.6.5.10.C, TMC278-NC113). Plasma samples were collected up to 24 hours after dosing, and urine and feces samples were collected up to 96 hours after dosing. Plasma concentrations of TMC278 were measured by LC-MS/MS. The biliary metabolite profile of TMC278 was also investigated in male Sprague Dawley rats after a single dose of [^{14}C]TMC278 base in PEG400/CA (10%) at 40 mg/kg (Tabulated Summary 2.6.5.10.D, TMC278-NC145). In both studies, the total radioactivity was measured by scintillation counting. Metabolite profiles were determined by reversed-phase radio-HPLC, and identification of the metabolites was carried out using co-chromatography with synthesized metabolites, enzymatic hydrolysis, and LC-MS/MS analysis.

After oral administration of [^{14}C]TMC278 base, the radioactivity was predominantly excreted in feces (93% of the dose), and 0.45% (males) to 1.8% (females) was excreted in urine. Unchanged TMC278 accounted for 47% and 43% of the administered dose in the overall pooled 0- to 48-hour feces in males and females, respectively. Urinary excretion of unchanged TMC278 was negligible.

TMC278 was metabolized to a moderate extent. Metabolic pathways included oxidative pathways (aliphatic and aromatic hydroxylation), glutathione conjugation, and metabolites derived thereof (Figure 10).

In feces, dimerization of a thiol intermediate resulting from glutathione conjugation (M47) was the most predominant metabolic pathway in male and female rats (4.0% and 3.8% of the dose, respectively). M41/M42 and M43/M45, which co-eluted pairwise, resulted from hydroxylation, isomerization, or glutathione conjugation and accounted on average for 2.4% to 3.6% of the dose in both genders. A variety of minor metabolites (M21, M24/M27/M28/M29, M33, M38, and M46) were also observed in rat feces; each represented less than 2% of the administered dose. M30 accounted for 0.47% and 0.05% of the dose in male and female rats, respectively.

In urine, only the mercapturic acids (M17 and M18) were detected and accounted for 1.1% and 0.45% in female rats and for 0.02% and 0.03% of the dose in male rats, respectively. Several unknown metabolites were also present in urine but accounted in total for < 0.4% of the dose.

In plasma, unchanged TMC278 accounted for the largest fraction of the circulating radioactivity. Only 2 minor metabolites, M12, a cysteinylglycine-S-conjugate, which co-eluted with M14, a cysteinyl-S-conjugate, were present in plasma. They accounted for 4% to 14% of the plasma radioactivity in total, at all time points.

The metabolic profile of TMC278 is qualitatively comparable in male and female rats.

In bile, the amount of radioactivity excreted within 24 hours after a dose of [^{14}C]TMC278 base was rather low, i.e., 18% and 25% of the radioactive dose, in restrained and nonrestrained rats, respectively. The percentage of unchanged TMC278 excreted in the bile during this time period was negligible (~0.2%). The most important biotransformation pathway involved conjugation of glutathione to TMC278 to form M10 followed by formation of the cysteinylglycine-S-conjugate M12 and the cysteine-S-conjugate M14. They accounted for 6.4% in total. From this pathway, other metabolites were generated. The most abundant ones were M9 (thiol glucuronide conjugate) and M18 (mercapturic acid S-conjugate of TMC278) and each accounted for less than 2.8%. The minor one was M1 (oxidation and glucuronidation of M14) and accounted for 0.63%. Other minor metabolites were M25 (1.4%) and M30 (0.4%) (Tabulated Summary 2.6.5.10.D, TMC278-NC145).

Multiple Dose Administration:

In an attempt to understand the time-dependent decrease in exposure in male rats (see Table 2), the metabolic profiles of plasma samples obtained after oral administration of TMC278 at 1500 mg/kg/day in male and female rats (carcinogenicity study) were assessed by LC/UV and LC/MS (Report TMC278-NC290; Tabulated Summary 2.6.5.4.N, TMC278-NC123; see Section 2.6.4.3.4.5).

In all samples, unchanged TMC278 was by far the major circulating compound. The comparison of the plasma profiles from male and female rats at Day 1, Week 27, and Week 39 did not show a relevant increase in metabolites after repeated administration.

Dogs

Male beagle dogs were dosed orally with a single dose of [^{14}C]TMC278 base in PEG400/CA (10%) at 5 mg/kg (Tabulated Summary 2.6.5.8.G, TMC278-NC114). Plasma, urine, and feces were collected up to 168 hours (1 week) after dosing. Plasma concentrations of TMC278 were measured by LC-MS/MS. Radioactivity levels were determined by liquid scintillation counting and metabolite profiles were investigated by radio-HPLC and LC-MS/MS.

After oral administration of [^{14}C]TMC278 base, radioactivity was excreted predominantly in feces (95% of the dose), while a very low amount of radioactivity was excreted in urine (1.7% of the dose). Unchanged drug represented 45% of the administered dose in the overall pooled 0- to 72-hour feces. Unchanged TMC278 was not detected in urine.

TMC278 was not extensively metabolized in dogs. The most important biotransformation pathway of TMC278 in dogs was oxidation at various positions of the molecule. In addition, but less significant, direct N-glucuronidation of TMC278 and further metabolism of the oxidized metabolites via dehydration (ring closure), glucuronidation, and sulfation occurred (Figure 10). The most abundant fecal metabolites included M33 (hydroxymethyl-TMC278), M42 (hydroxyl metabolite at the 5-position of the pyrimidinyl moiety of TMC278), and M44 (monooxygenated-TMC278) and represented 8.7%, 5.3%, and 4.3% of the dose, respectively. In addition, M30 (carboxylic acid) and M48 (unknown), which co-eluted, represented 3.1% of the dose. Other minor fecal metabolites (M23 co-eluted with M27, M37, M40, M46, and M49) were also detected and individually these metabolites did not represent more than 2% of the administered dose.

In urine, several minor metabolites (M3, M12, M14, M19, M25, M30, and M36) were identified; none of which represented more than 0.08% of the radioactive dose.

In plasma, unchanged TMC278 was the only radioactive component detected. Minor metabolites that were present in trace amounts and were detected only by LC-MS included M15 (*N*-glucuronide), M19 (glucuronide), M27, M30, and M33.

Humans

Six healthy male subjects received a single oral dose of 150 mg [^{14}C]TMC278 base (administered as a PEG400 formulation) (Tabulated Summary 2.6.5.8.H, TMC278-NC157/TMC278-NC119). Urine and plasma were collected for up to 1 week after dosing; feces were collected for up to 2 weeks after dosing. Plasma concentrations of TMC278 were measured by LC-MS/MS. Radioactivity levels were determined by liquid scintillation counting. Metabolite profiles were determined by reversed-phase radio-HPLC and identification of the metabolites was carried out using co-chromatography with synthesized metabolites, LC-MS/MS, and NMR analysis. In plasma, the more sensitive method LC-ARC (liquid chromatography-accurate radioisotope counting) was used for metabolite profiles.

After oral administration, radioactivity was mainly excreted in feces (85% of the dose over the 14-day period), while in urine a low amount of radioactivity was excreted (6.1% of the dose over the 7-day period). Unchanged TMC278 represented on average 26% of the administered dose in feces. Unchanged TMC278 was not detected in urine.

TMC278 was extensively metabolized. The most important biotransformation pathway of TMC278 was oxidation.

The most abundant fecal metabolite was M42, which accounted on average for 16% of the dose. M33 (hydroxymethyl-TMC278) accounted for 3.0% of the administered dose, M30 (carboxylic acid derivative) for 2.7%, and a metabolite of unknown structure (M35) accounted for 2.2% of the administered dose. Some minor metabolites resulting from further biotransformation of M33 (M27, M11 and M23) were also detected (each < 1.6%).

In urine, apart from M30 (0.03%), metabolites were Phase II metabolites (glucuronides (0.9% of the administered dose) or glutathione-derived (1.2% of the administered dose) conjugates).

In plasma unchanged drug accounted for the major part of the total radioactivity. Several minor metabolites were detected, namely, the glucuronide of TMC278 (M15), the tricyclic metabolite (M27), and hydroxymethyl TMC278 (M33); others (glucuronide of hydroxymethyl TMC278, and of hydroxylated TMC278) were only present in trace amounts.

In general, all identified metabolites in human matrices were also detected in at least 1 animal species (see Figure 10).

The antiviral activities of M33 and M42 were tested on a panel of wild-type and mutant HIV-1 virus strains to determine their in vitro antiviral activity. The 50% effective concentration values for the wild-type virus were 0.4 nM for M33, 18 nM for M42, and 0.5 nM for TMC278 (Module 2.7.2).

Metabolite Plasma Profile of TMC278 Across Species

In a pilot study, the comparative metabolite profile of TMC278 was investigated in mouse, rat, rabbit, dog, and human plasma after single and/or repeated oral administration of cold TMC278 base (Report TMC278-NC155). A modified LC-MS/MS method was used for the quantification (when the authentic substance was available) and comparative metabolite profiling, by analytical responses, of TMC278.

In mouse plasma after single dose administration of TMC278 base at 2000 mg/kg, (micronucleus test) only 1 metabolite (a metabolite with a molecular mass 18 amu higher) was detected. In rat plasma after single and repeated administration of TMC278 base, the cysteinyl glycine conjugate, the cysteine conjugate, the N-acetyl-cysteine conjugate, and a metabolite with a molecular mass 18 amu higher were detected. In rabbit plasma after repeated oral administration of TMC278, 1 metabolite with a molecular mass 18 amu higher and traces of *N*-glucuronide were detected. No metabolites were found in dog plasma. In human plasma after single and repeated administration of TMC278 base, *N*-glucuronide (probably M15), cysteinyl glycine (probably M12), and cysteine conjugates (probably M14) and a metabolite unidentified at that time (probably M27) were detected.

Presystemic Metabolism (Gastrointestinal/Hepatic First-Pass Effects)

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

2.6.4.5.2.3. Tenofovir DF/Tenofovir: Metabolism In Vivo

Tenofovir DF has been demonstrated to be metabolized to TFV by way of the monoester, tenofovir soproxil, and is anabolized intracellularly to an active diphosphorylated species (PMPApp). No metabolites other than TFV and tenofovir soproxil have been detected in vivo. Details of these experiments are summarized below.

Rats

Studies utilizing [^{14}C]TDF and TFV have been conducted in male rats (Tabulated Summaries 2.6.5.8.I and 2.6.5.12.A; 97-DDM-4331-003/4 and 96-DDM-1278-001, respectively). In each of these studies, a single oral (TDF) or IV dose (TFV) was administered that was equivalent to 10 mg/kg of TFV. In 1 experiment, following oral TDF administration, bile was collected via cannula at predose, 0 to 0.5, 0.5 to 2.2, 2.2 to 3, 3 to 4, 4 to 5, 5 to 21.5, 21.5 to 22.5, and 22.5 to 24 hours postdose. The samples were collected into acidified acetonitrile to prevent degradation of the prodrug, and analyzed by HPLC with radiometric detection. Tenofovir was the major radioactive species detected and was present in bile collected from 0.5 to 5 hours postdose. Tenofovir disoproxil accounted for 37% of the radioactivity in the 0.5 to 2.2 hour fraction and was not detected at later time points. The total percent of the dose excreted in bile was 0.12%, which was consistent with findings in other studies that biliary excretion is a minor route of elimination for this drug.

In a second experiment, stomach, intestinal tissues, and contents were analyzed 1 hour following a single oral dose of [^{14}C]TDF (Tabulated Summary 2.6.5.8.I, 97-DDM-4331-003/4). Tenofovir was detected in the stomach, duodenum, jejunum, and ileum, and tenofovir disoproxil was found in the stomach and intestinal homogenate. No other metabolites were detected.

A mass-balance study was conducted in male Sprague-Dawley rats administered a single IV 10- or 50-mg/kg dose of [^{14}C]TFV (Tabulated Summary 2.6.5.12.A, 96-DDM-1278-001). The total radioactivity recovered from urine, feces, and cage wash was 97.3% ± 6.01 % (n = 4) and 92.5 (n = 2) for the 2 dose groups, respectively. Tenofovir was the only drug-related species observed.

The potential for TDF to induce CYP450s was determined using livers from rats that had been administered daily oral doses of TDF for 28 days in an acute toxicity study (Tabulated Summary 2.6.5.11.F, R2001024). To assess induction potential, livers were collected from female Sprague-Dawley rats treated for 28 days with 40 or 400 mg/kg/day TDF or with the control vehicle (Tabulated Summary 2.6.5.11.F, R2000036). Microsomes were prepared separately from each liver (n = 6 per dose group). Phenacetin O-deethylase and testosterone 6β-hydroxylase activities were measured. CYP1A2 and CYP3A4 mediate these activities in humans. Formation of androstenedione catalyzed by CYP2B1, CYP2B2, and CYP2C11 in the rat was also measured. The activity of microsomes from treated rats was compared to the activity from rats treated with only vehicle. Activities of phenacetin O-deethylase and androstenedione formation were increased in the high-dose group compared to control and low dose groups. Mean (± SD) velocities observed for the O-deethylation of phenacetin were 0.133 ± 0.023, 0.110 ± 0.026, and 0.214 ± 0.015 nmol/min/mg protein, for the control, 40-, and 400-mg/kg dose groups, respectively. Mean (± SD) velocities observed for the formation of androstenedione were 0.562 ± 0.157, 0.536 ± 0.141, and 0.811 ± 0.142 nmol/min/mg protein, for the control, 40-, and 400-mg/kg dose groups, respectively. The 6β-hydroxylation of testosterone in treated animals was not significantly different from controls with group mean velocities of

1.29 ± 0.55, 1.35 ± 0.49, and 1.9 ± 0.54 nmol/min/mg protein. Although these activities exist in humans, the enzymes responsible may not be direct correlates of those in rats, and studies have revealed substantial species related differences in the ligand binding domains associated with CYP450 induction {2625}.

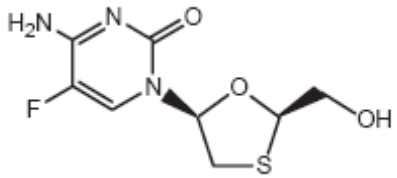
Monkeys

The kinetics of intracellular TFV anabolism in PBMCs, red blood cells (RBCs), and lymph nodes were studied in monkeys that received a single dose of either 15, 30, or 60 mg/kg of [^{14}C]TFV subcutaneously (Report P2001025). Tenofovir was efficiently taken up by PBMCs and anabolized to tenofovir diphosphate (PMPApp) with intracellular concentrations of the active antiviral anabolite reaching 1.6 μM (60 mg/kg dose group). The half-life of PMPApp in this experiment was > 50 hours. Similar concentrations of TFV anabolites were observed in RBCs. Significant intracellular concentrations of TFV and its anabolites were observed in axillary, inguinal, and mesenteric lymph nodes. This long intracellular half-life of the active diphosphate form observed both in vitro and in vivo supports the proposed once daily clinical dosing regimen.

2.6.4.5.3. Possible Metabolic Pathways for Emtricitabine, Rilpivirine, and Tenofovir DF

2.6.4.5.3.1. FTC: Possible Metabolic Pathways

The biotransformation of FTC was similar across species and the principal metabolite identified was a 3'-sulfoxide diastereomer, accounting for 2% to 11% of dose in mice, rats, and monkeys. Several other urinary metabolites were identified, none accounting for more than 2% of dose. In addition to the diastereomeric sulfoxide, they included a glucuronide conjugate and deaminated metabolites (Tabulated Summary 2.6.5.10.A).



Emtricitabine

2.6.4.5.3.2. Rilpivirine (RPV/TMC278): Possible Metabolic Pathways

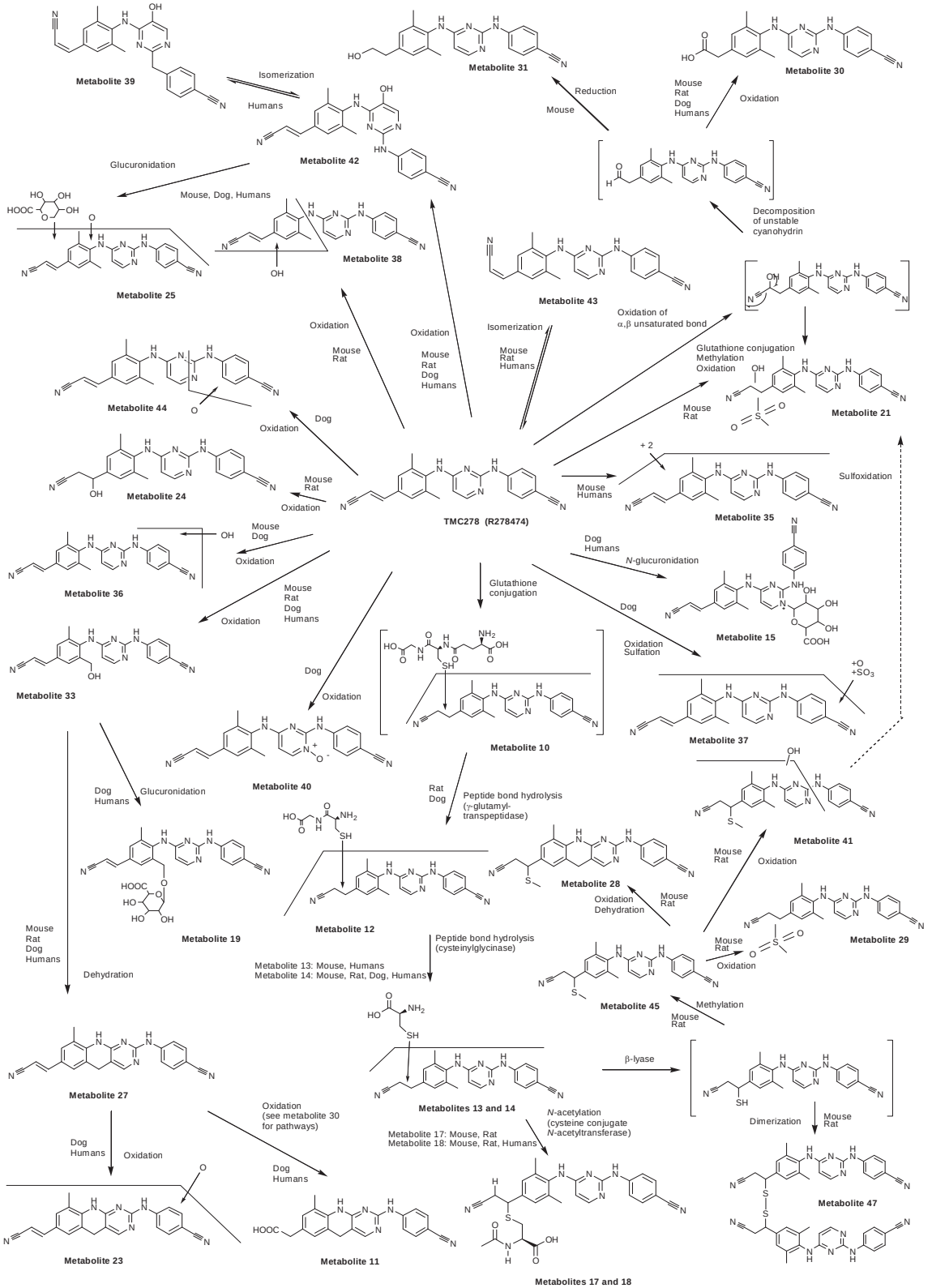
A number of TMC278 metabolites were identified in the in vivo studies in mice, rats, dogs, and humans (Tabulated Summaries 2.6.5.10.B, 2.6.5.10.C, 2.6.5.10.E, and 2.6.5.10.F; TMC278-NC190, TMC278-NC113, TMC278-NC114, and TMC278-NC157, respectively). The structures of these metabolites and the in vivo metabolic pathways are represented in Figure 10. TMC278 is metabolized via Phase I and Phase II reactions and the most important pathways are hydroxylation and glutathione conjugation. The contribution of the different metabolic pathways to the overall disposition of TMC278 is represented in Table 8.

Table 8. Total Percentage of the Administered Dose Metabolized per Major Pathways in Man and its Corresponding Percentages in Mice, Rats, and Dogs After Oral Administration of [^{14}C]TMC278

Metabolites	Mice		Rats	Dogs	Man
	20 mg/kg	320 mg/kg	40 mg/kg	5 mg/kg	150 mg
5-Hydroxyl TMC278 at the pyrimidinyl moiety (M42)	18–26 [a]	9.2–13[a]	2.8–3.6[b]	5.3	16
Hydroxymethyl of TMC278 (M33)	0.5–0.7	1.3–1.0	0.54–0.54	8.7 (traces in plasma)	3.0 (seen in plasma)
Carboxylic acid metabolite of the cyanoethenyl moiety (M30)	1.6–3.1	1.5–1.2	0.47 - 0.05	3.1[c]	2.7
Unknown (M35)	< 0.2	< 0.2	—	—	2.2
Tricyclic metabolite (M27) and carboxylic metabolite of M27 (M11)	0.3–< 0.2 [d]	< 0.2–0.1 [d]	0.99–1.60 [f]	3.1[g] (traces of M27 in plasma)	2.2 (M27 seen in plasma)
Glutathione derived conjugates (M13, M14, and M18)	9.6–7.9[e]	8.7–7.3 [e]	0.03–0.46 [i]	< 0.08 [h]	1.2
Unchanged compound	8.8–7.9	33–34	47–43	45	26
N-glucuronide of TMC278 (M15)	—	—	—	traces in plasma	0.6 (seen in plasma)

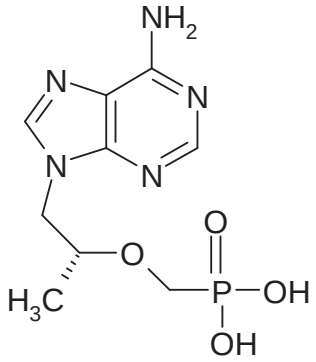
a co-eluted with M41, M42 was estimated at 13.9-16.6% (20 mg/kg) and at 5.9-8.0% (320 mg/kg)
b co-eluted with M41
c co-eluted with M48
d co-eluted with M28 and M29
e includes M1
f co-eluted with M24, M28 and M29
g including M23
h each of them
i M14 co-eluted with M12
In mice and rats, the first number is male data

Figure 10. In Vivo Metabolic Pathways of TMC278 in Animals and Humans (Excluding Rat Bile)



2.6.4.5.3.3. Tenofovir DF: Possible Metabolic Pathways

The prodrug, TDF is metabolized to tenofovir disoproxil and TFV through cleavage of the phosphoester linkages by nonspecific esterases in blood and tissues. No other metabolic pathways have been observed (Tabulated Summary 2.6.5.10.G).



Tenofovir

2.6.4.5.4. Emtricitabine/Tenofovir DF: Metabolism

Tenofovir is not a substrate or inhibitor of any CYP450 enzymes. Only a small fraction (14%) of the dose eliminated in urine is metabolized. Emtricitabine is subject to Phase 1 metabolism (oxidation to a diastereomeric sulfoxide) and to some direct conjugation (glucuronidation of hydroxymethyl group), both to a limited extent. Thus, it is unlikely when the drugs are coadministered that there will be any metabolism-mediated drug interaction. This has been confirmed in clinical studies by the detection of similar plasma concentrations of TFV and FTC after coadministration or after separate administration of the individual drugs.

2.6.4.5.5. Emtricitabine/Rilpivirine/Tenofovir DF: Metabolism

Based on the distinct routes of elimination displayed by FTC/RPV/TDF, drug-drug interactions are unlikely.

2.6.4.6. Excretion

2.6.4.6.1. Route and Extent of Excretion of Emtricitabine, Rilpivirine, and Tenofovir DF

2.6.4.6.1.1. Emtricitabine: Mouse

Emtricitabine excretion data in mice were obtained from study TEIN/93/0015 and are described in Section 2.6.4.5.2.1.

2.6.4.6.1.2. Emtricitabine: Rat

Emtricitabine excretion data in rats were obtained from study TOX092 and are described in Section 2.6.4.4.2.2.

2.6.4.6.1.3. Emtricitabine: Monkey

Emtricitabine excretion data in monkeys were obtained from study TOX063 and are described in Section 2.6.4.5.2.1.

2.6.4.6.1.4. Rilpivirine: Mouse, Rat, Dog, and Human

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, in Sprague Dawley rats at 40 mg/kg, and in male beagle dogs at 5 mg/kg (Tabulated Summaries 2.6.5.10.B, 2.6.5.10.C, 2.6.5.10.D, and 2.6.5.10.E; TMC278-NC190, TMC278-NC113, TMC278-NC145, and TMC278-NC114, respectively). Healthy male subjects were dosed orally with 150 mg of [^{14}C]TMC278 base (Tabulated Summary 2.6.5.10.F, TMC278-NC157). 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). Total radioactivity was measured by scintillation counting.

In rodents, the total radioactivity 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 total radioactivity was eliminated in feces as unchanged TMC278 in mice (33% to 34% at 320 mg/kg), in rats (43% to 47%), and in dogs (43%) at 48 hours after dosing. Only in mice at 20 mg/kg, 1 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 9). 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. Eighty-five percent 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 total radioactivity 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 9. 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[a]				Rat[b]		Dog[c]	Human[d]
	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[e]**

a Study TMC278-TiDP6-NC190
b Study TMC278-NC113
c Study TMC278-NC114
d Study TMC278-TiDP6-NC157
e Expressed as percent of the administered dose in the 0-168h urine and 0-336h feces

2.6.4.6.1.5. Tenofovir: Rat, Intravenous

The route and effect of dose on excretion of [^{14}C]TFV was evaluated in Sprague-Dawley rats following IV administration (Tabulated Summary 2.6.5.12.A, 96-DDM-1278-001). Four male rats (Group 1) received a single IV injection of [^{14}C]TFV at 10 mg/kg (400 μCi/kg) and 2 male rats (Group 2) received a single IV injection of [^{14}C]TFV at 50 mg/kg (400 μCi/kg). Following IV dosing at 10 mg/kg, the mean (± SD) cumulative recovery in the urine/cage wash was 85.2% ± 7.63 % by 24 hours and 92.7% ± 6.77 % by 7-days postdose (N = 4). The mean (± SD) terminal elimination half-life from urine data was 15.82 ± 1.79 hours (N = 4). The mean (± SD) recovery of the administered dose in the feces was 3.18% ± 1.85% by 24 hours and 4.48% ± 1.89% by 7-days postdose (N = 4). Following IV dosing at 50 mg/kg, the mean (± SD) cumulative recovery of the administered dose in urine/cage wash was 77.5% ± 4.15% by 24 hours and 84.0% ± 4.60 % by 7-days postdose. The mean (± SD)

terminal half-life from urine data was 20.38 ± 0 hours (N = 4). The corresponding mean (± SD) recovery of the administered dose in the feces was 7.39% ± 0.56% by 24 hours and 8.46% ± 0.01% by 7-days postdose. Tenofovir was the only species present in the urine and feces; no metabolites were detected. These results indicate that TFV is primarily excreted by renal clearance of the unchanged drug. In addition, renal clearance values of 1580 and 782 mL/h/kg for the 10- and 50-mg/kg cohorts, respectively, were calculated based on AUC values from a single dose IV study (Tabulated Summary 2.6.5.5.F, R2000075). These values are 2 to 4 times the glomerular filtration rate in rats (314 mL/h/kg) and suggest active renal secretion of TFV.

2.6.4.6.1.6. Tenofovir DF/Tenofovir: Dog, Oral/Intravenous

The routes of excretion were evaluated in beagle dogs following oral administration of [^{14}C]-radio-labeled TDF. Dogs received a single dose of [^{14}C]TDF (10 mg/kg; 25 µCi/kg) by oral gavage (Tabulated Summary 2.6.5.5.E, 97-DDM-4331-001). Animals were housed in metabolism cages and urine and feces were cage-collected through 24 hours at which time the animals were euthanized and tissues were collected. Total recovery of the dose at 24 hours was > 90% (24% to 44% in feces, 4% to 24% GI contents, 24% in urine, 1% to 4% in cage wash, and 15% to 20% in tissues). The amount of dose recovered in urine and the cage wash (25% to 28%) combined with the amount remaining in tissues of (15% to 20%) were consistent with an oral bioavailability of 31% to 44% measured from a previous study (Tabulated Summary 2.6.5.3.P, D2000076). These data are consistent with renal elimination as the primary path of excretion of TFV following absorption of TDF and metabolism to TFV.

Tenofovir excretion was evaluated following IV administration (Tabulated Summary 2.6.5.12.C, 96-DDM-1278-002). The primary route of elimination was via the kidneys, as 70.03% of the total ^{14}C dose was recovered in the urine during the first 48 hours following dosing. Total fecal ^{14}C recovery was 0.42% of the total ^{14}C dose.

2.6.4.6.2. Excretion into Bile

2.6.4.6.2.1. Tenofovir DF/Tenofovir: Excretion into Bile

The extent of biliary excretion of [^{14}C]TFV following a single IV administration to a beagle dog was evaluated over 48 hours (Tabulated Summary 2.6.5.12.C, 96-DDM-1278-002). The study consisted of 1 male beagle dog, surgically cannulated to permit the continuous collection of bile via the common bile duct. The animal received a single IV administration of [^{14}C]TFV at a dose level of 10 mg/kg (50 mCi/kg). Total biliary recovery of TFV through 48 hours was 0.26% of the total dose.

The extent of biliary excretion of TFV following oral administration of TDF in a single male Sprague-Dawley rat was evaluated over 24 hours (97-DDM-4331-003/4). Bile fluids were collected from a bile duct catheterized male rat following oral administration of TDF at a dose of 10 mg-equivalent of TFV /kg (containing 25 µCi [^{14}C]TDF/kg). The total percentage dose excreted in the bile was 0.12%.

2.6.4.6.3. Excretion into Milk

2.6.4.6.3.1. Rilpivirine: Excretion into 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 2.6.4.4.2.5), some radioactivity was seen in the mammary glands (tissue/blood $AUC_{0–8h}$ ratio = 3), which indicates the potential for excretion of TMC278-related radioactivity via the milk.

In a dose range-finding study for a prenatal and postnatal developmental study (Tabulated Summary 2.6.5.4.O, TMC278-NC168), 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_{0-24h}) 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 2.6.4.3.4.5).

2.6.4.6.3.2. Tenofovir DF/Tenofovir: Excretion into Milk

Rats

Milk samples from a developmental and perinatal/postnatal reproduction toxicity study of TDF in female Sprague-Dawley rats (Tabulated Summary 2.6.5.7.F, R990202-PK) were analyzed for TFV. The samples were collected at approximately 1 hour postdose on Day 11 postpartum. The dose of TDF administered was 50, 150, 450, or 600 mg/kg/day. Tenofovir concentrations in milk represented 13.5%, 11.0%, 20.8%, and 23.5% of the TFV plasma concentration at similar times. Although there was a trend towards increasing percent in milk with increasing dose, the data is inconclusive due to the comparison of results from different rats and the different numbers of observations between plasma data (n = 3 to 4) and milk (n = 6 to 11). The results indicated that TFV was excreted but not concentrated in milk upon multiple dosing in lactating rats.

Monkeys

Milk was also obtained from 2 lactating adult female rhesus monkeys following a single 30 mg/kg subcutaneous dose of TFV (Tabulated Summary 2.6.5.7.I, P2000116). Both milk and serum samples were collected over time up to 24 hours postdose. Concentrations of TFV in milk reached an apparent maximum at 4 hours for 1 animal and at 1 hour in the second. Tenofovir C_{max} in milk was 4.04% and 2.02% of the observed Cmax in plasma, for the 2 animals, respectively, and declined with apparent half-lives of 10.3 and 10.9 hours. The tenofovir AUC(0-∞) in milk was 18.6% and 21.5% of the observed $AUC_{(0-\infty)}$ in plasma, for the 2 animals, respectively.

2.6.4.6.4. Emtricitabine/Tenofovir DF: Excretion

Both TFV and FTC are primarily excreted by the renal pathway and the clearance of both of these molecules is by glomerular filtration and active tubular secretion. Clinical studies comparing renal elimination of TFV with agents primarily eliminated by the renal pathway (such as adefovir) have resulted in no change in renal clearance of the drug. In light of all these data, it is unlikely that there would be an interaction affecting elimination for the combination product.

2.6.4.6.5. Emtricitabine/Rilpivirine/Tenofovir DF: Excretion

No formal study has been conducted to evaluate the excretion of these drugs when administered in combination. Based on the available data for each compound, the excretion profiles are unlikely to be altered in combination.

2.6.4.7. Pharmacokinetic Drug Interactions

2.6.4.7.1. Emtricitabine: Pharmacokinetic Drug Interactions

No drug interaction studies were performed with FTC. However, the fact that FTC is primarily eliminated in urine as unchanged drug with very little metabolism, especially very minor Phase 1 oxidative metabolism, suggests that this drug presents a low potential for pharmacokinetic drug interactions at the level of hepatic or other organ metabolism in humans (see Section 2.6.4.5.2.1).

2.6.4.7.2. Rilpivirine: 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 (Tabulated Summary 2.6.5.14.A, TMC278-NC194).

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) these results are compared to the clinical data.

2.6.4.7.3. Tenofovir DF: Pharmacokinetic Drug Interactions

Coadministration of high doses of TDF (300 mg/kg/day) and adefovir dipivoxil (ADV) (40 mg/kg/day) to rats for 1 or 6 days reduced plasma adefovir levels (TDF levels not determined [Tabulated Summary 2.6.5.14]). Coadministration of clinically relevant doses of TDF (5 mg/kg/day) and ADV (5 mg/kg/day) did not alter biodistribution of either compound (Report R2000096). No other nonclinical pharmacokinetic drug interaction studies have been performed with TDF. Tenofovir has not been shown to be metabolized in any species tested and has been shown to have no significant interaction with the major P450 enzymes from humans in vitro or rats in vivo. Therefore, the potential for adverse interactions with other drugs used clinically is anticipated to be low.

The route of elimination of TFV is renal excretion by a combination of glomerular filtration and tubular secretion. In order to understand the role of transporters in the renal secretion of TFV and to explore potential drug interactions based on these transport systems, the interactions of TFV with a variety of both uptake and efflux transporters were studied in vitro.

Results of in vitro transport studies indicate that the active tubular secretion of TFV is mediated by hOAT1 and MRP4 acting in series as the major uptake and efflux transporters in proximal tubules, respectively (Reports PC-103-2001, AD-104-2001, AD-104-2002), {2520}, {7299}, {8418}. Human organic anion transporter type 3 may play a secondary role in the tubular uptake of TFV. Neither MDR1 nor MRP2 appear to be involved in the tubular efflux of TFV. As the primary transporter handling the tubular uptake of TFV, hOAT1 has been assessed for its potential role in drug interactions between TFV and other renally secreted therapeutics including antibiotics, anti-inflammatory agents, and other antivirals (including PIs). Under physiologically relevant conditions, none of the tested drugs affected hOAT1-mediated transport of TFV, indicating a low potential for renal interactions with TFV due to inhibition of this pathway (Reports PC-104-2010 and PC-104-2011), {2520}. Similarly, PIs atazanavir, lopinavir, and ritonavir did not exhibit any effect on the active cellular elimination of TFV mediated by MRP4 efflux pump {8418}. The results of in vitro drug interaction studies indicate that PIs are unlikely to exert any substantial effect on the accumulation of TFV in renal proximal tubules with consequential changes in the renal safety profile of TDF.

The results from in vitro studies investigating the contribution from MRP1 in tubular reabsorption of TFV (Report PC-104-2014) indicated that MRP1 is not involved in the reabsorption of TFV at the basolateral membrane of proximal tubule cells.

After the lack of interaction between P-gp and TFV was established, efforts continued to assess the potential transport of TDF by P-gp. In vitro studies have shown that the intestinal

absorption of the oral prodrug of TFV (TDF) is limited by a combination of P-gp-mediated efflux transport and esterase degradation {5939}. Further studies in human intestinal S9 fractions, the human colon carcinoma cell line Caco-2, and Madin-Darby canine kidney (MDCKII) cells stably transfected with the human gene that encodes P-gp have suggested that the relative ability of PIs to inhibit esterase activity and inhibit or induce intestinal P-gp may account for the modest changes in plasma TFV levels when TDF is coadministered with some PIs (Tabulated Summary 2.6.5.14.J, AD-104-2010), {11255}.

2.6.4.7.4. Emtricitabine/Tenofovir DF: Pharmacokinetic Drug Interactions

In vitro protein binding studies with the individual agents suggest that drug interactions due to altered protein binding are unlikely to occur when these agents are used in combination. Based on metabolism data available for the individual agents, it is unlikely when the 2 drugs are coadministered that there will be any metabolism-mediated drug interaction.

The findings of in vitro pharmacodynamic investigations suggest a low potential for intracellular drug antagonism between TDF or FTC and other antiretroviral compounds (see Module 2.6.2).

With the exception of the interaction of TDF with didanosine and atazanavir (without ritonavir), no clinically significant drug interactions that involve either TDF or FTC have been identified with a range of antiretrovirals and other medications tested to date.

Overall, the body of evidence supports a low potential for clinically relevant drug interactions between FTC and TDF and other medications frequently used by the HIV population (see Modules 2.5 and 2.7.2).

2.6.4.7.5. Emtricitabine/Rilpivirine/Tenofovir DF: Pharmacokinetic Drug Interactions

For the FTC/RPV/TDF FDC, the clinical PK interaction studies with the components should be taken into consideration. Full details are described in Modules 2.5 and 2.7.2. The conduct of additional preclinical studies to evaluate potential pharmacokinetic drug interactions is unlikely to inform clinical use and was therefore considered unwarranted.

2.6.4.8. Other Pharmacokinetic Studies

2.6.4.8.1. Emtricitabine/Rilpivirine/Tenofovir DF

There are no additional studies to report under this heading.

2.6.4.9. Discussion and Conclusions

Emtricitabine, RPV (TMC278), and TDF have been assessed individually in comprehensive nonclinical studies.

Results from pharmacokinetic studies of FTC, RPV (TMC278), TDF, and the FDCs of FTC/TDF and FTC/RPV/TDF are summarized below.

Emtricitabine (FTC)

Emtricitabine was rapidly and extensively absorbed after oral administration in mice, rats, and cynomolgus monkeys, with oral bioavailability ranging from 58% to 97% over the dose range of 10 to 600 mg/kg. Maximum plasma concentrations observed after oral dosing ranged from 2.4 to 139 mg/mL, and were generally 6- to 10-fold lower than the peak concentrations after comparable IV doses. Peak plasma concentrations (C_{max}) and AUC increased nearly dose proportionally over the 10- to 600-mg/kg dose range. There was a trend towards slower and less extensive absorption at the highest doses; however, these doses represented large multiples of the proposed human dose (approximately 2–4 mg/kg), and therefore absorption of FTC may be expected to be rapid and complete in humans over the clinical dose range. Since FTC was not extensively metabolized in these species, its bioavailability is likely to be governed only by its absorption, with little or no first-pass metabolism in the gut wall or liver. A summary of the pharmacokinetic parameters obtained in these studies is presented in Table 10 and Table 11.

The tissue distribution of FTC has been characterized in mice, rats, and cynomolgus monkeys after single oral and IV doses ranging from 10 mg/kg to 600 mg/kg, and in studies of orally administered [^{14}C]FTC (200 mg/kg) in rats and monkeys.

Protein binding studies of FTC in mouse, rabbit, monkey, and human plasma using equilibrium dialysis method showed that FTC has little or no measurable reversible binding to plasma proteins at concentrations between 0.02 and 200 mg/mL. Plasma protein binding did not exceed 3.6% in any species or at any concentration. Thus, the fraction unbound in plasma is > 96% for FTC in all species. The observed volume of distribution for FTC (~ 0.9 L/kg) suggests that FTC may be somewhat more extensively bound to tissue proteins than to plasma proteins. The low level of protein binding for FTC suggests that drug interactions due to altered protein binding will not occur for this drug.

Emtricitabine was widely distributed in the body, with measurable concentrations (radioactivity) found in all tissues after administration. Following single oral and IV doses of FTC to mice, rats, and cynomolgus monkeys, the Vd_{ss} were similar across species (mice 0.9–1.1 L/kg; rats 1.2–1.5 L/kg; and monkeys 0.8 L/kg), showing little if any dose dependence over a wide range of doses (10–600 mg/kg). These volumes of distribution are slightly larger than total body water in all species, suggesting that FTC is distributed to both the intracellular and extracellular fluid spaces. After oral administration, the highest concentrations are found in the gut and kidneys, consistent with its absorption and elimination via these tissues. Levels in CNS tissues reach ~ 2% to 9% of those in plasma.

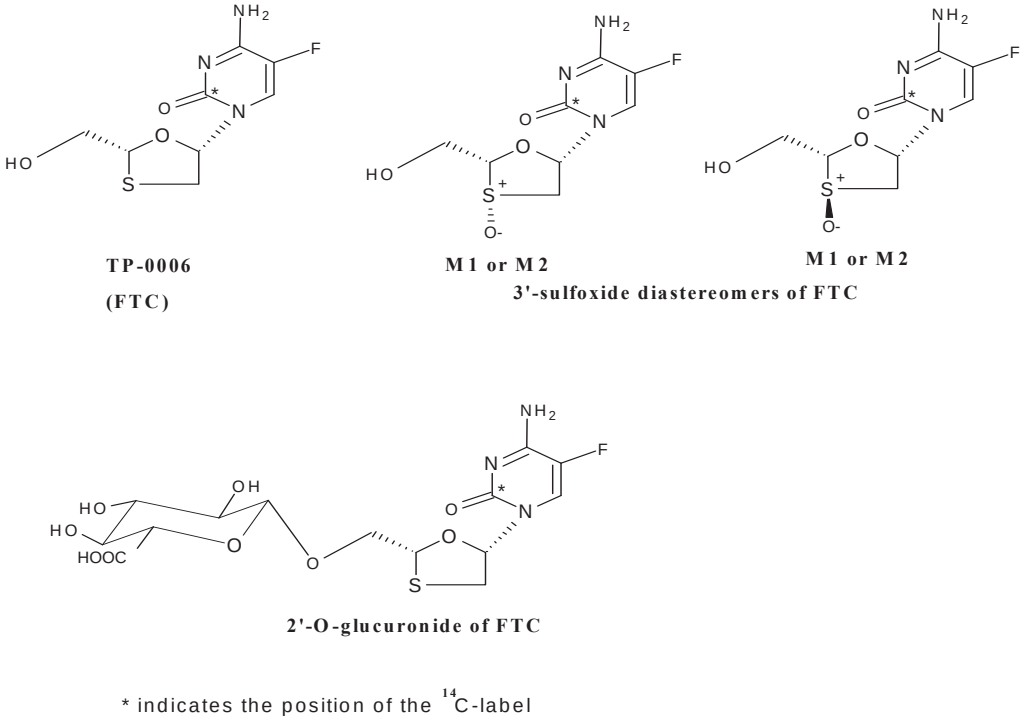
The metabolism of FTC was studied in mice, rats, and cynomolgus monkeys after a single oral or IV administration of [^{3}H]FTC at doses of 10 to 120 mg/kg, and in rats and monkeys after a single oral administration of [^{14}C]FTC at 200 mg/kg. In all 3 species, metabolism accounted for only a minor percentage of FTC elimination. Only trace levels of metabolites were found in feces. Over 90% of the radioactivity in mouse and rat urine, and 64% of the radioactivity in monkey urine was unchanged drug. The principal metabolite was a 3'-sulfoxide, accounting for approximately 2% of the dose in mice, 2.6% in rats, and 6% to

11% in monkeys. Seven other metabolites were detected in the urine of rats and 2 others in cynomolgus monkeys, none accounting for more than 2% of the dose. These minor metabolites may include 5-fluorocytosine, a diastereomeric 3'-sulfoxide, a glucuronide conjugate, and deaminated metabolites. No 5-fluorouracil was detected in urine or feces after FTC administration. In contrast to other nucleoside analogues, FTC is not extensively metabolized and is eliminated primarily as unchanged drug by renal excretion. Based on these observations, it is unlikely that FTC will be subject to significant first-pass metabolism, or to changes in clearance due to hepatic disease or metabolic drug interactions.

The biotransformation of FTC in humans is similar to that in monkeys. The same 3 putative metabolites were isolated as shown in Figure 11 for monkeys, with structures tentatively identified by mass spectrometry. Metabolism in humans includes oxidation of the thiol moiety (Phase 1 metabolism) to form the 3'-sulfoxide diastereomers (designated as metabolites M1 and M2 in Figure 11) and conjugation with glucuronic acid (Phase 2 metabolism) to form the 2'-O-glucuronide (designated as metabolite M3 in Figure 11). These metabolites were generally present in low levels, or were not detectable, and were only quantifiable in urine samples. Metabolite M2 was the predominant metabolite of the 3, with its urinary recovery accounting for 8.7% of the dose administered. M2 was sporadically quantifiable in plasma samples of 2 out of 5 subjects and when measurable, plasma M2 concentrations were 30- to 50-fold lower than plasma FTC concentrations at corresponding time points. Urinary recoveries of metabolites M1 and M3 accounted for only 0.3% and 4% of the dose administered, respectively. These 3 metabolites along with unchanged FTC in urine accounted for essentially the entire dose recovered in urine (~ 86%).

The excretion of FTC has been characterized after single dose oral and IV administration in mice, rats, and cynomolgus monkeys. Absorbed (systemic) FTC is excreted primarily into the urine as unchanged drug. Fecal recovery observed after oral dosing most likely represents unabsorbed drug. Emtricitabine metabolites are also excreted almost entirely into the urine, with only trace levels detected in feces. Since FTC is largely excreted unchanged and does not bind significantly to plasma proteins, the renal clearance of FTC should be similar to its total body clearance. Emtricitabine clearances generally exceeded the GFR and approached the renal plasma flow, suggesting that the kidneys not only filter, but also actively secrete FTC into the urine, a phenomenon observed with other pyrimidine nucleosides {4570}.

Figure 11. Structure of Emtricitabine (TP-0006, FTC) and the Proposed Structures for Putative Metabolites M1, M2, and M3, from Metabolism in Monkey (TOX063)



Rilpivirine (RPV/TMC278)

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 the 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 citric acid 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 citric acid

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 the 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 monkeys, after repeated administration of the HCl salt, exposure had a tendency to increase, but due to the high interindividual 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, interindividual variability was high especially after administration of the HCl salt.

In rats, tissue distribution of [^{14}C]TMC278 and its metabolites after a 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. Total radioactivity 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, total radioactivity 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 total radioactivity eliminated was unchanged TMC278. Renal excretion of total radioactivity 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 was 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, as 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. P-gp 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-naive patients is 25 mg once daily. At this dose level, mean C_{max} (Week 4 to 8) was 0.13 µg/mL and mean $AUC_{0\text{-}24h}$ (Week 48; population pharmacokinetics) was 2.4 µg.h/mL (Clinical Study TMC278-TiDP6-

C209 and TMC278-TiDP6-C215 (Module 2.7.2, Sections 2.7.2.2.2.6.4 and 2.7.2.2.2.6.5). 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.

Tenofovir DF (TDF)

A comparison of TFV pharmacokinetics following single-dose administration of TDF in Sprague-Dawley rats, beagle dogs, rhesus monkeys, and HIV-infected humans is shown in Table 12. In general the overall pharmacokinetic behavior was similar between species with rapid absorption and conversion to TFV. Maximum TFV plasma concentrations were reached generally within 0.25 to 1.5 hours followed by a biphasic decline. Relative to administration in the fasted state, the bioavailability of TFV is enhanced when TDF is taken with a high fat meal, but is unaffected when TDF is administered with a light meal. Neither rhesus monkeys nor dogs showed any substantial changes in pharmacokinetics of TFV with food. Clearance/F values showed the greatest difference between species indicating differences in either absorption or clearance with rat > monkey > dog ≅ humans (Figure 12). A similar relationship was observed following IV administration of TFV suggesting that the main difference is in clearance rates between species (Table 13). The terminal half-life of TFV in dogs following oral administration of TDF of approximately 60 hours is substantially greater than observed in rats (7 hours), monkeys (9 hours), or humans (13 hours) and results in substantial accumulation upon daily repeat dosing in dogs (Figure 12). Excretion appears similar across species, with renal clearance of TFV as the primary route of drug elimination from plasma.

Figure 12. Comparison of Tenofovir CL/F Between Species Following Oral Administration of TDF

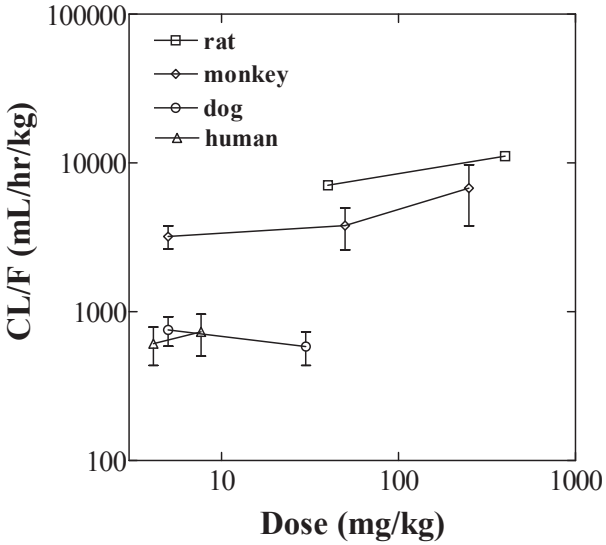
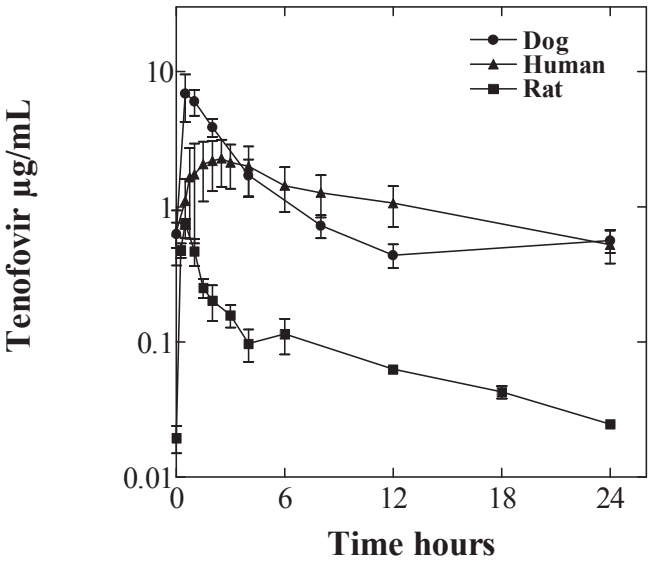


Figure 13. Comparison of Tenofovir Plasma Concentrations at Steady State Between Species Following Oral Administration of Tenofovir DF (dose normalized to 30 mg/kg, mean ± SD)



Human 3.61 mg/kg n = 8 Day 28 (901) Fed
Dog 30 mg/kg n= 4M & 4 F Week 13
Rat 40 mg/kg n= 18M, 3 per time point Day 28

Plasma pharmacokinetics of TFV following oral administration of TDF have been evaluated in mice, rats, woodchucks, dogs, and monkeys. Rapid absorption and conversion of TDF to TFV was observed in all species tested. Oral bioavailability ranged from 10% to 20% in rats and 30% to 40% in monkeys and dogs.

Dose proportional pharmacokinetics of TFV were observed following oral administration of TDF at doses of up to 100 mg/kg. Repeat dose studies at these doses demonstrated similar pharmacokinetic parameters to single-dose studies.

Following oral administration of TDF, extensive tissue distribution of TFV was observed in all species with the liver and kidney as the major sites of distribution in dogs.

TDF is metabolized via nonspecific esterases, as well as chemical hydrolysis at pH 7.4, to TFV via the monoester (tenofovir soproxil). No other metabolites have been observed in vitro or in vivo. Tenofovir is anabolized intracellularly to the active species, tenofovir diphosphate which was found to have an intracellular half-life of > 50 hours in PBMCs of rhesus monkeys.

Tenofovir was excreted unchanged in the urine of all animal species tested and renal excretion was identified as the primary route of elimination by a combination of glomerular filtration and tubular secretion. In vitro transport studies indicate that the active tubular secretion of TFV in humans is mediated by hOAT1 and MRP4 acting in series as the major uptake and efflux transporters in proximal tubules, respectively. Human organic anion transporter type 3 may play a secondary role in the tubular uptake of TFV. MDR1, MRP1, and MRP2 do not appear to be involved in the tubular efflux of TFV. As the primary transporter handling the tubular uptake of TFV, hOAT1 has been assessed for its potential role in drug interactions between TFV and other renally secreted therapeutics including antibiotics, anti-inflammatory agents, and other antivirals (including PIs). Under physiologically relevant conditions, a number of renally excreted drugs showed no effect in vitro on the hOAT1-mediated transport of TFV. Similarly, PIs did not exhibit any effect on the in vitro active cellular elimination of TFV mediated by MRP4 efflux pump, indicating that PIs are unlikely to exert any substantial effect on the accumulation of TFV in renal proximal tubules.

In vitro studies have shown that the intestinal absorption of the oral prodrug of TFV, tenofovir disoproxil, can be slightly affected by a combination of P-gp-mediated efflux transport and esterase degradation in GI tissue. Further studies in human intestinal S9 fractions, the human colon carcinoma cell line Caco-2, and MDCKII cells stably transfected with the human gene that encodes P-gp have suggested that the relative ability of PIs to inhibit esterase activity and inhibit or induce intestinal P-gp may account for the modest changes in plasma TFV levels when TDF is coadministered in humans with some PIs.

Emtricitabine/Tenofovir DF

The absorption kinetics of the FTC/TDF combination have not been evaluated after single or multiple doses in any preclinical studies because clinical studies have shown absorption characteristics to be unaffected when the 2 actives are dosed together. After single and multiple doses of the combined drugs in clinical studies, the time to maximum drug concentration and the maximum drug concentration in plasma are similar to the parameters observed when these drugs are administered separately.

Preclinical studies to evaluate the distribution kinetics of the FTC/TDF combination have not been conducted as both TFV and FTC exhibit extremely low protein binding to plasma proteins of < 0.7% and < 4%, respectively. Interaction, in terms of changes in the distribution profile when the drugs are coadministered, seems unlikely. This conclusion has been confirmed in clinical trials when the actives are coadministered, and the apparent volumes of distribution for TFV and FTC are unaltered relative to the estimates when administered separately.

Tenofovir is not a substrate or inhibitor of any CYP450 enzymes. Only a small fraction (14%) of the dose eliminated in urine is metabolized. Emtricitabine is subject to Phase 1 metabolism (oxidation to a diastereomeric sulfoxide) and to some direct conjugation (glucuronidation of hydroxymethyl group), both to a limited extent. Thus, it is unlikely when the drugs are coadministered that there will be any metabolism-mediated drug interaction. Again, this has been confirmed in clinical studies by the detection of similar plasma concentrations of TFV and FTC after coadministration or after separate administration of the individual drugs.

Both TFV and FTC are primarily excreted by the renal pathway and the clearance of both these molecules is by glomerular filtration and active tubular secretion. Clinical studies comparing renal elimination of TFV with agents primarily eliminated by the renal pathway (such as adefovir) have resulted in no change in renal clearance of the drug. In light of all these data, it is unlikely that there would be an interaction affecting elimination for the FTC/TDF tablet.

In conclusion, in view of the results of extensive nonclinical and clinical pharmacokinetic studies of FTC and TDF, and clinical experience with these agents, no additional nonclinical studies are warranted with the FTC/TDF tablet.

Emtricitabine/Rilpivirine/Tenofovir DF

Based on the data supporting the individual components, the extensive clinical data with the FTC/TDF combination within HIV-1 therapy, and the clinical data with RPV administered with FTC/TDF in Phase 2 and 3 studies, adverse pharmacokinetic interactions that would negatively affect pharmacological efficacy are not anticipated. This assumption is based on the discrete routes of absorption and elimination demonstrated for each compound and the differences in physicochemical properties between the compounds which influence drug distribution. Single dose pharmacokinetic studies in dogs demonstrate that comparable

exposures for each component can be achieved through co-formulation in a bilayer tablet relative to the clinical formulations administered separately. More compelling support for this assumption may be derived from the human clinical data.

2.6.4.10. Summary Tables

Table 10. Summary of Pharmacokinetics Parameters for Emtricitabine After Single Dose Administration

Study	Species/ Strain	Dose mg/kg	Route	$AUC_{0-\infty}$		F	C_{max}		T_{max}	MRT	MAT	$t_{1/2}$ (minutes)		Cl	Vd_{ss}
				µM•h	µg •h/ mL	%	µM	µg/mL	min	hours	hours	Absorption	Distribution/ Elimination	L/kg/h	L/kg
TEIN/93/0003	Mouse/ CD-1	10	iv	17.4	4.3*	—	—	—	—	0.38	—	—	4 / 23	2.33	0.89
TEIN/93/0003	Mouse/ CD-1	10	Oral	16.7	4.1*	96	9.8	2.4*	25.4	—	—	14	16 / 137	—	—
TEIN/93/0004	Mouse/ CD-1	100	iv	181	44.7*	—	—	—	—	0.42	—	ND	1.7 / 15.5 / 82	2.23	0.94
TEIN/93/0004	Mouse/ CD-1	100	Oral	143	35.3*	79	89	22*	24.5	—	—	10.4	26 / 120	—	—
IUW00101	Mouse/ CD-1	600	iv	—	473	—	—	1560	—	0.86	—	—	248	1.28	1.1
IUW00101	Mouse/ CD-1	600	Oral	—	296	62.7	—	139	40	2.90	2.04	—	190	—	—
TEZZ/93/0019	Monkey/ Cynomolgus	10	iv	59.9	14.8*	—	129	—	—	1.2	—	—	3.6 / 60	0.7	0.8
TEZZ/93/0019	Monkey/ Cynomolgus	10	Oral	37.4	9.2*	62.7	14.1	3.5*	78	—	—	—	—	1.1	—
TEZZ/93/0019	Monkey/ Cynomolgus	80	iv	493	122*	—	1093	—	—	1.1	—	—	3.6 / 61	0.7	0.8
TEZZ/93/0019	Monkey/ Cynomolgus	80	Oral	285	70.4	57.5	111	—	138	—	—	—	—	1.2	—
IUW00301	Monkey/ Cynomolgus	80	iv	—	86.1	—	—	238	–	0.802	—	—	46.5	0.97	0.77
IUW00301	Monkey/ Cynomolgus	80	Oral	—	83.6	97.4		39.4	53	1.75	0.953	—	56.2	—	—
TOX063	Monkey/ Cynomolgus	200	Oral	—	133	—	—	46.7	60	—	—	—	443	1.57	—

All values are mean values $AUC_{0-\infty}$ = area under the plasma concentration-time curve, extrapolated from time zero to infinity;
* = µg/mL values calculated from (µM x 0.2472 = µg/mL) F = Bioavailability MRT = mean residence time
MAT = mean absorption time Vdss = steady state volume of distribution — = not determined / not applicable

Table 11. Disposition of Radioactivity after Oral Single Dose Administration of Radiolabeled Emtricitabine in Animals

Study	Species / Strain	Dose (mg/kg) / Radiolabel	Plasma			Recovery (% dose)			Metabolites (% dose)		Urinary Metabolites (tentative identification) as % of Recovered Dose	Tissues With Highest Radioactivity Exposures
			C_{max} (µg/ml)	T_{max} (hours)	$t_{1/2}$ (hours)	Total *	Urine	Feces	Urine	Feces		
TEIN/93/ 0015	Mouse/ CD-1	120 ^{3}H	—	—	—	85 (72 h)	66.8	18.1	9	< 1	- 64% unchanged drug - 5-fluorocytosine1.4% - two 3′-sulfoxides 1.7% and 2% 5-fluorouracil not found (< LOD 0.1 %)	—
TOX092	Rat/SD	200 ^{14}C	51.9	1.0	2.7	98.6	79.3 (73.3% parent drug)	18.4 (17.7% parent drug)	7	4	—	Gut, gut contents, kidney, liver, bladder (lowest in CNS)
TOX092	Rat/LE	200 ^{14}C	59.2	1.0	3.4	ND	ND	ND	ND	ND	—	—
TEIN/93/ 0016	Monkey/ Cynomolgus	80 ^{3}H	—	—	—	84 (72 h)	41	33	36	2	- 64% unchanged drug - 11% 3′-sulfoxide - 1.7% other metabolites (5-fluorocytosine, 2′-glucuronidated and deaminated metabolites) 5-fluorouracil not found, (< LOD 0.02 %)	—
TOX063	Monkey/ Cynomolgus	200 ^{14}C	46.7	1.0	7.38	84.5 (120 h)	40.8 (29.2% parent drug)	35.4 (34.4% parent drug)	11.6	1.3	- 11% total metabolites - Two 3′-sulfoxides, - 2′-O-glucuronide	Mainly gut + contents Some in kidney, liver (lowest in CNS + spinal cord)

a Includes urine, faeces, and cage washes All values presented are mean values — = not determined

Table 12. Comparison of Tenofovir Non-Compartmental Pharmacokinetic Parameters Between Species Following Single Dose Oral Administration of TDF

Species	Rat P4331-00025 R2000036-PK		Dog D2000076, 97-DDM-4331-001		Rhesus P4331-00033, P2000031			Human	
Dose (mg/kg)	**40**	**400**	**5**	**30**	**5***	**50***	**250***	**4.12***	**7.66***
N	18M (3/tp)**	21M (3/tp)**	4M	4M	3M/3F	3M/3F	3M/3F	7M/1F	9
C_{max} (µg/mL)	1.06	3.17	0.446	4.09	0.113	1.15	2.15	0.362	0.612
T_{max} (h)	0.250	0.500	1.25	0.875	0.830	1.00	0.585	2.1	1.4
$AUC_{0-\infty}$ (µg·h/mL)	2.55	16.3	3.14	24.4	0.727	6.40	19.0	3.19	5.03
$t_{1/2}\lambda z$ (h)	6.88	19.7	79.9	47.4	9.27	9.40	8.94	13.0	12.7
CL/F (mL/h/kg)	7090	11100	757	586	3210	3810	6770	614	734
C_{last} (µg/mL)	0.016	0.235	0.0040	0.0279	0.0017	0.0118	0.0366	0.0413	0.0627
T_{last} (h)	24.0	24.0	120	120	44.0	48.0	48.0	24.1	24.0
V_z/F (mL/kg)	70400	314000	90000	41900	42200	50900	90800	11544	13303
F%	18.6	11.9	31.0	43.8	32.3	23.7	14.5	39.7	34.4

* Fed data only.

** tp = timepoint

Dog combined fed and fasted.

Human doses are mean values calculated from fixed doses of 300 and 600 mg using individual patient weights.

Table 13. Comparison of Tenofovir Non-Compartmental Pharmacokinetic Parameters Between Species Following IV Administration of Tenofovir

Species	Rat R2000075		Dog D2000076, 97-DDM-4331-001		Rhesus P4331-00033, P2000031		Human	
Dose (mg/kg)	**10**	**50**	**1**	**10**	**5**	**30**	**1**	**3**
N	4M	4M	4M	4M	3M/3F	3M/3F	8M	8M
C_{max} (µg/mL)	22.0	162	2.99	31.0	13.8	79.0	2.71	8.52
$AUC_{0-\infty}$ (µg·h/mL)	5.86	53.7	4.48	41.2	5.12	38.4	4.41	15.2
$t_{1/2}\lambda z$ (h)	4.02	5.41	45.3	38.6	5.37	8.79	5.27	7.80
CL (mL/h/kg)	1706	931	226	244	1031	888	236	229
C_{last} (µg/mL)	0.0309	0.0428	0.0017	0.0103	0.0042	0.0062	0.0426	0.0508
T_{last} (h)	12.0	24.0	120	120	24.0	38.0	12.0	24.0
V_{ss} (mL/kg)	2810	1120	3090	2510	1280	969	763	975

2.6.4.10. References

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SECTION 2.6 NONCLINICAL SUMMARY

Section 2.6.5 Pharmacokinetics Tabulated Summaries

EMTRICITABINE/ RILPIVIRINE/ TENOFOVIR DISOPROXIL FUMARATE FIXED-DOSE COMBINATION

03 August 2010

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2.6. NONCLINICAL SUMMARY

2.6.5. PHARMACOKINETICS TABULATED SUMMARIES

Type of Study	Test System	Method of Administration	Testing Facility	Study Number
2.6.5.1. Pharmacokinetics				
Absorption After a Single Dose (FTC)				
Absorption, bioavailability	Mouse / CD-1	Oral and IV	Burroughs Wellcome Co., Research Triangle Park, NC 27709 USA	TEIN/93/0003[B]
Absorption, bioavailability	Mouse / CD-1	Oral and IV	Burroughs Wellcome Co., Research Triangle Park, NC 27709 USA	TEIN/93/0004[B]
Absorption, bioavailability	Mouse / CD-1	Oral and IV	[redacted]	IUW00101[B]
Absorption, bioavialability	Monkey / Cynomolgus	Oral and IV	[redacted] USA	TEZZ/93/0019[B]
Absorption, bioavialability	Monkey / Cynomolgus	Oral and IV	[redacted] UK	IUW00301

A = Study includes tenofovir as test article
B = Report contains a GLP Compliance Statement
CA: citric acid; CYP: cytochrome P450; GST: glutathione S-transferase; HPMC: hydroxyproply-methlycellulose; PEG400: polyethylene glycol 400

2.6.5.1. Pharmacokinetics

Overview

Test Article: Emtricitabine, Rilpivirine, Tenofovir, or Tenofovir disoproxil fumarate

Type of Study	Test System	Method of Administration	Testing Facility	Study Number
In Vitro Absorption (RPV)				
Drug Permeability and Transport	Caco-2 cells	In vitro	J&J PRD	R278474-JRF FK4155
Drug Permeability and Transport	Caco-2 cells	In vitro	J&J PRD	TMC278-NC104
Absorption After a Single Dose (RPV)				
Absorption	Mouse/CD-1	Oral/Gavage (base in PEG400/CA [10%])	J&J PRD	TMC278-FK4259[B]
Absorption	Rat/Sprague Dawley	Intravenous (base in PEG400/sterile water (25%) Oral/Gavage (base in PEG400 or PEG400/CA [10%])	J&J PRD	TMC278-FK4195

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B = Report contains a GLP Compliance Statement
CA: citric acid; CYP: cytochrome P450; GST: glutathione S-transferase; HPMC: hydroxyproply-methlycellulose; PEG400: polyethylene glycol 400

2.6.5.1. Pharmacokinetics

Overview

Test Article: Emtricitabine, Rilpivirine, Tenofovir, or Tenofovir disoproxil fumarate

Type of Study	Test System	Method of Administration	Testing Facility	Study Number
Absorption	Rat/Sprague Dawley	Oral/Gavage (base in PEG400; HCl and fumarate salt in Tween 20/HPMC/water)	J&J PRD	TMC278-NC106
Absorption	Rat/Sprague Dawley	Oral/Gavage (base in PEG400)	J&J PRD	TMC278-FK4278
Absorption	Rabbit/New Zealand white	Intravenous (base in PEG400/sterile water [25%])	J&J PRD	TMC278-FK4293
Absorption	Dog/beagle	Intravenous (base in PEG400/sterile water [25%]) Oral/Gavage (base in PEG400 or PEG400/CA [10%])	J&J PRD	TMC278-FK4231

A = Study includes tenofovir as test article
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CA: citric acid; CYP: cytochrome P450; GST: glutathione S-transferase; HPMC: hydroxyproply-methlycellulose; PEG400: polyethylene glycol 400

2.6.5.1. Pharmacokinetics

Overview

Test Article: Emtricitabine, Rilpivirine, Tenofovir, or Tenofovir disoproxil fumarate

Type of Study	Test System	Method of Administration	Testing Facility	Study Number
Absorption	Dog/beagle	Oral/Gavage (base in PEG400 or PEG400/CA [10%])	J&J PRD	TMC278-FK4102
Absorption	Dog/beagle	Oral/Gavage (base in PEG400/CA [10%])	J&J PRD	TMC278-NC163
Absorption	Monkey/Cynomolgus	Intravenous (base in PEG400/sterile water [25%])	J&J PRD	TMC278-DM02403
		Oral/Gavage (base in PEG400/CA [10%])		
Absorption	Monkey/Cynomolgus	Oral/Gavage (HCl salt in HPMC (1%) with Tween 20 [0.5%])	[redacted]	TMC278-NC326

A = Study includes tenofovir as test article
B = Report contains a GLP Compliance Statement
CA: citric acid; CYP: cytochrome P450; GST: glutathione S-transferase; HPMC: hydroxyproply-methlycellulose; PEG400: polyethylene glycol 400

	Type of Study	Test System	Method of Administration	Testing Facility	Study Number
Absorption After a Single Dose (TDF)					
	Absorption	Mouse CD1-l (ICR) BR	Oral gavage	[redacted], USA (In-life phase), [redacted], USA (Analysis)	97-TOX-4331-008-PK
	Absorption, bioavailability	Woodchuck	Oral gavage, IV bolus	[redacted], USA (In life phase), [redacted], Canada, (Analysis)	W2000108
A	Absorption, bioavailability	Dog, Beagle	IV Bolus	[redacted], USA (In-life phase), [redacted] Canada (Analysis)	D2000076
	Absorption, bioavailability	Monkey, Rhesus	Oral gavage, IV bolus	[redacted], USA, (In-life phase), [redacted] Canada (Analysis)	P2000031

A = Study includes tenofovir as test article
B = Report contains a GLP Compliance Statement
CA: citric acid; CYP: cytochrome P450; GST: glutathione S-transferase; HPMC: hydroxyproply-methlycellulose; PEG400: polyethylene glycol 400

2.6.5.1. Pharmacokinetics

Overview

Test Article: Emtricitabine, Rilpivirine, Tenofovir, or Tenofovir disoproxil fumarate

Type of Study	Test System	Method of Administration	Testing Facility	Study Number
Absorption After a Single Dose (FTC, RPV, TDF)				
Absorption, bioavailability	Dog/beagle	Oral	[redacted]	AD-264-2001
Absorption, bioavailability	Dog/beagle	Oral	[redacted]	AD-264-2023

A = Study includes tenofovir as test article
B = Report contains a GLP Compliance Statement
CA: citric acid; CYP: cytochrome P450; GST: glutathione S-transferase; HPMC: hydroxyproply-methlycellulose; PEG400: polyethylene glycol 400

Type of Study	Test System	Method of Administration	Testing Facility	Study Number
Absorption After a Repeated Dose (FTC)				
Absorption	Mouse, CD 1	Oral gavage	[redacted] & Gilead Sciences Inc., USA	TOX-109[B]
Absorption	Mouse, CD 1	Oral gavage	[redacted], UK	IUW00701
Absorption	Mouse, CD 1	Oral gavage	Burroughs Wellcome, USA	TOX 599
Absorption	Mouse, CD 1	Oral gavage	[redacted], UK	TOX 022
Absorption	Mouse, CD 1	Oral gavage	Burroughs Wellcome	TOX 628
Absorption	Rat, CD	Oral gavage	[redacted] & Gilead Sciences Inc., USA	TOX 108[B]
Absorption	Rat, CD	Oral gavage	[redacted] & Gilead Sciences Inc., USA	TOX 097

A = Study includes tenofovir as test article
B = Report contains a GLP Compliance Statement
CA: citric acid; CYP: cytochrome P450; GST: glutathione S-transferase; HPMC: hydroxyproply-methlycellulose; PEG400: polyethylene glycol 400

2.6.5.1. Pharmacokinetics

Overview

Test Article: Emtricitabine, Rilpivirine, Tenofovir, or Tenofovir disoproxil fumarate

Type of Study	Test System	Method of Administration	Testing Facility	Study Number
Absorption	Cynomolgus Monkeys	Oral	[redacted], USA	TOX 600
Absorption	Cynomolgus Monkeys	Oral	[redacted], USA	TOX 627
Absorption	Cynomolgus Monkeys	Oral	[redacted], USA	TOX 032
Absorption After a Repeated Dose (RPV)				
Absorption, Repeat Dose (2 weeks)	Mouse/CD-1	Oral/Gavage (HCl salt in HPMC [0.5%]) Oral/Diet (HCl salt)	J&J PRD	TMC278-NC118
Absorption, Repeat Dose (1 month)	Mouse/CB6F1-nonTgrasH2	Oral/Gavage (HCl salt in HPMC [0.5%])	[redacted]	TMC278-NC121 [B]
Absorption, Repeat Dose (3 months)	Mouse/CD-1	Oral/Gavage (HCl salt in HPMC [0.5%])	J&J PRD	TMC278-NC119 [B]

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B = Report contains a GLP Compliance Statement
CA: citric acid; CYP: cytochrome P450; GST: glutathione S-transferase; HPMC: hydroxyproply-methlycellulose; PEG400: polyethylene glycol 400

2.6.5.1. Pharmacokinetics

Overview

Test Article: Emtricitabine, Rilpivirine, Tenofovir, or Tenofovir disoproxil fumarate

Type of Study	Test System	Method of Administration	Testing Facility	Study Number
Absorption, Repeat Dose (2 years)	Mouse/CD-1	Oral/Gavage (HCl salt in HPMC [0.5%])	[redacted]	TMC278-NC120 [B]
Absorption, Repeat Dose (5 days)	Rat/Sprague Dawley	Oral/Gavage (base in PEG400)	J&J PRD	TMC278-FK4103
Absorption, Repeat Dose (2 weeks)	Rat/Sprague Dawley	Oral/Gavage (base in PEG400/CA [10%])	J&J PRD	TMC278-FK4243 [B]
Absorption, Repeat Dose (2 weeks)	Rat/Sprague Dawley	Oral/Gavage (HCl salt in HPMC [0.5%]) Oral/Diet (base and HCl salt)	J&J PRD	TMC278-NC136
Absorption, Repeat Dose (2 weeks)	Rat/Sprague Dawley	Oral/Gavage (HCl salt in HPMC [0.5%])	J&J PRD	TMC278-NC177
Absorption, Repeat Dose (1 month)	Rat/Sprague Dawley	Oral/Gavage (base in PEG400/CA [10%])	[redacted]	TMC278-TOX5692 [B]

A = Study includes tenofovir as test article
B = Report contains a GLP Compliance Statement
CA: citric acid; CYP: cytochrome P450; GST: glutathione S-transferase; HPMC: hydroxyproply-methlycellulose; PEG400: polyethylene glycol 400

2.6.5.1. Pharmacokinetics

Overview

Test Article: Emtricitabine, Rilpivirine, Tenofovir, or Tenofovir disoproxil fumarate

Type of Study	Test System	Method of Administration	Testing Facility	Study Number
Absorption, Repeat Dose (1 month)	Rat/Sprague Dawley	Oral/Gavage (base in PEG400/CA (10%), HCl salt in HPMC [0.5%])	J&J PRD	TMC278-NC117 [B]
Absorption, Repeat Dose (6 months)	Rat/Sprague Dawley	Oral/Gavage (base in PEG400/CA [10%])	J&J PRD	TMC278-NC101 [B]
Absorption, Repeat Dose (2 years)	Rat/Sprague Dawley	Oral/Gavage (HCl salt in HPMC [0.5%])	[redacted]	TMC278-NC123 [B]
Absorption, Repeat Dose (2 weeks)	Juvenile rat/Sprague Dawley	Oral/Gavage (HCl salt in HPMC [0.5%])	[redacted]	TMC278-NC168 [B]
Absorption, Repeat Dose (5 days)	Rabbit/New Zealand white	Oral/Gavage (base in HPMC [0.5%])	J&J PRD	TMC278-NC126
Absorption, Dose Escalating (5 days)	Dog/beagle	Oral/Gavage (base in PEG400/CA [10%])	J&J PRD	TMC278-FK4102

A = Study includes tenofovir as test article
B = Report contains a GLP Compliance Statement
CA: citric acid; CYP: cytochrome P450; GST: glutathione S-transferase; HPMC: hydroxyproply-methlycellulose; PEG400: polyethylene glycol 400

2.6.5.1. Pharmacokinetics

Overview

Test Article: Emtricitabine, Rilpivirine, Tenofovir, or Tenofovir disoproxil fumarate

Type of Study	Test System	Method of Administration	Testing Facility	Study Number
Absorption, Repeat Dose (7 days)	Dog/beagle	Oral/Gavage (base in PEG400/CA [10%])	J&J PRD	TMC278-FK4244 [B]
Absorption, Repeat Dose (1 month)	Dog/beagle	Oral/Gavage (base in PEG400/CA [10%])	J&J PRD	TMC278-TOX5650 [B]
Absorption, Repeat Dose (1 month)	Dog/ beagle	Oral/Gavage (base in PEG400/CA (10%), HCl salt in HPMC [0.5%])	J&J PRD	TMC278-NC116 [B]
Absorption, Repeat Dose (6 months)	Dog/beagle	Oral/Gavage (base in PEG400/CA [10%])	J&J PRD	TMC278-NC115 [B]
Absorption, Repeat Dose (12 months)	Dog/beagle	Oral/Gavage (base in PEG400/CA [10%])	[redacted]	TMC278-NC107 [B]

A = Study includes tenofovir as test article
B = Report contains a GLP Compliance Statement
CA: citric acid; CYP: cytochrome P450; GST: glutathione S-transferase; HPMC: hydroxyproply-methlycellulose; PEG400: polyethylene glycol 400

2.6.5.1. Pharmacokinetics

Overview

Test Article: Emtricitabine, Rilpivirine, Tenofovir, or Tenofovir disoproxil fumarate

Type of Study	Test System	Method of Administration	Testing Facility	Study Number
Absorption, Repeat Dose (7 days)	Monkey/Cynomolgus	Oral/Gavage (HCl salt in HPMC [0.5%])	[redacted]	TMC278-NC249
Absorption, Repeat Dose (14 days)	Monkey/Cynomolgus	Oral/Gavage (HCl salt in HPMC (1%) with Tween 20 [0.5%])	[redacted]	TMC278-NC326
Absorption, Repeat Dose (8 weeks)	Monkey/Cynomolgus	Oral/Gavage (HCl salt in HPMC (1%) with Tween 20 [0.5%])	[redacted]	TMC278-NC248
Absorption, Repeat Dose (gestation day 6 to 16)	Pregnant rat/Sprague Dawley	Oral/Gavage (base in PEG400CA [10%])	J&J PRD	TMC278-NC105 [B]
Absorption, Repeat Dose (gestation day 6 to 19)	Pregnant rabbit/New Zealand white	Oral/Gavage (base in HPMC [0.5%])	J&J PRD	TMC278-NC128
Absorption, Repeat Dose (gestation day 6 to 19)	Pregnant rabbit/New Zealand white	Oral/Gavage (base in HPMC [0.5%])	J&J PRD	TMC278-NC130 [B]

A = Study includes tenofovir as test article
B = Report contains a GLP Compliance Statement
CA: citric acid; CYP: cytochrome P450; GST: glutathione S-transferase; HPMC: hydroxyproply-methlycellulose; PEG400: polyethylene glycol 400

2.6.5.1. Pharmacokinetics

Overview

Test Article: Emtricitabine, Rilpivirine, Tenofovir, or Tenofovir disoproxil fumarate

Type of Study	Test System	Method of Administration	Testing Facility	Study Number
Absorption After a Repeated Dose (TDF)				
Absorption	Mice, albino	Oral gavage	[redacted] Canada (In-life phase), [redacted], USA (Analysis)	M990203-PK[B]
Absorption	Mice, albino	Oral	[redacted] Canada	M990205-PK
Absorption	Rat, Sprague Dawley	Oral gavage	[redacted] Canada (In-life phase), [redacted], USA (Analysis)	96-TOX-4331-003-PK[B]
Absorption	Rat, Sprague Dawley	Oral gavage	[redacted] Canada (In-life phase), [redacted], USA (Analysis)	R2000036-PK[B]
Absorption	Rat, Sprague Dawley	Oral gavage	[redacted] Canada (In-life phase), [redacted], USA (Analysis)	97-TOX-4331-002-PK[B]

A = Study includes tenofovir as test article
B = Report contains a GLP Compliance Statement
CA: citric acid; CYP: cytochrome P450; GST: glutathione S-transferase; HPMC: hydroxyproply-methlycellulose; PEG400: polyethylene glycol 400

2.6.5.1. Pharmacokinetics

Overview

Test Article: Emtricitabine, Rilpivirine, Tenofovir, or Tenofovir disoproxil fumarate

Type of Study	Test System	Method of Administration	Testing Facility	Study Number
Absorption	Rat, Sprague Dawley	Oral, gavage	[redacted] Canada (In-life phase), [redacted] Canada (Analysis)	R990204[B]
Absorption	Dog, Beagle	Oral gavage	[redacted], USA (In-life phase), [redacted], USA (Analysis)	96-TOX-4331-001-PK
Absorption	Dog, Beagle	Oral gavage	[redacted] Canada (In-life phase), [redacted], USA (Analysis)	96-TOX-4331-004-PK[B]
Absorption	Dog, Beagle	Oral gavage	[redacted] Canada (In-life phase), [redacted], USA (Analysis)	98-TOX-4331-003-PK[B]
Absorption	Dog, Beagle	Oral gavage	[redacted] Canada (In-life phase), [redacted], USA (Analysis)	97-TOX-4331-001-PK[B]

A = Study includes tenofovir as test article
B = Report contains a GLP Compliance Statement
CA: citric acid; CYP: cytochrome P450; GST: glutathione S-transferase; HPMC: hydroxyproply-methlycellulose; PEG400: polyethylene glycol 400

2.6.5.1. Pharmacokinetics

Overview

Test Article: Emtricitabine, Rilpivirine, Tenofovir, or Tenofovir disoproxil fumarate

Type of Study	Test System	Method of Administration	Testing Facility	Study Number
Absorption	Monkey, Rhesus	Oral gavage & SC for (tenofovir)	[redacted], USA, (In-life phase), [redacted] Canada (Analysis)	P2000078-PK[B]

A = Study includes tenofovir as test article
B = Report contains a GLP Compliance Statement
CA: citric acid; CYP: cytochrome P450; GST: glutathione S-transferase; HPMC: hydroxyproply-methlycellulose; PEG400: polyethylene glycol 400

2.6.5.1. Pharmacokinetics

Overview

Test Article: Emtricitabine, Rilpivirine, Tenofovir, or Tenofovir disoproxil fumarate

Type of Study	Test System	Method of Administration	Testing Facility	Study Number
Distribution (including normal & pregnant animals) (FTC)				
Plasma protein binding	Human, monkey, mouse, and rabbit plasma	In vitro	Burroughs Wellcome Co., Research Triangle Park, NC 27709 USA	TBZZ/93/0025[B]
Tissue Distribution, Excretion	Rat, SD/Long Evans	Oral	[redacted]	TOX092

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2.6.5.1. Pharmacokinetics

Overview

Test Article: Emtricitabine, Rilpivirine, Tenofovir, or Tenofovir disoproxil fumarate

Type of Study	Test System	Method of Administration	Testing Facility	Study Number
Studies in pregnant or nursing animals				
Repeat-dose tissue distribution	Pregnant Female Mouse and Fetus / CD-1	Repeat Oral	[redacted] USA	TOX103[B]
Repeat-dose tissue toxicokinetics on Embryo/fetus	Female Rabbit / New Zealand White	Repeat Oral	[redacted] USA	TOX038
Distribution (including normal & pregnant animals) (RPV)				
Tissue Distribution (Single dose)	Rat/pigmented Long Evans	Oral/Gavage (^{14}C-TMC278 in PEG400/CA [10%])	J&J PRD	TMC278-NC108
Tissue Distribution (Single dose)	Pregnant rat/Sprague Dawley	Oral/Gavage (^{14}C-TMC278 in PEG400/CA [10%])	J&J PRD	TMC278-NC109

A = Study includes tenofovir as test article
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CA: citric acid; CYP: cytochrome P450; GST: glutathione S-transferase; HPMC: hydroxyproply-methlycellulose; PEG400: polyethylene glycol 400

2.6.5.1. Pharmacokinetics

Overview

Test Article: Emtricitabine, Rilpivirine, Tenofovir, or Tenofovir disoproxil fumarate

Type of Study	Test System	Method of Administration	Testing Facility	Study Number
Tissue Distribution (Single dose)	Rat/Sprague Dawley	Intravenous (base in PEG400/sterile water [25%])	J&J PRD	TMC278-FK4195
		Oral/Gavage (base in PEG400 or PEG400/CA [10%])		
Tissue Distribution (Repeat dose)	Dog/beagle	Oral/Gavage (base in PEG400/CA [10%])	J&J PRD	TMC278-NC115 [B]
Tissue Distribution (Repeat dose)	Dog/beagle	Oral/Gavage (base in PEG400/CA [10%])	J&J PRD	TMC278-TOX5650 [B]
Protein Binding Blood Distribution	Mouse, rat, dog, human	In vitro	J&J PRD	TMC278-FK4217
Protein Binding Blood Distribution	Mouse, rat, rabbit, dog, human	In vitro (^{3}H-TMC278)	J&J PRD	TMC278-NC112
Protein Binding Blood Distribution	Guinea pig, monkey	In vitro (^{14}C-TMC278)	J&J PRD	TMC278-NC332

A = Study includes tenofovir as test article
B = Report contains a GLP Compliance Statement
CA: citric acid; CYP: cytochrome P450; GST: glutathione S-transferase; HPMC: hydroxyproply-methlycellulose; PEG400: polyethylene glycol 400

2.6.5.1. Pharmacokinetics

Overview

Test Article: Emtricitabine, Rilpivirine, Tenofovir, or Tenofovir disoproxil fumarate

Type of Study		Test System	Method of Administration	Testing Facility	Study Number
Distribution (including normal & pregnant animals) (TDF)					
A	Plasma protein binding in vitro	Human plasma/serum	In vitro	Gilead Sciences, Inc., USA	P0504-00039.1
	Single dose tissue distribution	Rat, Sprague Dawley	Oral & IV	[redacted], USA	95-DDM-1278-002
	Single dose tissue distribution	Dog, Beagle	Oral	[redacted], USA	97-DDM-4331-001[B]
A	Absorption	Rat, Sprague Dawley	IV Bolus	Gilead Sciences, Inc., USA (In-life phase), [redacted] Canada (Analysis).	R2000075
A	Absorption	Monkey, Rhesus	SC	[redacted], USA, [redacted] Canada (Analysis)	P2000117

A = Study includes tenofovir as test article
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CA: citric acid; CYP: cytochrome P450; GST: glutathione S-transferase; HPMC: hydroxyproply-methlycellulose; PEG400: polyethylene glycol 400

2.6.5.1. Pharmacokinetics

Overview

Test Article: Emtricitabine, Rilpivirine, Tenofovir, or Tenofovir disoproxil fumarate

Type of Study		Test System	Method of Administration	Testing Facility	Study Number
A	Absorption	Monkey (SIV infected/ non-infected)	SC (group 1) iv (group 2)	[redacted], USA [redacted], USA	T1278-00034
Studies in pregnant or nursing animals					
	Repeat Dose Distribution (plasma, milk)	Rat, Sprague Dawley	Oral , gavage	[redacted] (In-life phase), [redacted] Canada (Analysis)	R990202-PK[B]
	Repeat Dose tissue distribution (presumed pregnant animals)	Rabbit, New Zealand White	Oral gavage	[redacted] (In-lifephase), [redacted], USA (Analysis)	98-TOX-4331-005-PK[B]

A = Study includes tenofovir as test article
B = Report contains a GLP Compliance Statement
CA: citric acid; CYP: cytochrome P450; GST: glutathione S-transferase; HPMC: hydroxyproply-methlycellulose; PEG400: polyethylene glycol 400

2.6.5.1. Pharmacokinetics

Overview

Test Article: Emtricitabine, Rilpivirine, Tenofovir, or Tenofovir disoproxil fumarate

Type of Study		Test System	Method of Administration	Testing Facility	Study Number
A	Single/ Repeat dose tissue distribution to evaluate placental transfer	Pregnant Monkey, Rhesus & Foetus	SC	[redacted], USA (In-life phase), Gilead Sciences (Analysis)	96-DDM-1278-005
A	Single dose tissue distribution	Monkey, Lactating Rhesus	SC	[redacted], USA, [redacted] Canada (Analysis)	P2000116

A = Study includes tenofovir as test article
B = Report contains a GLP Compliance Statement
CA: citric acid; CYP: cytochrome P450; GST: glutathione S-transferase; HPMC: hydroxyproply-methlycellulose; PEG400: polyethylene glycol 400

2.6.5.1. Pharmacokinetics

Overview

Test Article: Emtricitabine, Rilpivirine, Tenofovir, or Tenofovir disoproxil fumarate

Type of Study	Test System	Method of Administration	Testing Facility	Study Number
Metabolism (FTC)				
Metabolism, excretion	Mouse / CD1	Oral	Burroughs Wellcome Co., Research Triangle Park, NC 27709 USA	TEIN/93/0015[B]
Metabolism, excretion	Monkey / Cynomolgus	Oral	[redacted] USA.	TEIN/93/0016[B]
Metabolism, excretion	Monkey / Cynomolgus	Oral	[redacted]	TOX063
Metabolism (RPV)				
Metabolism Excretion (Single dose)	Mouse/CD-1	Oral/Gavage (^{14}C-TMC278 in PEG400/CA (10%)	J&J PRD	TMC278-NC190
Metabolism Excretion (Single dose)	Rat/Sprague Dawley	Oral/Gavage (^{14}C-TMC278 in PEG400/CA (10%)	J&J PRD	TMC278-NC113

A = Study includes tenofovir as test article
B = Report contains a GLP Compliance Statement
CA: citric acid; CYP: cytochrome P450; GST: glutathione S-transferase; HPMC: hydroxyproply-methlycellulose; PEG400: polyethylene glycol 400

2.6.5.1. Pharmacokinetics

Overview

Test Article: Emtricitabine, Rilpivirine, Tenofovir, or Tenofovir disoproxil fumarate

Type of Study	Test System	Method of Administration	Testing Facility	Study Number
Metabolism Excretion (Single dose)	Dog/beagle	Oral/Capsule (^{14}C-TMC278 in PEG400/CA (10%)	J&J PRD	TMC278-NC114
Metabolism Excretion (Single dose)	Human	Oral/Gavage (^{14}C-TMC278 in PEG400)	J&J PRD	TMC278-NC157
Metabolism Excretion in Bile (Single dose)	Rat/Sprague Dawley	Oral/Gavage (^{14}C-TMC278 in PEG400/CA (10%)	J&J PRD	TMC278-NC145
Metabolism (Repeat dose)	Rat/Sprague Dawley	Oral/Gavage (HCl salt in HPMC [0.5%])	J&J PRD	TMC278-NC290
Metabolism (Single and/or repeat dose)	Mouse, rat, rabbit, dog, human	Plasma samples from different studies	J&J PRD	TMC278-NC155
Metabolism	Mouse, rat, rabbit, dog, human	In vitro: hepatocytes, subcellular liver fractions	J&J PRD	TMC278-FK4152

A = Study includes tenofovir as test article
B = Report contains a GLP Compliance Statement
CA: citric acid; CYP: cytochrome P450; GST: glutathione S-transferase; HPMC: hydroxyproply-methlycellulose; PEG400: polyethylene glycol 400

2.6.5.1. Pharmacokinetics

Overview

Test Article: Emtricitabine, Rilpivirine, Tenofovir, or Tenofovir disoproxil fumarate

Type of Study	Test System	Method of Administration	Testing Facility	Study Number
Metabolism	Mouse, rat, rabbit, dog, human	In vitro: hepatocytes, subcellular liver fractions (^{14}C-TMC278)	J&J PRD	TMC278-NC102
Metabolism	Guinea pig, monkey	In vitro: hepatocytes, subcellular liver fractions (^{14}C-TMC278)	J&J PRD	TMC278-NC333
Metabolism	Human	In vitro: human liver microsomes, *E. coli* expressed CYP isoforms	J&J PRD	TMC278-FK4151
Metabolism	Human	In vitro: human liver microsomes, *E. coli* expressed CYP isoforms, supersomes	J&J PRD	TMC278-NC141

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B = Report contains a GLP Compliance Statement
CA: citric acid; CYP: cytochrome P450; GST: glutathione S-transferase; HPMC: hydroxyproply-methlycellulose; PEG400: polyethylene glycol 400

2.6.5.1. Pharmacokinetics

Overview

Test Article: Emtricitabine, Rilpivirine, Tenofovir, or Tenofovir disoproxil fumarate

Type of Study	Test System	Method of Administration	Testing Facility	Study Number
Metabolism	Human	In vitro: heterologous expressed GST isoforms	J&J PRD	TMC278-FK4789
Induction	Human	In vitro: cryopreserved human hepatocytes (CYP activity and mRNA level)	J&J PRD	TMC278-NC186
Induction	Human	In vitro: human hepatocytes (GST activity)	J&J PRD	TMC278-FK4824
Metabolism Induction/Inhibition (3 months)	Mouse/CD-1	Ex vivo: hepatic microsomes	[redacted]	TMC278-NC192 [B]
Metabolism Induction/Inhibition (6 months)	Rat/Sprague Dawley	Ex vivo: hepatic microsomes	[redacted]	TMC278-NC193 [B]
Metabolism Induction/Inhibition (6 months)	Dog/beagle	Ex vivo: hepatic microsomes	[redacted]	TMC278-NC140 [B]

A = Study includes tenofovir as test article
B = Report contains a GLP Compliance Statement
CA: citric acid; CYP: cytochrome P450; GST: glutathione S-transferase; HPMC: hydroxyproply-methlycellulose; PEG400: polyethylene glycol 400

2.6.5.1. Pharmacokinetics

Overview

Test Article: Emtricitabine, Rilpivirine, Tenofovir, or Tenofovir disoproxil fumarate

Type of Study	Test System	Method of Administration	Testing Facility	Study Number
Induction/Inhibition (2 weeks)	Rat/Sprague Dawley	Ex vivo: hepatic microsomes	J&J PRD	TMC278-FK4247
Inhibition	Human	In vitro: human liver microsomes	J&J PRD	TMC278-FK4123
Inhibition	Human	In vitro: human liver microsomes	J&J PRD	TMC278-NC283
Effect Adrenal Gland	Dog	In vitro: adrenal cortex cell-free extracts	J&J PRD	TMC278-FK4790

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B = Report contains a GLP Compliance Statement
CA: citric acid; CYP: cytochrome P450; GST: glutathione S-transferase; HPMC: hydroxyproply-methlycellulose; PEG400: polyethylene glycol 400

	Type of Study	Test System	Method of Administration	Testing Facility	Study Number
Metabolism (TDF)					
A	In vitro metabolism	Rat, dog, human tissues	In vitro	Gilead Sciences, Inc., USA	96-DDM-1278-003
	In vitro metabolism	Rat, dog, human tissues	In vitro	Gilead Sciences, Inc., USA	97-VIT-1278-001
	Metabolism, epithelial transport	Caco 2 cells (colon adenocarcinoma; human) monolayers	In vitro	Gilead Sciences, Inc., USA	98-VIT-4331-001
	In vitro Cytochrome P450 inhibition	Human hepatic microsomes	In vitro	Gilead Sciences, Inc., USA	V990172-104
	Cytochrome P450 enzyme induction	Rat, Sprague Dawley	Oral gavage	[redacted], [redacted], USA	R2001024[B]

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B = Report contains a GLP Compliance Statement
CA: citric acid; CYP: cytochrome P450; GST: glutathione S-transferase; HPMC: hydroxyproply-methlycellulose; PEG400: polyethylene glycol 400

2.6.5.1. Pharmacokinetics

Overview

Test Article: Emtricitabine, Rilpivirine, Tenofovir, or Tenofovir disoproxil fumarate

Type of Study	Test System	Method of Administration	Testing Facility	Study Number
Excretion (RPV)				
Excretion (Single dose) Metabolism	Mouse/CD-1	Oral/Gavage (^{14}C-TMC278 in PEG400/CA (10%)	J&J PRD	TMC278-NC190
Excretion (Single dose) Metabolism	Rat/Sprague Dawley	Oral/Gavage (^{14}C-TMC278 in PEG400/CA (10%)	J&J PRD	TMC278-NC113
Excretion (Single dose) Metabolism	Dog/beagle	Oral/Capsule (^{14}C-TMC278 in PEG400/CA (10%)	J&J PRD	TMC278-NC114
Excretion (Single dose) Metabolism	Human	Oral/Gavage (^{14}C-TMC278 in PEG400)	J&J PRD	TMC278-NC157
Excretion in Bile (Single dose) Metabolism	Rat/Sprague Dawley	Oral/Gavage (^{14}C-TMC278 in PEG400/CA (10%)	J&J PRD	TMC278-NC145

A = Study includes tenofovir as test article
B = Report contains a GLP Compliance Statement
CA: citric acid; CYP: cytochrome P450; GST: glutathione S-transferase; HPMC: hydroxyproply-methlycellulose; PEG400: polyethylene glycol 400

2.6.5.1. Pharmacokinetics

Overview

Test Article: Emtricitabine, Rilpivirine, Tenofovir, or Tenofovir disoproxil fumarate

Type of Study	Test System	Method of Administration	Testing Facility	Study Number
Excretion (TDF)				
Absorption & excretion	Rat, Sprague Dawley	IV bolus	Gilead Sciences, Inc., USA	96-DDM-1278-001
Absorption & excretion	Rat, Sprague Dawley	Oral gavage	Gilead Sciences, Inc., USA	97-DDM-4331-003/4
Absorption & excretion	Dog, Beagle	IV bolus	TSI Mason Labs, MA, USA	96-DDM-1278-002
Pharmacokinetic Drug Interactions Studies (RPV)				
Drug-drug Interactions	Human	In vitro: human liver microsomes	J&J PRD	TMC278-NC194

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B = Report contains a GLP Compliance Statement
CA: citric acid; CYP: cytochrome P450; GST: glutathione S-transferase; HPMC: hydroxyproply-methlycellulose; PEG400: polyethylene glycol 400

Type of Study	Test System	Method of Administration	Testing Facility	Study Number
Pharmacokinetic Drug Interactions Studies (TDF)				
Drug drug interaction study	Rat, Sprague Dawley	Oral gavage	Gilead Sciences, Inc., USA	R2000096 appendices E and F containing reports R20001032 & R2001008
Drug drug interaction study	In vitro		Gilead Sciences, Inc., USA	PC-103-2001
Drug drug interaction study	In vitro		Gilead Sciences, Inc., USA	AD-104-2001
Drug drug interaction study	In vitro		Gilead Sciences, Inc., USA	AD-104-2002
Drug drug interaction study	In vitro		Gilead Sciences, Inc., USA	PC-104-2010
Drug drug interaction study	In vitro		Gilead Sciences, Inc., USA	PC104-2011
Drug drug interaction study	In vitro		Gilead Sciences, Inc., USA	PC-104-2014
Drug drug interaction study	In vitro		Gilead Sciences, Inc., USA	AD-104-2010

A = Study includes tenofovir as test article
B = Report contains a GLP Compliance Statement
CA: citric acid; CYP: cytochrome P450; GST: glutathione S-transferase; HPMC: hydroxyproply-methlycellulose; PEG400: polyethylene glycol 400

2.6.5.2. Bioanalytical Methods

2.6.5.2.A. Emtricitabine

Bioanalytical methods for FTC are described in Module 2.6.4, Section 2.6.4.2.1.

2.6.5.2.B. Rilpivirine

Type of Study: Bioanalytical methods

	Anticoagulant	Volume (mL)	RPV LLOQ-ULOQ (ng/mL)	Study Number
Species				
Mouse	EDTA[b]	0.05	2.00 – 4000	R278474/FK4240
Rat	EDTA[a]	0.1	1.00 – 2000	R278474/FK4170
Rabbit	EDTA[b]	0.1	1.00 – 2000	BA1014
Dog	EDTA[a]	0.1	1.00 – 2000	R278474/FK4169
	Heparin[b]	0.1	1.00 – 2000	
Monkey	EDTA[b]	0.05	1.00 – 2000	BA1062

a full validation;
b partial validation
LLOQ: lower limit of quantification; ULOQ: upper limit of quantification

Species	Short-term Storage		Long-term Storage	Processed QC Samples	Study Number
	Blood	Plasma	Plasma		
Mouse (EDTA)	2 h at refrigerator temp.	24 h at room temp. 3 freeze/thaw cycles	914 days in freezer	3 days	R278474/FK4240
Rat (EDTA)	2 h at room temp. 2 h at 37°C		581 days in freezer	2 days	R278474/FK4170
Dog (EDTA or heparin)	2 h at refrigerator temp. 4 h at room temp. 2 h at 37°C		1085 days in freezer	5 days	R278474/FK4169
Rabbit (EDTA)	2 h on melting ice 4 h at room temp. 2 h at 37°C		1119 days in freezer	6 days	BA1014
Monkey EDTA)			361 days in freezer	2 days	BA1062

2.6.5.2.C. Tenofovir

Bioanalytical methods for TFV are described in Module 2.6.4, Section 2.6.4.2.3.

2.6.5.3. Pharmacokinetics: In Vitro and In Vivo Absorption

2.6.5.3.A. TEIN/93/0003: Pharmacokinetics of 524W91 in Male CD-1 Mice Following Oral and Intravenous Administration

Type of Study: Pharmacokinetics of 524W91 in Male CD-1 Mice Following Oral and Intravenous Administration

Study Number: TEIN/93/0003

Species	**Mouse / CD-1**
Feeding Condition	-
Vehicle/Formulation	Solution in 0.9% sodium chloride
Method of Administration	Oral and intravenous
Sample (e.g. whole blood, plasma, serum)	Plasma
Analyte	L-(-)-2',3'-Dideoxy-5-Fluoro-3'-Thiacytidine (FTC)
Assay	Validated HPLC with UV detection
Dose (mg/kg)	10 oral/ 10 IV
Gender (M/F)/Number of Animals	120 M / dose

PK Parameters

	Oral	IV
T_{max} (min)	25.4	-
C_{max} (μM)	9.8	-
$AUC_{0\rightarrow\infty}$(μM•hr)	16.7	17.4
CL (l/hr/kg)	-	2.33
T ½β (min)	-	23
V_{ss} (l/kg)	-	0.89

Additional Information:
Absolute bioavailability = 96%

2.6.5.3.B. TEIN/93/0004: Pharmacokinetics of 100 mg/kg Oral and Intravenous 524W91 in Male CD-1 Mice

Type of Study: Pharmacokinetics of 100 mg/kg or Oral and Intravenous 524W91 in Male CD-1 Mice

Study Number: TEIN/93/0004

Species	**Mouse / CD-1**
Feeding Condition	-
Vehicle/Formulation	Solution in 0.9% sodium chloride
Method of Administration	Oral and intravenous
Sample (e.g. whole blood, plasma, serum)	Plasma
Analyte	L-(-)-2',3'-Dideoxy-5-Fluoro-3'-Thiacytidine (FTC)
Assay	Validated HPLC with UV detection
Dose (mg/kg)	100 oral/ 100 IV
Gender (M/F)/Number of Animals	120 M / dose

PK Parameters

	Oral	**IV**
T_{max} (min)	24.5	-
C_{max} (µM))	89	-
$AUC_{0\rightarrow\infty}$ (µM•hr)	143	181
CL (l/hr/kg)	-	2.23
T ½ (min) beta	-	15.5
lambda	-	82
V_{ss} (l/kg)	-	0.94

Additional Information:
Absolute bioavailability = 79%

2.6.5.3.C. IUW00101: Pharmacokinetic Study in Male Mice Following Single Oral and Intravenous Administration of L-(-)-2',3'-Dideoxy-5-Fluoro-3'-Thiacytidine

Type of Study: Pharmacokinetic Study in Male Mice Following Single Oral and Intravenous Administration of L-(-)-2',3'-Dideoxy-5-Fluoro-3'-Thiacytidine

Study Number: IUW00101

Species	**Mouse / CD-1**
Gender (M/F)/Number of Animals	Oral – 36 (numbered 4-39, 1-3 were bled pre-dose) (male) Intravenous – 36 (numbered 43-78, 40-42 were bled pre-dose) (male)
Feeding Condition	-
Vehicle/Formulation	Oral – 60 mg/mL suspension with 0.5% hydroxypropylmethyl cellulose Intravenous – 60 mg/mL in phosphated buffered saline using distilled water, pH 7.2
Method of Administration	Oral and intravenous
Dose (mg/kg)	600
Sample (e.g. whole blood, plasma, serum)	Plasma
Analyte	L-(-)-2',3'-Dideoxy-5-Fluoro-3'-Thiacytidine (FTC)
Assay	Validated HPLC with UV detection

PK Parameters

	Oral	**IV**
T_{max} (hr)	0.667	0
C_{max} (µg/mL))	139	1560
$AUC_{0\rightarrow last}$ (µg•hr/mL)	270	465
$AUC_{0\rightarrow\infty}$ (µg•hr/mL)	296	473
CL (l/hr/kg)	-	1.28
T ½ (h) lambda	3.17	4.14
λ_z (hr^{-1})	0.219 (apparent terminal rate constant)	0.167 (apparent terminal rate constant)
V_{ss} (l/kg)	-	1.10
Mean Absorption Time (hr)	2.04 (calculated as MRT_{po}-MRT_{IV})	-
Mean Residence Time (hr)	2.90 (calculated from AUMC/ $AUC_{0\rightarrow\infty}$)	0.860 (calculated from AUMC/ $AUC_{0\rightarrow\infty}$)
Absolute Bioavailability	62.7%	-

Additional Information: none

NC = not calculated due to insufficient data NA = data not available

2.6.5.3.D. TEZZ/93/0019: A Pharmacokinetic Study of 524W91 in Cynomolgus Monkeys Following Oral and Intravenous Administration

Type of Study: A Pharmacokinetic Study of 524W91 in Cynomolgus Monkeys Following Oral and Intravenous Administration

Study Number: TEZZ/93/0019

Species	**Monkey / Cynomolgus**
Gender (M/F)/Number of Animals	4 M / dose
Feeding Condition	-
Vehicle/Formulation	Solution in 0.9% sodium chloride
Method of Administration	Oral and intravenous
Dose (mg/kg)	10 and 80
Sample (e.g. whole blood, plasma, serum)	Plasma
Analyte	L-(-)-2',3'-Dideoxy-5-Fluoro-3'-Thiacytidine (FTC)
Assay	Validated HPLC with UV detection

PK Parameters

Plasma (oral)	**10 mg/kg**	**80 mg/kg**
C max (μM)	14.1 ± 2.00	111 ± 34.0
T max (hr)	1.3 ± 0.50	2.30 ± 0.29
AUC (0-∞) (μM•hr)	37.4 ± 6.5	285 ± 82.6
CL / f (l/kg/hr)	1.1 ± 0.19	1.2 ± 0.31
Plasma (intravenous)	**10 mg/kg**	**80 mg/kg**
AUC (0-∞) (μM•hr)	59.9 ± 11.4	493 ± 65.4
CLt(l/h/kg)	0.70 ± 0.14	0.70 ± 0.08
Vss (l/kg)	0.80 ± 0.02	0.80 ± 0.09
T 1/2 beta(min)	1.00 ± 0.19	1.02 ± 0.13

Additional Information: Absolute bioavailability 44 to 69%.

2.6.5.3.E. IUW00301: Pharmacokinetic Study in Male cynomolgus Monkeys Following Single Oral and Intravenous Administration of L-(-)-2',3'-Dideoxy-5-Fluoro-3'-Thiacytidine

Type of Study: Pharmacokinetic Study in Male cynomolgus Monkeys Following Single Oral and Intravenous Administration of L-(-)-2',3'-Dideoxy-5-Fluoro-3'-Thiacytidine	**Study Number:** IUW00301
Species	**Monkey / Cynomolgus**
Gender (M/F)/Number of Animals	4 M / dose
Feeding Condition	-
Vehicle/Formulation	Oral – 32.0 mg/mL aqueous solution using double-distilled water for oral administration Intravenous – 81.8 mg/mL in phosphate buffered saline prepared using double-distilled water, pH 7.2
Method of Administration	Oral and intravenous
Dose (mg/kg)	80
Sample (e.g. whole blood, plasma, serum)	Plasma
Analyte	L-(-)-2',3'-Dideoxy-5-Fluoro-3'-Thiacytidine (FTC)
Assay	Validated HPLC with UV detection
PK Parameters	

Oral			**IV**		
Plasma		Mean ± Standard Deviation	**Plasma (intravenous)**		Mean ± Standard Deviation
C max	**(µg/mL)**	39.4 ± 4.87	**C max**	**(µg/mL)**	238 ± 46.0 (end of infusion)
T max	**(h)**	0.884 (median)	**T max**	**(h)**	Not applicable
AUC (0 - last)	**(µg•hr/mL)**	83.0 ± 11.1	**AUC (0 - last)**	**(µg•hr/mL)**	85.5 ± 17.3
AUC (0 - ∞)	**(µg•hr/mL)**	83.6 ± 11.2	**AUC (0 - ∞)**	**(µg•hr/mL)**	86.1 ± 17.3
CL / f	**(l/h/kg)**	Not calculated	**CL**	**(l/h/kg)**	0.970 ± 0.158
T 1/2 lambda	**(h)**	0.936 ± 0.0909	**T 1/2 lambda**	**(h)**	0.775 ± 0.0586
λ_z	**(h^{-1})**	0.746 ± 0.0793 (apparent terminal rate constant)	**λ_z**	**(h^{-1})**	0.898 ± 0.0640 (apparent terminal rate constant)
Further Parameters :			**Further parameters :**		
Degree of absorption			**Degree of absorption**		
Mean absorption time (h)		0.953 ± 0.154 (the apparent mean absorption time, calculated as MRT_{po} - MRT_{iv})	**Mean residence time (h)**		0.802 ± 0.0793 (calculated from AUMC/$AUC_{0-\infty}$)
Mean residence time (h)		1.75 ± 0.156 (calculated from AUMC/$AUC_{0-\infty}$)	**V_{ss} (l/kg)**		0.769 ± 0.0743
Abs. bioavailability	**(%)**	97.4 ± 6.98			

NC = not calculated due to insufficient data NA = data not available

2.6.5.3.F. TMC278-NC104: Determination of the In Vitro Transport Characteristics of TMC278, Evaluation of the Possible Role of P-glycoprotein in TMC278 Transport and Assessment of Possible Inhibition of P-glycoprotein Activity by TMC278: A Study in Caco-2 Monolayers

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

2.6.5.3.G. TMC278-FK4259: In Vivo Micronucleus Test on Bone Marrow Cells of Mice

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						
C1h (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

2.6.5.3.H. TMC278-FK4195: Pharmacokinetics and tissue distribution in male Sprague-Dawley rats after single intravenous administration (slow bolus injection) of a PEG400/sterile water (75/25) solution at 4 mg R278474/kg and absorption and tissue distribution after single oral administration of a solution of PEG400 + citric acid (10%) at 40, 160 and 400 mg R278474/kg and of a solution of PEG400 at 40 mg R278474/kg

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

2.6.5.3.I. TMC278-NC106: Pharmacokinetics and relative bioavailability of R278474 in SPF Sprague Dawley rats after single oral administration of R278474 at 40 mg base eq./kg given as an oral PEG400 solution R278474.HCl (R314585) and R278474-fumarate salt (R366650) given as a suspension

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

2.6.5.3.J. TMC278-FK4278: Single Dose Oral Toxicity Study in the Rat

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

2.6.5.3.K. TMC278–FK4293: Pharmacokinetics of R278474 in female white New Zealand rabbits after single intravenous administration of a 75 % PEG400 / 25 % sterile water solution of R278474 at 1.25, 2.9 and 4 mg/kg

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

2.6.5.3.L. TMC278-FK4231: Pharmacokinetics and Absolute and Relative Bioavailability in Male Beagle Dogs After Single Intravenous Administration of a 75 % PEG400/25 % Sterile Water Solution of R278474 at 1.25 mg/kg and After Single Oral Administration of Different Formulations of R278474 at 5 mg/kg

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

2.6.5.3.M. TMC278-DM02403: Evaluation of the Oral Bioavailability and Pharmacokinetics of R278474 in Male Cynomolgus Monkeys following Single Oral and Intravenous Bolus Administration

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

2.6.5.3 Pharmacokinetics: In Vitro and In Vivo Absorption **Test Article:** Tenofovir

2.6.5.3.N. 97-TOX-4331-008-PK: In Vivo Micronucleus Assay

Species	**Mouse**		
	97-TOX-4331-008-PK		
Feeding condition	Fasted		
Vehicle formulation	Suspension vehicle		
Method of administration	Oral		
Sample	Plasma		
Analyte	Tenofovir		
Assay	HPLC		
Dose mg/kg	**500**	**1000**	**2000**
Gender (M/F) No. of Animals	5M	5M	5M
PK parameters			
Tenofovir concentration µg/mL (mean & SD)			
1 hr	3.727 (1.189)	8.129 (2.685)	28.180 (14.25)
24 hr	0.682 (0.257)	1.214 (0.199)	2.059 (0.558)
48 hr	0.045 (0.013)	0.152 (0.044)	0.921 (0.339)

Additional information
The limited data precluded determination of half life and AUC

NC = not calculated due to insufficient data NA = data not available

2.6.5.3.O. W2000108: Single Dose Oral Bioavailability of Tenofovir DF in Woodchucks

Species	**Woodchuck**		
	W2000108		
Feeding Condition	-		
Vehicle Formulation	Tenofovir (supplied as pre formulated stock solution). TDF (formulated in cane syrup).		
Method of Administration	IV (tenofovir) Oral (TDF)		
Sample	Plasma		
Analyte	Tenofovir		
Assay	HPLC/MS		
Dose mg/kg (TDF oral dose)	**5**	**15**	**25**
Gender (M/F) No. of Animals	6 (3M, 3F)	6 (3M, 3F)	6 (3M, 3F)
PK Parameters (oral) mean +/-SD			
Parameter* Dose TDF equivalent to	**2.26 mg/kg Tenofovir**	**6.78 mg/kg Tenofovir**	**11.3 mg/kg Tenofovir**
C_{max} (µg/mL)	0.171 ± 0.0687	0.377 ± 0.217	0.524 ± 0.229
T_{max} (hr)	1.08 ± 0.376	1.00 ± 0.632	0.667 ± 0.258
$AUC_{0\rightarrow\infty}$ (µg•hr/mL)	0.635 ± 0.162	1.42 ± 0.413	1.85 ± 0.687
AUC % Extrapolated	11.4 ± 4.80	5.37 ± 5.67	4.01 ± 1.16
$t_{1/2}\lambda_z$ (hr)	4.87 ± 1.70	5.53 ± 1.02	6.35 ± 0.782
CL/F (mL/hr/kg)	3810 ± 1240	5090 ± 1380	7130 ± 3320
$MRT_{0\rightarrow\infty}$ (hr)	5.29 ± 0.815	6.33 ± 1.68	5.96 ± 0.782
V_z/F (mL/kg)	26800 ± 11400	41600 ± 16600	67100 ± 36400
F (%)	20.6 ± 12.9	32.4 ± 16.2	10.0 ± 4.16

Additional information
* Pharmacokinetic analysis based on mg/kg dose of Tenofovir

Species	**Woodchuck**
	W2000108 (cont)

NC = not calculated due to insufficient data NA = data not available

2.6.5.3 Pharmacokinetics: In Vitro and In Vivo Absorption **Test Article:** Tenofovir

Feeding Condition			
Vehicle Formulation	Tenofovir (supplied as pre-formulated stock solution). TDF (formulated in cane syrup).		
Method of Administration	IV (tenofovir) Oral (TDF)		
Sample	Plasma		
Analyte	Tenofovir		
Assay	HPLC/MS		
Dose mg/kg (Tenofovir IV dose)	**2.5**	**7.5**	**12.5**
Gender (M/F) No. of Animals	6 (3M, 3F)	6 (3M, 3F)	6 (3M, 3F)
PK Parameters (IV) mean +/-SD			
C_{max} (µg/mL)	8.49 ± 5.33	8.55 ± 4.74	47.5 ± 37.6
$AUC_{0\rightarrow\infty}$ (µg•hr/mL)	4.21 ± 1.68	5.39 ± 2.47	21.0 ± 3.04
AUC % Extrapolated	0.462 ± 0.300	0.531 ± 0.502	0.263 ± 0.171
$t_{1/2}\lambda_z$ (hr)	1.86 ± 1.05	3.62 ± 1.96	4.92 ± 2.75
CL/F (mL/hr/kg)	712 ± 384	1600 ± 591	606 ± 81.9
$MRT_{0\rightarrow\infty}$(hr)	0.927 ± 0.351	1.04 ± 0.136	1.08 ± 0.231
V_z/F (mL/kg)	1970 ± 1900	7870 ± 5460	4190 ± 2180
Additional information			

NC = not calculated due to insufficient data NA = data not available

2.6.5.3.P. D2000076: Single Dose Oral Bioavailability of Tenofovir DF in Beagle Dogs

Species	**Dog**			
	D2000076			
Feeding Condition	Fed (IV) Fasted/Fed (oral)			
Vehicle Formulation	Tenofovir (physiological buffered solution) TDF (50 mM Citric acid)			
Method of Administration	Tenofovir (IV) TDF (oral).			
Sample	Plasma			
Analyte	Tenofovir			
Assay	LC/MS/MS assay			
Dose mg/kg (TDF oral dose)	**5**		**30**	
Gender (M/F) No. of Animals	4M	4M	4M	4M
	Fasted	**Fed**	**Fasted**	**Fed**
PK Parameters mean (SD)				
C_{max} (µg/mL)	0.401 (0.141)	0.490 (0.211)	3.49 (1.88)	4.69 (2.03)
T_{max} (hr)	1.38 (1.75)	1.13 (0.629)	1.13 (0.629)	0.625 (0.25)
$AUC_{(0\text{-}last)}$ (µg.hr/mL)	2.68 (0.635)	2.69 (0.822)	21.4 (4.91)	23.6 (6.89)
$AUC_{(0\text{-}\infty)}$ (µg.hr/mL)	3.07 (0.620)	3.21 (0.905)	23.0 (4.76)	25.8 (6.64)
% AUC Extrapolated	12.9 (5.71)	16.4 (5.88)	7.07 (4.18)	9.39 (4.55)
$t_{1/2}\lambda z$ (hr)	78.5 (34.3)	81.3 (23.6)	45.8 (17.2)	49.0 (14.8)
CL/F (mL/hr/kg)	762 (148)	752 (212)	608 (114)	563 (193)
C_{last} (µg/mL)	0.00354 (0.00128)	0.00450 (0.00172)	0.0235 (0.00630)	0.0323 (0.00494)
T_{last} (hr)	120 (0.0)	120 (0.0)	120 (0.0)	120 (0.0)
Vz/F (mL/kg)	89368 (48289)	90608 (41067)	41018 (17769)	42840 (29540)
F %	30.2 (4.08)	31.8 (9.21)	41.2 (8.28)	46.4 (13.0)

Additional information

Absorption following single oral doses were compared with single IV bolus administration of TDF. Absolute oral bioavailibility was calculated at 32% & 46% in fed animals for 5 & 30 mg/kg & 30% & 41% for the same doses in fasted animals

NC = not calculated due to insufficient data NA = data not available

2.6.5.3 Pharmacokinetics: In Vitro and In Vivo Absorption

Test Article: Tenofovir disoproxil fumarate

Species	**Dog**			
	D2000076 (continued)			
Feeding Condition	Fed (IV) Fasted/Fed (oral)			
Vehicle Formulation	Tenofovir (physiological buffered solution) TDF (50 mM Citric acid)			
Method of Administration	Tenofovir (IV) TDF (oral).			
Sample	Plasma			
Analyte	Tenofovir			
Assay	LC/MS/MS assay			
Dose mg/kg (Tenofovir IV dose)	**1**		**10**	
Gender (M/F) No. of Animals	4M		4M	
Tabulated PK Results for IV Dose mean (SD)				
C_{max} (µg/mL)	2.99	(0.410)	31.0	(4.91)
$AUC_{(0-\infty)}$ (µg.hr/mL)	4.48	(0.584)	41.2	(3.19)
% AUC Extrapolated	2.41	(0.242)	1.43	(0.633)
$t_{1/2}\lambda z$ (hr)	45.3	(5.18)	38.6	(5.47)
CL (mL/hr/kg)	226	(29.3)	244	(19.9)
C_{last} (µg/mL)	0.0017	(0.0003)	0.0103	(0.0031)
T_{last} (hr)	120	(0.0)	120	(0.0)
V_{ss} (mL/kg)	3090	(275)	2510	(574)
Additional information				

NC = not calculated due to insufficient data NA = data not available

2.6.5.3.Q. P2000031: A Single Dose Oral Bioavailability Study of Tenofovir DF in Rhesus Monkeys

Species	**Monkey** **P2000031**					
Feeding Condition	Fed (IV) Fed & Fasted (oral)					
Vehicle Formulation	Tenofovir (physiological buffered solution) TDF (Citric acid)					
Method of Administration	Tenofovir (IV) TDF (oral)					
Sample	Plasma					
Analyte	Tenofovir					
Assay	HPLC/MS					
Dose mg/kg (TDF oral dose)	**5***		**50***		**250****	
Gender (M/F) No. of Animals	6 (3M, 3F)		6 (3M, 3F)		12 (6M, 6F)	
PK Parameters (oral) mean (SD)						
C_{max} (μg/mL)	0.113	(0.042)	1.15	(0.676)	1.68	(1.05)
T_{max} (hr)	0.83	(0.408)	1.00	(0.548)	1.08	(0.56)
$AUC_{0\rightarrow\infty}$ (μg•hr/mL)	0.725	(0.125)	6.38	(1.74)	14.8	(7.81)
AUC % Extrapolated	2.82	(0.768)	2.26	(1.00)	2.19	(0.710)
$t_{1/2}\lambda_z$ (hr)	8.23	(1.06)	8.54	(1.14)	8.41	(1.20)
CL/F (mL/hr/kg)	3202	(566)	3807	(1191)	6569	(2996)
C_{last} (μg/mL)	0.00169	(0.00027)	0.0118	(0.00615)	0.0366	(0.0102)
T_{last} (hr)	44.0	(6.20)	48.0	(0.0)	48.0	(0.0)
V_z/F (mL/kg)	38000	(8538)	47250	(18111)	82600	(50113)
F (%)	32.4	(7.90)	23.7	(7.82)	17.0	(5.66)

Additional information *=Fed **=Fed and fasted combined. No statistically significant differences were observed between male and female animals in any treatment group and none were observed between fed and fasted states in the 250 mg/kg TDF dose group. Oral bioavailabilities for the 5, 50 & 250 mg/kg groups were 32.4, 23.7 & 17.0%.

NC = not calculated due to insufficient data NA = data not available

2.6.5.3 Pharmacokinetics: In Vitro and In Vivo Absorption

Test Article: Tenofovir disoproxil fumarate

Species	**Monkey**			
	P2000031 (continued)			
Feeding Condition	Fed (IV) Fed & Fasted (oral)			
Vehicle Formulation	Tenofovir (physiological buffered solution) TDF (Citric acid)			
Method of Administration	Tenofovir (IV) TDF (oral)			
Sample	Plasma			
Analyte	Tenofovir			
Assay	HPLC/MS			
Dose mg/kg (Tenofovir IV dose)	**5**		**30**	
Gender (M/F) No. of Animals	6 (3M/3F)		6 (3M/3F)	
Tabulated PK for IV Dose Results mean (SD)				
C_{max} (µg/mL)	13.8	(3.08)	79.0	(12.6)
$AUC_{0\rightarrow\infty}$ (µg•hr/mL)	5.12	(1.15)	38.4	(16.2)
AUC % Extrapolated	0.520	(0.394)	0.199	(0.0841)
$t_{1/2}\,\lambda_z$ (hr)	5.37	(1.35)	8.79	(2.79)
CL (mL/hr/kg)	1031	(301)	888	(315)
C_{last} (µg/mL)	0.00419	(0.00455)	0.00620	(0.00317)
T_{last} (hr)	24.0	(7.59)	38.0	(11.8)
V_{ss} (mL/kg)	1188	(312)	930	(146)
Additional information				

NC = not calculated due to insufficient data NA = data not available

2.6.5.3.R. AD-264-2001: Pharmacokinetics of Rilpivirine (TMC-278)/Tenofovir/Emtricitabine after Oral Doses in Various Formulations in Beagle Dogs

Species	**Dog (Beagle)**		
	AD-264-2001		
Feeding Condition	Fasted/Fed		
Vehicle Formulation	Tablet		
Method of Administration	Oral		
Sample	Plasma		
Analyte	Rilpivirine/Tenofovir/Emtricitabine		
Assay	LC/MS/MS		
Dose mg (RPV HCl/TDF/FTC)	6.25/75/50 mg		
Gender (M/F) No. of Animals	M (12)		
	Fasted		
Parameter	**AUC_{0-t} (ng hr/mL)[a]**	**C_{max} (ng/mL)[a]**	**T_{max} (hr)[a]**
Clinical tablets	1206/3065/11424 (381/431/2015)	132/879/4081 (29/187/594)	2.4/0.7/0.7 (1.0/0.5/0.3)
Co-blend Tablet	1792/3040/13301 (359/467/1835)	182/833/4642 (34/202/1090)	2.3/0.9/0.9 (1.1/0.2/0.2)
Co-dried Tablet	1653/8115/1184 (399/1306/2019)	170/1837/4067 (46/646/973)	2.3/0.9/0.9 (0.8/0.2/0.2)
	Fed		
Parameter	**AUC_{0-t} (ng hr/mL)**	**C_{max} (ng/mL)**	**T_{max} (hr)**
Clinical tablets	1520/2838/13100 (428/521/2186)	130/897/3816 (46/333/556)	2.5/0.6/0.9 (1.4/0.3/0.2)
Co-blend Tablet	1870/5290/10600 (376/499/1330)	163/1570/3370 (35.1/331/247)	2.8/0.7/0.7 (1.3/0.3/0.3)
Co-dried Tablet	1998/8788/13767 (276/1402/1779)	188/2153/4335 (29/596/610)	2.6/0.8/0.9 (0.8/0.3/0.2)

a Data are for rilpivirine/tenfovir/emtricitabine respectively, mean (SD)

NC = not calculated due to insufficient data NA = data not available

2.6.5.3.S. AD-264-2023: Oral Exposure of Rilpivirine (TMC-278)/Tenofovir/Emtricitabine after Oral Administration of a Bilayer Tablet Formulation in Beagle Dogs

Species	**Dog (Beagle)**			
	AD-264-2023			
Feeding Condition	Fasted			
Vehicle Formulation	Tablet			
Method of Administration	Oral			
Sample	Plasma			
Analyte	Rilpivirine/Tenofovir/Emtricitabine			
Assay	LC/MS/MS			
Dose mg (RPV HCl/TDF/FTC)	6.25/75/50 mg			
Gender (M/F) No. of Animals	M (6-12)			
Parameter	**Fasted**			
	n	**AUC_{0-t} (ng hr/mL)[a]**	**C_{max} (ng/mL)[a]**	**T_{max} (hr)[a]**
Clinical tablets	12	1206/3065/11424 (381/431/2015)	132/879/4081 (29/187/594)	2.4/0.7/0.7 (1.0/0.5/0.3)
Bilayer Tablet	6	1310/2500/11600 (552/319/2510)	128/769/3510 (64/235/902)	2.5/0.7/1.1 (1.2/0.3/0.5)

a Data are for rilpivirine/tenfovir/emtricitabine respectively, mean (SD)

NC = not calculated due to insufficient data NA = data not available

2.6.5.4. Pharmacokinetics: Absorption After Repeated Doses

2.6.5.4.A. TOX-109: Two-year Oral Oncogenicity study of FTC in the Mouse

Species	**Mouse**
	TOX-109
Feeding Condition	Fed
Vehicle Formulation	0.5% aqueous methylcellulose
Method of Administration	Oral gavage
Sample	Plasma
Analyte	Emtricitabine
Assay	LC/MS/MS
Dose mg/kg	**0, 80, 250, 750**
Gender (M/F) No. of Animals	3/sex/group on Week 2 & Week 26

Parameters	**Week**		**Daily Dose**		
			80 mg/kg	**250 mg/kg**	**750 mg/kg**
	Week 2	Female	27.59	64.97	209.16
		Male	25.52	91.05	234.58
AUC_{0-24}		Mean	26.56	78.01	221.87
(µg•hr/mL)	**Week 26**	Female	23.74	91.71	322.65
		Male	27.48	90.94	287.3
		Mean	25.61	91.33	304.98
C_{max}	**Week 2**	Female	10.446	33.373	109.690
(µg/mL)		Male	16.200	46.609	88.693
		Mean	13.323	39.991	99.192
	Week 26	Female	20.487	49.058	176.950
		Male	16.265	57.682	143.229
		Mean	18.372	53.370	160.090

Additional Information none

NC = not calculated due to insufficient data NA = data not available

2.6.5.4 Pharmacokinetics: Absorption After Repeated Doses **Test Article:** Emtricitabine

2.6.5.4.B. TOX-108: Two-year Oral Oncogenicity study of FTC in the Rat

Species	**Rat**
	TOX-108
Feeding Condition	Fed
Vehicle Formulation	0.5% aqueous methylcellulose
Method of Administration	Oral gavage
Sample	Plasma
Analyte	Emtricitabine
Assay	LC/MS/MS
Dose mg/kg	**0, 60, 200, 600**
Gender (M/F) No. of Animals	3/sex/group on Week 2 & Week 26

Parameters	**Week**		**Daily Dose**		
			60 mg/kg	**200 mg/kg**	**600 mg/kg**
	Week 2	Female	30.91	155.99	260.02
		Male	29.91	97.26	279.68
AUC_{0-24}		Mean	30.41	126.62	269.85
(µg•hr/mL)	**Week 26**	Female	52.53	170.68	404.07
		Male	42.87	137.42	326.77
		Mean	47.70	154.05	365.42
C_{max}	**Week 2**	Female	11.044	30.991	63.813
(µg/mL)		Male	12.380	27.565	59.610
		Mean	11.712	29.278	61.712
	Week 26	Female	15.569	52.168	88.993
		Male	13.996	32.339	73.053
		Mean	14.783	42.254	81.023

Additional Information none

NC = not calculated due to insufficient data NA = data not available

2.6.5.4.C. TMC278-NC118: Two-Week Repeated Dose Oral Toxicity Study in the Swiss Mouse

Study No.	**TMC278-NC118**					
Species	**Mouse (CD-1)**					
Feeding Condition	**-**				**Not fasted**	
Vehicle/Formulation	**TMC278.HCl in dietary admixture**				**TMC278.HCl in HPMC (0.5%)**	
Route	**Oral (diet)**				**Oral (gavage)**	
Gender (M/F)/Number of Animals	M/6	F/6	M/6	F/6	M/9	F/9
Dose (mg/kg/day)	40		400		400	
Concentration (mg base eq./mL)	-		-		40	
Duration of Dosing (day)	14		14		14	
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)	8.9	8.3	57	63	63	94
t_{max} (hh:min [a] or h [b])	2:00	2:00	6:00	14:00	8.0	2.0
AUC (µg.h/mL)	150	148	1066	1320	687	1233
(Time for calculation –h)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)
$t_{1/2}$ (h)	NA	NA	NA	NA	NC	NC
(Time for calculation –h)	-	-	-	-	-	-

Additional Information

After diet administration, the sampling times were 6:00, 14:00, 18:00 and 2:00. After gavage administration, the sampling times were 1, 2, 4, 6, 8 and 24 hours after dosing.

a o'clock time; b ; after gavage

HPMC: hydroxypropyl methylcellulose; LC-MS/MS: liquid chromatography with tandem mass spectrometry; NA: not applicable; NC: not calculated

2.6.5.4 Pharmacokinetics: Absorption After Repeated Doses **Test Article:** Rilpivirine

2.6.5.4.D. TMC278–NC121: Four-Week Toxicity Study by Oral Route (Gavage) in CB6F1-NonTgrasH2 Mice

Study No.	**TMC278–NC121**											
Species	**Mouse (CB6F1-nonTgrasH2)**											
Feeding Condition	**Not fasted**											
Vehicle/Formulation	**TMC278.HCl in HPMC (0.5% w/v)**											
Route	**Oral (gavage)**											
Gender (M/F)/Number of Animals	M/15		F/15		M/15		F/15		M/15		F/15	
Dose (mg base eq./kg/day)	20				80				320			
Concentration (mg base eq./mL)	2				8				32			
Duration of Dosing (day)	1	29	1	29	1	29	1	29	1	29	1	29
Sample (whole blood, plasma, serum, etc.)	plasma		plasma		plasma		plasma		plasma		plasma	
Analyte	TMC278		TMC278		TMC278		TMC278		TMC278		TMC278	
Assay	LC-MS/MS		LC-MS/MS		LC-MS/MS		LC-MS/MS		LC-MS/MS		LC-MS/MS	
Pharmacokinetic Parameters												
C_{max} (µg/mL)	15	14	14	15	30	30	28	37	45	66	42	69
t_{max} (h)	1.0	2.0	1.0	1.0	1.0	2.0	1.0	1.0	6.0	6.0	1.0	1.0
AUC (µg.h/mL)	52	64	45	54	199	250	192	240	643	1090	667	942
(Time for calculation –h)	(0-∞)	(0-24)	(0-∞)	(0-24)	(0-∞)	(0-24)	(0-∞)	(0-24)	(0-∞)	(0-24)	(0-∞)	(0-24)
$t_{1/2}$ (h)	1.9	3.1	1.7	5.1	1.3	1.4	1.5	2.1	4.2	28	4.0	NC
(Time for calculation –h)	(6-12)	(12-24)	(6-12)	(12-24)	(12-24)	(12-24)	(12-24)	(12-24)	(12-24)	(12-24)	(12-24)	-

Additional Information

The sampling times were 1, 2, 6, 12 and 24 h after dosing

LC-MS/MS: liquid chromatography with tandem mass spectrometry; NC: not calculated; HPMC: hydroxypropyl methylcellulose

2.6.5.4.E. TMC278–NC119: Three-Month Repeated Dose Oral Toxicity Study in the Swiss Mouse

Study No.	**TMC278–NC119**											
Species	**Mouse (CD-1)**											
Feeding Condition	**Not fasted**											
Vehicle/Formulation	**TMC278.HCl in HPMC (0.5% w/v)**											
Route	**Oral (gavage)**											
Gender (M/F)/Number of Animals	M/15			F/15			M/15			F/15		
Dose (mg base eq./kg/day)	20						80					
Concentration (mg base eq./mL)	2						8					
Duration of Dosing (day)	1	31	87	1	31	87	1	31	87	1	31	87
Sample (whole blood, plasma, serum, etc.)	plasma			plasma			plasma			Plasma		
Analyte	TMC278			TMC278			TMC278			TMC278		
Assay	LC-MS/MS			LC-MS/MS			LC-MS/MS			LC-MS/MS		
Pharmacokinetic Parameters												
C_{max} (µg/mL)	14	14	18	13	18	19	28	38	34	32	37	42
t_{max} (h)	1.0	1.0	1.0	1.0	1.0	0.5	2.0	1.0	0.5	1.0	2.0	1.0
AUC (µg.h/mL)	71	61	80	59	74	61	236	263	210	250	313	313
(Time for calculation –h)	(0-∞)	(0-8)	(0-24)	(0-∞)	(0-24)	(0-24)	(0-∞)	(0-24)	(0-24)	(0-∞)	(0-24)	(0-24)
$t_{1/2}$ (h)	3.0	4.5	1.6	1.9	1.8	2.0	1.6	1.9	2.2	1.6	1.7	1.9
(Time for calculation –h)	(2-8)	(2-8)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)

Additional Information

The sampling times were 0.5, 1, 2, 8 and 24 h after dosing.

LC-MS/MS: liquid chromatography with tandem mass spectrometry; HPMC : hydroxypropyl methylcellulose

2.6.5.4 Pharmacokinetics: Absorption After Repeated Doses

Test Article: Rilpivirine

Study No.	**TMC278–NC119 (continued)**					
Species	**Mouse (CD-1)**					
Feeding Condition	**Not fasted**					
Vehicle/Formulation	**TMC278.HCl in HPMC (0.5% w/v)**					
Route	**Oral (gavage)**					
Gender (M/F)/Number of Animals	M/15			F/15		
Dose (mg base eq./kg/day)	320					
Concentration (mg base eq./mL)	32					
Duration of Dosing (day)	1	31	87	1	31	87
Sample (whole blood, plasma, serum, etc.)	plasma			plasma		
Analyte	TMC278			TMC278		
Assay	LC-MS/MS			LC-MS/MS		
Pharmacokinetic Parameters						
C_{max} (µg/mL)	63	63	61	55	84	90
t_{max} (h)	8.0	2.0	1.0	8.0	1.0	1.0
AUC (µg.h/mL)	1010	860	665	707	1170	1360
(Time for calculation –h)	(0-∞)	(0-24)	(0-24)	(0-∞)	(0-24)	(0-24)
$t_{1/2}$ (h)	6.8	4.9	2.9	4.1	5.8	7.5
(Time for calculation –h)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)

Additional Information

The sampling times were 0.5, 1, 2, 8 and 24 h after dosing.

LC-MS/MS: liquid chromatography with tandem mass spectrometry; HPMC : hydroxypropyl methylcellulose

2.6.5.4.F. TMC278–NC120: Carcinogenicity Study by Oral Gavage Administration to CD-1 mice for 104 Weeks

Study No.	**TMC278–NC120**											
Species	**Mouse (CD-1)**											
Feeding Condition	**Not fasted**											
Vehicle/Formulation	**TMC278.HCl in HPMC (0.5% w/v)**											
Route	**Oral (gavage)**											
Gender (M/F)/Number of Animals	M/15	M/9	F/15	F/9	M/15	M/9	F/15	F/9	M/15	M/9	F/15	F/9
Dose (mg base eq./kg/day)	20				60				160			
Concentration (mg base eq./mL)	2				6				16			
Duration of Dosing	Day 1	Week 28	Day 1	Week 28	Day 1	Week 28	Day 1	Week 28	Day 1	Week 28	Day 1	Week 28
Sample (whole blood, plasma, serum, etc.)	plasma		plasma		plasma		plasma		plasma		plasma	
Analyte	TMC278		TMC278		TMC278		TMC278		TMC278		TMC278	
Assay	LC-MS/MS		LC-MS/MS		LC-MS/MS		LC-MS/MS		LC-MS/MS		LC-MS/MS	
Pharmacokinetic Parameters												
C_{max} (µg/mL)	9.9	9.8	13	9.9	23	22	24	29	41	36	38	58
t_{max} (h)	2.0	1.0	1.0	1.0	2.0	1.0	2.0	1.0	6.0	2.0	2.0	1.0
AUC (µg.h/mL)	60	76	61	51	239	230	182	278	440	505	345	766
(Time for calculation –h)	(0-∞)	(0-24)	(0-∞)	(0-24)	(0-∞)	(0-24)	(0-∞)	(0-24)	(0-∞)	(0-24)	(0-∞)	(0-24)
$t_{1/2}$ (h)	2.0	2.2	2.1	2.7	1.5	2.8	1.7	2.5	1.7	4.6	1.5	3.7
(Time for calculation –h)	(12-24)	(12-24)	(12-24)	(12-24)	(12-24)	(12-24)	(12-24)	(12-24)	(12-24)	(12-24)	(12-24)	(12-24)

Additional Information

The sampling times were 1, 2, 6, 12 and 24 h after dosing

In the vehicle group, TMC278 plasma concentrations above the lower limit of quantification (0.002 µg/mL) were found in 10 out of 42 samples (concentrations ranged between 0.002 and 0.012 µg/mL)

LC-MS/MS: liquid chromatography with tandem mass spectrometry; HPMC : hydroxypropyl methylcellulose

2.6.5.4.G. TMC278-FK4103: Five-Day Repeated Dose Oral Toxicity Study in the Rat (Investigative Tolerance Study)

Study No.	**TMC278-FK4103** [a]			
Species	**Rat (Sprague Dawley)**			
Feeding Condition	**NS**			
Vehicle/Formulation	**TMC278 base in PEG400**			
Route	**Oral (gavage)**			
Gender (M/F)/Number of Animals	M/5		M/5	
Dose (mg/kg/day)	40		400	
Concentration (mg/mL)	5		50	
Duration of Dosing (day)	1	5	1	5
Sample (whole blood, plasma, serum, etc.)	plasma	plasma	plasma	plasma
Analyte	TMC278	TMC278	TMC278	TMC278
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Mean Pharmacokinetic Parameters				
C_{max} (µg/mL)	1.4		1.8	
t_{max} (h)	0.6		8	
C_{1h} (µg/mL)	1.0	1.8	1.5	2.8
C_{24h} (µg/mL)	0.039	0.15	0.18	0.56
AUC (µg.h/mL)	14		28	
(Time for calculation –h)	(0-∞)		(0-∞)	
$t_{1/2}$ (h)	3.6		5.7	
(Time for calculation –h)	(8-24)		(8-24)	

Additional Information

The sampling times on Day 1 were 0.5, 1, 4, 8 and 24h after gavage administration; on Day 5 samples were taken 1 and 24 h after administration.

a the toxicology study number is EXP5463

LC-MS/MS: liquid chromatography with tandem mass spectrometry; NS: not specified; PEG400: polyethylene glycol 400

2.6.5.4.H. TMC278-FK4243: Two-Week Repeated Dose Oral Toxicity Study in the Rat

Study No.	**TMC278-FK4243** [a]											
Species	**Rat (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		M/4		F/4		M/4		F/4	
Dose (mg/kg/day)	40		40		120		120		400		400	
Concentration (mg/mL)	4		4		12		12		40		40	
Duration of Dosing (day)	1	14	1	14	1	14	1	14	1	14	1	14
Sample (whole blood, plasma, serum, etc.)	Plasma		Plasma		Plasma		Plasma		Plasma		Plasma	
Analyte	TMC278		TMC278		TMC278		TMC278		TMC278		TMC278	
Assay	LC-MS/MS		LC-MS/MS		LC-MS/MS		LC-MS/MS		LC-MS/MS		LC-MS/MS	
Mean Pharmacokinetic Parameters												
C_{max} (µg/mL)	1.6	2.2	3.2	5.7	2.8	3.6	4.6	7.8	10	8.4	14	15
t_{max} (h)	2.0	1.0	1.5	2.5	3.3	2.0	4.5	4.5	8.0	8.0	8.0	8.0
AUC (µg.h/mL)	13	16	29	42	30	35	55	88	86	84	128	152
(Time for calculation –h)	(0-∞)	(0-24)	(0-∞)	(0-24)	(0-∞)	(0-24)	(0-∞)	(0-24)	(0-∞)	(0-24)	(0-∞)	(0-24)
$t_{1/2}$ (h)	2.7	3.7	3.3	3.8	3.9	3.1	5.0	5.5	2.7	3.2	2.9	3.6
(Time for calculation –h)	(8-24)	(3 or 8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)

Additional Information

The sampling times were 0.3, 1, 3, 8 and 24 h after gavage administration.

a the toxicology study number is TOX5535

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

2.6.5.4 Pharmacokinetics: Absorption After Repeated Doses **Test Article:** Rilpivirine

2.6.5.4.I. TMC278-NC136: Two-Week Repeated Dose Oral Toxicity Study in the Rat

Study No.	**TMC278-NC136**							
Species	**Rat (Sprague Dawley)**							
Feeding Condition	**Not fasted**		**Not fasted**				**Not fasted**	
Vehicle/Formulation	**TMC278 base in dietary admixture**		**TMC278.HCl in dietary admixture**				**TMC278.HCl in HPMC (0.5%)**	
Route	**Oral (diet)**		**Oral (diet)**				**Oral (gavage)**	
Gender (M/F)/Number of Animals	M/6	F/6	M/6	F/6	M/6	F/6	M/6	F/6
Dose (mg base eq./kg/day)	400		400		1200		400	
Concentration (mg base eq./mL)	-		-		-		40	
Duration of Dosing (day)	12		12		12		12	
Sample (whole blood, plasma, serum, etc.)	plasma		plasma		plasma		plasma	
Analyte	TMC278		TMC278		TMC278		TMC278	
Assay	NS		NS		NS		NS	
Pharmacokinetic Parameters								
C_{max} (µg/mL)	3.7	4.3	5.2	7.2	11	13	7.3	12
t_{max} (hh:min (diet) or h (gavage))	22:00	2:00	22:00	2:00	2:00	22:00	4	2
AUC (µg.h/mL)	57	86	82	137	180	266	51	103
(Time for calculation –h)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)
$t_{1/2}$ (h)	NA	NA	NA	NA	NA	NA	3.3	2.0
(Time for calculation –h)	-	-	-	-	-	-	(16-24)	(16-24)

Additional Information

The sampling times were 6:00, 10:00, 14:00, 18:00, 22:00 and 2:00 after diet administration and 0, 1, 2, 4, 8, 12, 16, 20 and 24 h after gavage administration.

LC-MS/MS: liquid chromatography with tandem mass spectrometry; HPMC: hydroxypropyl methylcellulose; NA: not applicable; NS: not specified.

2.6.5.4.J. TMC278-NC177: Two-Week Repeated Dose Oral Toxicity Study of R314585 in the Rat

Study No.	**TMC278-NC177**							
Species	**Rat (Sprague Dawley)**							
Feeding Condition	**Not fasted**							
Vehicle/Formulation	**TMC278.HCl in HPMC (0.5%)**							
Route	**Oral (gavage)**							
Gender (M/F)/Number of Animals	M/3		F/3		M/3		F/3	
Dose (mg base eq./kg/day)	400		400		1500		1500	
Concentration (mg base eq./mL)	40		40		150		150	
Duration of Dosing (day)	1	14	1	14	1	14	1	14
Sample (whole blood, plasma, serum, etc.)	plasma		plasma		plasma		plasma	
Analyte	TMC278		TMC278		TMC278		TMC278	
Assay	LC-MS/MS		LC-MS/MS		LC-MS/MS		LC-MS/MS	
Pharmacokinetic Parameters								
C_{max} (µg/mL)	5.4	5.2	12	12	18	9.5	12	12
t_{max} (h)	5.7	3.3	3.3	1.0	3.3	5.7	3.3	3.3
AUC (µg.h/mL)	50	42	78	96	153	86	152[a]	115
(Time for calculation –h)	(0-∞)	(0-24)	(0-∞)	(0-24)	(0-∞)	(0-24)	(0-∞)	(0-24)
$t_{1/2}$ (h)	2.1	3.1	3.3	3.7	5.5	2.5	2.9[a]	2.1[a]
(Time for calculation –h)	NS	NS	NS	NS	NS	NS	NS	NS

Additional Information

The sampling times were 1, 8, 12 and 24 h after dosing on Days 1 and 14

[a] n=2; AUC_{0-24h} (n=3) = 123 µg.h/mL

HPMC: hydroxypropyl methylcellulose; LC-MS/MS: liquid chromatography with tandem mass spectrometry; NS: not specified

2.6.5.4 Pharmacokinetics: Absorption After Repeated Doses

Test Article: Rilpivirine

Study No.	**TMC278-NC177 (continued)**			
Species	**Rat (Sprague Dawley)**			
Feeding Condition	**Not fasted**			
Vehicle/Formulation	**TMC278.HCl in HPMC (0.5%)**			
Route	**Oral (gavage)**			
Gender (M/F)/Number of Animals	M/3		F/3	
Dose (mg base eq./kg/day)	2000		2000	
Concentration (mg base eq./mL)	200		200	
Duration of Dosing (day)	1	14	1	14
Sample (whole blood, plasma, serum, etc.)	plasma	plasma	plasma	plasma
Analyte	TMC278	TMC278	TMC278	TMC278
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Pharmacokinetic Parameters				
C_{max} (µg/mL)	10	7.7	17	14
t_{max} (h)	8.0	3.3	8.0	3.3
AUC (µg.h/mL)	103	77	206[a]	147
(Time for calculation –h)	(0-∞)	(0-24)	(0-∞)	(0-24)
$t_{1/2}$ (h)	2.0	2.1	6.7	2.9
(Time for calculation –h)	NS	NS	NS	NS

Additional Information

The sampling times were 1, 8, 12 and 24 h after dosing on Days 1 and 14

a n=2; AUC_{0-24h} (n=3) = 208 µg.h/mL

HPMC: hydroxypropyl methylcellulose; LC-MS/MS: liquid chromatography with tandem mass spectrometry

2.6.5.4.K. TMC278-TOX5692: Four-Week Oral (Gavage) Immunotoxicity Study in the Rat

Study No.	**TMC278-TOX5692**											
Species	**Rat (Sprague Dawley)**											
Feeding Condition	**Not fasted**											
Vehicle/Formulation	**TMC278 base in PEG400/CA (10%)**											
Route	**Oral (gavage)**											
Gender (M/F)/Number of Animals	M/4		F/4		M/4		F/4		M/4		F/4	
Dose (mg/kg/day)	10		10		40		40		160		160	
Concentration (mg/mL)	1		1		4		4		16		16	
Duration of Dosing (day)	1	29	1	29	1	29	1	29	1	29	1	29
Sample (whole blood, plasma, serum, etc.)	Plasma		Plasma		Plasma		Plasma		Plasma		Plasma	
Analyte	TMC278		TMC278		TMC278		TMC278		TMC278		TMC278	
Assay	LC-MS/MS		LC-MS/MS		LC-MS/MS		LC-MS/MS		LC-MS/MS		LC-MS/MS	
Pharmacokinetic Parameters												
C_{max} (µg/mL)	0.94	0.88	2.0	1.6	2.0	2.6	3.2	5.8	6.5	6.7	9.6	8.8
t_{max} (h)	4.0	2.0	1.0	2.0	2.0	8.0	1.0	4.0	8.0	8.0	8.0	8.0
AUC (µg.h/mL)	7.5	7.2	14	14	23	27	30	42	52	51	77	89
(Time for calculation –h)	(0-∞)	(0-24)	(0-∞)	(0-24)	(0-∞)	(0-24)	(0-∞)	(0-24)	(0-∞)	(0-24)	(0-∞)	(0-24)
$t_{1/2}$ (h)	1.9	2.3	2.1	3.2	3.8	2.7	4.1	3.1	2.5	2.5	2.3	2.7
(Time for calculation –h)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)

Additional Information

The sampling times were 0.5, 1, 2, 4, 8 and 24 h after dosing on Days 1 and 29

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

2.6.5.4 Pharmacokinetics: Absorption After Repeated Doses **Test Article:** Rilpivirine

2.6.5.4.L. TMC278-NC117: One-Month Repeated Dose Oral Toxicity Study in the Rat

Study No.	**TMC278-NC117**							
Species	**Rat (Sprague Dawley)**							
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	
Dose (mg/kg/day)	10		10		400		400	
Concentration (mg/mL)	1		1		40		40	
Duration of Dosing (day)	1	24	1	24	1	24	1	24
Sample (whole blood, plasma, serum, etc.)	Plasma		Plasma		Plasma		Plasma	
Analyte	TMC278		TMC278		TMC278		TMC278	
Assay	LC-MS/MS		LC-MS/MS		LC-MS/MS		LC-MS/MS	
Pharmacokinetic Parameters								
C_{max} (µg/mL)	0.87	0.74	1.5	1.4	8.3	7.7	12	13
t_{max} (h)	2.0	2.0	2.0	2.0	8.0	12	8.0	8.0
AUC (µg.h/mL)	6.3	5.4	10	8.6	101	90	123	149
(Time for calculation –h)	(0-∞)	(0-24)	(0-∞)	(0-24)	(0-∞)	(0-24)	(0-∞)	(0-24)
$t_{1/2}$ (h)	2.9	2.8	3.6	3.1	3.02	2.59	2.21	2.50
(Time for calculation –h)	(8-12)	(6-24)	(8-24)	(8-24)	(12-24)	(12-24)	(12-24)	(12-24)

Additional Information

The sampling times were 0.5, 1, 2, 4, 6, 8, 12 and 24 h after dosing on Days 1 and 24

The dose was given in a daily volume of 1 mL/100g in two administrations (0.5 mL/administration and 1 hour separated).

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

2.6.5.4 Pharmacokinetics: Absorption After Repeated Doses

Test Article: Rilpivirine

Study No.	**TMC278-NC117 (continued)**							
Species	**Rat (Sprague Dawley)**							
Feeding Condition	**Not fasted**							
Vehicle/Formulation	**TMC278.HCl in HPMC (0.5%)**							
Route	**Oral (gavage)**							
Gender (M/F)/Number of Animals	M/6		F/6		M/6		F/6	
Dose (mg base eq./kg/day)	10		10		400		400	
Concentration (mg base eq./mL)	1		1		40		40	
Duration of Dosing (day)	1	24	1	24	1	24	1	24
Sample (whole blood, plasma, serum, etc.)	Plasma		Plasma		Plasma		Plasma	
Analyte	TMC278		TMC278		TMC278		TMC278	
Assay	LC-MS/MS		LC-MS/MS		LC-MS/MS		LC-MS/MS	
Pharmacokinetic Parameters								
C_{max} (µg/mL)	0.90	0.76	1.6	1.7	7.0	4.8	9.5	8.9
t_{max} (h)	2.0	2.0	2.0	2.0	4.0	2.0	4.0	2.0
AUC (µg.h/mL)	5.5	4.5	8.6	7.8	52	33	100	86
(Time for calculation –h)	(0-∞)	(0-24)	(0-∞)	(0-24)	(0-∞)	(0-24)	(0-∞)	(0-24)
$t_{1/2}$ (h)	1.8	2.7	2.1	3.4	1.9	2.5	1.7	1.9
(Time for calculation –h)	(8-12)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(12-24)	(12-24)

Additional Information

The sampling times were 0.5, 1, 2, 4, 6, 8, 12 and 24 h after dosing on Days 1 and 24

The dose was given in a daily volume of 1 mL/100g in two administrations (0.5 mL/administration and 1 hour separated).

HPMC: hydroxypropyl methylcellulose; LC-MS/MS: liquid chromatography with tandem mass spectrometry

2.6.5.4 Pharmacokinetics: Absorption After Repeated Doses **Test Article:** Rilpivirine

2.6.5.4.M. TMC278-NC101: Six-Month Repeated Dose Oral Toxicity Study with 1-Month Recovery in the Rat

Study No.	**TMC278-NC101**								
Species	**Rat (Sprague Dawley)**								
Feeding Condition	**Not fasted**								
Vehicle/Formulation	**TMC278 base in PEG400/CA (10%)**								
Route	**Oral (gavage)**								
Gender (M/F)/Number of Animals	M/6	M/5	M/3	F/6	F/6	F/5	M/6	M/5	M/3
Dose (mg/kg/day)		40			40			120	
Concentration (mg/mL)		4			4			12	
Duration of Dosing (day)	1	84	175 [a]	1	84	175 [a]	1	84	175 [a]
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)	2.9	3.3	1.7	6.5	8.2	6.6	6.4	3.4	3.0
t_{max} (h)	1.0	2.0	2.0	1.0	1.0	1.0	8.0	0.5	10
AUC (µg.h/mL)	19	19	12	32	41	50	53	41	35
(Time for calculation –h)	(0-∞)	(0-24)	(0-24)	(0-∞)	(0-24)	(0-24)	(0-∞)	(0-24)	(0-24)
$t_{1/2}$ (h)	2.3	3.9	3.9	4.3	5.3	4.3	2.2	6.3	3.9
(Time for calculation –h)	(8-24)	(8-24)	(10-24)	(8-24)	(8-24)	(10-24)	(8-24)	(8-24)	(10-24)

Additional Information

The sampling times were 0.5, 1, 2, 4, 8 and 24 h after dosing on Days 1 and 84 and 0.5, 1, 2, 4, 6, 10 and 24 h on Day 175.

a Total dosing volume of 10 mL/kg was changed after Day 84 and split in two administrations of 5 mL/kg each, with 1.5 h between the two administrations

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

2.6.5.4 Pharmacokinetics: Absorption After Repeated Doses

Test Article: Rilpivirine

Study No.	**TMC278-NC101 (continued)**								
Species	**Rat (Sprague Dawley)**								
Feeding Condition	**Not fasted**								
Vehicle/Formulation	**TMC278 base in PEG400/CA (10%)**								
Route	**Oral (gavage)**								
Gender (M/F)/Number of Animals	F/6	F/5	F/5	M/6	M/5	M/3	F/6	F/6	F/6
Dose (mg/kg)		120			400			400	
Concentration (mg/mL)		12			40			40	
Duration of Dosing (day)	1	84	175 [a]	1	84	175 [a]	1	84	175 [a]
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)	8.5	11	8.8	9.1	3.9	6.2	17	15	16
t_{max} (h)	0.50	0.50	0.50	8.0	0.50	10	8.0	8.0	10
AUC (µg.h/mL)	83	100	116	92	56	73	160	184	244
(Time for calculation –h)	(0-∞)	(0-24)	(0-24)	(0-∞)	(0-24)	(0-24)	(0-∞)	(0-24)	(0-24)
$t_{1/2}$ (h)	2.6	4.9	5.6	3.5	11	4.4	2.5	4.5	7.4
(Time for calculation –h)	(8-24)	(8-24)	(10-24)	(8-24)	(8-24)	(10-24)	(8-24)	(8-24)	(10-24)

Additional Information

The sampling times were 0.5, 1, 2, 4, 8 and 24 h after dosing on Days 1 and 84 and 0.5, 1, 2, 4, 6, 10 and 24 h on Day 175.

a Total dosing volume of 10 Ml/kg was changed after Day 84 and split in two administrations of 5 Ml/kg each, with 1.5 h between the two administrations

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

2.6.5.4.N. TMC278–NC123: TMC278-HCI: Carcinogenicity Study by Oral Gavage Administration to CD Rats for 104 Weeks

Study No.	**TMC278–NC123**											
Species	**Rat (Sprague Dawley)**											
Feeding Condition	**Not fasted**											
Vehicle/Formulation	**TMC278.HCl in HPMC (0.5%)**											
Route	**Oral (gavage)**											
Gender (M/F)/Number of Animals	M/9			F/9			M/9			F/9		
Dose (mg base eq./kg/day)	40						200					
Concentration (mg base eq./Ml)	4						20					
Duration of Dosing (day)	1	Week 27	Week 39	1	Week 27	Week 39	1	Week 27	Week 39	1	Week 27	Week 39
Sample (whole blood, plasma, serum, etc.)	plasma			plasma			plasma			plasma		
Analyte	TMC278			TMC278			TMC278			TMC278		
Assay	LC-MS/MS			LC-MS/MS			LC-MS/MS			LC-MS/MS		
Pharmacokinetic Parameters												
C_{max} (µg/Ml)	1.6	0.85	0.82	2.2	2.2	2.1	2.6	1.2	1.3	4.2	5.2	4.7
t_{max} (h)	2.0	2.0	2.0	2.0	0.50	0.50	2.0	2.0	2.0	4.0	4.0	2.0
AUC (µg.h/Ml)	19	4.5	6.3	17	15	14	34	11	8.2	40[b]	36	41
(Time for calculation –h)	(0-∞)	(0-24)	(0-24)	(0-∞)	(0-24)	(0-24)	(0-∞)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)
$t_{1/2}$ (h)	6.6	4.2	NC[a]	5.0	NC[a]	NC[a]	4.5	12	11	NC[a]	NC[a]	7.6
(Time for calculation –h)	(12-24)	(12-24)	-	(12-24)	-	-	(12-24)	(12-24)	(12-24)	-	-	(12-24)

Additional Information

The sampling times were 0.5, 2, 4, 8, 12 and 24 h after dosing.

In the vehicle group, on Day 1, 2 samples out of 18 (at 12 hours, 0.00132 µg/Ml and 0.00488 µg/Ml) were above the limit of quantification (LOQ, 0.001-0.002 µg/Ml), at Week 27, 17 samples (16 samples < 0.006 µg/Ml and 1 = 0.108 µg/Ml) out of 18 were above LOQ and at Week 39, 7 samples (< 0.019 µg/Ml) out of 18 were above the LOQ.

a not calculated since $C_{24h} > C_{12h}$; [b] $AUC_{0-\infty}$ = NC

HPMC: hydroxypropyl methylcellulose; LC-MS/MS: liquid chromatography with tandem mass spectrometry; NC: not calculated

2.6.5.4 Pharmacokinetics: Absorption After Repeated Doses

Test Article: Rilpivirine

Study No.	**TMC278–NC123 (continued)**											
Species	**Rat (Sprague Dawley)**											
Feeding Condition	**Not fasted**											
Vehicle/Formulation	**TMC278.HCl in HPMC (0.5%)**											
Route	**Oral (gavage)**											
Gender (M/F)/Number of Animals		M/9		F/9	F/9	F/8	M/9	M/8	M/7		F/9	
Dose (mg base eq./kg/day)			500						1500			
Concentration (mg base eq./mL)			50						150			
Duration of Dosing (day)	1	Week 27	Week 39	1	Week 27	Week 39	1	Week 27	Week 39	1	Week 27	Week 39
Sample (whole blood, plasma, serum, etc.)		plasma			plasma			plasma			plasma	
Analyte		TMC278			TMC278			TMC278			TMC278	
Assay		LC-MS/MS			LC-MS/MS			LC-MS/MS			LC-MS/MS	
Pharmacokinetic Parameters												
C_{max} (µg/mL)	4.5	2.0	1.8	6.5	5.7	8.5	6.1	2.7	2.2	7.0	10	9.4
t_{max} (h)	4.0	0.50	0.50	2.0	4.0	0.50	8.0	0.50	2.0	2.0	0.50	0.50
AUC (µg.h/mL)	45	14	14	63	70	46	58	20	18	81	81	84
(Time for calculation –h)	(0-∞)	(0-24)	(0-24)	(0-∞)	(0-24)	(0-24)	(0-∞)	(0-24)	(0-24)	(0-∞)	(0-24)	(0-24)
$t_{1/2}$ (h)	4.5	7.4	8.0	4.1	NC[a]	11	7.8	5.1	NC[a]	5.1	8.0	3.0
(Time for calculation –h)	(12-24)	(12-24)	(12-24)	(12-24)	-	(12-24)	(12-24)	(12-24)	-	(12-24)	(12-24)	(12-24)

Additional Information

The sampling times were 0.5, 2, 4, 8, 12 and 24 h after dosing.

In the vehicle group, on Day 1, 2 samples out of 18 (at 12 hours, 0.00132 µg/mL and 0.00488 µg/mL) were above the limit of quantification (LOQ, 0.001-0.002 µg/mL), at Week 27, 17 samples (16 samples < 0.006 µg/mL and 1 = 0.108 µg/mL) out of 18 were above LOQ and at Week 39, 7 samples (< 0.019 µg/mL) out of 18 were above the LOQ.

a not calculated since $C_{24h} > C_{12h}$

HPMC: hydroxypropyl methylcellulose; LC-MS/MS: liquid chromatography with tandem mass spectrometry; NC: not calculated

2.6.5.4 Pharmacokinetics: Absorption After Repeated Doses **Test Article:** Rilpivirine

2.6.5.4.O. TMC278–NC168: Oral (Gavage) Pre- and Post-Natal Developmental Toxicity and Juvenile Toxicity Dose Range Finding Study in the Rat

Study No.	**TMC278–NC168**							
Species	**Rat (Sprague Dawley)**							
Feeding Condition	**Not fasted**							
Vehicle/Formulation	**TMC278.HCl in HPMC (0.5%)**							
Route	**Oral (gavage)**							
Gender (M/F)/Number of Animals	M/8	F/8	M/7	F/8	M/7	F/7	M/8	F/8
Dose (mg base eq./kg/day)	40		120		400		400	
Concentration (mg base eq./mL)	4		12		40		40	
Age of pups (day)	25		25		25		25	
Duration of Dosing (days)	14		14		14		14	
Sample (whole blood, plasma, serum, etc.)	plasma		plasma		plasma		plasma	
Analyte	TMC278		TMC278		TMC278		TMC278	
Assay	LC-MS/MS		LC-MS/MS		LC-MS/MS		LC-MS/MS	
Pharmacokinetic Parameters								
C_{max} (µg/mL)	2.6	5.8	3.7	3.6	9.1	7.3	6.4	4.9
t_{max} (h)	1.0	1	4	4	4	4	4	1
AUC (µg.h/mL)	12	18	34	28	50	53	43	39
(Time for calculation –h)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)
$t_{1/2}$ (h)	2.7	2.3	4.4	2.6	2.8	2.0	4.1	3.2
(Time for calculation –h)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)

Additional Information

Pups in the first 400 mg/kg come from dams dosed with TMC278 HCl at 400 mg/kg. Pups in the second 400 mg/kg group come from dams dosed with vehicle.

The sampling times were predose, 1, 4 and 8 h after dose administration.

On Day 7 of lactation blood samples were taken from pups (4/group/sex/time point) at predose, 2 and 6 h after dose administration to the dams. In pups dosed by lactation AUC_{0-24h} 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.

HPMC: hydroxypropyl methylcellulose; LC-MS/MS: liquid chromatography with tandem mass spectrometry

2.6.5.4 Pharmacokinetics: Absorption After Repeated Doses **Test Article:** Rilpivirine

2.6.5.4.P. TMC278–NC126: Five-Day Repeated Dose Oral Toxicity Study in the Female Rabbit

Study No.	**TMC278–NC126**		
Species	**Rabbit (New Zealand white)**		
Feeding Condition	**-**		
Vehicle/Formulation	**TMC278 base in aqueous HPMC (0.5%)**		
Route	**Oral (gavage)**		
Gender (M/F)/Number of Animals	F/3	F/3	F/3
Dose (mg/kg/day)	100	300	1000
Concentration (mg/mL)	10	30	100
Duration of Dosing (day)	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)	58	124	138
t_{max} (h)	8.0	13	19
AUC (µg.h/mL)	1120	2695	2971
(Time for calculation –h)	(0-24)	(0-24)	(0-24)
$t_{1/2}$ (h)	ND	ND	ND
(Time for calculation –h)	-	-	-

Additional Information

The sampling times were 0 (predose), 0.5, 1, 2, 4, 8 and 24 h after dosing

HPMC: hydroxypropyl-methylcellulose; LC-MS/MS: liquid chromatography with tandem mass spectrometry; ND: could not be determined

2.6.5.4.Q. TMC278–FK4102: Single Dose Escalation Oral Toxicity Study Followed by a 5-day Repeated Dose Oral Toxicity Study in the Beagle Dog (Tolerence Study)

Study No.	**TMC278–FK4102** [a]									
Species	**Dog (beagle)**									
Feeding Condition	-									
Vehicle/Formulation	**TMC278 base in PEG400**		**TMC278 base in PEG400**		**TMC278 base in PEG400/CA (10%)**		**TMC278 base in PEG400/CA (10%)**			
Route	**Oral (gavage)**		**Oral (gavage)**		**Oral (gavage)**		**Oral (gavage)**			
Gender (M/F)/Number of Animals	M/1	F/1	M/1	F/1	M/1	F/1	M/1		F/1	
Dose (mg/kg/day)	40		80		80		80			
Concentration (mg/mL)	50		100		100		100			
Duration of Dosing (day)	1		1		1		1	5	1	5
Sample (whole blood, plasma, serum, etc.)	plasma		plasma		plasma		plasma			
Analyte	TMC278		TMC278		TMC278		TMC278			
Assay	LC-MS/MS		LC-MS/MS		LC-MS/MS		LC-MS/MS			
Pharmacokinetic Parameters										
C_{max} (µg/mL)	1.1	0.95	0.88	0.81	2.1	2.2	3.8	13	2.2	8.0
t_{max} (h)	7.0	4.0	7.0	4.0	7.0	7.0	7.0	24	2.0	24
AUC (µg.h/mL)	41	52	37	47	42	49	78	262	43	154
(Time for calculation –h)	(0-∞)	(0-∞)	(0-∞)	(0-∞)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)
$t_{1/2}$ (h)	12	20	16	24	ND	ND	ND	ND	ND	ND
(Time for calculation –h)	(24-72)	(24-144)	(24-120)	(24-120)	-	-	-	-	-	-

Additional Information

The sampling times were 0.5, 1, 2, 4, 7 and 24 h; additionally at 72 and 144 h after dosing at 40 mg/kg and at 120 h after dosing at 80 mg/kg (PEG400).

a the toxicology study number was Exp5461

CA: citric acid; LC-MS/MS: liquid chromatography with tandem mass spectrometry; ND: not determined; PEG400: polyethylene glycol.

2.6.5.4 Pharmacokinetics: Absorption After Repeated Doses **Test Article:** Rilpivirine

2.6.5.4.R. TMC278–FK4244: Seven-Day Repeated Dose Oral Toxicity Study in the Beagle Dog

Study No.	**TMC278–FK4244**[a]					
Species	**Dog (beagle)**					
Feeding Condition	**Not fasted**					
Vehicle/Formulation	**TMC278 base in PEG400/CA (10%)**					
Route	**Oral (gavage)**					
Gender (M/F)/Number of Animals	M/3		M/3		M/3	
Dose (mg/kg/day)	20		40		80	
Concentration (mg/mL)	20		40		80	
Duration of Dosing (day)	1	7	1	7	1	7
Sample (whole blood, plasma, serum, etc.)	plasma	plasma	plasma	plasma	plasma	plasma
Analyte	TMC278	TMC278	TMC278	TMC278	TMC278	TMC278
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Pharmacokinetic Parameters						
C_{max} (µg/mL)	1.6	3.7	1.7	7.7	1.5	7.8
t_{max} (h)	3.0	3.0	5.0	11	10	6.0
AUC (µg.h/mL)	18	39	27	159	19	147
(Time for calculation –h)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)
$t_{1/2}$ (h)	3.6	7.8	4.1	11	4.2	19
(Time for calculation –h)	(4-8)	(4-8)	(4-8)	(4-8)	(4-8)	(4-8)

Additional Information

The sampling times were 0, 0.5, 1, 2, 4, 8 and 24 h after dosing on Day 1 and Day 7..

a the toxicology study number was Exp5534

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

2.6.5.4 Pharmacokinetics: Absorption After Repeated Doses **Test Article:** Rilpivirine

2.6.5.4.S. TMC278–TOX5650: One-month Repeated Dose Oral Toxicity Study in the Beagle Dog with 1-Month Recovery

Study No.	**TMC278–TOX5650**											
Species	**Dog (beagle)**											
Feeding Condition	**Not fasted**											
Vehicle/Formulation	**TMC278 base in PEG400/CA (10%)**											
Route	**Oral (gavage)**											
Gender (M/F)/Number of Animals	M/3		F/3		M/3		F/3		M/3		F/3	
Dose (mg/kg/day)	5		5		10		10		40		40	
Concentration[b] (mg/mL)												
Duration of Dosing (day)	1	28	1	28	1	28	1	28	1	28	1	28
Sample (whole blood, plasma, serum, etc.)	plasma		plasma		plasma		plasma		plasma		plasma	
Analyte	TMC278		TMC278		TMC278		TMC278		TMC278		TMC278	
Assay	LC-MS/MS		LC-MS/MS		LC-MS/MS		LC-MS/MS		LC-MS/MS		LC-MS/MS	
Pharmacokinetic Parameters												
C_{max} (µg/mL)	0.94	1.5	1.3	2.0	1.3	5.6	1.3	2.5	2.8	12	2.4	9.5
t_{max} (h)	3.0	2.0	3.0	11	6.0	8.0	2.0	5.0	19	5.0	12	8.0
AUC (µg.h/mL)	13[a]	27	19	37	22[a]	103	14[a]	47	51[a]	204	40[a]	160
(Time for calculation –h)	(0-24)	(0-24)	(0-∞)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)
$t_{1/2}$ (h)	33	29	11	181	28	33	21	43	21	48	17	50
(Time for calculation –h)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)

Additional Information

The sampling times were 0, 0.5, 1, 2, 4, 8 and 24 h after dosing on Day 1 and Day 28. On Day 30, 31, 35, 44, 49 and 56 at 8:00.

During the 1-month recovery period the mean through levels decreased from Day 30 to Day 56.

The TMC278 levels were measured in the adrenal gland and plasma at autopsy. Tissue to plasma ratios for the adrenal gland were 1.7 and 1.3 at 5 mg/kg, 1.9 and 2.2 at 10 mg/kg and 3.6 and 1.7 at 40 mg/kg in males and females, respectively.

a $AUC_{0-\infty}$ extrapolation > 45%

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

2.6.5.4 Pharmacokinetics: Absorption After Repeated Doses **Test Article:** Rilpivirine

2.6.5.4.T. TMC278–NC116: One-Month Repeated Dose Oral Toxicity Study in the Beagle Dog

Study No.	**TMC278–NC116**							
Species	**Dog (beagle)**							
Feeding Condition	**Not fasted**							
Vehicle/Formulation	**TMC278 base in PEG400/CA (10%)**							
Route	**Oral (gavage)**							
Gender (M/F)/Number of Animals	M/3		F/3		M/3		F/3	
Dose (mg/kg/day)	5				40			
Concentration (mg/mL)	5				40			
Duration of Dosing (day)	1	28	1	28	1	28	1	28
Sample (whole blood, plasma, serum, etc.)	plasma		plasma		plasma		plasma	
Analyte	TMC278		TMC278		TMC278		TMC278	
Assay	LC-MS/MS		LC-MS/MS		LC-MS/MS		LC-MS/MS	
Pharmacokinetic Parameters								
C_{max} (µg/mL)	1.0	1.4	0.66	1.1	1.0	3.8	3.5	2.5
t_{max} (h)	3.0	2.7	7.3	2.3	17	1.8	5.3	2.7
AUC (µg.h/mL)	12	23	8.1	14	17	73	59	43
(Time for calculation –h)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)
$t_{1/2}$ (h)	ND	ND	ND	ND	ND	ND	ND	ND
(Time for calculation –h)	-	-	-	-	-	-	-	-

Additional Information

The sampling times were 0, 0.5, 1, 2, 4, 6, 8, 12 and 24 h after dosing on Day 1 and Day 28.

CA: citric acid; LC-MS/MS: liquid chromatography with tandem mass spectrometry; ND: not determined; PEG400: polyethylene glycol.

2.6.5.4 Pharmacokinetics: Absorption After Repeated Doses

Test Article: Rilpivirine

Study No.	**TMC278–NC116 (continued)**							
Species	**Dog (beagle)**							
Feeding Condition	**Not fasted**							
Vehicle/Formulation	**TMC278.HCl in HPMC (0.5%)**							
Route	**Oral (gavage)**							
Gender (M/F)/Number of Animals	M/3		F/3		M/3		F/3	
Dose (mg base eq./kg/day)	5				40			
Concentration (mg base eq./mL)	5				40			
Duration of Dosing (day)	1	28	1	28	1	28	1	28
Sample (whole blood, plasma, serum, etc.)	plasma		plasma		plasma		plasma	
Analyte	TMC278		TMC278		TMC278		TMC278	
Assay	LC-MS/MS		LC-MS/MS		LC-MS/MS		LC-MS/MS	
Pharmacokinetic Parameters								
C_{max} (µg/mL)	0.49	0.89	0.53	0.70	1.3	4.1	1.5	5.8
t_{max} (h)	11	2.0	2.7	1.3	9.3	3.0	18	2.0
AUC (µg.h/mL)	6.9	13	6.2	7.5	20	76	22	81
(Time for calculation –h)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)
$t_{1/2}$ (h)	ND	ND	ND	ND	ND	ND	ND	ND
(Time for calculation –h)	-	-	-	-	-	-	-	-

Additional Information

The sampling times were 0, 0.5, 1, 2, 4, 6, 8, 12 and 24 h after dosing on Day 1 and Day 28.

HPMC: hydroxypropyl methylcellulose; LC-MS/MS: liquid chromatography with tandem mass spectrometry; ND: not determined.

2.6.5.4 Pharmacokinetics: Absorption After Repeated Doses **Test Article:** Rilpivirine

2.6.5.4.U. TMC278–NC115: Six-Month Repeated Dose Oral Toxicity Study with 3-Month Interim Kill in the Beagle Dog

Study No.	**TMC278–NC115**					
Species	**Dog (beagle)**					
Feeding Condition	**Not fasted**					
Vehicle/Formulation	**TMC278 base in PEG400/CA (10%)**					
Route	**Oral (gavage)**					
Gender (M/F)/Number of Animals		M/3			F/3	
Dose (mg/kg/day)			5			
Concentration (mg/mL)			5			
Duration of Dosing (day)	1	86	177	1	86	177
Sample (whole blood, plasma, serum, etc.)		plasma			plasma	
Analyte		TMC278			TMC278	
Assay		LC-MS/MS			LC-MS/MS	
Pharmacokinetic Parameters						
C_{max} (µg/mL)	1.0	1.4	1.5	0.70	1.1	1.4
t_{max} (h)	2.0	2.3	1.7	2.3	3.3	1.7
AUC (µg.h/mL)	11	21	21	9.2	18	17
(Time for calculation –h)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)
$t_{1/2}$ (h)	ND	ND	ND	ND	ND	ND
(Time for calculation –h)	-	-	-	-	-	-

Additional Information

The sampling times were 0, 1, 2, 4, 8 and 24 h after dosing at Day 1, Day 86 and Day 177.

The TMC278 levels were measured in the liver, adrenal gland and plasma at autopsy at Day 93 and Day 184(males)/185(females). Tissue to plasma ratios for the liver were 8.1/8.9 and 13/13 at 5 mg/kg, 9.7/11 and 9.1/12 at 10 mg/kg and 7.7/9.6 and 9.9/9.4 at 40 mg/kg in males and females on Day93/Day184 or 185, respectively. Tissue to plasma ratios for the adrenal gland were 4.1/5.2 and 5.4/4.1 at 5 mg/kg, 8.1/4.7 and 4.6/4.2 at 10 mg/kg and 5.4/6.8 and 7.4/5.7 at 40 mg/kg in males and females on Day93/Day184 or 185, respectively.

CA: citric acid; LC-MS/MS: liquid chromatography with tandem mass spectrometry; ND: not determined; PEG400: polyethylene glycol.

Study No.	**TMC278–NC115 (continued)**					
Species	**Dog (beagle)**					
Feeding Condition	**Not fasted**					
Vehicle/Formulation	**TMC278 base in PEG400/CA (10%)**					
Route	**Oral (gavage)**					
Gender (M/F)/Number of Animals		M/3			F/3	
Dose (mg/kg/day)			10			
Concentration (mg/mL)			10			
Duration of Dosing (day)	1	86	177	1	86	177
Sample (whole blood, plasma, serum, etc.)		plasma			plasma	
Analyte		TMC278			TMC278	
Assay		LC-MS/MS			LC-MS/MS	
Pharmacokinetic Parameters						
C_{max} (µg/mL)	1.3	2.0	2.0	1.2	2.2	1.9
t_{max} (h)	6.0	2.3	4.0	2.7	2.0	4.0
AUC (µg.h/mL)	22	28	26	20	27	32
(Time for calculation –h)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)
$t_{1/2}$ (h)	ND	ND	ND	ND	ND	ND
(Time for calculation –h)	-	-	-	-	-	-

Additional Information

The sampling times were 0, 1, 2, 4, 8 and 24 h after dosing at Day 1, Day 86 and Day 177.

CA: citric acid; LC-MS/MS: liquid chromatography with tandem mass spectrometry; ND: not determined; PEG400: polyethylene glycol.

2.6.5.4 Pharmacokinetics: Absorption After Repeated Doses

Test Article: Rilpivirine

Study No.	**TMC278–NC115 (continued)**					
Species	**Dog (beagle)**					
Feeding Condition	**Not fasted**					
Vehicle/Formulation	**TMC278 base in PEG400/CA (10%)**					
Route	**Oral (gavage)**					
Gender (M/F)/Number of Animals		M/3			F/3	
Dose (mg/kg/day)			40			
Concentration (mg/mL)			40			
Duration of Dosing (day)	1	86	177	1	86	177
Sample (whole blood, plasma, serum, etc.)		plasma			plasma	
Analyte		TMC278			TMC278	
Assay		LC-MS/MS			LC-MS/MS	
Pharmacokinetic Parameters						
C_{max} (µg/mL)	1.5	2.5	4.0	0.82	3.1	2.9
t_{max} (h)	12	2.0	2.7	9.3	2.7	2.0
AUC (µg.h/mL)	23	41	68	10	52	43
(Time for calculation –h)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)
$t_{1/2}$ (h)	ND	ND	ND	ND	ND	ND
(Time for calculation –h)	-	-	-	-	-	-

Additional Information

The sampling times were 0, 1, 2, 4, 8 and 24 h after dosing at Day 1, Day 86 and Day 177.

CA: citric acid; LC-MS/MS: liquid chromatography with tandem mass spectrometry; ND: not determined; PEG400: polyethylene glycol.

2.6.5.4.V. TMC278–NC107: Fifty-Two Week Oral (Gavage) Toxicity Study in the Beagle Dog

Study No.	**TMC278–NC107**							
Species	**Dog (beagle)**							
Feeding Condition	**Not 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/day)	5							
Concentration (mg/mL)	5							
Duration of Dosing (day)	1	90	273	363	1	90	273	363
Sample (whole blood, plasma, serum, etc.)	plasma				plasma			
Analyte	TMC278				TMC278			
Assay	LC-MS/MS				LC-MS/MS			
Pharmacokinetic Parameters								
C_{max} (µg/mL)	0.70	1.2	1.1	1.1	0.75	1.4	1.1	1.5
t_{max} (h)	2.0	0.50	1.0	9.0	2.0	1.1	0.88	1.5
AUC (µg.h/mL)	11	18	16	17	9.7	21	18	19
(Time for calculation –h)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)
$t_{1/2}$ (h)	ND	ND	ND	ND	ND	ND	ND	ND
(Time for calculation –h)	-	-	-	-	-	-	-	-

Additional Information
The sampling times were 0, 0.5, 1, 2, 4, 8 and 24 h after dosing

CA: citric acid; LC-MS/MS: liquid chromatography with tandem mass spectrometry; ND: not determined; PEG400: polyethylene glycol.

2.6.5.4 Pharmacokinetics: Absorption After Repeated Doses

Test Article: Rilpivirine

Study No.	**TMC278–NC107 (continued)**							
Species	**Dog (beagle)**							
Feeding Condition	**Not 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/day)	10							
Concentration (mg/mL)	10							
Duration of Dosing (day)	1	90	273	363	1	90	273	363
Sample (whole blood, plasma, serum, etc.)	plasma				plasma			
Analyte	TMC278				TMC278			
Assay	LC-MS/MS				LC-MS/MS			
Pharmacokinetic Parameters								
C_{max} (µg/mL)	0.90	2.0	1.3 [b]	1.3 [c]	1.2 [a]	2.3 [b]	2.3 [d]	2.2 [e]
t_{max} (h)	1.5	0.38	1.3 [b]	4.0 [c]	2.8 [a]	0.33 [b]	2.3 [d]	7.0 [e]
AUC (µg.h/mL)	15	29	19 [b]	24 [c]	15 [a]	29 [b]	31 [d]	36 [e]
(Time for calculation –h)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)
$t_{1/2}$ (h)	ND	ND	ND	ND	ND	ND	ND	ND
(Time for calculation –h)	-	-	-	-	-	-	-	-

Additional Information

The sampling times were 0, 0.5, 1, 2, 4, 8 and 24 h after dosing

LC-MS/MS: liquid chromatography with tandem mass spectrometry; ND: not determined; PEG400: polyethylene glycol 400;

a n=5: one animal (number 133) replaced animal 124 that was killed on Day 20, and received the first dose on Day 28 and was sampled with the other animals at Day 89 (61 days of dosing) ;

b n=3;

c n=2;

d one animal was sampled at day 245; [e] one animal was sampled at day 335

2.6.5.4 Pharmacokinetics: Absorption After Repeated Doses

Test Article: Rilpivirine

Study No.	**TMC278–NC107 (continued)**							
Species	**Dog (beagle)**							
Feeding Condition	**Not fasted**							
Vehicle/Formulation	**TMC278 base in PEG400/CA (10%)**							
Route	**Oral (gavage)**							
Gender (M/F)/Number of Animals	M/4				F/4			
Dose[a] (mg/kg/day)	40							
Concentration (mg/mL)	40							
Duration of Dosing (day)	1	90	273	363	1	90	273	363
Sample (whole blood, plasma, serum, etc.)	plasma				plasma			
Analyte	TMC278				TMC278			
Assay	LC-MS/MS				LC-MS/MS			
Pharmacokinetic Parameters								
C_{max} (µg/mL)	2.4	3.6	3.2	4.1	2.5	5.5 [a]	3.5 [a]	5.5[a]
t_{max} (h)	14	1.8	3.0	3.7	8.0	0.67 [a]	4.3 [a]	2.7 [a]
AUC (µg.h/mL)	37	60	60	65	41	88 [a]	51 [a]	61 [a]
(Time for calculation –h)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)
$t_{1/2}$ (h)	ND	ND	ND	ND	ND	ND	ND	ND
(Time for calculation –h)	-	-	-	-	-	-	-	-

Additional Information

The sampling times were 0, 0.5, 1, 2, 4, 8 and 24 h after dosing

LC-MS/MS: liquid chromatography with tandem mass spectrometry; ND: not determined; PEG400: polyethylene glycol 400;
a n=3;

2.6.5.4.W. TMC278–NC249: Oral (Gavage) Pharmacokinetic Study of TMC278 HCl in the Female Cynomolgus Monkey

Study No.	**TMC278–NC249**			
Species	**Monkey (cynomolgus)**			
Feeding Condition	**Not fasted**			
Vehicle/Formulation	**TMC278.HCl in HPMC (0.5% w/v)**			
Route	**Oral (gavage)**			
Gender (M/F)/Number of Animals	F/3		F/3	
Dose (base eq./kg/day)	10		100	
Concentration (mg base eq./mL)	2		20	
Duration of Dosing (day)	1	7	1	7
Sample (whole blood, plasma, serum, etc.)	plasma	plasma	plasma	plasma
Analyte	TMC278	TMC278	TMC278	TMC278
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Pharmacokinetic Parameters				
C_{max} (µg/mL)	0.086	0.041	0.39	0.48
t_{max} (h)	2.7	3.3	5.3	4.7
AUC (µg.h/mL)	0.88 [a]	0.45	5.1 [b]	5.3
(Time for calculation –h)	(0-24)	(0-24)	(0-24)	(0-24)
$t_{1/2}$ (h)	7.3	13	121	11
(Time for calculation –h)	(8-24)	(8-24)	(8-24)	(8-24)

Additional Information

The sampling times were 0, 1, 2, 4, 8 and 24 h after dosing

a $AUC_{0-\infty}$ = 0.99 µg.h/mL; [b] $AUC_{0-\infty}$ extrapolation > 25%

HPMC: hydroxypropyl methylcellulose; LC-MS/MS: liquid chromatography with tandem mass spectrometry

2.6.5.4 Pharmacokinetics: Absorption After Repeated Doses **Test Article:** Rilpivirine

2.6.5.4.X. TMC278–NC326: Pharmacokinetics of TMC278 after Single and Repeated Oral Administration of TMC278 HCl in Female Monkeys

Study No.	**TMC278–NC326**					
Species	**Monkey (cynomolgus)**					
Feeding Condition	**Not fasted**					
Vehicle/Formulation	**TMC278.HCl in HPMC (1% w/v) with 0.5% Tween 20**					
Route	**Oral (gavage)**					
Gender (M/F)/Number of Animals	F/3	F/3	F/3		F/3	
Dose (mg base eq./kg/day)	250 b.i.d [a]	500 q.d.	100 b.i.d [a]		250 b.i.d [a]	
Concentration (mg base eq./mL)	50 b.i.d. [a]	50 q.d.	20 b.i.d. [a]		50 b.i.d. [a]	
Duration of Dosing (day)	1	1	1	14	1	14
Sample (whole blood, plasma, serum, etc.)	plasma	plasma	plasma	plasma	plasma	plasma
Analyte	TMC278	TMC278	TMC278	TMC278	TMC278	TMC278
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Pharmacokinetic Parameters						
C_{max1} (µg/mL)	0.33	0.58	0.094	0.29	0.25	0.50
t_{max1} (h)	6.7	6.7	8.0	3.0	5.3	4.0
C_{max2} (µg/mL)	0.67	-	0.19	0.21	0.38	0.56
t_{max2} (h)	5.3	-	5.3	3.7	6.7	4.3
AUC (µg.h/mL)	10.1	6.1	2.8	4.4	5.9	10
(Time for calculation –h)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)
$t_{1/2}$ (h)	8.6	8.2	33	19	5.8	9.5
(Time for calculation –h)	(16-24)	(8-24)	(16-24)	(16-24)	(16-24)	(16-24)

Additional Information

The sampling times were 0, 1, 2, 4, 8 (before second dose), 9, 10, 12, 16 and 24 h after b.i.d dosing and 0, 1, 2, 4, 8 and 24 h after q.d. dosing

In the control-dosed group, 3 plasma concentrations, only on day 1, were above the limit of quantification (0.001 µg/mL): at 1 h after dosing (0.00155 and 0.00194 µg/mL) and at 10 h after dosing (0.084 µg/mL)

a twice daily dosing with an interval of 8 hours;

HPMC: hydroxypropyl methylcellulose; LC-MS/MS: liquid chromatography with tandem mass spectrometry; q.d.: once daily; b.i.d.: twice daily;

C_{max1} and t_{max1}: after the first dose; C_{max2} and t_{max2}: after the second dose

2.6.5.4.Y. TMC278–NC248: Endocrinological Oral (Gavage) 8-Week Study in the Female Sexually Immature Cynomolgus Monkey

Study No.	**TMC278–NC248**	
Species	**Monkey (cynomolgus)**	
Feeding Condition	**Not fasted**	
Vehicle/Formulation	**TMC278.HCl in HPMC (1% w/v) with 0.5% Tween 20**	
Route	**Oral (gavage)**	
Gender (M/F)/Number of Animals	F/8	F/7
Dose[b] (mg/kg/day)	100 b.i.d.	250 b.i.d.
Concentration (mg/mL)	20 b.i.d.	50 b.i.d.
Duration of Dosing (day)	55	55
Sample (whole blood, plasma, serum, etc.)	plasma	plasma
Analyte	TMC278	TMC278
Assay	LC-MS/MS	LC-MS/MS
Pharmacokinetic Parameters		
C_{max1} (µg/mL)	0.14	0.31
t_{max1} (h)	2.5	4.6
C_{max2} (µg/mL)	0.18	0.31
t_{max2} (h)	5.5	4.6
AUC (µg.h/mL)	2.7	4.6
(Time for calculation –h)	(0-24)	(0-24)
$t_{1/2}$ (h)	7.8	7.5
(Time for calculation –h)	(16-24)	(16-24)

Additional Information

The sampling times were 0, 1, 2, 4, 8 (before second dose), 9, 10, 12, 16 and 24 h after first dose.

In the control-dosed group, plasma concentrations (26 out of 80) were above the limit of quantification (0.001 µg/mL) and ranged from 0.001 to 0.003 µg/mL

b.i.d.: twice daily; HPMC: hydroxypropyl methylcellulose; LC-MS/MS: liquid chromatography with tandem mass spectrometry

C_{max1} and t_{max1}: after the first dose; C_{max2} and t_{max2}: after the second dose.

2.6.5.4.Z. M990203-PK: Thirteen-Week Oral (Gavage) Toxicity Study of Tenofovir DF in the Mouse

Species	**Mouse**							
	M990203-PK							
Feeding Condition	Fed							
Vehicle Formulation	50mM Citric acid							
Method of Administration	Oral							
Sample	Plasma							
Analyte	Tenofovir							
Assay	HPLC							
Dose mg/kg	**100**		**300**		**600**			
Gender (M/F) No. of Animals	14M		14M		14M		14F	
PK parameters	**Single Dose**	**Day 91**	**Single Dose**	**Day 91**	**Single Dose**	**Day 91**	**Single Dose**	**Day 91**
C_{max} (µg/mL)	3.38	8.82	5.89	17.9	24.8	28.7	20.6	38.9
T_{max} (hr)	0.25	0.0830	0.500	0.0830	0.0830	0.25	0.500	0.0830
AUC 0-∞ (µg•hr/mL)	51.3	-	46.3	-	29.8	-	48.1	-
AUC % Extrapolated	87.4	-	62.8	-	27.1	11.9	23.0	-
AUC 0-τ (µg•hr/mL)	-	14.6	-	35.8	-	68.1	-	55.2
$t_{1/2}\lambda_z$ (hr)	42.2	10.2	16.4	10.7	5.00	7.50	3.28	8.22
CL/F (mL/hr/kg)	884	-	2940	-	9130	-	5660	-
CLss/F (mL/hr/kg)	-	3730	-	4700	-	4670	-	5540
MRT 0-∞ (hr)	60.6	11.6	21.0	13.2	5.75	10.3	4.80	8.82
V_z/F (mL/kg)	53800	54700	69500	72900	65800	50600	26800	65700

Additional Information

NC = not calculated due to insufficient data NA = data not available

2.6.5.4.AA. M990205: Carcinogeniticy Study of Tenofovir DF in the Mouse (Oral Gavage)

Species	**Mouse**
	M990205-PK
Feeding Condition	Fed
Vehicle Formulation	50mM Citric acid
Method of Administration	Oral
Sample	Plasma
Analyte	Tenofovir
Assay	HPLC
Dose mg/kg	600
Gender (M/F) No. of Animals	60 M / 60F

PK parameters	**Single Dose**	**Day 180**	**Single Dose**	**Day 180**
C_{max} (µg/mL)	11.5	8.80	17.6	16.05
T_{max} (hr)	0.289	0.500	0.094	0.258
AUC 0-24 (µg•hr/mL)	30.9	44.3	35.1	50.9
AUC 0-∞ (µg•hr/mL)	50.4	NC	48.7	NC
$t_{1/2}\lambda_z$ (hr)	20.7	10.2	17.9	11.2

Additional Information

NC = not calculated due to insufficient data NA = data not available

2.6.5.4.BB. 96-TOX-4331-003-PK: A 28-Day Oral Gavage Toxicity Study of GS-4331-05 in the Albino Rat

Species	**Rat**
	96-TOX-4331-003-PK
Feeding Condition	Fasted
Vehicle Formulation	0.9% benzyl alcohol, 0.5% polysorbate 20, 0.5% carboxymethyl cellulose, 0.9% NaCl
Method of Administration	Oral
Sample	Plasma
Analyte	Tenofovir
Assay	HPLC

Dose mg/kg	**20**		**100**		**500**	
Gender (M/F) No. of Animals	8M		8M		8M & 8F	
PK Parameters	**Single Dose**	**Day 28**	**Single Dose**	**Day 28**	**Single Dose**	**Day 28**
C_{max} (µg/mL)	0.207	0.221	0.857	0.835	1.52	1.79
T_{max} (hr)	1.00	1.00	0.500	0.500	0.25	0.50
$AUC_{0\rightarrow\tau}$ (µg•hr/mL)	NC	NC	NC	NC	NC	15.4
$AUC_{0\rightarrow24}$ (µg*hr/mL)	NC	-	NC	-	7.75	-
$AUC_{0\rightarrow\infty}$ (µg*hr/mL)	NC	-	NC	-	19.1	-
AUC % Extrapolated	NC	-	NC	-	56.3	-
$t_{1/2}\,\lambda_z$ (hr)	NC	NC	NC	NC	26.6	10.3
CL/F (mL/hr/kg)	NC	NC	NC	NC	13445	14900
C_{last} (µg/mL)	0.046	0.046	0.212	0.143	0.279	0.239
T_{last} (hr)	8.00	8.00	2.00	8.00	24.0	24.0
Vz/F (mL/kg)	NC	NC	NC	NC	481500	220500
F %	NC	-	NC	-	9.84	-

Additional Information

NC = not calculated due to insufficient data NA = data not available

2.6.5.4.CC. R2000036-PK: Mechanistic Study of Tenofovir DF in the Rat (Oral Gavage)

Species	**Rat**
	R2000036-PK
Feeding Condition	Fed
Vehicle Formulation	50mM Citric acid
Method of Administration	Oral
Sample	Plasma
Analyte	Tenofovir
Assay	LC-MS

Dose mg/kg		**40**			**400**	
Gender (M/F) No. of Animals		18M			21M	
PK Parameters	**Single Dose**	**Day 13**	**Day 28**	**Single Dose**	**Day 13**	**Day 28**
C_{max} (µg/mL)	1.06	0.770	1.00	3.17	2.55	4.01
T_{max} (hr)	0.250	0.500	0.500	0.500	0.500	1.00
$AUC_{0\rightarrow24}$ (µg*hr/mL)	2.39	2.53	3.13	9.67	11.9	19.6
$AUC_{0\rightarrow\infty}$ (µg*hr/mL)	2.55	-	-	16.3	-	-
AUC % Extrapolated	6.23	-	-	40.8	-	-
$t_{1/2}\lambda_z$ (hr)	6.88	6.82	8.34	19.7	13.5	21.8
CL/F (mL/hr/kg)	7090	7160	5770	11100	15200	9210
C_{last} (µg/mL)	0.016	0.018	0.033	0.235	0.189	0.267
T_{last} (hr)	24.0	24.0	24.0	24.0	24.0	48.0
Vz/F (mL/kg)	70400	70500	69400	314000	295000	290000
F %	18.6	-	-	11.9	-	-

Additional Information

NC = not calculated due to insufficient data NA = data not available

2.6.5.4.DD. 97-TOX-4331-002-PK: A 13- and 42-Week Oral Gavage Toxicity Study (with a 13-Week Recovery Period) of Bis-POC PMPA (GS-4331-05) in the Albino Rat

Species	**Rat**
	97-TOX-4331-002-PK
Feeding Condition	Fed
Vehicle Formulation	0.9% benzyl alcohol, 0.5% polysorbate 20, 0.5% carboxymethyl cellulose sodium
Method of Administration	Oral
Sample	Plasma
Analyte	Tenofovir
Assay	HPLC

Dose mg/kg			**30**				**100**	
Gender (M/F) No. of Animals	2F & 2M	2F & 2M	2F & 2M	2F & 2M	2F & 2M	2F & 2M	2F &2 M	2F & 2M
PK Parameters	**Single Dose**	**Week 13**	**Week 26**	**Week 42**	**Single Dose**	**Week 13**	**Week 26**	**Week 42**
C_{max} (µg/mL)	0.46	0.56	0.66	0.74	1.17	1.40	1.36	1.86
T_{max} (hr)	0.38	0.75	0.50	0.50	0.75	0.75	0.50	1.00
$AUC_{0\rightarrow24}$ (µg*hr/mL)	NA	2.65	3.93	3.78	NA	7.00	6.89	8.34
$AUC_{0\rightarrow\infty}$ (µg*hr/mL)	2.87	-	-	-	6.64	-	-	-
AUC % Extrapolated	NA	-	-	-	NA	-	-	-
$t_{1/2}\lambda_z$ (hr)	6.21	4.93	8.30	7.24	10.7	7.68	8.91	9.34
CL/F (mL/hr/kg)	4724	5120	3450	3590	6806	6460	6560	5420
C_{last} (µg/mL)	0.007	0.012	0.038	0.031	0.052	0.072	0.081	0.123
T_{last} (hr)	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0
F %	27.9	-	-	-	19.4	-	-	-

Additional Information

NC = not calculated due to insufficient data NA = data not available

Species	**Rat (cont)**
	97-TOX-4331-002-PK
Feeding Condition	Fed
Vehicle Formulation	0.9% benzyl alcohol, 0.5% polysorbate 20, 0.5% carboxymethyl cellulose sodium
Method of	Oral
Sample	Plasma
Analyte	Tenofovir
Assay	HPLC

Dose mg/kg			**300**				**1000**	
Gender (M/F) No. of Animals	2F & 2M	2F & 2M	2F & 2M	2F & 2M	2F & 2M	2F & 2M	2F & 2M	2F &2 M
PK Parameters	**Single Dose**	**Week13**	**Week 26**	**Week 42**	**Single Dose**	**Week 13**	**Week 26**	**Week 42**
C_{max} (µg/mL)	1.51	2.86	2.32	2.58	2.71	4.52	5.82	6.57
T_{max} (hr)	0.75	2.50	1.00	1.00	0.75	0.50	1.00	1.50
$AUC_{0\rightarrow24}$ (µg*hr/mL)	NA	18.4	13.8	17.6	NA	34.1	42.8	64.9
$AUC_{0\rightarrow\infty}$ (µg*hr/mL)	17.7	-	-	-	43.5	-	-	-
AUC % Extrapolated	NA	-	-	-	NA	-	-	-
$t_{1/2}\lambda_z$ (hr)	17.3	7.21	11.0	9.10	29.6	25.6	12.1	9.66
CL/F (mL/hr/kg)	7660	7370	9830	7700	10389	13300	10600	6960
C_{last} (µg/mL)	0.200	0.151	0.209	0.300	0.447	0.699	0.758	1.42
T_{last} (hr)	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0
F %	17.2	-	-	-	12.7	-	-	-

Additional Information

NC = not calculated due to insufficient data NA = data not available

2.6.5.4.EE. R990204: An Oral Carcinogenicity Study of Tenofovir Disoproxil Fumarate (Tenofovir DF) in the Albino Rat

Species	**Rat**
	R990204
Feeding Condition	Fed
Vehicle Formulation	0.9% benzyl alcohol, 0.5% polysorbate 20, 0.5% carboxymethyl cellulose, Sterile Water
Method of Administration	Oral
Sample	Plasma
Analyte	Tenofovir
Assay	LC/MS

Dose mg/kg	**30**		**100**		**300**	
Gender (M/F) No. of Animals	3/sex/timepoint		3/sex/timepoint		3/sex/timepoint	
PK Parameters	**Day 1**	**Day 180**	**Day 1**	**Day 180**	**Day 1**	**Day 180**
C_{max} (µg/mL)	0.66	0.69	1.37	1.37	3.91	2.87
T_{max} (hr)	0.50	0.50	0.5	0.50	0.25	0.50
$AUC_{0\rightarrow24}$ (µg*hr/mL)	2.32	2.89	5.11	6.99	10.70	16.20
$AUC_{0\rightarrow\infty}$ (µg*hr/mL)	2.46	NC	5.52	NC	15.82	NC
$t_{1/2}\lambda_z$ (hr)	6.52	6.24	7.02	6.85	17.60	7.93
C_{last} (ng/mL)	14.81	18.01	40.12	51.44	201.35	154.13
T_{last} (hr)	24.0	24.0	24.0	24.0	24.0	24.0
Vz/F (mL/kg)	NC	NC	NC	NC	NC	NC
F %	NC	NC	NC	NC	NC	NC
Additional Information	Data for combined sexes					

NC = not calculated due to insufficient data NA = data not available

2.6.5.4.FF. 96-TOX-4331-001-PK: Five-Day Toxicity Study of Tenofovir DF in the Dog (Oral Gavage)

Species	**Dog** **96-TOX-4331-001-PK**
Feeding Condition	Fed
Vehicle Formulation	50 mM citric acid
Method of Administration	Oral
Sample	Plasma
Analyte	Tenofovir
Assay	HPLC

Dose mg/kg	**9**		**45**		**180**	
Gender (M/F) No. of Animals	2M		2M		2M	
PK Parameters	**H0039** **Day 5**	**H0046**	**H00398** **Day 5**	**H00400**	**H00403** **Day 5**	**H00404**
C_{max} (µg/mL)	0.943	1.54	6.16	7.31	79.7	11.5
T_{max} (hr)	0.250	0.250	0.250	0.250	0.250	0.250
$AUC_{0\rightarrow\infty}$ (µg*hr/mL)	4.19	4.98	30.5	62.9	8.00	1.00
Cl/F	868	731	596	289	39.5	817
T_{last} (hr)	24.0	24.0	24.0	24.0	24.0	24.0
$t_{1/2}\lambda_z$ (hr)	15.5	4.25	15.3	18.8	NC	19.2
Vz/F (mL/kg)	19403	4487	13200	7859	NC	22604
F %						
Additional Information						

NC = not calculated due to insufficient data NA = data not available

2.6.5.4.GG. 96-TOX-4331-004-PK: Twenty-eight Day Toxicity Study of Tenofovir DF in the Dog (Oral Gavage)

Species	**Dog** 96-TOX-4331-004-PK							
Feeding Condition								
Vehicle Formulation	50 mM citric acid							
Method of Administration	Oral							
Sample	Plasma							
Analyte	Tenofovir							
Assay	HPLC							
Dose mg/kg			**3**				**10**	
Gender (M/F) No. of Animals	4M	4 F	4M	4F	4M	4F	4M	4F
PK Parameters mean +/- SD	**Single dose**		**Day 28**		**Single dose**		**Day 28**	
C_{max} (µg/mL)	0.864 ± 0.908	0.459 ± 0.676	1.0 ± 1.14	0.141 ± 0.0877	0.487 ± 0.0437	0.985 ± 0.628	0.984 ± 0.951	0.699 ± 0.396
T_{max} (hr)	0.750 ± 0.289	0.750 ± 0.289	1.25 ± 0.866	0.500 ± 0.354	0.375 ± 0.144	0.313 ± 0.125	0.375 ± 0.144	0.438 ± 0.125
$AUC_{0\to\tau}$ (µg•hr/mL)	-	-	NC	NC	-	-	3.84 ± 2.66	NC
$AUC_{0\to\infty}$ (µg*hr/mL)	NC	NC	-	-	NC	NC	-	-
$t_{1/2}\lambda_z$ (hr)	NC	NC	NC	NC	NC	NC	20.1 ± 11.8	NC
CL_{ss}/F (mL/hr/kg)	-	-	NC	NC	-	-	1520 ± 674	NC
CL/F (mL/hr/kg)	NC	NC	-	-	NC	NC	-	-
$MRT_{0\to\infty}$ (hr)	NC	NC	NC	NC	NC	NC	23.1 ± 12.4	NC
Vz/F (mL/kg)	NC	NC	NC	NC	NC	NC	82300 ± 31200	NC
Additional Information								

NC = not calculated due to insufficient data NA = data not available

2.6.5.4 Pharmacokinetics: Absorption After Repeated Doses

Test Article: Tenofovir disoproxil fumarate

Species	**Dog**			
	96-TOX-4331-004-PK (cont)			
Feeding Condition				
Vehicle f|Formulation	50 mM citric acid			
Method of Administration	Oral			
Sample	Plasma			
Analyte	Tenofovir			
Assay	HPLC			
Dose mg/kg			**30**	
Gender (M/F) No. of Animals	4M	4F	4M	4F
PK Parameters mean +/- SD	**Single Dose**		**Day 28**	
C_{max} (µg/mL)	3.45 ± 2.62	2.39 ± 0.932	1.83 ± 0.219	5.31 ± 1.36
T_{max} (hr)	0.313 ± 0.125	0.563 ± 0.315	1.13 ± 0.629	0.875 ± 0.250
$AUC_{0\rightarrow\tau}$ (µg•hr/mL)	-	-	14.4 ± 2.35	24.6 ± 6.12
$AUC_{0\rightarrow\infty}$ (µg•hr/mL)	11.2 ± 5.34	7.95 ± 1.94	-	-
$t_{1/2}\lambda_z$ (hr)	11.8 ± 2.30	10.7 ± 1.47	16.4 ± 5.38	21.8 ± 10.1
CL_{ss}/F (mL/hr/kg)	-	-	967 ± 184	574 ± 116
CL/F (mL/hr/kg)	1420 ± 598	1790 ± 492	-	-
MRT $_{0\rightarrow\infty}$ (hr)	12.4 ± 1.21	10.6 ± 0.467	21.6 ± 7.02	23.3 ± 10.7
Vz/F (mL/kg)	52200 ± 24000	161000 ± 189000	49900 ± 15500	41000 ± 23600
Additional Information				

NC = not calculated due to insufficient data NA = data not available

2.6.5.4.HH. 98-TOX-4331-003-PK: Twenty-eight Day Toxicity Study of Tenofovir DF in the Dog (Oral Gavage)

Species	**Dog**								
	98-TOX-4331-003-PK								
Feeding Condition									
Vehicle Formulation	50 mM citric acid								
Method of Administration	Oral								
Sample	Plasma								
Analyte	Tenofovir								
Assay	HPLC								
			1 day					**28 days**	
Dose mg/kg	**15 bid**	**30 qd**	**60 q2d**	**210 q7***	**210 q7****	**15 bid**	**30 qd**	**60 q2d**	**210 q7d**
Gender (M/F) No. of Animals	4M	4M	4M	2M	2M	4M	4M	4M	2M
PK Parameters Mean									
C_{max} (µg/mL)	2.64	4.48	8.04	26.9	49.8	2.62 (1.67)	7.54 (1.11)	13.0 (2.97)	32.9 (9.25)
T_{max} (hr)	0.500	0.625	0.750	0.750	1.00	0.875 (0.250)	0.750 (0.289)	1.38 (0.750)	1.00 (0.0)
$AUC_{0\rightarrow\tau}$ (µg•hr/mL)	-	-	-	-	-	NC	29.4 (5.87)	67.0 (15.1)	219 (61.6)
$AUC_{(0\text{-}last)}$ (µg.hr/mL)	4.31	12.1	24.1	127	201	-	-	-	-
$AUC_{0\rightarrow\infty}$ (µg*hr/mL)	5.20	14.9	32.2	134	NC	-	-	-	-
AUC % Extrapolated	18.2	18.9	25.1	4.72	NC	-	-	-	-
$t_{1/2}\lambda_z$ (hr)	3.72	13.4	17.1	42.5	NC	NC	29.4 (9.59)`	41.6 (19.9)	40.1 (6.13)
CL_{ss}/F (mL/hr/kg)	-	-	-	-	-	NC	473 (78.9)	422 (101)	452 (127)
CL/F (mL/hr/kg)	1360	932	932	765	NC	-	-	-	-
C_{last} (µg/mL)	0.180	0.144	0.329	0.107	10.3	NC	0.575 (0.158)	0.447 (0.0958)	0.127 (0.0665)
T_{last} (hr)	6.0	24.0	24.0	168	24.0	NC	24.0 (0.0)	48.0 (0.0)	168 (0.0)
Vz/F (mL/kg)	7810	18200	23300	44900	NC	NC	20500 (8660)	27300 (19800)	25556 (3367)
F %	NC	25.7	27.9	33.2	NC	-	-	-	-

NC = not calculated due to insufficient data NA = data not available

Additional Information * Mean values calculated from dogs 501 and 503. , ** Mean values calculated from dogs 502 and 504. These dogs were euthanized on days 5 and 6 due to apparent renal failure associated with the test article, NC – not calculated due to insufficient data

2.6.5.4.II. 97-TOX-4331-001-PK: Thirteen- and 42-Week Toxicity Study of Tenofovir DF in the Dog (Oral Gavage)

Species	**Dog** **97-TOX-4331-001-PK**					
Feeding Condition	Fed					
Vehicle Formulation	50mM Citric acid					
Method of Administration	Oral					
Sample	Plasma					
Analyte	Tenofovir					
Assay	HPLC					
Dose mg/kg	**3**	**10**	**30**	**3**	**10**	**30**
Gender (M/F) No. of Animals	4M	4M	4M 4F	4M	4M	4M 4F
		Single Dose			**Week 13**	
PK Parameters						
C_{max} (µg/mL)	0.214	0.659	2.95	0.211 (0.0620)	1.38 (0.0850)	7.38 (1.87)
T_{max} (hr)	0.625	0.50	0.625	0.750 (0.289)	0.625 (0.250)	0.875 (0.518)
$AUC_{(0\rightarrow tau)}$ (µg•hr/mL)	-	-	-	NC	5.71 (0.1587)	28.1 (3.52)
$AUC_{(0\text{-}last)}$ (µg*hr/mL)	NC	NC	8.17	-	-	-
$AUC_{0\rightarrow\infty}$ (µg*hr/mL)	NC	NC	12.8	-	-	-
AUC % Extrapolated	NC	NC	31.5	-	-	-
$t_{1/2}\lambda_z$ (hr)	NC	NC	22.1	NC	27.6 (10.0)	55.8 (61.8)
CL_{ss}/F (mL/hr/kg)	-	-	-	-	792 (22.3)	490 (67.5)
CL/F (mL/hr/kg)	NC	NC	1304	-	-	-
C_{last} (µg/mL)	0.0390	0.0363	0.128	0.0305 (0.0027)	0.112 (0.0050)	0.564 (0.109)
T_{last} (hr)	2.75	18.0	24.0	12.0 (8.64)	24.0 (0.00)	24
Vz/F (mL/kg)	NC	NC	37900	NC	31600 (11400)	42550 (55320)
F %	NC	NC	22.2	-	-	-

Additional Information
Values are mean (SD)

NC = not calculated due to insufficient data NA = data not available

Species	**Dog**					
	97-TOX-4331-001-PK (cont)					
Feeding Condition	Fed					
Vehicle Formulation	50mM Citric acid					
Method of Administration	Oral					
Sample	Plasma					
Analyte	Tenofovir					
Assay	HPLC					
Dose mg/kg	**3**	**10**	**30**	**3**	**10**	**30**
Gender (M/F) No. of Animals	4M	4M	4M 4F	6M 6F	6M 6F	6M 6F
		Week 26			**Week 42**	
PK Parameters						
C_{max} (µg/mL)	0.262 (0.0476)	1.03 (0.172)	7.42 (1.77)	0.305 (0.0748)	1.46 (0.365)	8.17 (2.39)
T_{max} (hr)	1.00 (0.000)	0.75 (0.289)	0.750 (0.267)	0.792 (0.257)	0.667 (0.444)	0.875 (0.226)
$AUC_{(0\rightarrow tau)}$ (µg•hr/mL)	NC	5.83 (1.42)	28.5 (6.47)	2.06 (0.428)	6.84 (1.32)	31.1 (7.27)
$AUC_{(0\text{-}last)}$ (µg*hr/mL)	-	-	-	-	-	-
$AUC_{0\rightarrow\infty}$ (µg*hr/mL)	-	-	-	-	-	-
AUC % Extrapolated	-	-	-	-	-	-
$t_{1/2}\lambda_z$ (hr)	NC	46.1 (27.1)	32.6 (17.6)	NC	36.9 (8.68)	32.9 (13.8)
CL_{ss}/F (mL/hr/kg)	NC	814 (220)	500 (127.5)	NC	686 (149)	454 (88.0)
CL/F (mL/hr/kg)	-	-	-	-	-	-
C_{last} (µg/mL)	0.0310 (0.0053)	0.130 (0.0306)	0.580 (0.153)	0.0493 (0.0176)	0.136 (0.0417)	0.573 (0.146)
T_{last} (hr)	17.0 (8.25)	24	24	26.7 (14.1)	32.0 (11.8)	32.0 (11.8)
Vz/F (mL/kg)	NC	53400 (29600)	24500 (16700)	NC	36300 (10700)	21800 (10900)
F %	-	-	-	-	-	-

Additional Information
Values are mean (SD)

NC = not calculated due to insufficient data NA = data not available

2.6.5.4.JJ. P2000078-PK: Mechanistic Study of Tenofovir DF /Tenofovir in the Rhesus Monkey (Oral/Subcutaneous)

Species **Monkey**
P2000078-PK

Feeding Condition	Fed							
Vehicle Formulation	50mM citric acid							
Method of Administration	Oral (nasogastric gavage)							
Sample	Plasma							
Analyte	Tenofovir							
Assay	Reverse phase HPLC mass spectroscopy							
Dose mg/kg	**30**			**250**			**600**	
Gender (M/F) No. of Animals	3M & 3F			3M & 3F			3M & 3F	
PK Parameters (oral data)	**Single Dose**	**Day 27**	**Day 55**	**Single Dose**	**Day 27**	**Day 55**	**Single Dose**	**Day 21-22***
N	6	6	6	6	6	6	6	4
C_{max} (µg/mL)	0.424 (0.239)	0.587 (0.199)	0.768 (0.28)	2.17 (1.13)	1.64 (0.504)	3.74(2.46)	3.40 (2.61)	7.97 (6.37)
$T_{(max)}$ (hr)	1.0 (0.5)	1.2 (0.4)	1.1 (0.5)	2 (2)	1.3 (0.6)	1.5 (0.5)	0.5 (0.0)	1.8 (0.5)
$AUC_{0-\infty/\tau}$ (µg•hr/mL)	3.64 (0.902)	3.77 (1.07)	4.05 (0.958)	16.0 (3.55)	14.4 (4.54)	20.4 (10.2)	28.8 (19.5)	56.3 (22.1)
AUC Extrapolated. (%)	17.0 (5.81)	-	-	23.9 (11.9)	-	-	34.0 (21.4)	-
$t_{1/2}\lambda_z$ (hr)	9.79 (2.43)	11.0 (6.24)	8.26(1.79)	12.5 (4.86)	9.28(1.12)	14.3 (5.63)	22.2 (19.0)	12.7 (2.48)
CL/F (L/hr/kg)	4.04 (1.49)	3.87 (1.15)	3.53 (0.887)	7.47 (2.18)	8.37(2.02)	6.86 (3.55)	13.3 (7.68)	5.60 (2.68)
$MRT_{0-\infty}$	13.4 (2.85)	-	-	16.9 (6.22)	-	-	29.0 (24.3)	-
C_{last} (µg/ml))	0.0433 (0.0126)	0.0526 (0.0237)	0.0650 (0.0442)	0.206 (0.0710)	0.310 (0.304)	0.373 (0.237)	0.307 (0.150)	1.03 (0.427)
T_{last} (hr)	24.0 (0.0)	24 (0)	22 (5)	24 (0)	22 (5)	24 (0)	24 (0)	24 (0)
Vz/F (L/kg)	55.8 (18.1)	62.4 (41.9)	42.5 (15.6)	129 (41.8)	114 (36.1)	130 (55.1)	307 (134)	109 (70.0)

Additional Information * Group euthanized earlier than planned (day 20-22) due to poor condition. SC pharmacokinetic data is shown overleaf.

NC = not calculated due to insufficient data NA = data not available

Species	**Monkey**
	P2000078-PK (cont)
Feeding Condition	Fed
Vehicle Formulation	50mM citric acid
Method of Administration	SC
Sample	Plasma
Analyte	Tenofovir
Assay	Reverse phase HPLC mass spectroscopy

Dose mg/kg		**30**	
Gender (M/F) No. of Animals		3M & 3F	
PK Parameters (oral data)	**Single Dose**	**Day 27**	**Day 55**
N	6	4**	4**
C_{max} (µg/mL)	31.3 (3.75)	43.6 (18.6)	33.7 (3.15)
$T_{(max)}$ (hr)	0.5 (0.0)	0.5 (0.0)	0.5 (0.0)
$AUC_{0-\infty/\tau}$ (µg•hr/mL)	44.1 (10.1)	62.3 (19.2)	44.2 (5.86)
AUC Extrapolated. (%)	0.456 (0.182)	-	-
$t_{1/2}\lambda_z$ (hr)	4.75 (0.753)	3.91 (1.25)	5.48 (0.168)
CL/F (L/hr/kg)	0.708 (0.150)	0.510 (0.122)	0.312 (0.0442)
$MRT_{0-\infty}$	2.06 (0.266)	-	-
C_{last} (µg/mL))	0.0290 (0.0125)	0.0377 (0.00633)	0.0315 (0.00740)
T_{last} (hr)	24 (0)	24 (0)	24 (0)
Vz/F (L/kg)	4.88 (1.33)	2.98 (1.34)	2.47 (0.433)

Additional Information ** Data from 2 animals in this group have been excluded from the mean data as they achieved C_{max} and AUC values 2-3 fold higher than other animals and exhibited signs of renal failure.

NC = not calculated due to insufficient data NA = data not available

2.6.5.5. Pharmacokinetics: Organ Distribution

2.6.5.5.A. TOX092 : [14C]TP-0006: A Tissue Distribution and Excretion Study in Rats

[14C]TP-0006: A Tissue Distribution and Excretion Study in Rats

Study Number: TOX092

Species	**Rat**
Gender (M/F) No. of Animals	20 M SD group 1, 6 M Long-Evans group 2
Feeding Condition	
Vehicle Formulation	
Method of Administration	Oral
Dose (mg/kg)	200, single dose
Radionuclide	^{14}C
Specific Activity	135 μCi/mg
Sampling Time	1, 4, 8, 24, 72 and 144 hours

Tissues/Organs	Mean Tissue:Plasma Concentration Ratios Post Dosing											
	1 hour		4 hours		8 hours		24 hours		72 hours		144 hours	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Blood	0.762	0.057	0.829	0.041	NA	NA	NA	NA	NA	NA	NA	NA
Large Intestine Content	0.742	0.037	1.01	0.273	15.0	7.00	NA	NA	NA	NA	NA	NA
Kidney	2.45	0.388	2.10	0.431	2.62	NA	NA	NA	NA	NA	NA	NA
Liver	1.17	0.055	1.09	0.079	NA	NA	NA	NA	NA	NA	NA	NA
Renal Cortex	2.21	0.393	2.05	0.410	2.52	NA	NA	NA	NA	NA	NA	NA
Small Intestine Content	1.27	0.359	1.31	0.596	NA	NA	NA	NA	NA	NA	NA	NA
Cerebellum	0.068	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Cerebrum	0.066	0.001	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

Data for SD rats only.

NA – Undetected or not sampled.

Additional Information: none

2.6.5.5.B. TMC278–NC108: Tissue Distribution of ^{14}C-R278474, as Studied by Whole-Body Autoradiography, in the Pigmented Male Rat After Single Oral Administration of ^{14}C-R278474 at 40 mg/kg

Study No.	**TMC278–NC108**
Species	**Rat (pigmented Long Evans)**
Feeding Condition	**Not fasted**
Vehicle/Formulation	**TMC278 base in PEG400/CA (10%)**
Route	**Oral (gavage)**
Gender (M/F)/Number of Animals	**M/5**
Dose (mg/kg)	**40**
Radionuclide	**^{14}C-TMC278**
Specific Activity (kBq/mg)	**233**

Sampling Times (h)	**1**		**4**		**24**		**96**		**336**	
Tissues/Organs	**Conc. (µg eq./g)**	**Tissue/Blood**	**Conc. (µg eq./g)**	**Tissue/Blood**	**Conc. (µg eq./g)**	**Tissue/Blood**	**Conc. (µg eq./g)**	**Tissue/Blood**	**Conc. (µg eq./g)**	**Tissue/Blood**
Adrenal gland	3.29	4.64	7.93	5.13	1.21	8.66	0.351	6.15	BLQ	-
Blood (LSC)	0.710	1.00	1.34	0.867	0.139	1.00	0.057	1.00	0.026	1.00
Blood (RLG)	0.708	1.00	1.55	1.00	BLQ	-	BLQ	-	BLQ	-
Bone	BLQ	-	0.348	0.225	BLQ	-	BLQ	-	BLQ	-
Bone marrow	1.10	1.55	2.00	1.29	BLQ	-	BLQ	-	BLQ	-
Brain	0.506	0.715	0.981	0.634	BLQ	-	BLQ	-	BLQ	-
Brown fat	2.95	4.17	6.00	3.88	BLQ	-	BLQ	-	BLQ	-
Eye ball (LSC)	0.967	1.37	5.08	3.29	3.73	26.8	1.78	31.2	1.03	39.8
Heart	1.42	2.00	2.77	1.79	BLQ	-	BLQ	-	BLQ	-
Kidney	2.78	3.93	5.27	3.41	0.707	5.07	BLQ	-	BLQ	-
Liver	9.52	13.4	16.6	10.7	2.28	16.4	0.390	6.82	BLQ	-
Lung	1.03	1.45	3.54	2.29	BLQ	-	BLQ	-	BLQ	-

Additional Information

Tissue/ blood concentration ratios were calculated preferably with blood total radioactivity levels as determined with RLG till 4 hours after dosing and as determined with LSC from 24 hours onwards.

-: not applicable; BLQ: below limit of quantification (0.207 µg/g); CA: citric acid; LSC: liquid scintillation counting; PEG400: polyethylene glycol 400; RLG: radioluminography

Study No.	**TMC278–NC108 (continued)**
Species	**Rat (pigmented Long Evans)**
Feeding Condition	**Not fasted**
Vehicle/Formulation	**TMC278 base in PEG400/CA (10%)**
Route	**Oral (gavage)**
Gender (M/F)/Number of Animals	**M/5**
Dose (mg/kg)	**40**
Radionuclide	**^{14}C-TMC278**
Specific Activity (kBq/mg)	**233**

Sampling Times (h)	**1**		**4**		**24**		**96**		**336**	
Tissues/Organs	**Conc. (µg eq./g)**	**Tissue/Blood**	**Conc. (µg eq./g)**	**Tissue/Blood**	**Conc. (µg eq./g)**	**Tissue/Blood**	**Conc. (µg eq./g)**	**Tissue/Blood**	**Conc. (µg eq./g)**	**Tissue/Blood**
Meninges	1.84	2.60	5.80	3.75	1.73	12.4	1.57	27.5	1.21	46.6
Muscle	0.555	0.783	1.23	0.792	BLQ	-	BLQ	-	BLQ	-
Pancreas	2.20	3.11	4.23	2.73	BLQ	-	BLQ	-	BLQ	-
Prostate	0.865	1.22	2.07	1.34	BLQ	-	BLQ	-	BLQ	-
Skin pigmented	0.915	1.29	3.73	2.41	1.60	11.5	1.67	29.3	0.657	-
Skin white	1.10	1.55	2.93	1.89	0.761	5.47	0.470	8.24	BLQ	-
Spleen	NA	-	4.18	2.70	BLQ	-	BLQ	-	BLQ	-
Testis	0.306	0.433	1.25	0.807	BLQ	-	BLQ	-	BLQ	-
Thyroid	1.58	2.23	2.26	1.46	BLQ	-	BLQ	-	BLQ	-
Uveal tract	5.45	7.70	28.2	18.2	38.3	275	10.9	191	6.86	265
White fat	0.945	1.33	5.63	3.64	BLQ	-	BLQ	-	BLQ	-

Additional Information

Tissue/ blood concentration ratios were calculated preferably with blood total radioactivity levels as determined with RLG till 4 hours after dosing and as determined with LSC from 24 hours onwards.

-: not applicable; BLQ: below limit of quantification (0.207 µg/g); CA: citric acid; LSC: liquid scintillation counting; NA: not analyzed; PEG400: polyethylene glycol 400; RLG: radioluminography

Study No.	**TMC278–NC108 (continued)**
Species	**Rat (pigmented Long Evans)**
Feeding Condition	**Not fasted**
Vehicle/Formulation	**TMC278 base in PEG400/CA (10%)**
Route	**Oral (gavage)**
Gender (M/F)/Number of Animals	**M/5**
Dose (mg/kg)	**40**
Radionuclide	**^{14}C-TMC278**
Specific Activity (kBq/mg)	**233**
Sampling Times (h)	**1, 4, 24, 96 and 336**

Tissues/Organs	**AUC_{0-4h} (µg.h/g)**	**Tissue/Blood AUC_{0-4h} ratio**
Adrenal gland	18.5 (140 [a])	4.95 (6.76 [b])
Blood (LSC)	3.43 [c]	0.92
Blood (RLG)	3.74	1.00
Bone	NC [d]	-
Bone marrow	5.20	1.39
Brain	2.48	0.66
Brown fat	14.9	3.98
Eye ball (LSC)	9.55 (616 [e])	2.55 (20.5 [f])
Heart	6.98	1.87
Kidney	13.5 (58.9 [g])	3.61 (4.21 [h])
Liver	43.9 (265 [a])	11.7 (12.8 [b])
Lung	7.38	1.97

Additional Information

a AUC_{0-96h}; b calculated with AUC_{0-96h}; c AUC_{0-24h} = 14.0 µg.h/g, AUC_{0-96h} = 20.7 µg.h/g and AUC_{0-336h} = 30.1 µg.h/g; d NC: not calculated, too limited data; [e] AUC_{0-336h}; [f] calculated with AUC_{0-336h}; [g] AUC_{0-24h}; [h] calculated with AUC_{0-24h}

-: not applicable; CA: citric acid; LSC: liquid scintillation counting; PEG400: polyethylene glycol 400; RLG: radioluminography

Study No.	**TMC278–NC108 (continued)**	
Species	**Rat (pigmented Long Evans)**	
Feeding Condition	**Not fasted**	
Vehicle/Formulation	**TMC278 base in PEG400/CA (10%)**	
Route	**Oral (gavage)**	
Gender (M/F)/Number of Animals	**M/5**	
Dose (mg/kg)	**40**	
Radionuclide	**^{14}C-TMC278**	
Specific Activity (kBq/mg)	**233**	
Sampling Times (h)	**1, 4, 24, 96 and 336**	
Tissues/Organs	**AUC_{0-4h} (µg.h/g)**	**Tissue/Blood AUC_{0-4h} ratio**
Meninges	12.4 (530 [c])	3.32 (17.6 [d])
Muscle	2.95	0.79
Pancreas	10.7	2.86
Prostate	4.83	1.29
Skin pigmented	7.42 (436 [c])	1.98 (14.5 [d])
Skin white	6.59 (82.2 [a])	1.76 (3.97 [b])
Spleen	-	-
Testis	2.48	0.66
Thyroid	6.55	1.75
Uveal tract	53.2 (4380 [e])	14.2 (146 [f])
White fat	10.3	2.75

Additional Information

a AUC_{0-96h}; b calculated with AUC_{0-96h}; c AUC_{0-336h}; d calculated with AUC_{0-336h}
-: not applicable; CA: citric acid; LSC: liquid scintillation counting; PEG400: polyethylene glycol 400; RLG: radioluminography

2.6.5.5.C. TMC278–NC109: Tissue Distribution and Placental Transfer of ^{14}C-TMC278, as Studied by Whole-Body Autoradiography, in the Pregnant Sprague-Dawley Rat after Single Oral Administration at 40 mg/kg

Study No.	**TMC278–NC109**
Species	**Rat (Sprague Dawley, pregnant)**
Feeding Condition	**Not fasted**
Vehicle/Formulation	**TMC278 base in PEG400/CA (10%)**
Route	**Oral (gavage)**
Gender (M/F)/Number of Animals	**F/4**
Dose (mg/kg)	**40**
Radionuclide	**^{14}C-TMC278**
Specific Activity (kBq/mg)	**233**

Sampling Times (h)	**1**		**4**		**8**		**24**	
Tissues/Organs	**Conc. (µg eq./g)**	**Tissue/Blood**	**Conc. (µg eq./g)**	**Tissue/Blood**	**Conc. (µg eq./g)**	**Tissue/Blood**	**Conc. (µg eq./g)**	**Tissue/Blood**
Adrenal gland	4.52	3.59	9.76	3.47	7.37	4.05	1.75	17.0
Blood (LSC)	1.01	0.802	2.32	0.83	1.40	0.769	0.102	1.00
Blood (RLG)	1.26	1.00	2.81	1.00	1.82	1.00	BLQ	-
Brain	0.849	0.674	1.92	0.683	1.24	0.681	BLQ	-
Fat brown	3.55	2.82	7.29	2.59	6.22	3.42	BLQ	-
Fat white	1.37	1.09	8.18	2.91	8.12	4.46	BLQ	-
Fetus	0.627	0.498	1.87	0.665	1.19	0.654	BLQ	-
Heart	1.85	1.47	3.76	1.34	2.73	1.50	BLQ	-
Kidney	3.91	3.10	5.78	2.06	5.76	3.17	0.582	5.70
Lachrymal gland	3.87	3.07	9.56 [a]	3.40	7.48	4.11	BLQ	-
Liver	7.73	6.14	14.4	5.13	13.3	7.31	0.800	7.84
Lung	2.42	1.92	5.19	1.85	3.46	1.90	BLQ	-
Mammary gland	2.46	1.95	7.70	2.74	7.72	4.24	0.400	3.92
Muscle	0.643	0.510	1.39	0.495	1.14	0.626	BLQ	-
Ovary	NA [b]	-	5.90	2.10	4.03	2.21	BLQ	-
Pancreas	2.59	2.06	4.34	1.54	4.08	2.24	BLQ	-
Placenta	1.10	0.87	2.63	0.936	1.88	1.03	BLQ	-
Salivary gland	1.96	1.56	4.00	1.42	2.94	1.62	BLQ	-
Spleen	1.84	1.46	2.82	1.00	2.37	1.30	BLQ	-
Uterine epithelium	1.49	1.18	10.6	3.77	10.6	5.82	5.51	54.0
Uterus	1.18 [a]	0.937	2.63 [a]	0.936	2.36	1.30	0.303 [c]	3.00
Vagina	0.857	0.680	2.36	0.840	1.95	1.07	0.249 [a]	2.44

Additional Information : Tissue/ blood concentration ratios were calculated preferably with blood total radioactivity levels as determined with RLG till 8 hours after dosing and as determined with LSC at 24 hours.

a n=2; b NA: not analyzed due to flare effect of the high radioactive concentration in the formulation in stomach; c n=1

-: not applicable; BLQ: below limit of quantification (0.196 µg eq./g); CA: citric acid; LSC: liquid scintillation counting; PEG400: polyethylene glycol 400; RLG: radioluminography.

Study No.	**TMC278–NC109 (continued)**
Species	**Rat (Sprague Dawley, pregnant)**
Feeding Condition	**Not fasted**
Vehicle/Formulation	**TMC278 base in PEG400/CA (10%)**
Route	**Oral (gavage)**
Gender (M/F)/Number of Animals	**F/4**
Dose (mg/kg)	**40**
Radionuclide	**^{14}C-TMC278**
Specific Activity (kBq/mg)	**233**
Sampling Times (h)	**1, 4, 8 and 24**

Tissues/Organs	**$AUC_{0\text{-}8h}$ (µg.eq.h/g)**	**Tissue/Blood $AUC_{0\text{-}8h}$ ratio**
Adrenal gland	57.7 (120[a])	3.6 (5.8[c])
Blood (LSC)	12.8 (20.7[a])	0.81
Blood (RLG)	15.9	1.0
Brain	10.8	0.68
Fat brown	45.0	2.8
Fat white	47.6	3.0
Fetus	10.1	0.64
Heart	22.2	1.4
Kidney	39.6 (75.7[a])	2.5 (3.7[c])
Lachrymal gland	56.0	3.5
Liver	92.3 (163[a])	5.8 (7.9[c])
Lung	29.7	1.9
Mammary gland	47.3 (86.9[a])	3.0 (4.2[c])
Muscle	8.41	0.53
Ovary	31.4[b]	2.0
Pancreas	28.5	1.8
Placenta	15.1	0.95
Salivary gland	23.7	1.5
Spleen	18.3	1.2
Uterine epithelium	61.2 (186[a])	3.8 (9.0[c])
Uterus	16.3 (32.3[a])	1.0 (1.6[c])
Vagina	13.9 (27.1[a])	0.87 (1.3[c])

CA: citric acid; LSC: liquid scintillation counting; PEG400: polyethylene glycol 400; RLG: radioluminography

a $AUC_{0\text{-}24h}$; b AUC was calculated with concentration versus time data at 4 and 8 hours. For this tissue, no data were available at 1 hour post dose; c $AUC_{0\text{-}24h}$ ratio calculated with blood (LSC) = 20.7 µg eq.h/g)

2.6.5.5.D. 95-DDM-1278-002: Determination of Distribution of [14C]-PMPA in Male Rats Following Single Administration Using Whole Body Autoradiography

Study Number: 95-DDM-1278-002

Species	Rat
Gender (M/F) No. of Animals	2 (1M per group)
Feeding Condition	IV-Fed; Oral –Fasted
Vehicle Formulation	Sterile aqueous solution. (10mg/mL)
Method of Administration	Single oral dose (Group 1), single IV dose (Group 2)
Dose (mg/kg)	10 (tenofovir)
Radionuclide	^{14}C
Specific Activity	10 µCi/mg
Sampling Time	24 hours

Tissues/Organs

	Organs and tissues in order of decreasing concentrations (autoradiography qualitative analysis)
t 1=24 hours IV administration	Renal cortex> large intestine> bladder> liver> cecum
t 2=24 hours po administration	Large intestine> cecum> small intestine> renal cortex> bladder> liver

2.6.5.5.E. 97-DDM-4331-001: Tissue Distribution of [14C] GS-4331 in Bealge Dogs Following Oral Administration

Study Number: 97-DDM-4331-001

Species	Dog
Gender (M/F) No. of Animals	12 (4 animals per time point, 2M/ 2F)
Feeding Condition	Fasted
Vehicle Formulation	Aqueous solution
Method of Administration	Single oral dose
Dose (mg/kg)	10 (TDF)
Radionuclide	^{14}C
Specific Activity	2.45 µCi/mg
Sampling Time	1, 6 & 24 hours (3 groups)

Tissues/Organs	Ct 1 (1 hr after admin.) (µg equiv/g)		Ct 2 (6 hrs after admin.) (µg equiv/g)		Ct 3 (24 hrs after admin. Last measurement) (µg equiv/g)		C x / C pl.		t/2 (hrs)
	1M*	2F	2M	2F	2M	2F	2M	2F	
Plasma	2.325	1.541	0.332	0.574	0.201	0.201	1	1	15.4
Large Intestine Content	0.272	173.066	961.973	1077.522	26.095	70.804	129.8	352.3	4.09
Kidney	127.382	85.341	103.073	140.603	87.938	89.686	437.5	446.2	39.3
Liver	52.176	37.727	38.986	46.764	38.316	33.657	190.6	167.4	71.1
Rectum	3.731	1.568	37.895	4.444	3.398	10.580	16.9	52.6	11.3
Small Intestine Content	677.381	635.520	32.104	58.263	19.913	18.076	99.1	89.9	14.4
Colon	1.137	4.934	26.466	45.094	2.573	9.426	12.8	46.9	6.98
Bile	144.248	32.223	18.965	47.007	9.633	6.538	47.9	32.5	8.87
Duodenum	25.484	15.783	13.977	11.455	4.960	4.332	24.7	21.6	12.4

* = data from one animal only. The following organs & tissues were analysed but not listed above: Blood, gall bladder, lymph nodes, salivary glands, heart, lungs, abdominal fat, spleen, adrenals, pancreas, testes/ovaries, epididymis, prostate, urinary bladder, thyroid, whole brain, pituitary, spinal cord, eyes, skeletal muscle, skin, femoral bone, femoral bone marrow, stomach, and stomach contents.

2.6.5.5.F. R2000075: Single Dose IV Pharmacokinetics of Tenofovir at Two Doses in Sprague-Dawley Rats

Type of Study: Single dose pharmacokinetic study

Study Number: R2000075

Methods		
Species	Rat	
Feeding Condition	Fed	
Vehicle Formulation	Physiological buffered solution	
Method of Administration	IV bolus	
Sample	Plasma	
Assay	HPLC (MS detection)	
Dose mg/kg	**10**	**50**
Gender (M/F) No. of Animals	8 per group (4M/ 4F)	
Tabulated Results PK Results:		
n per time point	4	4
C_{max} (µg/mL)	22.0	162
$AUC_{0\rightarrow\infty}$ (µg•hr/mL)	5.86	53.7
AUC % Extrapolated	3.06	0.622
$t_{1/2}\lambda_z$ (hr)	4.02	5.41
CL (mL/hr/kg)	1706	931
C_{last} (µg/mL)	0.0309	0.0428
T_{last} (hr)	12.0	24.0
V_{ss} (mL/kg)	2810	1122

2.6.5.5.G. T1278-00034: PMPA in SIV-Infected and Uninfected Rhesus Macaques: Studies From Martin and Tsai Laboratories

Type of Study: Safety/efficacy with pharmacokinetic parameters

Study Number: T1278-00034

Methods:

Species	Monkey (SIV infected/ non-infected)	
Method of Administration	SC (group 1) iv (group 2)	
Sample	Plasma	
Dose mg/kg/day	**30, 14 months (group 1)**	**10, single dose (group 2)**
No. of Animals/Condition	5 adult SIV infected	4 adult non-infected

Tabulated PK Results :	**Individual animals**					**Mean data**
	1	**2**	**3**	**4**	**5**	
C_{max} (μg/mL)	47.7	52.9	33.7	39.8	53.5	27.7
AUC (μg•hr/mL)	144	145	97.9	154	240	14.7
$t_{1/2}$ (hr)	6.04	6.72	3.94	5.97	6.27	10.6
CL/F (mL/hr/kg)	209	207	306	195	125	710

2.6.5.5.H. P2000117: Pharmacokinetics of Tenofovir in Healthy and Infected Rhesus Monkeys Administered Chronic Subcutaneous Doses of Tenofovir

Type of Study: Pharmacokinetic study

Study Number: P2000117

Methods:

Species	Monkey (SIV infected/ non-infected)															
Method of Administration	SC chronic dosing															
Sample	Plasma															
Dose mg/kg/day	**2.5**	**5**				**10**						**20**				
No of Animals	1	1				6						8				
Individual Animal ID	**1**	**1**	**1**	**2[1]**	**3**	**4**	**5[1]**	**6[1]**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Tabulated PK Results at Steady State																
AUC 0-τ (μg•hr/mL)	10.9	51.5	94.0	57.5	44.9	13.1	18.0	9.86	33.2	26.5	31.5	24.0	18.8	39.2	21.2	26.3
Cl_{ss}/F mL/kg/hr	230	97.0	106	174	223	765	556	1015	603	755	634	834	1064	510	945	760
C_{max} (μg/mL)	6.09	14.2	28.9	24.4	21.1	10.4	17.4	10.0	24.4	19.8	21.7	21.0	18.3	27.4	18.2	20.7
C_{min}(μg/mL)	0.0343	0.316	0.530	0.180	0.126	0.00882	0.00648	0.00738	0.0224	0.017	0.0199	0.0212	0.0181	0.0514	0.0203	0.0168
MRT (0-∞)	3.97	6.62	6.05	4.20	4.32	1.91	1.70	2.59	2.23	1.96	2.01	2.04	1.97	2.52	2.29	1.90
$t_{1/2}\lambda_z$ (hr)	7.18	7.42	7.49	6.74	7.94	4.82	3.77	10.5	4.56	4.89	5.22	6.83	6.69	6.21	5.64	5.23
T_{max} (hr)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
V_z/F	2376	1039	1149	1689	2551	5320	3024	15418	3968	5328	4777	8216	10272	4568	7692	5733
Time on Dose (months)	5	31	7	38	13	27	30	30	4	4	4	3	5	5	5	5
Dose Reduction	30→10→5 →2.5	30→10 →5	30→10 →5→10	30→10	10→20 →10	-	-	-	10→20	10→20	10→20	10→20	10→20	10→20	10→20	10→20

1= non SIV-infected animals

2.6.5.5 Pharmacokinetics: Organ Distribution **Test Article:** Tenofovir or Tenofovir disoproxil fumarate

2.6.5.5.I. 96-DDM-1278-005: Placental Transfer and Pharmacokinetics of PMPA (GS-1278) in Infant Rhesus Monkeys

Type of Study: Pharmacokinetic study in infant animals

Study Number: 96-DDM-1278-005

Method:

Species	Monkey (infant)
Method of Administration	SC
Sample	Plasma
Assay	HPLC
Dose mg/kg	30 (single dose)
No of Animals	8 (n=2 per group)

Tabulated PK Results	**Newborn (0.54kg)**	**1 month (0.73 kg)**	**3 Month (0.90 kg)**	**12 Month (2.26 kg)**
AUC 0-∞ (μg•hr/mL)	164	58.4	77.0	30.1
C_{max} (μg/mL)	51.8	30.7	34.6	18.8
T_{max} (hr)	0.5	0.5	0.5	0.5
Terminal Half Life (hr)	3.34	4.39	3.62	3.16
CL/F (L/hr/kg)	0.184	0.544	0.406	1.02
CL/F (mL/min)	1.6	6.5	6.2	38.0
Vss/F (L/kg)	0.587	1.066	0.782	1.43
MRT (hr)	3.17	2.01	1.93	1.41

2.6.5.6. Pharmacokinetics: Plasma Protein Binding

2.6.5.6.A. TBZZ/93/0025: Protein Binding of 524W91 in Human, Monkey, Mouse and Rabbit Plasma

Protein Binding of 524W91 in Human, Monkey, Mouse and Rabbit Plasma.

Study Number: TBZZ/93/0025

Study system: ***In vitro***

Target Entity, Test System and Method: Plasma serum, Ultrafiltration

Species	Drug concentration		% Bound
	µg/mL	µM	
Human	0.020	0.08	3.3
	0.101	0.41	0.8
	0.501	2.03	2.7
	2.51	10.2	2.2
	10.0	40.4	3.4
	49.9	202	2.0
	200	808	0.0
Monkey	0.020	0.08	0.0
	0.101	0.41	0.0
	0.501	2.03	0.0
	2.51	10.2	0.0
	10.0	40.4	0.0
	49.9	202	2.0
	200	808	0.0
Mouse	0.020	0.08	0.7

	0.101	0.41	3.6
	0.501	2.03	0.0
	2.51	10.2	2.3
	10.0	40.4	0.0
	49.9	202	3.0
	200	808	3.4
Rabbit	0.020	0.08	2.3
	0.101	0.41	0.0
	0.501	2.03	1.3
	2.51	10.2	0.0
	10.0	40.4	0.0
	49.9	202	0.0
	200	808	0.4

Additional Information: none

2.6.5.6.B. TMC278–NC112: The Plasma Protein Binding and Blood Distribution of TMC278 in Animals and Man

Study No. TMC278–NC112

Method The plasma protein binding of TMC278 was studied by equilibrium dialysis of plasma samples after fortification with ^{3}H-labeled TMC278. Plasma was subjected to equilibrium dialysis against a 0.067 M phosphate buffer, pH 7.17, at 37°C for 3 hours. Concentration of ^{3}H –TMC278 in dialysis compartments was determined by liquid scintillation counting. The binding of TMC278 to purified human serum albumin and α_1-acid glycoprotein was also investigated by equilibrium dialysis. In blood distribution studies, samples of whole blood were combusted in an oxidizer and $^{3}H_2O$ captured and counted by liquid scintillation.

Species	Swiss CD-1 Mouse[a] (Male)		Swiss CD-1 Mouse[a] (Female)		Sprague Dawley Rat[a] (Male)		Sprague Dawley Rat[a] (Female)		New Zealand White Rabbit[b] (Female)		Beagle Dog[b] (Male)		Human[b] (Male)	
Parameters Measured														
Concentration Tested in Plasma (µg/mL)	0.01-100		0.01-100		0.01-100		0.01-100		0.01-100		0.01-100		0.01-3	
Plasma Bound % at 1 µg/mL[c]	99.93		99.94		99.84		99.86		99.97		99.35		99.67	
Concentration Tested in Blood (µg/mL)	0.1	1	0.1	1	0.1	1	0.1	1	0.1	1	0.1	1	0.1	1
Blood-to-Plasma Ratio	0.60	0.60	0.58	0.58	0.69	0.67	0.67	0.67	0.61	0.61	0.69	0.68	0.67	0.66
Distribution to: Plasma Water (%)	0.12	0.07	0.06	0.07	0.16	0.15	0.13	0.14	0.02	0.03	0.5	0.5	0.3	0.2
Plasma Proteins (%)	101	100	102	103	90.5	93.0	94.1	94.1	102	103	72.6	73.4	77.3	78.1
Blood Cells (%)	-0.9	-0.2	-1.7	-3.0	9.3	6.8	5.8	5.8	-1.8	-2.8	26.9	26.2	22.4	21.7
Hematocrit (%)	40		41		38		38		38		51		48	

Additional Information

Preliminary studies evaluated: (a) length of time needed to reach equilibrium in the dialysis cells (3 hours was deemed sufficient); (b) the effect of pH (as pH increased from 5.1 to 8.4, the percentage bound increased from 99.59 to 99.80, so pH was standardized at 7.4 for human plasma); and (c) binding to purified human plasma protein (at physiological concentrations, TMC278 (0.01 to 3 µg/mL) bound to serum albumin (99.50%) and α_1-acid glycoprotein (between 25.9% and 55.0%).

a for rat and mouse, each value represents a mean of 4 observations (2 pools x 2);

b for rabbit, dog and man, each value represents a mean of 10 observations (5 individual samples x 2);

c Over the concentration range tested the fraction bound to plasma remained nearly the same

2.6.5.6.C. TMC278–NC332: The Distribution of ^{14}C-TMC278 in Blood and Protein Binding of ^{14}C-TMC278 in Plasma from Monkey and Guinea Pig

Study No. TMC278–NC332

Method The plasma protein binding of TMC278 was studied by equilibrium dialysis of plasma samples after fortification with ^{14}C-labeled TMC278. Plasma was subjected to equilibrium dialysis against a 0.067 M phosphate buffer, pH 7.17, at 37°C for 4 hours. Concentration of ^{14}C –TMC278 in dialysis compartments was determined by liquid scintillation counting. In blood distribution studies, samples of whole blood were combusted in an oxidizer and $^{14}CO_2$ captured and counted by liquid scintillation.

Species	Dunkin Hartley Guinea Pig		Cynomolgus Monkey	
Parameters Measured				
Concentration Tested in Plasma (µg/mL)	2.5	8	2.5	5
Plasma Bound %	99.87	99.87	99.14	99.08
Concentration Tested in Blood (µg/mL)	2.5	8	2.5	5
Blood-to-Plasma Ratio	0.64	0.63	0.96	0.94
Distribution to: Plasma Water (%)	0.11	0.11	0.53	0.59
Plasma Proteins (%)	84.71	85.23	61.84	63.46
Blood Cells (%)	15.18	14.66	37.63	35.96
Hematocrit (%)	46		40	

Additional Information

2.6.5.6 Pharmacokinetics: Plasma Protein Binding

Test Article: Tenofovir

2.6.5.6.D. P0504-00039.1: Protein Binding of Tenofovir

Study System In vitro

Target Entity, Test System & Method Plasma/ serum, ultrafiltration

Species	Conc. Tenofovir Tested	% Unbound	Study Number: P0504-00039.1
Human			
Plasma			
	0.01 µg/mL	96.9	
	2.01 µg/mL	99.9	
	5.01 µg/mL	101.0	
	10.01 µg/mL	95.0	
	25.01 µg/mL	103.5	
		99.3 (3.3) Mean (SD)	
Human			
Serum			P0504-00039.1
	0.01 µg/mL	90.6	
	2.01 µg/mL	92.4	
	5.01 µg/mL	97.7	
	10.01 µg/mL	88.5	
	25.01 µg/mL	94.7	
		92.8 (3.6) Mean (SD)	

2.6.5.7. Pharmacokinetics: Study in Pregnant or Nursing Animals

2.6.5.7.A. TOX103: Toxicokinetic Study to Determine Fetal Exposures in CD-1 Mice Given TP-0006 Orally

Study Type: Toxicokinetic Study to Determine Fetal Exposures in CD-1 Mice Given TP-0006 Orally

Study Number: TOX103

Species	Mouse / CD-1
Gestation Day/Number of Animals	Gestation days 6-15
Vehicle/Formulation	0.5% aqueous methylcellulose
Method of Administration	Oral gavage
Dose (mg/kg)	1000 (plus 500 only on GD 15)
Analyte	L-(-)-2',3'-Dideoxy-5-Fluoro-3'-Thiacytidine (FTC)
Assay	Mass spectrometry using selected ion monitoring (SIM)

Plasma:

The mean ± standard deviation for plasma concentrations:

Pregnant mice	137.1 ± 28.0 µg/mL
Pooled fetal homogenate	57.7 ± 10.4 µg/mL

The mean fetal/maternal concentration ration was 0.41 ± 0.04 µg/mL

Additional Information:

One female in the low dose group was not pregnant and was not included in the mean values.

2.6.5.7.B. TOX038: A Study of the Effects of TP-0006 on Embryo/Fetal Development in Rabbits

Study Type: A Study of the Effects of TP-0006 on Embryo/Fetal Development in Rabbits

Study Number: TOX038

Species	Rabbit / New Zealand White
Gestation Day/Number of Animals	Treated on gestation day 7-19, 20 females / group
Vehicle/Formulation	0.5% aqueous methylcellulose
Method of Administration	Oral
Dose (mg/kg)	0, 100, 300, 1000
Analyte	L-(-)-2',3'-Dideoxy-5-Fluoro-3'-Thiacytidine (FTC)
Assay	-

Toxicokinetics

Emtricitabine was rapidly absorbed with C_{max} occurring generally within 1 hour post-dose. AUC and C_{max} increased linearly with dose. Plasma elimination $t_{1/2}$ was 3–4 hours at all dose levels. Fetal/maternal exposure ratios were around 0.4–0.5 one hour after dosing (at t_{max}) for all dose levels. Emtricitabine is readily
transferred across the placenta.

Dose (mg/kg/day)	C_{max} (µg/mL)	T_{max} (h)	AUC_{0-12} (hr•µg/mL)	AUC_{0-24} (hr•µg/mL)	Fetal/Maternal Ratio
100	16.0	1.0	43.6	87.3	0.42
300	44.2	1.4	157.6	315.2	0.51
1000	143.3	1.7	628.9	1257.8	0.41

Conclusion

A NOEL of 100 mg/kg/day was established for maternal toxicity.
No effect on developmental toxicity was noted at dose levels up to 1000 mg/kg/day.

Additional Information: none

2.6.5.7.C. TMC278-NC105: Oral Developmental Toxicity Study in the Rat

Study No.	**TMC278-NC105**					
Species	**Rat (pregnant Sprague Dawley)**					
Feeding Condition	**Not fasted**					
Vehicle/Formulation	**TMC278 base in PEG400/CA (10%)**					
Route	**Oral (gavage)**					
Gender (M/F)/Number of Animals	F/6	F/4	F/6	F/4	F/6	
Dose (mg/kg/day)	40		120		400	
Concentration (mg/mL)	4		12		40	
Duration of Dosing (day)	1 (GD6)	11 (GD16)	1 (GD6)	11 (GD16)	1 (GD6)	11 (GD16)
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)	4.9	5.6	6.0	7.2	14	13
t_{max} (h)	1.0	2.0	0.5	1.0	8.0	8.0
AUC (µg.h/mL)	33	37	65	63	182	152
(Time for calculation –h)	(0-∞)	(0-24)	(0-∞)	(0-24)	(0-∞)	(0-24)
$t_{1/2}$ (h)	3.3	3.8	4.2	4.0	5.5	5.2
(Time for calculation –h)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)

Additional Information

The sampling times were 0.5, 1, 2, 4, 8 and 24 h after dose administration

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

2.6.5.7.D. TMC278–NC128: Pilot Oral Developmental Toxicity Study in the Rabbit

Study No.	**TMC278–NC128 (pilot study)**		
Species	**Rabbit (pregnant New Zealand white)**		
Feeding Condition	**-**		
Vehicle/Formulation	**TMC278 base in aqueous HPMC (0.5%)**		
Route	**Oral (gavage)**		
Gender (M/F)/Number of Animals	F/3	F/3	F/3
Dose (mg/kg/day)	25	75	150
Concentration (mg/mL)	5	15	30
Duration of Dosing [a] **(day)**	12 (GD17)	12 (GD17)	12 (GD17)
Sample (whole blood, plasma, serum, etc.)	plasma	plasma	plasma
Analyte	TMC278	TMC278	TMC278
Assay	NS	NS	NS
Pharmacokinetic Parameters			
C_{max} (µg/mL)	18	39	63
t_{max} (h)	6.7	6.7	13
AUC (µg.h/mL)	327	685	1252
(Time for calculation –h)	(0-24)	(0-24)	(0-24)
$t_{1/2}$ (h)	ND	ND	ND
(Time for calculation –h)	-	-	-

Additional Information
The sampling times were 0 (predose), 1, 2, 4, 8 and 24 h on Day 12 of dosing (GD17)

a pregnant animals were dosed once daily from Day 6 to Day 19 inclusive
GD: gestation day; HPMC: hydroxypropyl-methylcellulose; LC-MS/MS: liquid chromatography with tandem mass spectrometry; ND: not determined; NS: not specified.

2.6.5.7.E. TMC278–NC130: Oral Developmental Toxicity Study in the Rabbit

Study No.	**TMC278–NC130 (main study)**					
Species	**Rabbit (New Zealand white)**					
Feeding Condition	**Not fasted**					
Vehicle/Formulation	**TMC278 base in aqueous HPMC (0.5%)**					
Route	**Oral (gavage)**					
Gender (M/F)/Number of Animals	F/3		F/3		F/3	
Dose (mg/kg/day)	5		10		20	
Concentration (mg/mL)	1		2		4	
Duration of Dosing[a] (day)	1 (GD 6)	14 (GD 19)	1 (GD 6)	14 (GD 19)	1 (GD 6)	14 (GD 19)
Sample (whole blood, plasma, serum, etc.)	plasma	plasma	plasma	plasma	plasma	plasma
Analyte	TMC278	TMC278	TMC278	TMC278	TMC278	TMC278
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Pharmacokinetic Parameters						
C_{max} (µg/mL)	6.4	6.7	9.7	10	13	15
t_{max} (h)	11	11	11	8.0	9.3	11
AUC (µg.h/mL)	95	105	162	170	219	232
(Time for calculation –h)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)
$t_{1/2}$ (h)	8.2	7.6	12	8.4	11	8.6
(Time for calculation –h)	-	-	-	-	-	-

Additional Information
The sampling times were 0 (predose), 1, 2, 4, 8, 12 and 24 h after dosing.

a pregnant animals were dosed once daily from Day 6 to Day 19 of gestation inclusive.
GD: gestation day; HPMC: hydroxypropyl-methylcellulose; LC-MS/MS: liquid chromatography with tandem mass spectrometry

2.6.5.7.F. R990202-PK: Oral (Gavage) Developmental and Perinatal/Postnatal Reproduction Toxicity Study of GS-4331-05 (bis-POC PMPA) in Rats Including a Postnatal Behavioral/Functional Evaluation

Excretion into Milk **Study Number: R990202-PK**

Species	Rat
Number of Animals	12F per group
Feeding Condition	Fed
Vehicle Formulation	Citric acid (50 mM)
Method of Administration	Oral gavage
Analyte	Tenofovir
Assay (tenofovir in milk)	HPLC mass spectroscopy method (LC/MS/MS)

Dose (mg/kg)/ day	**50**	**150**	**450**	**600**
Concentration Tenofovir (ng/mL) at day 11				
Milk	154	249	532	730
Milk/Plasma	0.135	0.11	0.208	0.235

Additional Information:

There was a trend towards an increasing percentage of tenofovir in milk with increasing dose of TDF. The data are however weak due to the comparison of data from different rats and the different number of observations between plasma data (n= 2–4) and milk data (n=5–11). It was concluded that postpartum data on day 11 confirmed the presence of tenofovir in milk with results suggesting that tenofovir was not concentrated in milk. Plasma Pharmacokinetic data is shown overleaf.

Excretion into Milk *cont*

Study Number: R990202-PK
Cont

Species	Rat
Number of Animals	12F per group
Feeding Condition	Fed
Vehicle Formulation	Citric acid (50 mM)
Method of Administration	Oral gavage
Analyte	Tenofovir
Assay (tenofovir in plasma	HPLC mass spectroscopy method (LC/MS/MS)

Dose (mg/kg)/ day	**50**		**150**		**450**		**600**	
PK parameters	**Single Dose**	**Day 20 Postpartum**	**Single Dose**	**Day 20 Postpartum**	**Single Dose**	**Day 20 Postpartum**	**Single Dose**	**Day 20 Postpartum**
C_{max} (µg/mL)	1.271	1.461	2.525	2.256	3.434	4.933	4.710	2.892
T_{max} (hr)	0.25	0.67	0.25	1.0	0.67	0.67	0.25	0.67
AUC $_{last}$ (µg*hr/mL)	3.263	-	7.312	-	14.820	-	19.007	-
AUC $_{(0-\infty)}$ (µg*hr/mL)	3.699	-	7.840	-	54.373	-	28.961	-
AUC % Extrap	11.8	-	6.73	-	72.7	-	34.4	-
AUC $_{(0-\tau)}$	-	5.883	-	11.716	-	26.761	-	27.286
Cl/F (mL/hr/kg)	6110	-	8648	-	3733	-	9357	-
Clss/F	-	3842	-	5787	-	7586	-	9932
T_{last} (hr)	12	24	24	24	24	24	24	12
T ½ λ_z (hr)	4.06	5.12	6.75	7.53	61.2	12.6	18.4	7.24
V_z/F (mL/kg)	35764	28374	84184	62827	329618	138328	248392	103691

2.6.5.7.G. 98-TOX-4331-005-PK: Developmental Toxicity Study of Tenofovir DF in Pregnant Rabbits

Study Number: 98-TOX-4331-005-PK

Pregnant Animals			
Species	Rabbit		
Feeding Condition	Fed		
Vehicle Formulation	TDF Oral suspension		
Method of Administration	Oral		
Sample	Plasma		
Analyte	Tenofovir		
Assay	HPLC		
Dose mg/kg/day	**30**	**100**	**300**
Gender (M/F) No. of Animals	4F	4F	4F
PK Parameters (on day 18)			
T_{max} (hr)	0.75 (0.29)	0.50 (0.00)	0.75(0.29)
C_{max} (µg/mL)	4.92 (1.34)	20.65 (4.09)	47.62 (9.51)
$AUC_{0\rightarrow\tau}$ (µg•hr/mL)	19.15 (4.40)	65.30 (16.07)	212.48 (97.14)
$t_{1/2}\lambda_z$ (hr)	8.61 (1.64)	10.34 (3.08)	9.67 (3.23)
MRT (hr)	7.53 (1.38)	7.11 (1.57)	7.50 (0.72)
T_{last} (hr)	24	24	24
Cl_{ss}/F (mL/hr/kg)	740 (170)	730 (210)	720 (230)

Additional Information: Results indicate that tenofovir C_{max} & AUC appeared to increase in a dose proportional manner following repeated administration of TDF indicating linear pharmacokinetics following multiple daily administration to pregnant animals.

2.6.5.7.H. 96-DDM-1278-005: Tenofovir DF: Placental Transfer and Pharmacokinetics of PMPA (GS-1278) in Infant Rhesus Monkeys

Placental Transfer **Study Number: 96-DDM-1278-005**

Species	Monkey					
Gestation Day/ Number of Animals	Daily dosing beginning at gestational day 111, one animal					
Vehicle Formulation	Aqueous suspension					
Method of Administration	SC					
Dose (mg/kg)/ day	30					
Analyte	Tenofovir					
Assay	HPLC					
Time (gestational day)	**115**	**127**	**134**	**140**	**151**	**Mean (SD) [CV%]**
Serum Concentration Tenofovir (µg/mL) 30 mins After Administration						
Fetal	7.9	9.1	10.1	15	5.9	9.6 (3.4) [35.4]
Maternal	45.6	61.2	69.4	53.7	56.3	57.2 (8.84) [15.4]
Fetal/Maternal Ratio	0.17	0.15	0.15	0.28	0.11	0.17 (0.07) [38.6]

Additional Information:

Based upon the data above it was concluded that placental transfer of tenofovir appeared to be significant.

2.6.5.7.I. P2000116: Pharmacokinetics of Tenofovir in Healthy Adult Female Lactating Rhesus Monkeys Following a Single 30 mg/kg Subcutaneous Dose of Tenofovir

Lactating Animals	**Study Number: P2000116**
Species	Monkey
Gestation Day/ Number of Animals	Healthy adult female lactating animals
Vehicle Formulation	-
Method of Administration	SC
Dose (mg/kg)/ day	30 single dose
Analyte	Tenofovir
Assay	LC/MS/MS assay

PK Parameters (n=2)

	Animal 1		**Animal 2**	
	Milk	**Serum**	**Milk**	**Serum**
C_{max} (μg/mL)	0.808	18.3	0.610	30.2
T_{max} (hr)	4	0.5	1	0.5
$AUC_{(0-\infty)}$ (μg*hr/mL)	12.8	68.9	12.1	56.2
AUC Extrapolated (%)	21.7	0.219	23.3	0.193
C_{last} (μg/mL)	0.188	0.0264	0.179	0.0264
T_{last} (hr)	24	24	24	24
Cl/F (mL/hr/kg)	2338	435	2482	534
$MRT_{(0-\infty)}$ (hr)	16.1	2.79	17.0	3.14
$t_{1/2}\lambda_z$ (hr)	10.3	3.97	10.9	2.85
V_z/F (mL/kg)	34740	2489	39133	2191

2.6.5.8. Pharmacokinetics: Metabolism In Vivo

2.6.5.8.A. TEIN/93/0015: Metabolic Disposition and Balance Studies in Male CD-1 Mice Following Oral Administration of 120 mg/kg [6-^{3}H]524W91

Study Type: Metabolic Disposition and Balance Studies in Male CD-1 Mice Following Oral Administration of 120 mg/kg [6-^{3}H]524W91	**Study Number: TEIN/93/0015**
Species	Mouse, CD-1
Gender (M/F)/Number of Animals	15 M,
Feeding Condition	-
Vehicle/Formulation	Solution in Water
Method of Administration	Oral
Dose (mg/kg)	120 (single dose)
Radionucleotide	^{3}H
Specific Activity	2.6 µCi/mg

Metabolic Data: Cumulative (0–72 hour post-dose) recovery of emtricitabine and its tentatively identified metabolites from the urine of male CD1 mice dosed orally.

		% of Compound in Sample (Mean +/- Standard Deviation)					
Sample	**Sampling Time or Period**	**5-FC**	**3742W92**	**3743W92**	**524W91**	**M1800**	**M1870[a]**
Urine	0–72 hours	1.4 ± 0.2	1.7 ± 0.3	2.0 ± 0.4	64 ± 7.1	0.5 ± 0.4	0.8 ± 0.2

a Unidentified metabolites.

Excretion Data: The recovery of radioactivity in the urine and feces of male CD1 mice dosed orally.

	Mean ± Standard Deviation		
Sample	**Sampling period (hours)**	**Percent of dose in sample**	**Total recovery (%; 0-72 hours)**
Urine	0–24	62.3 ± 7.6	
	24–48	3.4 ± 2.1	66.8 ± 7.0
	48–72	1.5 ± 0.4	
Feces	0–24	15.9 ± 3.0	
	24–48	0.7 ± 0.4	18.1 ± 3.1
	48–72	1.5 ± 0.6	

Feces and Urine	0–72	--	85.0 ± 4.2

Additional Information:
Analytical method: Liquid scintillation counting, HPLC radiochromatogram.
Mean ± SD percent of dose excreted in urine (0–72 hours) as parent drug = 64 ± 7

2.6.5.8.B. TOX063: Metabolism and excretion of [^{14}C]-TP-0006 Following Oral Administration to Male Cynomolgus Monkeys

Study Type: Integrated metabolism and excretion study report: Metabolism and excretion of [14C]-TP-0006 following oral administration to male Cynomolgus monkeys

Study Number: TOX063

Species	Monkey, Cynomolgus
Gender (M/F)/Number of Animals	4 M
Feeding Condition	Fasted
Vehicle/Formulation	Sterile water
Method of Administration	Oral
Dose (mg/kg)	200 (single dose)
Radionucleotide	^{14}C
Specific Activity	42.9 µCi/mg and 57.6 µCi/mg

Metabolic Data:

Plasma	**FTC**	**Metabolite 1**
C max (µg/mL)	46.7	15.9
T max (h)	1	2
AUC (0 - last) (µg•hr/mL)	129	56.6
AUC (0 - ∞) (µg•hr/mL)	133	86.6
CL / f (l/h/kg)	1.57	-

Excretion Data:

40.8% of the administered radioactivity was recovered in the urine, 35.3% in the faeces, and 8.3% in cage washes/wipes. Unchanged parent drug represented the great majority of radioactivity present in urine (approximately 74%) and faeces (97%). The recovery of large amounts of radioactivity in the gut contents following a second dose of radioactivity indicates that much of the faecal recovery represented unabsorbed rather than excreted drug.

Distribution Data:

22 tissues obtained 1 hour post dose. Highest levels: kidneys (596 equivalents µg/g); liver (121 µg/ equivalents /g.); CSF/blood ratio 0.031.

2.6.5.8.C. TEIN/93/0016: Metabolic Disposition of 80 mg/kg Orally Administered [6-^{3}H]524W91 in Cynomolgus Monkeys

Metabolic Disposition of 80 mg/kg Orally Administered [6-^{3}H]524W91 in Cynomolgus Monkeys **Study Number:** TEIN/93/0016

Species	Monkey, cynomolgus
Gender (M/F)/Number of Animals	4 F
Feeding Condition	-
Vehicle/Formulation	Solution in Water
Method of Administration	Oral
Dose (mg/kg)	80 (single dose)
Radionucleotide	^{3}H
Specific Activity	1.8 µCi/mg

Metabolic Data: Cumulative (0–72 hour post-dose) urinary recovery of emtricitabine and its metabolites from Cynomolgus Monkeys dosed orally. Fecal recovery of emtricitabine and its tentatively identified metabolites from Cynomolgus Monkeys dosed orally.

Sample	Percent of Dose (Mean ± Standard Deviation)								
	M200	M350	M950	M1030	M1100	M1500	524W91	M1940	M1980
Urine	0.2 ± 0.1	0.3 ± 0.04	11 ± 4	1.2 ± 0.2	0.3 ± 0.2	1.6 ± 1.9	28.3 ± 4.1	0.1 ± 0.1	1.1 ± 0.3
Feces	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.03 ± 0.1	--	--	34.5 ± 10.7	--	0.5 ± 0.1

Excretion Data: Examined (pool) samples – The recovery of radioactivity in the urine, feces, and cage washes of Cynomolgus Monkeys dosed orally.

		Mean ± Standard Deviation	
	Sampling period (hours)	Percent of dose in sample	Total recovery (% ; 0–72 hours)
Urine	0–8	32.9 ± 8.6	
	8–24	5.5 ± 1.1	41.2 ± 6.4
	24–48	2.2 ± 1.7	
	48–72	0.6 ± 0.9	
Feces	0–24	23.6 ± 15.9	
	24–48	4.7 ± 5.7	33.1 ± 10.0
	48–72	4.8 ± 9.1	
Cage Wash	0–8	3.0 ± 1.9	
	8–24	5.0 ± 4.0	9.6 ± 6.7
	24–48	0.8 ± 0.6	
	48–72	0.8 ± 0.7	
Overall Recovery	0–72	--	83.8 ± 3.8

Monkey (continued), study number - TEIN/93/0016

Additional Information:
Analytical method: Liquid scintillation counting, reverse-phase HPLC

Mean ± SD percent of dose excreted in urine (0–24) as parent drug	= 28 ± 4
Mean ± SD percent of dose excreted in urine (0–24) as M950 (putative 524W91 sulfoxide)	= 11 ± 4

2.6.5.8.D. TMC278-NC190: The Metabolism, Excretion and Plasma Kinetics of TMC278 in the Male and Female CD-1 Mouse After Single Oral Administration of ^{14}CTMC278 at 20 and 320 mg/kg

Study No.		**TMC278-NC190**						
Species		**Mouse (CD-1)**						
Gender (M/F)/Number of Animals		**M12/F12 (plasma profile) - M12/F12 (excretion mass balance)**						
Feeding condition		**Not fasted**						
Vehicle/Formulation		**PEG400/CA (10%)**						
Route		**Oral**						
Dose of TMC278 base (mg/kg)		**20 and 320**						
Radionuclide		**^{14}C**						
Specific Activity (kBq/mg)		**345 and 23**						
Dose (mg/kg)		**20**				**320**			
Sample		**Urine**		**Fecal Extract**		**Urine**		**Fecal Extract**	
		Male	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
Time (h)		**0-24**	**0-24**	**0-48**	**0-48**	**0-24**	**0-24**	**0-48**	**0-48**
% of Administered Radioactivity in Excreta		**2.82**	**3.46**	**64.05**	**65.38**	**1.27**	**2.88**	**75.2**	**71.3**
Parent (UD)		0.63	0.08	8.2	7.8	0.11	0.02	33	34
M13	Cysteine-S-conjugate	0.14	0.39			0.09	0.31		
M14	Cysteinyl-S-conjugate	0.08	0.58			0.06	0.55		
M13+M14				5.0	4.4			5.1	2.8
M17	Mercapturic acid conjugate	0.19	0.15			0.08	0.14		
M18	Mercapturic acid conjugate	0.13	0.07			0.04	0.10		
M17+M18				4.2	2.3			3.3	3.4
M21	Hydroxylated sulfonyl conjugate			1.4	1.0			0.7	0.3
M25	Oxidation combined with glucuronidation	0.42	1.6			0.42	1.6		
M24+M25	Hydroxylation on the cyanoethenyl moiety, Oxidation combined with glucuronidation			3.4	2.7			1.6	1.6
M27+M28+ M29	Dehydration of M33; Aliphatic hydroxylation and dehydration of M45; Sulphoxidation of M45			0.3	<0.2			<0.2	0.1

Study No.		**TMC278-NC190**							
Species		**Mouse (CD-1)**							
Gender (M/F)/Number of Animals		**M12/F12 (plasma profile) - M12/F12 (excretion mass balance)**							
Feeding condition		**Not fasted**							
Vehicle/Formulation		**PEG400/CA (10%)**							
Route		**Oral**							
Dose of TMC278 base (mg/kg)		**20 and 320**							
Radionuclide		**^{14}C**							
Specific Activity (kBq/mg)		**345 and 23**							
Dose (mg/kg)		**20**				**320**			
Sample		**Urine**		**Fecal Extract**		**Urine**		**Fecal Extract**	
		Male	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
Time (h)		**0-24**	**0-24**	**0-48**	**0-48**	**0-24**	**0-24**	**0-48**	**0-48**
M30	Carboxylic acid metabolite			1.6	3.1			1.5	1.2
M33	Aliphatic hydroxylation			0.5	0.7			1.3	1.0
M35	Unknown structure			<0.2	<0.2			<0.2	<0.2
M38	Hydroxylation			1.4	1.4			1.0	0.8
M41+M42[a]	Hydroxylation of M45; Aromatic hydroxylation			18	26			9.2	13
M42	Aromatic hydroxylation	0.37	0.20			0.11	0.06		
M43+M45	Z-isomerization , S-Methyl conjugate			2.2	1.8			1.3	1.2
M46	Unknown structure			0.5	0.7			0.5	0.6
M47	Dimerization			0.3	<0.2			0.8	0.2

a Metabolite fraction mainly composed of M42, estimated at about 14% and 17% of the 20 mg/kg dose, and at 5.9% and 8.0% of the 320 mg/kg dose in male and female mice, respectively

CA: citric acid; F: female; M: male; PEG400: polyethylene glycol; UD: unchanged drug

Study No.		**TMC278-NC190 (continued)**											
Species		**Mouse (CD-1)**											
Gender (M/F)/Number of Animals		**M12/F12 (plasma profile) - M12/F12 (excretion mass balance)**											
Feeding condition		**Not fasted**											
Vehicle/Formulation		**PEG400/CA (10%)**											
Route		**Oral**											
Dose of TMC278 base (mg/kg)		**20 and 320**											
Radionuclide		**^{14}C**											
Specific Activity (kBq/mg)		**345 and 23**											
Dose (mg/kg)		**20**						**320**					
Sample		**Plasma**											
		Male			**Female**			**Male**			**Female**		
Time (h)		**1**	**3**	**8**	**1**	**3**	**8**	**1**	**3**	**8**	**1**	**3**	**8**
% of Plasma radioactivity		**11.1 [b]**	**6.85 [b]**	**9.46 [b]**	**8.28 [b]**	**5.70 [b]**	**12.5 [b]**	**30.7 [b]**	**26.3 [b]**	**42.5 [b]**	**26.8 [b]**	**32.2 [b]**	**49.4 [b]**
Parent (UD)		105	98	97	99	97	95	93	86	95	95	95	91
M13+M14	Cysteine-S-conjugate; Cysteinyl-S-conjugate	0.5	0.5	0.6	0.5	<0.4	0.6	<0.8	0.9	1.0	<1.2	1.8	<0.8
M27	Dehydration of M33	0.6	0.8	0.8	0.6	0.9	0.6	<0.8	1.2	<0.8	<1.2	<0.7	<0.8
M30	Carboxylic acid metabolite	0.6	0.5	0.6	0.7	0.8	1.0	<0.8	0.9	1.4	<1.2	1.3	1.5
M33	Aliphatic hydroxylation	4.5	3.1	3.5	3.4	6.6	4.3	2.7	2.1	1.5	2.8	4.1	2.2
M36	Hydroxylation of TMC278	0.3	0.8	0.4	0.6	1.3	0.5	<0.8	<0.9	<0.8	1.2	<0.7	<0.8

Additional Information

96 h after administration of TMC278, 88% and 87% of the dose was excreted in feces and 3.5% and 4.2% in urine at 20 mg/kg and 96% and 89% of the dose was excreted in feces and 1.84% and 3.6% in urine at 320 mg/kg, in males and females, respectively. Over the 4-day collection period, more than 95% of the administered dose was recovered.

b Total radioactivity levels in µg-eq./mL.

CA: citric acid; F: female; M: male; PEG400: polyethylene glycol; UD: unchanged drug

2.6.5.8.E. TMC278-NC113: The Metabolism and Excretion of ^{14}C-TMC278 in the Male and Female Sprague-Dawley Rat After Single Oral Administration of ^{14}CTMC278 at 40 mg/kg

Study No.	**TMC278-NC113**
Species	**Rat (Sprague Dawley)**
Gender (M/F)/Number of Animals	**M12/F12 (plasma profile) – M5/F5 (excretion mass balance)**
Feeding Condition	**Not fasted**
Vehicle/Formulation	**TMC278 base in PEG400/CA (10%)**
Route	**Oral**
Dose of TMC278 base (mg/kg)	**40**
Radionuclide	**^{14}C**
Specific Activity (kBq/mg base eq.)	**37**

Sample		**Urine**		**Fecal Extract**		**Plasma**					
		Male	**Female**	**Male**	**Female**		**Male**			**Female**	
Time (h)		**0-24**	**0-24**	**0-48**	**0-48**	**1**	**4**	**8**	**1**	**4**	**8**
% of Administered Radioactivity in Excreta or µg-eq./mL in Plasma		0.39[a)]	1.6[a)]	74[a)]	65[a)]	1.4	1.1	0.88	4.0	3.7	2.0
Parent (UD)		n.d.	0.01	47	43	60[b)]	72[b)]	71[b)]	83[b)]	78[b)]	64[b)]
M12+M14	Cysteinylglycine-S-conjugate; Cysteinyl-S-conjugate	n.d.	n.d.	n.d.	n.d.	7.0[c)]	3.8[c)]	5.6[c)]	14[c)]	13[c)]	10[c)]
M17	Mercapturic acid conjugate	0.02	1.1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
M18	Mercapturic acid conjugate	0.03	0.45	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
M21	Hydroxylated sulfonyl conjugate	n.d.	n.d.	0.20	0.44	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
M24+M27+M28+M29	Hydroxylation on cyanoethenyl moiety; Dehydration of M33; Aliphatic hydroxylation and dehydration of M45; Oxidation of M45	n.d.	n.d.	0.99[d)]	1.6[d)]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
M30	Carboxylic acid metabolite	n.d.	n.d.	0.47	0.05	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
M33	Aliphatic hydroxylation	n.d.	n.d.	0.54	0.54	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
M38	Hydroxylation	n.d.	n.d.	0.70	0.82	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
M41+M42	Hydroxylation of M45; Aromatic hydroxylation	n.d.	n.d.	2.8[e)]	3.6[e)]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
M43+M45	Isomerization; S-Methyl conjugate	n.d.	n.d.	2.4[f)]	2.9[f)]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
M46	Unknown	n.d.	n.d.	0.99	0.92	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
M47	Dimerization	n.d.	n.d.	4.0	3.8	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Study No.	TMC278-NC113										
Species	Rat (Sprague Dawley)										
Gender (M/F)/Number of Animals	M12/F12 (plasma profile) – M5/F5 (excretion mass balance)										
Feeding Condition	Not fasted										
Vehicle/Formulation	TMC278 base in PEG400/CA (10%)										
Route	Oral										
Dose of TMC278 base (mg/kg)	40										
Radionuclide	^{14}C										
Specific Activity (kBq/mg base eq.)	37										
Sample	**Urine**		**Fecal Extract**			**Plasma**					
	Male	**Female**	**Male**	**Female**		**Male**			**Female**		
Time (h)	**0-24**	**0-24**	**0-48**	**0-48**	**1**	**4**	**8**	**1**	**4**	**8**

Additional Information

a Percentage of administered dose.b Percentage of injected sample radioactivity. c Sum of % of administered dose of M12 and M14, which co-eluted together in rat plasma samples.

d Sum of % of administered dose of M24, M27, M28 and M29, which co-eluted together in rat feces samples. e Sum of % of administered dose of M41 and M42, which co-eluted together in rat feces samples.

f Sum of % of administered dose of M43 and M45, which co-eluted together in rat feces samples.

n.d. – Not detected in sample or under the limit of quantification. 96 h after administration of TMC278, 93% of the dose was excreted in feces and 0.45% and 1.8% in urine in males and females, respectively. Over the 4-day collection period, 94% and 95% of the administered dose was recovered in males and females, respectively.

2.6.5.8.F. TMC278-NC145: Biliary Excretion and Identification of Biliary Metabolites of TMC278 in Male Sprague-Dawley Rats After a Single Oral Dose of ^{14}C-TMC278 at 40 mg/kg

Study No.		**TMC278-NC145**	
Species		**Rat (Sprague Dawley)**	
Gender (M/F)/Number of Animals		**M/3 (restrained); M/3 (non-restrained)**	
Feeding condition		**Not fasted**	
Vehicle/Formulation		**TMC278 base in PEG400/CA (10%)**	
Route		**Oral**	
Dose of TMC278 base (mg/kg)		**40**	
Radionuclide		**^{14}C**	
Specific Activity (kBq/mg)		**37**	
Sample		**Bile (Restrained Rats)**	**Bile (Non-Restrained Rats)**
Time (h)		**0-24**	**0-24**
% of Administered Radioactivity in Bile		**18***	**25**
Metabolite Code		**Metabolite Profile - % Dose Radioactivity in 0-24 h Bile**	**Samples Not Profiled**
Parent (UD)		0.19	
M1	Glucuronidation and oxidation of M14	0.63	
M9	Thiol glucuronide conjugate	2.8	
M10+ M12+ M14	Cysteinylglycine-S-conjugate; Cysteinyl-S-conjugate	6.4	
M18	Mercapturic acid conjugate	2.7	
M25	Oxidation combined with glucuronidation	1.4	
M30	Carboxylic acid metabolite	0.4	
	Sum	**14***	

* The calculated total % dose value (14%) varies from the actual value (18%) due to an error arising from integration of noise in a radiochromatogram.

2.6.5.8.G. TMC278-NC114: The Absorption, Metabolism and Excretion of TMC278 in the Male Beagle Dog after a Single Oral Dose of ^{14}C-TMC278 at 5 mg/kg

Study No.		**TMC278-NC114**									
Species		**Dog (beagle)**									
Gender (M/F)/Number of Animals		**3M**									
Feeding condition		**Fed**									
Vehicle/Formulation		**TMC278 base in PEG400/CA (10%)**									
Route		**Oral (Capsule)**									
Dose of TMC278 base (mg/kg)		**5**									
Radionuclide		**^{14}C**									
Specific Activity (kBq/mg)		**99**									
Sample		**Urine**	**Feces**					**Plasma (mean)**			
Time (h)		**0-168h**	**0-72**	**0.25**	**0.5**	**1**	**2**	**4**	**6**	**8**	**24**
% of Administered Radioactivity in Excreta or µg-eq./mL in Plasma		1.73	81	0.06	0.21	0.37	0.57	0.65	0.54	0.46	0.40
Pooled Sample Results: % Dose Recovered		**0-24 h**	**0-72 h**	**Pooled plasma[a)]**		**1h**		**4h**		**8h**	
Parent (UD)		n.d	45			94		73		91	
M11 cyanoethenyl	Carboxylic acid metabolite on the moiety of M27	n.d	0.98			n.d.		n.d.		n.d.	
M23+M27 originating most probably of	Monooxy-M27; Tricyclic metabolite, from oxidation and dehydration M33	n.d	2.1			n.d.		n.d.		n.d.	
M30+M48	Carboxylic acid metabolite; Unknown	0.03, n.d.	3.1			n.d.		n.d.		n.d.	
M33	Aliphatic hydroxylation	n.d	8.7			n.d.		n.d.		n.d.	
M37 TMC278	Sulfate conjugate of monooxygenated-	n.d	0.31			n.d.		n.d.		n.d.	
M40	N-oxide-TMC278	n.d	0.50			n.d.		n.d.		n.d.	
M42	Aromatic hydroxylation	n.d	5.3			n.d.		n.d.		n.d.	
M44	Monooxygenated TMC278	n.d	4.3			n.d.		n.d.		n.d.	
M46	Unknown structure	n.d	0.09			n.d.		n.d.		n.d.	
M49	Unknown structure	n.d	1.4			n.d.		n.d.		n.d.	

Study No.	**TMC278-NC114**									
Species	**Dog (beagle)**									
Gender (M/F)/Number of Animals	**3M**									
Feeding condition	**Fed**									
Vehicle/Formulation	**TMC278 base in PEG400/CA (10%)**									
Route	**Oral (Capsule)**									
Dose of TMC278 base (mg/kg)	**5**									
Radionuclide	**^{14}C**									
Specific Activity (kBq/mg)	**99**									
Sample	**Urine**	**Feces**	**Plasma (mean)**							
Time (h)	**0-168h**	**0-72**	**0.25**	**0.5**	**1**	**2**	**4**	**6**	**8**	**24**

Additional Information

[a] Percentage of injected sample radioactivity.

n.d. = Not detected by radiometric detector.

In urine, minor metabolites (M3, M12, M14, M19, M25 and M36) were detected which accounted for less than 0.08% of the dose. In plasma, minor metabolites were present in trace amounts and included M15, M19, M27, M30, and M33

168 h after administration of TMC278, 95% and 1.7% of the administered dose was excreted in feces and urine, respectively. Over the 7-day collection period, 97% of the administered dose was recovered.

2.6.5.8.H. TMC278-NC157/TMC278-NC119: The Absorption, Metabolism and Excretion of TMC278 After a Single Oral Dose of 150 mg in Healthy Male Subjects (Clinical Trial TMC278-C119)/Three-Month Repeated Dose Oral Toxicity Study in the Swiss Mouse

Study No.		**TMC278-NC157/TMC278-C119**							
Species		**Male Subjects**							
Gender (M/F)/Number of Subjects		**6M**							
Feeding condition		**Fed (Breakfast)**							
Vehicle/Formulation		**TMC278 base in PEG400 (25 mg/mL)**							
Route		**Oral**							
Dose of TMC278 (mg)		**150**							
Radionuclide		**^{14}C**							
Specific Activity (kBq/mg)		**11.8**							
Sample		**Urine**	**Feces**	**Plasma Total Radioactivity (% of Sample Radioactivity in Plasma)**					
Time (h)		**0-168h**	**0-168h**	**1h**	**2h**	**4h**	**8h**	**12h**	**24h**
% of Administered Radioactivity or Conc. (µg-eq./mL)		6.13	76	0.40	0.57	0.77	0.56	0.36	0.32
Average Sample Results: % Dose Recovered			**Selection of Samples**	**1h**	**2h**	**4h**	**8h**	**12h**	**24h**
Parent (UD)		Traces	25.5	58.8	55.7	65.7	47.2	45.7	44.1
M3	Unknown structure		0.3						
M11	Carboxylic Acid Metabolite of M27		1.6						
M13+M14 and M18	Glycine conjugates of TMC278; mercapturic acid conjugate of TMC278	1.2							
M15	*N*-Glucuronide of TMC278	0.6		4.2	4.1	5.3	8.7	9.5	6.2
M19	Glucuronide of M33	0.3							
M23	Oxidized Metabolite of M27		0.7						
M25	Glucuronide of M42	0.6							
M27	Tricyclic Metabolite		0.6	6.6	6.6	9.7	6.5	5.7	8.0
M30	Carboxylic Acid Metabolite	0.03	2.7						
M33	Hydroxymethyl TMC278		3.0	LOQ	1.8	2.9	3.4	3.5	5.1
M35	Unknown structure		2.2						
M39	Cis 5-Hydroxy Pyrimidinyl (Cis of M42)		0.4						
M42	5-Hydroxyl Pyrimidinyl		16.1						
M43	Cis TMC278		0.6						
M46	Unknown structure		0.5						

Study No.	**TMC278-NC157/TMC278-C119**							
Species	**Male Subjects**							
Gender (M/F)/Number of Subjects	**6M**							
Feeding condition	**Fed (Breakfast)**							
Vehicle/Formulation	**TMC278 base in PEG400 (25 mg/mL)**							
Route	**Oral**							
Dose of TMC278 (mg)	**150**							
Radionuclide	**^{14}C**							
Specific Activity (kBq/mg)	**11.8**							
Sample	**Urine**	**Feces**	**Plasma Total Radioactivity (% of Sample Radioactivity in Plasma)**					
Time (h)	**0-168h**	**0-168h**	**1h**	**2h**	**4h**	**8h**	**12h**	**24h**

Additional Information

a Expressed as percent of the administered dose in the 0-168h urine and 0-336h faeces.

LOQ = Below the limit of quantification.

168 h after administration of TMC278, 76% and 6.1% of the administered dose was excreted in feces and urine, respectively. Over the 14-day collection period, 91% of the administered dose was recovered.

2.6.5.8.I. 97-DDM-4331-003/4: Determination of Tenofovir Disoproxil and Metabolite Concentrations in Bile and Gastrointestinal Tract Following Oral Administration of Tenofovir Disoproxil to Rats

Study Number: 97-DDM-4331-003/4 (*bile component*)

Species	Rat
Gender (M/F) No. of Animals	1M
Feeding Condition	Fed
Vehicle Formulation	Citric acid 50mM
Method of Administration	Oral
Dose mg/kg/day	^{14}C-tenofovir DF at 10 mg-equivalent of tenofovir/kg, single administration
Radionuclide	^{14}C
Specific Activity	2.5 µCi/mg

Sample	Sample Time or Period (hours)	Total Radioactivity (µg-equiv. Of tenofovir/mL)	% of Dose in Sample [1]	Metabolite Analysis
Bile	2.6.5.8.I.1.			
	Predose	L	0	ND
	0 – 0.5	L	0	ND
	0.5 – 2.2	0.664	0.014	tenofovir 63% [2]; t-soproxil 37% [2]
	2.2 – 3.0	0.534	0.009	tenofovir only
	3.0 – 4.0	0.518	0.009	tenofovir only
	4.0 – 5.0	0.319	0.005	tenofovir only
	5.0 – 21.5	0.065	0.002	ND
	21.5 – 22.5	0.065	0.002	ND
	22.5 – 24.0	0.267	0.076	ND
	Total	-	0.117	-

L = Less than the limit of quantitation (0.01 µg-equiv. Of tenofovir/mL), ND = Not Detected, 1 = Based on volume of bile collected/time-point, 2 = % total peak area

Continued over

Study Number: 97-DDM-4331-003/4 (*Gastrointestinal component*)

Species	Rat
Gender (M/F) No. of Animals	1M
Feeding Condition	Fasted
Vehicle Formulation	Citric acid 50mM
Method of Administration	Oral
Dose mg/kg/day	^{14}C-tenofovir DF at 10 mg-equivalent of tenofovir/kg, single administration
Radionuclide	^{14}C
Specific Activity	2.5 μCi/mg

Gastrointestinal Contents (10 mg-equiv. Tenofovir/kg)

Sample	Amount of Radioactivity Remaining at 1 Hour		Metabolic Profile (% of Peak Area)		
	μg-equiv. of tenofovir	% of Dose [1]	Tenofovir DF	Tenofovir soproxil	Tenofovir
Stomach	138	4.5	85.3	8.1	6.6
Duodenum	27	0.9	L	L	100
Jejunum	124	4.0	L	L	100
Ileum	1707	55.1	L	28.2	71.8
Cecum	L	0.0	L	L	L
Colon	L	0.0	L	L	L
Total	1996	64.5	5.9	24.7	69.4

L = Less than the limit of quantitatation (0.01 μg-equiv. Of tenofovir/mL), 1 = Based on body weight of 314 g.

2.6.5.9. Pharmacokinetics: Metabolism In Vitro

2.6.5.9.A. PDM-007: Identification of the Principal Human Cytochrome P450 Isoenzyme(s) and Potential Glucuronidation Responsible for the Metabolism of Emtricitabine (FTC) using Pooled Human Liver Microsomes and Bactosomes Containing cDNA-expressed Human Cytochrome P450 (CYP) Isoenzymes

Study Type: Identification of the Principal Human Cytochrome P450 Isoenzyme(s) and Potential Glucuronidation Responsible for the Metabolism of Emtricitabine (FTC) using Pooled Human Liver Microsomes and Bactosomes Containing cDNA-expressed Human Cytochrome P450 (CYP) Isoenzymes

Study Number: PDM-007

Study System	Study system 1) cDNA-Expressed Human Liver Microsomes (CYP 1A2, 2A6, 2B6, 2D6, 2E1, 2C8, 2C9 2C19 and 3A4 to evaluate the metabolism of emtricitabine Study system 2) Pooled Human Liver Microsomes.
Results	• One minor metabolite (~1%) was detected in cDNA-expressed CYP 3A4 incubations • No metabolites were formed by CYP 1A2, 2A6, 2B6, 2D6, 2E1, 2C8, 2C9 or 2C19 • Microsomal incubations in the presence and absence of selective inhibitors of various CYPs confirmed the low rate of emtricitabine metabolism, and also suggests the possible involvement of FAD-containing monooxygenase enzymes in the metabolism of emtricitabine • No glucuronidation of emtricitabine was observed in *in vitro* incubations with pooled human liver microsomes in the presence of NADPH-generating system and UDPGA
Conclusions	• Emtricitabine was relatively stable in the presence of cytochrome P450 isozymes

2.6.5.9.B. PDM-006: In Vitro Evaluation of Emtricitabine (FTC) as an Inhibitor of Human Cytochrome P450 Enzymes and 5'-Uridine Diphosphate Glucuronosyl Transferase (UGT)

Study Type: In Vitro Evaluation of Emtricitabine (FTC) as an Inhibitor of Human Cytochrome P450 Enzymes and 5'-Uridine Diphosphate Glucuronosyl Transferase (UGT)

Study Number: PDM-006

Study System

Study system Pooled human liver microsomes were utilized to measure the activities associated with 7-ethoxyresorufin *O*-deethylation (CYP1A2), coumarin 7-hydroxylation (CYP2A6), 7-benzyloxyresorufin *O*-dealkylation (CYP2B6), tolbutamide methyl hydroxylation (CYP2C9), *S*-mephenytoin 4'-hydroxylation (CYP2C19), dextromethorphan *O*-demethylation (CYP2D6), chlorzoxazone 6-hydroxylation (CYP2E1), testosterone 6β-hydroxylation (CYP3A4/5) and 7-hydroxycoumarin glucuronidation (UGT), in the presence of various concentrations of emtricitabine. Selective inhibitors of the corresponding cytochrome P450s were tested at one concentration to confirm the sensitivity of the assays

Tabulated Results

Enzyme	Enzyme Reaction	Control/ Selective Inhibitor	Emtricitabine		Control/Selective Inhibitor
			K_i (μM)	Type of Inhibition	K_i (μM)
CYP1A2	7-ethoxyresorufin *O*-deethylation	α-naphthoflavone	-	No inhibition	Competitive 0.011
CYP2A6	coumarin 7-hydroxylation	tranylcypromine	-	No inhibition	Mixed: Competitive 1.2/Uncompetitive 0.72
CYP2B6	7- benzyloxyresorufin *O*-dealkylation	orphenadrine	-	No inhibition	Competitive 200
CYP2C9	tolbutamide methyl-hydroxylation	sulfaphenazole	-	No inhibition	Mixed: Competitive 24/Uncompetitive 61
CYP2C19	*S*-mephenytoin 4'-hydroxylation	ticlopidine	-	No inhibition	Complete inhibition
CYP2D6	dextromethorphan *O*-demethylation	quinidine	-	No inhibition	Competitive 0.046
CYP2E1	chlorzoxazone 6-hydroxylation	4-methylpyrazole	1788	Competitive inhibition	Mixed: Competitive 16/Uncompetitive 7.4
CYP3A4/5	testosterone 6β-hydroxylation	ketoconazole	-	No inhibition	Competitive 0.044
UDP-GT	7-hydroxycoumarin glucuronidation	N/A	N/A	No inhibition	N/A

Conclusion:

- Emtricitabine was not an inhibitor for human CYP1A2, 2A6, 2B6, 2C9, 2C19, 2D6, 2E1 and 3A4/5
- Emtricitabine did not show inhibition of 7-hydroxycoumarin glucuronidation

2.6.5.9.C. TMC278-NC102: The In-Vitro Metabolism of ^{14}C-TMC278 in Hepatocytes and Liver Subcellular Fractions of Male and Female Swiss Albino Mice, Male and Female Black Agouti rasH2 Microinjected Mice, Male and Female Rats, Female Rabbit, Male Dog and Man

Study No. TMC278-NC102

Type of study: In vitro metabolism of TMC278 in hepatocytes and liver subcellular fractions in different species and in man.

Methodology: ^{14}C-TMC278 (5 µM) was incubated for various time periods with hepatocytes (suspensions and primary cultures) and with liver subcellular fractions (microsomes and 12,000 x g supernatant fractions). Samples were analyzed by radio-HPLC. Co-chromatography, enzyme hydrolysis, LC-MS/MS and NMR techniques were used for identification of the metabolites.

Percentage of injected sample radioactivity for unchanged compound and its metabolites

Study System	**Mouse (male, Swiss albino)**				**Mouse (female, Swiss albino)**				**Mouse (male, black Agouti rasH2)**				**Mouse (female, black Agouti rasH2)**			
Metabolites	**SK**	**PCK**	**12,000g**	**MICR**	**SK**	**PCK**	**12,000g**	**MICR**	**SK**	**PCK**	**12,000g**	**MICR**	**SK**	**PCK**	**12,000g**	**MICR**
Parent (UD)	11.8	3.9	57.1	85.3	3.2	4.4	71.9	81.6	13.1	3.9	49.1	72.4	6.1	1.9	57.7	77.3
2	-	0.5	-	0.6	-	0.7	-	1.0	-	-	-	0.9	-	-	0.7	0.6
3	0.6	1.0	-	-	1.0	0.6	-	-	-	-	-	-	-	-	-	-
4+5	4.1	2.0	-	0.5	7.2	5.2	-	-	6.6	4.6	0.7	-	8.6	4.6	0.6	-
6	4.8	2.9	0.7	-	3.5	2.2	-	0.7	8.2	3.1	0.8	-	4.1	3.6	0.7	-
7	2.8	1.4	1.1	-	1.0	-	0.6	-	-	-	-	-	-	-	-	-
8	1.8	2.9	1.4	-	3.3	6.7	0.8	-	3.0	3.5	2.3	-	4.5	5.3	4.0	-
10	61.9	49.7	35.9	-	7.6	12.6	17.1	-	-	-	-	-	-	-	-	-
10 (+12* or +14)**									28.3	43.9	36.0*	-	15.0**	36.4	23.8*	-
13	-	-	-	0.9	-	-	-	1.3	-	-	3.3	0.8	-	-	0.7	0.5
14	-	18.4	-	-	6.0	9.2	-	-	5.3	18.0	-	-	-	15.3	-	-
17	1.5	5.3	-	0.7	3.2	8.9	-	-	5.2	7.9	-	0.9	5.7	6.7	-	0.6
18	-	10.5	-	-	3.0	0.6	-	-	-	11.1	-	-	-	5.2	-	-
22	-	-	0.9	2.4	-	-	0.6	3.9	-	-	-	5.5	-	-	-	3.2
25	13.3	7.3	-	-	49.3	46.9	-	-	25.5	6.5	-	-	45.2	23.7	-	-
27	1.8	-	0.9	1.1	-	-	1.1	2.5	2.2	1.0	0.8	2.4	1.2	-	1.3	2.0
33	0.8	-	0.9	2.2	1.8	-	1.2	2.8	2.4	-	0.8	2.6	3.5	-	1.5	1.9
35+36	0.9	-	0.7	2.6	1.8	-	1.2	4.1	1.4	-	0.5	1.9	1.8	-	2.3	2.9
38	-	-	-	-	0.9	0.7	0.8	0.6	-	-	0.6	-	-	-	1.3	-
42	-	0.5	1.3	1.4	0.9	1.6	5.4	2.2	-	0.7	2.9	2.1	0.9	0.7	9.6	1.5
43	0.9	1.5	0.8	0.5	-	-	1.6	0.4	0.7	0.7	1.9	0.9	0.4	-	2.7	0.4
Sum	**107.0**	**107.8**	**101.7**	**98.2**	**93.7**	**100.3**	**102.3**	**101.1**	**101.9**	**104.9**	**99.7**	**90.4**	**97.0**	**103.4**	**106.9**	**90.9**

- : not detected; 12,000g: 12,000 x g supernatant fractions; HPLC: high performance liquid chromatography; LC-MS/MS: liquid chromatography with tandem mass spectrometry; MICR: microsomes; NMR: nuclear magnetic resonance; PCK: primary culture; SK: suspension culture; UD: unchanged drug
* The figures represent the sum of the % of M10 and M12; ** The figures represent the sum of the % of M10 and M14

Study No. **TMC278-NC102 (continued)**
Type of study: In vitro metabolism of TMC278 in hepatocytes and liver subcellular fractions in different species and in man.
Methodology: ^{14}C-TMC278 (5 µM) was incubated for various time periods with hepatocytes (suspensions and primary cultures) and with liver subcellular fractions (microsomes and 12,000 x g supernatant fractions). Samples were analyzed by radio-HPLC. Co-chromatography, enzyme hydrolysis, LC-MS/MS and NMR techniques were used for identification of the metabolites.

Percentage of injected sample radioactivity for unchanged compound and its metabolites

Study System	Rat (male, Sprague-Dawley)				Rat (female, Sprague-Dawley)				Rabbit (female, New Zealand)				Dog (male, Beagle)				Man			
Metabolites	**SK**	**PCK**	**12,000 g**	**MICR**	**SK**	**PCK**	**12,000 g**	**MICR**	**SK**	**PCK**	**12,000 g**	**MICR**	**SK**	**PCK**	**12,000 g**	**MICR**	**SK**	**PCK**	**12,000g**	**MICR**
Parent (UD)	59.5	8.2	42.2	90.2	47.7	18.2	64.0	96.4	20.2	1.0	28.7	87.4	72.3	25.4	91.5	90.7	75.8	23.1	34.8	43.5
2	-	-	-	-	-	-	-	-	1.3	-	1.3	-	-	0.9	-	-	-	-	4.8	1.6
3	-	-	-	-	-	-	-	-	0.8	-	1.1	-	-	0.5	-	-	0.2	1.5	-	-
4	0.6	2.1	-	-	0.3	1.4		-	-	-	-	-	-	-	-	-	-	-	-	-
5	-	-	5.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	0.5	1.0	3.5	-	-	0.5	0.6	-	-	-	-	-	-	-	-	-	-	-	-	-
7	-	-	-	-	-	-	-	-	1.5	0.7	3.9	-	0.7	2.0	-	-	0.4	1.8	2.3	1.3
8	0.5	1.6	4.3	-	0.7	2.1	1.5	-				-	-	-	-	-	-	-	-	-
10	35.6	56.4	40.7	-	46.7	58.7	25.7	-	3.8	3.0	2.1	1.0	1.2	6.4	1.4	-	-	-	-	-
13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2.2	4.8	-	-
14	-	5.6	-	-	-	-	-	-	-	-	-	-	1.5	4.2	-	-	1.5	4.9	-	-
15	-	-	-	-	-	-	-	-	15.8	11.3	-	-	-	-	-	-	1.1	4.6	-	-
17	0.8	7.1	0.6	1.1	3.2	22.5	-	-	5.3	8.8	-	-	-	-	-	-	0.5	6.8	-	-
18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.7	-	-
19	-	-	-	-	-	-	-	-	7.6	11.9	4.8	1.3	3.7	9.4	-	-	0.2	2.1	-	-
20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.5	0.9	-	-	-	-
22	-	-	-	-	-	-	0.4	0.7	-	-	-	-	-	-	-	-	-	-	3.6	7.8
25	0.8	1.1	-	-	1.7	3.5	-	-	38.8	46.2	5.4	-	8.6	22.0	-	-	11.8	31.0	-	-
26	-	-	-	-	-	-	-	-	-	-	-	-	2.6	6.7	-	-	-	-	-	-
27	0.4	0.7	0.9	0.7	0.6	-	-	-	-	-	11.8	0.4	-	-	2.0	0.8	2.4	7.2	5.6	4.9

- : not detected; 12,000g: 12,000 x g supernatant fractions; HPLC: high performance liquid chromatography; LC-MS/MS: liquid chromatography with tandem mass spectrometry; MICR: microsomes; NMR: nuclear magnetic resonance; PCK: primary culture; SK: suspension culture; UD: unchanged drug

Study No. **TMC278-NC102 (continued)**
Type of study: In vitro metabolism of TMC278 in hepatocytes and liver subcellular fractions in different species and in man.
Methodology: ^{14}C-TMC278 (5 µM) was incubated for various time periods with hepatocytes (suspensions and primary cultures) and with liver subcellular fractions (microsomes and 12,000 x g supernatant fractions). Samples were analyzed by radio-HPLC. Co-chromatography, enzyme hydrolysis, LC-MS/MS and NMR techniques were used for identification of the metabolites.

Percentage of injected sample radioactivity for unchanged compound and its metabolites

Study System	Rat (male, Sprague-Dawley)				Rat (female, Sprague-Dawley)				Rabbit (female, New Zealand)				Dog (male, Beagle)				Man			
Metabolites	**SK**	**PCK**	**12,000g**	**MICR**	**SK**	**PCK**	**12,000g**	**MICR**	**SK**	**PCK**	**12,000g**	**MICR**	**SK**	**PCK**	**12,000g**	**MICR**	**SK**	**PCK**	**12,000g**	**MICR**
30+31	-	-	-	-	-	-	-	-	-	-	4.6	1.2	-	-	0.5 + 0.8	-	0.9*	1.6 *	3.6	0.5*
32	-	1.3	-	-	-	6.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
33	-	-	1.0	1.1	-	-	-	-	-	-	4.6	1.2	-	-	2.3	1.0	1.4	1.1	2.5	2.9
34	-	-	-	-	-	-	-	-	-	-	-	-	1.7	6.0	-	-	-	-	-	-
35+36	-	-	-	-	-	-	-	-	-	-	-	-	-	1.3	0.5	1.2	0.4		9.8	7.9
36	-	-	1.1	2.1	-	-	0.5	1.2	2.5		6.0	1.4	-	-	-	-	-	-	-	-
38	-	-	0.7	2.0	-	-	-	0.5	-	-	6.9	0.4	-	-	0.6	-	-	-	3.8	-
42	-	-	1.1	2.0	-	-	1.2	1.1	-	-	5.1	0.8	1.7	2.6	3.5	1.2	0.5	1.0	6.6	6.0
43	2.8	-	3.8	0.9	2.0	0.8	3.8	1.4	-	-	1.2	1.4	-	0.6	0.9	0.9	1.8	0.6	Trace	-
Sum	**101.5**	**85.1**	**105.3**	**101.4**	**102.9**	**113.8**	**97.7**	**101.3**	**97.6**	**82.9**	**87.5**	**96.5**	**94.0**	**88.0**	**105.0**	**96.7**	**100.9**	**93.6**	**73.8**	**76.4**

- : not detected; 12,000g: 12,000 x g supernatant fractions; HPLC: high performance liquid chromatography; LC-MS/MS: liquid chromatography with tandem mass spectrometry; MICR: microsomes; NMR: nuclear magnetic resonance; PCK: primary culture; SK: suspension culture; UD: unchanged drug.
* Figure represents only M30

2.6.5.9.D. TMC278-NC333: The In Vitro Metabolism of ^{14}C-TMC-278 in Liver 12000 x g Supernatant and Hepatocytes of Guinea Pig and Monkey

Study No. TMC278-NC333
Type of study: In vitro metabolism of TMC278 in hepatocytes (primary cell cultures) and 12000 x g liver supernatant fractions of monkey and Guinea pigs.
Methodology: ^{14}C-TMC278 (5 µM) was incubated with hepatocytes (primary cultures) for approximately 24 hours and with liver subcellular fractions (12,000 x g supernatant fractions) for 120 minutes. Samples were analyzed by radio-HPLC and metabolites identified by LC-MS/MS.

Percentage of injected sample radioactivity for unchanged compound and its metabolites

Study System	Guinea pig (female, Dunking Hartley)		Monkey (male, Cynomolgus)	Monkey (female, Cynomolgus)
Metabolites	**PCK**	**12,000g**	**PCK**	**12,000g**
Parent (UD)	2.5	30.9	9.1	12.6
M10	ND	55.0	ND	ND
M12	45.8	ND	10.7	ND
M13+M14	19.1	ND	ND	ND
M17+M18	11.1	ND	ND	ND
M19	ND	ND	7.4	ND
M22	ND	ND	ND	8.3
M25	7.1	ND	53.5	ND
M27	ND	ND	ND	10.6
M30	2.6	2.8	ND	ND
M33	ND	ND	ND	12.7
M38	ND	ND	ND	9.2
M42	ND	ND	ND	9.4
Sum	**88.2**	**88.7**	**80.6**	**62.7**

12,000g: 12,000 x g supernatant fractions; HPLC: high performance liquid chromatography; LC-MS/MS: liquid chromatography with tandem mass spectrometry; ND: not detected; PCK: primary culture; UD: unchanged drug

2.6.5.9.E. TMC278-NC141: An In Vitro Study to (a) Identify the Microsomal Cytochrome P-450 Iso-enzymes Mediating TMC278 Metabolism (Reaction Phenotyping) and to (b) Determine the Kinetics of TMC278 Metabolism in Human Liver Microsomes

Study No. TMC278-NC141

Type of study: Enzyme kinetics of TMC278 metabolism in human liver microsomes

Method: ^{14}C-TMC278 was incubated at various concentrations in human liver microsomes for 15 minutes at a protein concentration of 0.25 mg/ml. The amount of unchanged TMC278 remained in the samples was determined by radio-HPLC and the % metabolized (substrate turnover rate) was calculated. The kinetic parameters were calculated by a Michaelis-Menten equation using validated Winnonlin software (Pharsight, Winnonlin 4.0.1).

Results

I. Protein concentration: 0.25 mg/ml

Substrate Conc. (µM)	**Product rate (pmol/mg/min)[a]**
0.5	32.2, 36.8, 28.5
1	60.7, 57.2, 56.2
3	146, 124, 150
5	206, 222, 248
7.5	281, 199, 205
10	327, 420, 223
15	287, 299, 231
20	270, 308, 350
30	317, 341, 429
50	356, 396, 222
K_m (± std error)	**4.17 (± 1.06) µM**
V_{max} (± std error)	**381 (± 26) pmol/min/mg protein**

Additional information

a Triplicate values are used in the determination of K_m and V_{max}

Study No. **TMC278-NC141 (continued)**
Type of study: CYP reaction phenotyping – Effect of diagnostic CYP inhibitors on the metabolism of TMC278
Method: Inhibition of the metabolism of TMC278 in human liver microsomes by diagnostic inhibitors was carried out with ^{14}C-TMC278 (5 µM) for 15 minutes at a protein concentration of 0.25 mg/ml. The amounts of unchanged TMC278 and its metabolites (M27, M33, M35+M36[a] and M42[a]) were determined by radio-HPLC. The values represent the percentage of inhibition obtained for each inhibitor in comparison to a control incubate (without inhibitor). Each value represents mean of three observations.
Results:

		% Inhibition of Metabolism[b]				
Diagnostic Inhibitor	**CYP P450 Form**	**Overall[c]**	**M27[c]**	**M33[c]**	**M35+M36[a]**	**M42[a,b]**
Furafylline (10 µM)	CYP1A2	-10.9	-10.9	-13.9	66.7	-22.3
Coumarin (100 µM)	CYP2A6	-9.3	-25.0	-44.4	75.0	-13.4
Sulphaphenazole (10 µM)	CYP2C8/9/10	-3.0	0.0	30.6	86.1	-25.1
Quinidine (10 µM)	CYP2D6	4.4	1.56	-5.56	69.4	-3.24
4-methylpyrazole (20 µM)	CYP2E1	-6.5	1.56	-8.33	63.9	-21.1
Ticlopidine (5 µM)	CYP2C19/D6	-3.1	-2.94	-27.8	25.0	-7.91
Ketoconazole (1 µM)	CYP3A4	107	100	100	100	100
Troleandomycin (200 µM)	CYP3A4	107	100	100	100	100
Clarithromycin (15 µM)	CYP3A	57.2	45.3	38.9	58.3	57.1
Ritonavir (0.15 µM)	CYP3A	91.3	100	100	100	80.2
1-aminobenzotriazole	CYP P450	104	100	100	100	100

Additional Information
a Major metabolite in human liver microsomes (> 5 % of the sample radioactivity)
b Calculated from control incubation (without inhibitor); higher the positive value and higher the extent of inhibition
c Negative values indicates higher % product formation in test sample compared to the control. This was more prominent with the minor metabolites. For all qualitative purposes, all negative values were considered as no inhibition.

Study No. **TMC278-NC141 (continued)**
Type of study: CYP reaction phenotyping – Metabolism of ^{14}C-TMC278 in *E. coli* expressed CYP isoforms
Method: the metabolism of TMC278 in *E. coli* expressed CYP systems (prepared in-house) was carried out with ^{14}C-TMC278 (5 µM) for 60 minutes at a CYP P450 concentration of 100 pmol/ml of incubation. The amounts of unchanged TMC278 and its metabolites (M50, M2, M22 [a], M27, M33 [a], M35+M36 [a], M51, and M42[a]) were determined by radio-HPLC. Each value represents mean ± S.D of three observations.
Results:

Cytochrome P-450 Form (100 pmol/ml)	Overall % Metabolism[b]	Product formation rate (pmol/min. 100 pmol P450) M50	M2	M22	M27	M33	M35+M36	M51	M42
CYP1A2	1.40 ± 0.26	-	-	-	-	-	-	-	-
CYP2A6	1.07 ± 1.44	-	-	-	-	-	-	-	-
CYP2B6	1.30 ± 1.76	-	-	-	-	-	-	-	-
CYP2C8	0.43 ± 0.38	-	-	-	-	-	-	-	-
CYP2C9	0.47 ± 0.42	-	-	-	-	-	-	-	-
CYP2C19	0.37 ± 0.32	-	-	-	-	-	-	-	-
CYP2D6	1.07 ± 1.85	-	-	-	-	-	-	-	-
CYP2E1	0.00 ± 0.00	-	-	-	-	-	-	-	-
CYP3A4	86.87 ± 1.40	4.11 ± 0.35	9.03 ±0.71	15.6 ± 1.30	2.58 ± 0.95	3.00 ± 0.58	20.4 ± 2.95	-	6.28 ± 0.75
CYP3A5	0.53 ± 0.68	-	-	-	-	-	-	-	0.31 ± 0.34

Additional Information
a Major metabolites in human liver microsomes (> 5 % of the sample radioactivity)
b Overall % metabolism of TMC278 calculated from % drug that remained in the sample at the end of the incubation
- No measurable product observed in radio-HPLC profile (LLOQ = 211 dpm)

Study No. **TMC278-NC141 (continued)**
Type of study: CYP reaction phenotyping – Metabolism of ^{14}C-TMC278 in CYP isoforms (Supersomes®)
Method: The metabolism of TMC278 in expressed CYP systems (Supersomes®) was carried out with ^{14}C-TMC278 (5 µM) for 60 minutes at a CYP P450 concentration of 100 pmol/ml of incubation. The amounts of unchanged TMC278 and its metabolites (M52, M2, M22[a], M27, M33[a], M35+M36[a], M51[a] and M42[a]) were determined by radio-HPLC. Each value represents mean ± S.D of three observations.
Results:

Cytochrome P450 Form (100 pmol/mL)	Overall Metabolism[b]	Product formation rate (pmol/min. 100 pmol P450)							
		M52	M2	M22	M27	M33	M35+M36	M51	M42
CYP1A2	1.17 ± 0.64	-	-	-	-	-	-	-	-
CYP2A6	0.37 ± 0.32	-	-	-	-	-	-	-	-
CYP2B6	0.50 ± 0.44	-	-	-	-	-	-	-	-
CYP2C8	0.00 ± 0.00	-	-	-	-	-	-	-	-
CYP2C9	0.43 ± 0.38	-	-	-	-	-	-	-	-
CYP2C19	0.43 ± 0.40	-	-	-	-	-	-	-	-
CYP2D6	2.37 ± 2.45	-	-	-	-	-	-	-	-
CYP2E1	0.23 ± 0.40	-	-	-	-	-	-	-	-
CYP3A4	39.5 ± 2.1	0.97 ± 0.84	4.44 ± 0.86	3.39 ± 0.32	4.14 ± 0.68	4.00 ± 0.46	7.72 ± 1.73	-	5.19 ± 1.13
CYP3A5	28.3 ± 2.7	0.61 ± 0.21	1.64 ± 0.21	5.00 ± 0.52	0.25 ± 0.43	5.08 ± 0.36	2.56 ± 0.21	2.61 ± 0.91	3.81 ± 1.14
CYP3A7	26.3 ± 2.3	-	1.17 ± 0.14	3.50 ± 1.39	-	4.33 ± 1.15	6.14 ± 0.60	-	6.39 ± 0.59

Additional Information
a Major metabolites of human liver microsomes.
b Overall % metabolism of TMC278 calculated from % drug that remained in the sample at the end of the incubation.
No measurable product observed in radio-HPLC profile (LLOQ = 211 dpm).

Study No. **TMC278-NC141 (continued)**

Type of study: CYP reaction phenotyping – Correlation analysis of TMC278 metabolites with CYP activities.

Methodology: The metabolism of ^{14}C-TMC278 (5 µM) was examined with a characterized panel of 10 human liver microsomal preparations. The protein concentration of the samples was 0.25 mg/ml and the time of incubation was 15 min. The amounts of unchanged TMC278 and its metabolites (M27, M33 [a], M35 + M36 [a] and M42 [a]) were determined by radio-HPLC. The rate of product formation was calculated for TMC278 metabolites and were correlated (pair-wise) with the CYP isoform dependent enzyme activities of corresponding batches of human liver microsomes.

Results:

Enzyme activities (CYP isoform)	Overall TMC278 metabolism Correlation (r^2)	TMC278 metabolite correlation coefficient (r^2)			
		M27	M33	M35 + M36	M42
7-ethoxyresorufine *O*-deethylase **(1A2)**	0.119	-0.050	0.523	0.093	0.073
Phenacetin *O*-deethylase **(1A2)**	0.052	-0.186	0.347	0.102	0.047
Coumarin 7-hydroxylase **(2A6)**	-0.071	0.055	-0.297	0.201	-0.080
Taxol 6-α-hydroxylase **(2C8)**	-0.437	-0.613	-0.324	-0.660	-0.289
Tolbutamide methyl hydroxylase **(2C9, 10)**	-0.711	-0.842	-0.530	-0.468	-0.608
S-mephenytoin 4-hydroxylase **(2C19)**	**0.748**	**0.704**	**0.878**	0.107	**0.790**
Dextromethorphan *O*-demethylase **(2D6)**	-0.413	-0.538	-0.310	-0.611	-0.306
Bufuralol hydroxylase **(2D6)**	-0.442	-0.578	-0.348	-0.659	
Chlorozoxazone 6-hydroxylase **(2E1)**	0.030	-0.098	-0.303	-0.389	0.215
Lauric acid ω-1 hydroxylase **(2E1)**	-0.543	-0.709	-0.393	-0.450	-0.424
Testosterone 6-β-hydroxylase **(3A4)**	**0.819**	**0.749**	0.485	-0.003	**0.881**
Cyclosporine oxidase **(3A)**	**0.716**	**0.744**	0.336	0.015	**0.746**
Taxol 3'- hydroxylase **(3A4)**	**0.889**	**0.938**	**0.503**	0.383	**0.872**
Midazolam 4-hydroxylase **(3A4/A5)**	**0.864**	**0.817**	**0.611**	0.055	**0.876**
Midazolam 1'- hydroxylase **(3A5/A4)**	**0.577**	**0.594**	0.329	-0.310	**0.638**
Lauric acid ω- hydroxylase **(4A)**	-0.001	-0.207	-0.185	-0.499	0.173

Additional Information

a Major metabolite in human liver microsomes.

Bolded numbers: Positive correlations higher than 0.500

2.6.5.9.F. 96-DDM-1278-003: In Vitro Metabolism of Tenofovir in Dog Plasma, in Control and Induced (Araclor 1254) Rat Liver Microsomes, and in Dog Liver and Intestinal Homogenates

Study Number: 96-DDM-1278-003

Study Systems

Study system 1) In vitro metabolism of tenofovir determined in rat liver microsomes from control data or following induction with Aroclor 1254 (an inducer of cytochrome P450 isoenzymes 1A & 1B).

Study system 2) Stability of tenofovir in plasma, intestinal & liver homogenates from human and dog tissues.

Half life t ½ (min)
(% tenofovir remaining following 60 min incubation at 37 °C)

	Liver microsomes/ homogenates	Liver microsomes Aroclor induced	Plasma	Intestine
Rat	> 60 (117%) [1]	> 60 (93%) [1]	-	-
Rat with Co Factors	> 60 (111%) [1]	> 60 (99%) [1]	-	-
Dog	> 60 (92%) [2]	-	> 60 (98%) [2]	> 60 (110%) [2]
Human	> 60 (108%) [2]	-	> 60 (111%) [2]	> 60 (116%) [2]

Additional Information: No metabolites were detected. 1= data from study system 1, 2= data from study system 2.

2.6.5.9.G. 97-VIT-1278-001: In Vitro Stability of Bis-POC PMPA (GS4331) in the Biological Fluids

Study Number: 97-VIT-1278-001

Study System	Stability of TDF in rat, human & dog plasma & dog & human liver & intestinal homogenates, with an NADPH regenerating system added to the liver homogenates to facilitate oxidative phosphorylation.

Half lives of tenofovir DF (min, at 37 °C)

Species	Plasma	Intestine	Liver
Rat	< 1	ND	ND
Dog	20.5	52.6	< 5
Human	< 5	< 5	< 5

Additional Information: TDF was rapidly converted to the monoester (tenofovir soproxil) with the half lives shown above. No other major metabolites were observed. ND = not determined

2.6.5.9.H. 98-VIT-4331-001: Epithelial Transport and Metabolism of Tenofovir Disoproxil (Bis-POC PMPA; GS-4331) in Caco-2 Cell Monolayers

Study Number: 98-VIT-4331-001

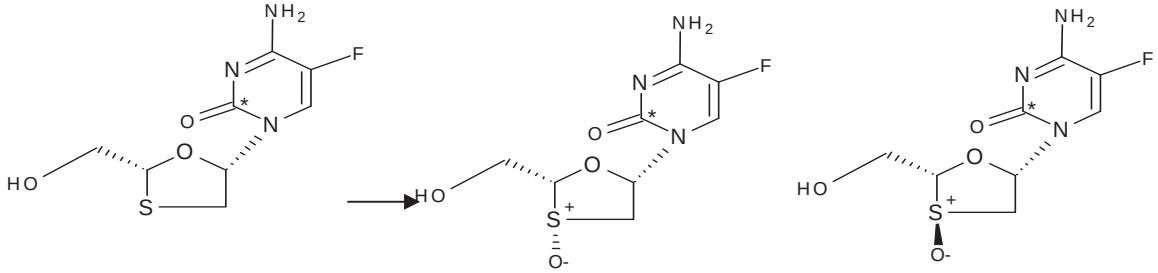
Study System TDF intracellular metabolism in Caco-2 cells (colon, adenocarcinoma; human). Cells incubated with TDF (100 μM).

Time (min)	**Metabolite concentrations (μM) inside Caco-2 cells following incubation with 100 μM TDF**		
	TDF	Tenofovir soproxil (monoester)	tenofovir
0	ND	ND	ND
30	0	ND	ND
60	20.3	349	177

Additional Information: Estimated cell volume approximately 1 μL, ND = not determined

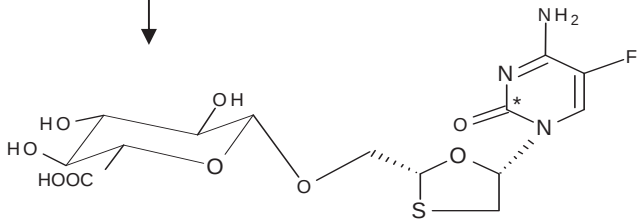
2.6.5.10.A. Emtricitabine

Diagram shows the conversion of Emtricitabine to its major metabolites, 3'-sulfoxide diastereomers, and 2'-o-gucuronide via conjugation.



TP-0006 (FTC) → **M1 or M2** **M1 or M2**

3'-sulfoxide diastereomers of FTC



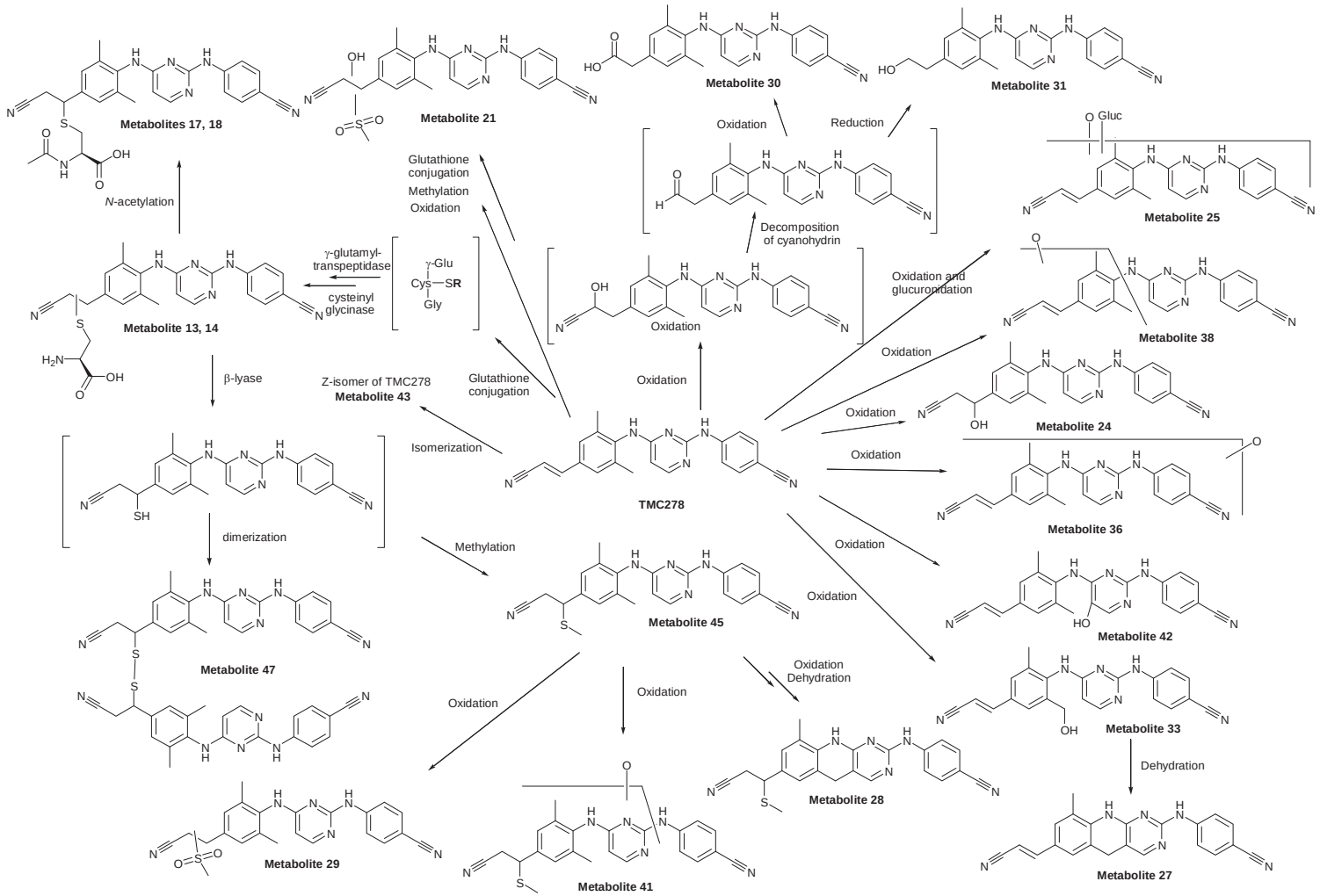
2'-O-glucuronide of FTC

* indicates the position of the ^{14}C-label

2.6.5.10.B. TMC278–NC190: The Metabolism, Excretion and Plasma Kinetics of TMC278 in the Male and Female CD-1 Mouse After Single Oral Administration of ^{14}C-TMC278 at 20 and 320 mg/kg

Study No. TMC278–NC190[a]

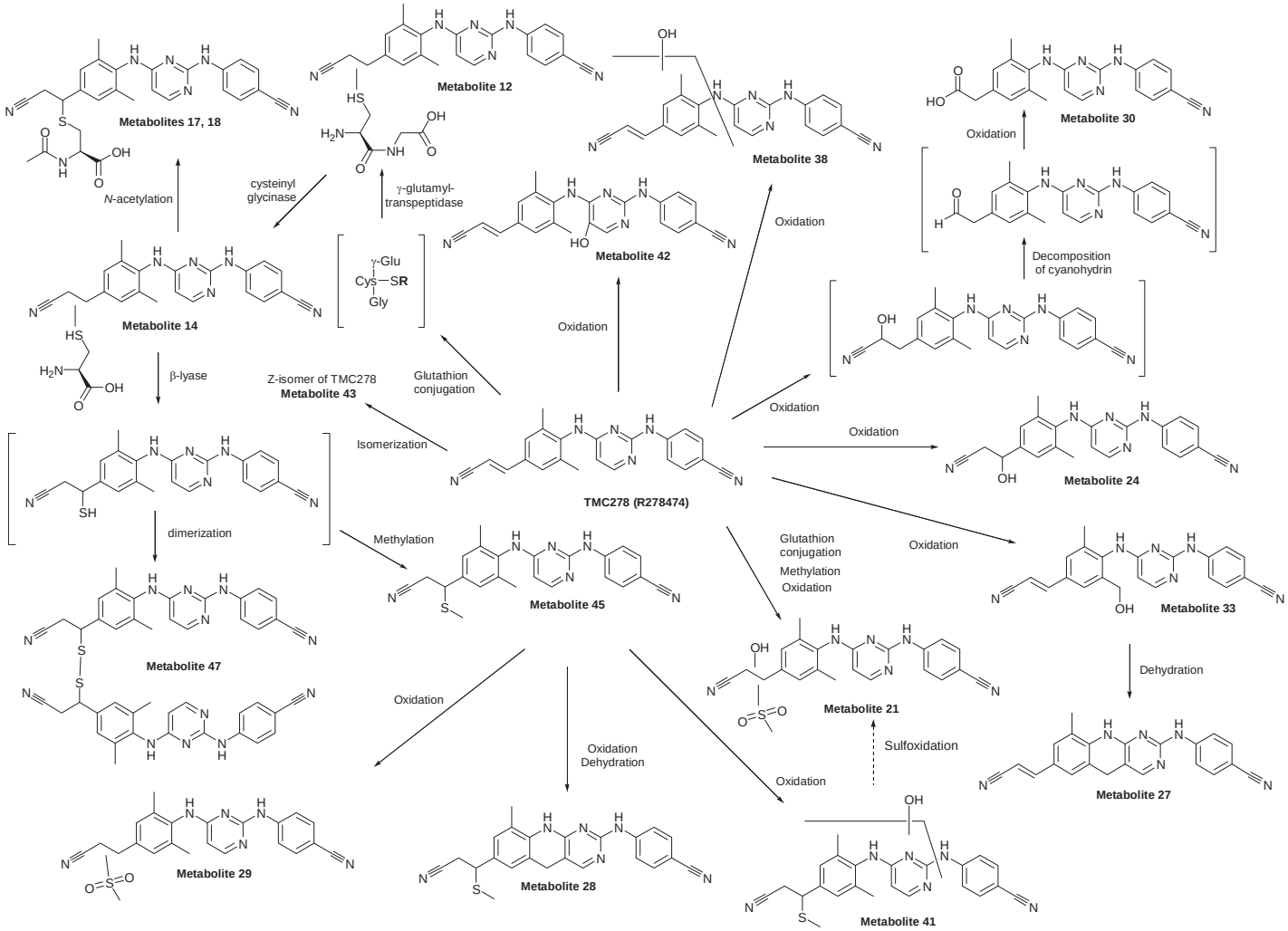
Species Mouse



a see Tabulated Summary 2.6.5.8.D

2.6.5.10.C. TMC278–NC113: The Metabolism and Excretion of ^{14}C-TMC278 in the Male and Female Sprague-Dawley Rat after Single Oral Administration of ^{14}C-TMC278 at 40 mg/kg

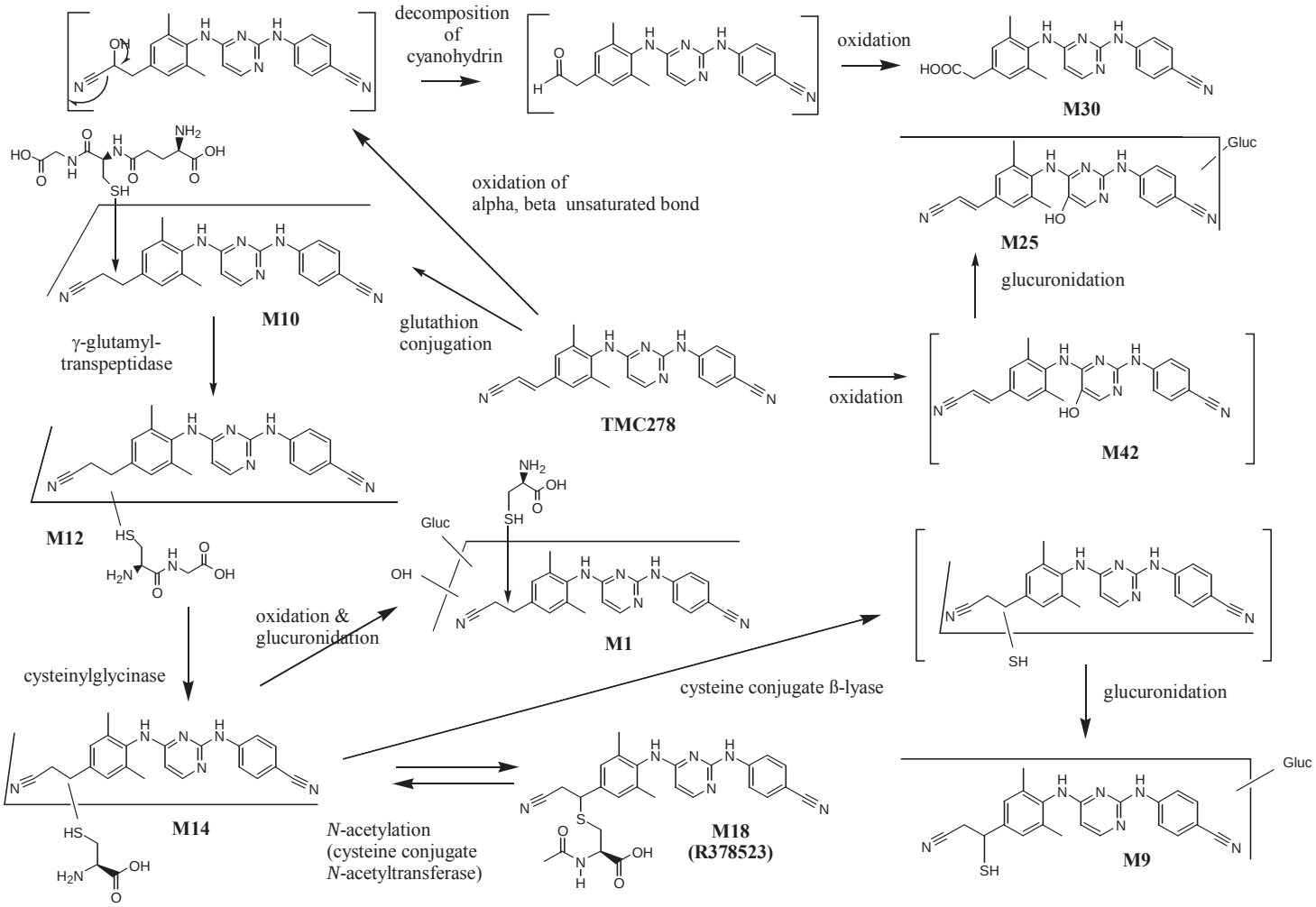
Study No. TMC278–NC113[a]
Species Rat



a see Tabulated Summary 2.6.5.8.E

2.6.5.10.D. TMC278–NC145: Biliary Excretion and Identification of Biliary Metabolites of TMC278 in Male Sprague-Dawley Rats After a Single Oral Dose of ^{14}C-TMC278 at 40 mg/kg

Study No. TMC278–NC145[a]
Species Rat

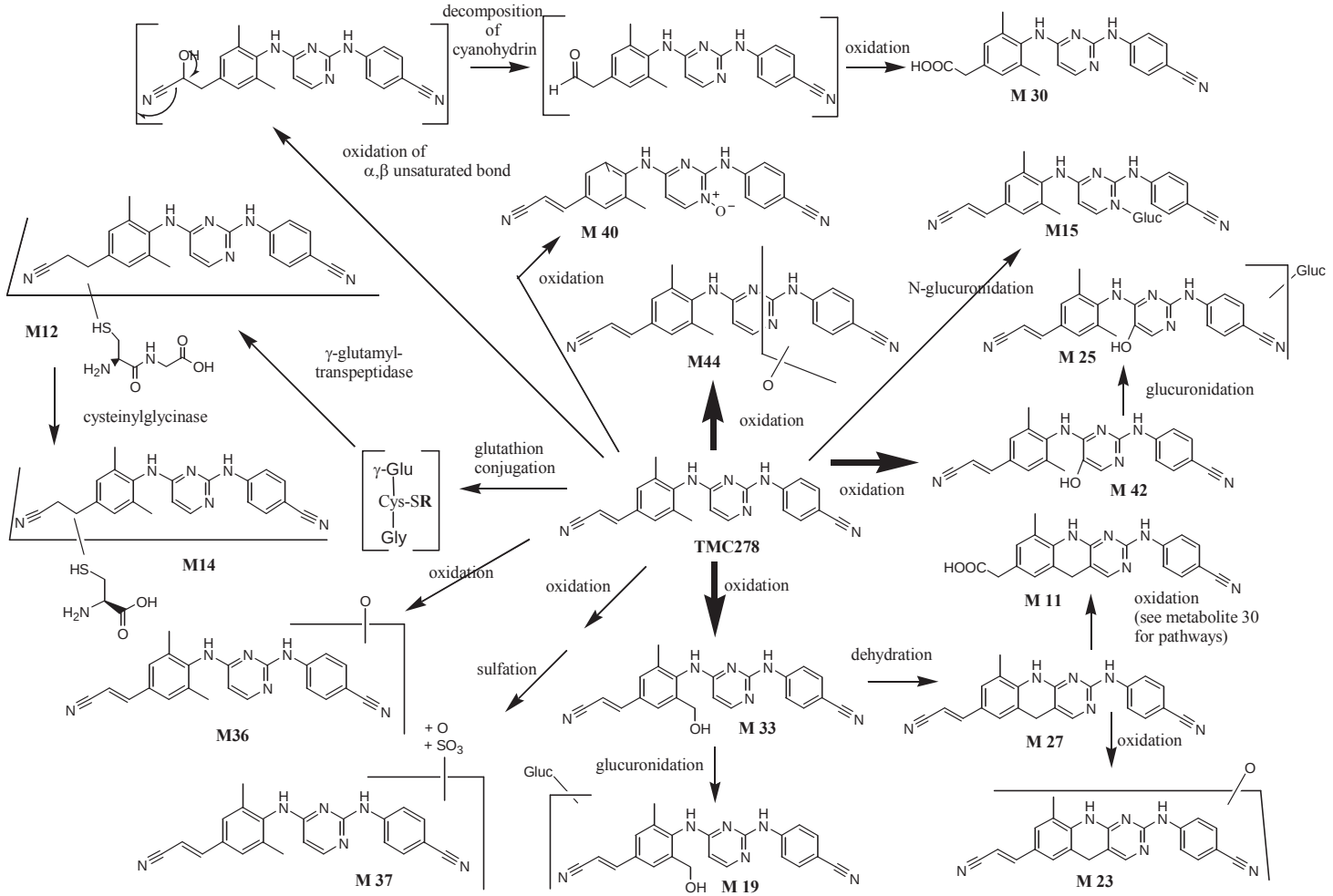


a see Tabulated Summary 2.6.5.8.F

2.6.5.10.E. TMC278–NC114: The Absorption, Metabolism and Excretion of TMC278 in the Male Beagle dog After a Single Oral Dose of ^{14}C-TMC278 at 5 mg/kg

Study No. TMC278–NC114[a]

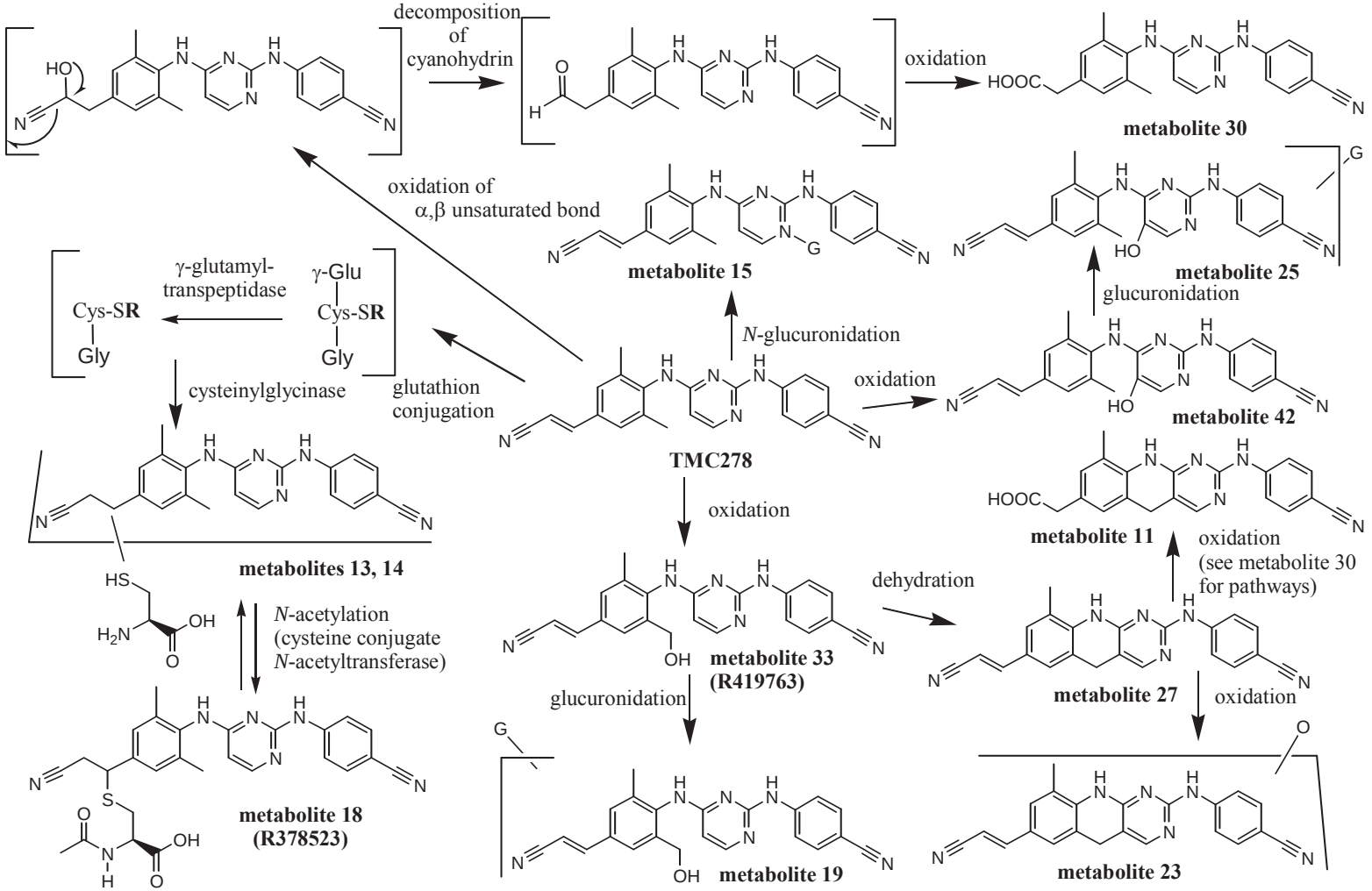
Species Dog (beagle)



[a] see Tabulated Summary 2.6.5.8.G

2.6.5.10.F. TMC278–NC157: The Absorption, Metabolism and Excretion of TMC278 After a Single Oral Dose of 150 mg in Healthy Male Subjects (Clinical Trial TMC278-C119)

Study No. TMC278–NC157[a]
Species Human



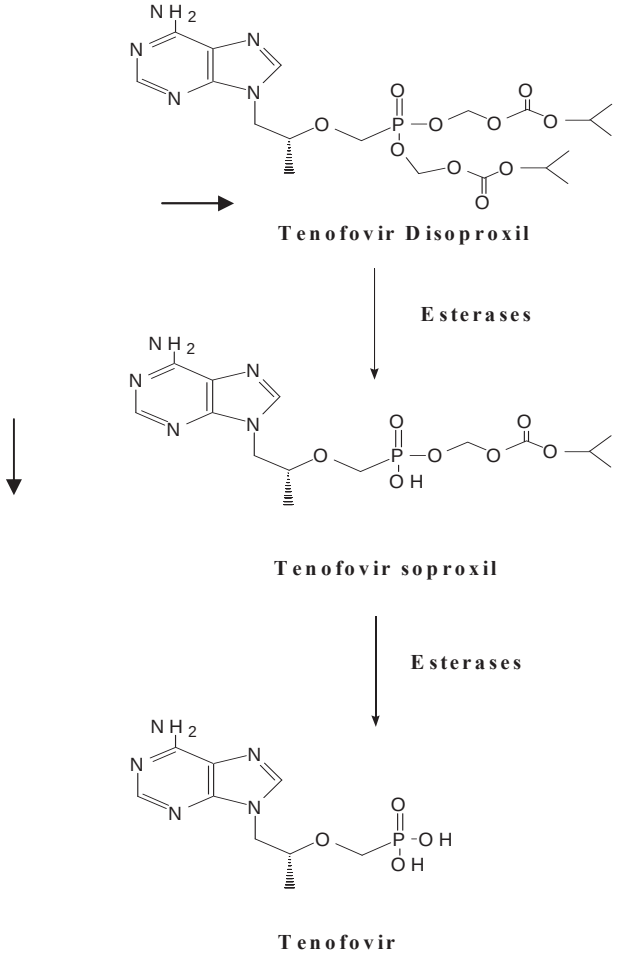
a see Tabulated Summary 2.6.5.10.F

2.6.5.10.G. Tenofovir DF

Diagram shows the conversion of TDF to tenofovir soproxil through to tenofovir illustrating the cleavage of the phosphoester linkages by esterases. No other pathways are known.

2.6.5.10. Pharmacokinetics: Possible Metabolic Pathways

Test Article: Tenofovir disoproxil fumarate



2.6.5.11. Pharmacokinetics: Induction/Inhibition of Drug Metabolizing Enzymes

2.6.5.11.A. TMC278-NC192: A Study of the Effects of TMC278 HCl on Some Hepatic Enzyme Activities After Oral Administration for 3 Months at Doses of 0, 20, 80, and 320 mg/kg/day to Make an Female Swiss Albino CD1 Mice

Study No. TMC278-NC192

Type of Study: Possible induction and/or inhibition of drug metabolizing enzymes by TMC278 evaluated ex vivo in liver microsomes

Method

Microsomal fractions of livers from TMC278 HCl treated animals were isolated. Swiss albino CD1 mice were treated with 0 (0.5% (w/v) HPMC, vehicle), 20, 80 and 320 mg.base eq./kg/day TMC278 for three months. Liver microsomal fractions were analyzed for protein and total cytochrome P450 (CYP) content and for the enzyme activities shown below. Liver cytosolic fractions were analyzed for protein and GSH *S*-transferase activity towards CDNB as substrate. Results are presented for groups of 5 mouse liver pools, each pool being prepared from the livers of 2 mice.

Results

	Control (vehicle only)		TMC278 (mg.base.eq/kg/day)					
			20		80		320	
Gender (M/F)	M	F	M	F	M	F	M	F
Microsomal protein[a]	17	16	18	16	19*	19**	25***	23***
CYP content[b]	1.2	0.83	1.2	0.99*	1.4*	1.0**	1.6***	1.2***
7-Ethoxyresorufin O-deethylase (CYP1A)[c]	315	223	378	284	362	322**	273	187
7-Pentoxyresorufin O-depentylase (CYP2B)[c]	86	150	103	168	92	191**	88	135
4-Nitrophenol hydroxylase (CYP2E)[d]	1.9	2.4	2.1	2.3	2.3**	2.6	1.8	2.1
Testosterone 6β-hydroxylase (CYP3A)[d]	2.7	2.1	3.0	3.2**	4.2***	3.6***	4.7***	3.6***
Lauric acid 11-hydroxylase[d]	1.1	1.2	1.1	1.4	1.4	2.6***	3.4***	4.3***
Lauric acid 12-hydroxylase (CYP4A)[d]	0.83	1.5	1.2*	1.9	4.4***	7.8***	21***	30***
UDPglucuronosyltransferase (substrate thyroxine)[c]	16	11	17	16*	23**	19***	33***	26***
Cytosolic protein[a]	97	106	93	102	97	108	115**	133***
GSH *S*-transferase (substrate CDNB)[e]	8.1	2.0	8.1	2.3	6.3*	2.2	3.6***	1.7

Additional Information

Values significantly different from control are: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

a Units : mg protein/g liver. b Units : nmol/mg protein. c Units : pmol/min/mg protein. d Units : nmol/min/mg protein. e Units : µmol/min/mg protein

CNDB: 1-chloro2,4-dinitrobenzene; CYP: cytochrome 450; GSH: glutathione; UDP: uridine diphosphate; HPMC: hydroxypropyl methylcellulose

2.6.5.11. Pharmacokinetics: Induction/Inhibition of Drug Metabolizing Enzymes **Test Article:** Rilpivirine

2.6.5.11.B. TMC278-NC193: A Study of the Effects of TMC278 on Some Hepatic Enzyme Activities After Oral Administration For 6 Months at Doses of 0, 40, 120, and 400 mg/kg/day to Male and Female Sprague-Dawley Rats

Study No. TMC278–NC193
Type of Study: Possible induction and/or inhibition of drug metabolizing enzymes by TMC278 evaluated ex vivo in liver microsomes
Method
Microsomal fractions of livers from TMC278 base treated animals were isolated. Sprague Dawley rats were treated with 0 (100 mg/ml citric acid in PEG400, vehicle), 40, 120 and 400 mg/kg/day TMC278 for 6 months. Liver microsomal fraction were analyzed for protein and GSH *S*-transferase activity towards CDNB as substrate. Results are presented for groups of 5 rats.

Results	Control (vehicle only)		TMC278 (mg/kg/day)					
			40		120		400	
Gender (M/F)	M	F	M	F	M	F	M	F
Microsomal protein[a]	61	45	65	45	66	47	72**	54***
CYP content[b]	0.84	0.56	0.83	0.56	0.81	0.63*	0.87	0.69***
7-Ethoxyresorufin O-deethylase (CYP1A)[c]	28	24	24	26	21	32*	18*	29
7-Pentoxyresorufin O-depentylase (CYP2B)[c]	50	11	43	11	52	14*	37	13*
4-Nitrophenol hydroxylase (CYP2E)[d]	0.64	0.73	0.62	0.69	0.60	0.77	0.46*	0.69
Testosterone 6β-hydroxylase (CYP3A)[d]	0.64	0.05	0.61	0.06	0.80	0.15***	0.77	0.30***
Lauric acid 11-hydroxylase[d]	0.42	0.34	0.47	0.30	0.57*	0.37	0.77***	0.42*
Lauric acid 12-hydroxylase (CYP4A)[d]	0.47	0.44	0.66	0.33	1.2**	0.41	2.2***	0.56*
UDPglucoronosyltransferase (substrate thyroxine)[c]	5.7	5.6	3.7**	7.1	4.4*	5.5	7.1*	7.5
Cystolic protein[a]	130	12	132	112***	132	111***	134	117*
GSH *S*-transferase [e]	2.6	1.2	2.5	1.7*	2.4	1.6	2.3	1.9**

Additional Information
Values significantly different from control are: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

a units: mg protein/g liver; b units: nmol/mg protein; c units: pmol/min/mg protein; d units: nmol/min/mg protein; e units: µmol/min/mg protein
CNDB: 1-chloro2,4-dinitrobenzene; CYP: cytochrome 450; GSH: glutathione; UDP: uridine diphosphate; PEG400: polyethylene glycol 400

2.6.5.11. Pharmacokinetics: Induction/Inhibition of Drug Metabolizing Enzymes **Test Article:** Rilpivirine

TMC278-NC140: A Study of the Effects of TMC278 on Some Hepatic Enzyme Activities After Oral Administration for 6 Months at Doses of 0, 5, 10, and 40 mg/kg/day to Male and Female Beagle Dogs

Study No. **TMC278–NC140**
Type of Study: Possible induction and/or inhibition of drug metabolizing enzymes by TMC278 evaluated ex vivo in liver microsomes
Method
Microsomal and cytosolic fractions of livers from TMC278 base treated animals were isolated. Male and female Beagle dogs were treated with 0 (citric acid (100 mg/ml) in PEG400, vehicle solution) 5, 10 and 40 mg/kg/day TMC278 for six months. Liver microsomes were analysed for protein and total cytochrome P450 (CYP) content and for the enzyme activities shown below. Cytosolic fractions were analyzed for protein content and GSH *S*-transferase activity. Groups of 2 male and 2 female control and TMC278 treated beagle dogs were combined for statistical analysis of data.

Results	Control (vehicle only)	TMC278 (mg/kg/day)		
		5	10	40
Microsomal protein[a]	40.7	42.3	44.4	43.2
CYP content[b]	0.72	0.72	0.75	0.70
7-Ethoxyresorufin O-deethylase (CYP1A)[c]	183	169	217	162
7-Pentoxyresorufin O-depentylase (CYP2B)[c]	76	84	96	87
4-Nitrophenol hydroxylase (CYP2E)[d]	0.50	0.59	0.53	0.51
Testosterone 6β-hydroxylase (CYP3A)[d]	0.58	0.49	0.33**	0.43*
Lauric acid 11-hydroxylase[d]	0.21	0.22	0.22	0.22
Lauric acid 12-hydroxylase (CYP4A)[d]	0.83	0.83	0.85	0.94
UDPglucuronosyltransferase (substrate thyroxine)[c]	2.8	2.3	2.1	1.9
Cytosolic protein[a]	128	129	124	124
GSH *S*-transferase (substrate CDNB)[e]	1.24	1.02	1.04	1.00

Additional Information
Values significantly different from control are: * $p < 0.05$; ** $p < 0.01$
a units: mg protein/g liver; [b] units: nmol/mg protein; [c] units: pmol/min/mg protein; [d] units: nmol/min/mg protein; [e] units: µmol/min/mg protein
CNDB: 1-chloro2,4-dinitrobenzene; CYP: cytochrome 450; GSH: glutathione; UDP: uridine diphosphate; PEG400: polyethylene glycol 400

2.6.5.11.C. TMC278-NC186: An In Vitro Study to Asess the Potential of TMC278 to Induce CYP Enzyme Activities in Cryopreserved Human Hepatocytes

Study No. TMC278–NC186

Type of Study: An in vitro study to assess the potential of TMC278 to induce CYP enzyme activities in cryopreserved human hepatocytes.

Method

After establishment of the hepatocyte cultures, human hepatocytes were treated either with vehicle (DMSO), with various concentrations of TMC278 or with the positive control compounds, omeprazole, rifampicin, or ethanol for 48 h. At the end of the treatment period, induction of CYP activities (CYP1A2, CYP2B6, CYP2C19, CYP2E1, CYP3A4) was measured based on the probe substrate metabolism. Mean fold induction of the different CYP-isoforms in cryopreserved human hepatocytes treated with TMC278 and positive controls was expressed against the vehicle control. In addition, induction of CYP activities was also determined by measurement of mRNA expression levels by TaqMan real-time RT-PCR. In total, three different individual batches of cryopreserved human hepatocytes were used in this study. The results are tabulated in the table below and each value is mean of three observations..

Test Condition	**Mean fold induction in enzyme activity levels**				
	CYP1A2	**CYP2B6**	**CYP2C19**	**CYP2E1**	**CYP3A4**
Control (Vehicle)	1.00	1.00	1.00	1.00	1.00
TMC278 (2.5 µM)	1.06	1.28	1.44	0.98	0.24
TMC278 (10 µM)	0.62	0.71	1.20	1.37	0.04
TMC278 (25 µM)	0.51	0.58	1.25	0.93	0.05
Rifampicin (50 µM)	NA	2.60	3.07	NA	14.43
Rifampicin (50 µM) + TMC278 (25 µM) [a]	NA	0.43	0.28	NA	0.10
Omeprazole (25 µM)	4.95	NA	NA	NA	NA
Omeprazole (25 µM) + TMC278 (25 µM) [a]	2.35	NA	NA	NA	NA
Ethanol (100 mM)	NA	NA	NA	1.24	NA
Ethanol (100 mM) + TMC278 (25 µM) [a]	NA	NA	NA	0.76	NA
	NA	2.60	3.07	NA	14.43
Test Condition	**Mean fold change in mRNA expression levels**				
	CYP1A2	**CYP2B6**	**CYP2C19**	**CYP2E1**	**CYP3A4**
Control (Vehicle)	1.00	1.00	1.00	1.00	1.00
TMC278 (2.5 µM)	2.55	2.89	1.19	0.81	27.12
TMC278 (10 µM)	3.17	2.96	1.12	0. 56	25.95
TMC278 (25 µM)	3.58	1.18	0.60	1.12	5.08
Rifampicin (50 µM)	NA	6.80	1.69	NA	54.88
Omeprazole (25 µM)	15.07	NA	NA	NA	NA
Ethanol (100 mM)	NA	NA	NA	1.01	NA

Additional Information

a Inhibition control to investigate interference of TMC278 with measurement of CYP activities;

CYP: cytochrome 450; mRNA: messenger ribonucleic acid; NA: not applicable; RT-PCR: reverse transcriptase-polymerase chain reaction; DMSO: dimethylsulfoxide

2.6.5.11. Pharmacokinetics: Induction/Inhibition of Drug Metabolizing Enzymes

Test Article: Tenofovir or tenofovir disoproxil fumarate

2.6.5.11.D. V990172-104: Probe Substrates Specific for the CYP450 Isoforms were Utilized to Examine the Potential for Tenofovir and Tenofovir DF to Inhibit CYP-450 Mediated Drug Metabolism In Vitro Using Human Hepatic Microsomes

Study Number: V990172-104

Type of Study

Method

Probe substrates specific for the CYP–450 isoforms were utilized to examine the effect of tenofovir and tenofovir DF on the activities of the distinct isoforms. The metabolism of the probe substrates for CYP 3A4 (terfenadine), CYP 2D6 (dextromethorphan), CYP 2C9 (tolbutamide), CYP 2E1 (chlorzoxazone) and CYP 1A (7-ethoxycoumarin) was evaluated in the presence and absence of 100 μM tenofovir or tenofovir DF.

Tabulated Results:

CYP-450 Isoform (substrate)	**Control** nmol/mg/min	**Tenofovir** nmol/mg/min	**Tenofovir DF** nmol/mg/min
CYP 3A4 (terfenadine hydroxylation)	0.018 ± 0.009	0.016 ± 0.009	0.018 ± 0.10
CYP 2D6 (dextromethorphan O-demethylation)	0.066 ± 0.041	0.064 ± 0.043	0.065 ± 0.045
CYP 2C9 (tolbutamide 3-hydroxylation)	0.218 ± 0.093	0.216 ± 0.096	0.209 ± 0.105
CYP 2E1 (chlorzoxazone 6-hydroxylation)	1.48 ± 0.58	1.50 ± 0.65	1.42 ± 0.77
CYP 1A (7-ethoxycoumarin O-deethylation)	0.481 ± 0.182	0.487 ± 0.19	0.450 ± 0.180*

Additional Information:

Significant compared to control, $p < 0.05$

$n = 3–4$

2.6.5.11.E. R2001024 and R2000036: Ex-Vivo Study to Examine the Potential of Tenofovir DF to Alter the Specific Cytochrome P450 Activities Using Liver Microsomes Prepared from Liver Tissues from Rats Previously Treated with Tenofovir DF in a 28-Day Toxicity Study

Study Number: R2001024, R2000036 (*in vivo* portion)

Type of Study

An ex-vivo study to examine the potential of TDF to alter the specific cytochrome P450 activities using liver microsomes prepared from liver tissues from rats previously treated with TDF in a 28 day toxicity study R2000036.

Method

To assess induction potential, livers were collected from female Sprague-Dawley rats treated for 28 days with 40 or 400 mg/kg/day TDF or with the control vehicle. Microsomes were prepared separately from each liver (n=6 per dose group). CYP1A2 and CYP3A4 activities were evaluated by measuring the activity of Phenacetin O-deethylase and testosterone 6β-hydroxylase. Formation of androstenedione calculated by CYP2B1, CYP2B2, and CYP2C11 in the rat was also measured. The activity of microsomes from treated rats was compared to the activity from rats treated with vehicle.

Tabulated Results:

	Phenacetin O-deethylase activity/ Mean velocity of APAP formation nmol/min/mg protein (SD)	Mean testosterone 6β-hydroxylase activity nmol/min/mg protein (SD)	Mean velocity of androstenedione formation nmol/min/mg protein (SD)
Control	0.133 (0.023)	1.29 (0.55)	0.562 (0.157)
40 mg/kg/Day Treated Animals	0.110 (0.026)	1.35 (0.49)	0.536 (0.141)
400 mg/kg/Day Treated Animals	0.214 (0.015)	1.90 (0.54)	0.811 (0.142)

Additional Information:

Treatment with high doses of TDF 400 mg/kg/day significantly increased the activities measured by O-deethylation of phenacetin and the formation of androstenedione. These activities are mediated by CYP1A and by CYP2B and CYP2C11, respectively in the rat. The activity of CYP3A as measured by testosterone 6β-hydroxylase activity was similar in treated and untreated rats. Although these activities exist in humans, the enzymes responsible may not be direct correlates of those in rats.

2.6.5.12. Pharmacokinetics: Excretion

Please refer to Sections 2.6.5.8.A, 2.6.5.8.B, and 2.6.5.8.C where these studies for FTC have been included.

The excretion study results for RPV in mice, rats, dogs, and humans are described in the Tabulated Summaries 2.6.5.8.D, 2.6.5.8.E, 2.6.5.8.G and 2.6.5.8.H, respectively.

Excretion study results for TFV and TDF are described below in Tabulated Summaries 2.6.5.12.A, 2.6.5.12.B, 2.6.5.12.C, and 2.6.5.12.D.

2.6.5.12.A. 96-DDM-1278-001: Effect of Dose on the Recovery of ^{14}C-PMPA Following Intravenous Administration to Sprague-Dawley Rats

Study Number: **96-DDM-1278-001**

Species	Rat
Gender (M/F) No. of Animals	6M (4M group 1, 2M group 2)
Feeding Condition	Fasted
Vehicle Formulation	Sterile saline or phosphate buffered saline
Method of Administration	IV single bolus
Dose mg/kg/day	10 (group 1), 50 (group 2)
Analyte (Radionuclide)	Total radioactivity, % recovery ^{14}C
Specific Activity	400 µCi/kg
Assay	Liquid scintillation counting (LCS)

10 mg/kg

Excretion Route	**Plasma (% dose in sample)**	**Urine (+ cage wash) (% dose in sample)**	**Feces (% dose in sample)**	**% Total recovery**	
Time				**Urine**	**Feces**
0–24 hr		85.2	3.18		
24–168 hr		7.6	1.30	92.7	4.48

50 mg/kg

0–24 hr		77.5	7.39		
24–168 hr		6.5	1.07	84.0	8.46

2.6.5.12.B. 97-DDM-4331-003/4: Determination of Tenofovir Disoproxil and Metabolite Concentrations in Bile and Gastrointestinal Tract Following Oral Administration of Tenofovir Disoproxil to Rats

Study Number: 97-DDM-4331-003/4

Rat
1M
Fed
Citric acid 50mM

Oral

^{14}C-tenofovir DF at 10 mg-equivalent of tenofovir/kg, single administration
^{14}C

2.5µCi/mg

Liquid scintillation counting (LCS)

Excretion Route	Bile (% dose in sample) [1]	Urine (% dose in sample)
Time (hr)		
Predose	0	ND
0–0.5	0	ND
0.5–2.2	0.014	ND
2.2–3.0	0.009	ND
3.0–4.0	0.009	ND
4.0–5.0	0.005	ND
5.0–21.5	0.002	ND
21.5–22.5	0.002	ND
22.5–24.0	0.076	ND
0–48	ND	ND
Total	**0.117**	

1 Based upon volume of bile collected/time point. ND= not done

2.6.5.12.C. 96-DDM-1278-002: A Pilot Study of Biliary Excretion of [^{14}C]-PMPA in the Beagle Dog

Study Number: 96-DDM-1278-002

Species	Dog
Gender (M/F) No. of Animals	1M
Feeding Condition	Fasted
Vehicle Formulation	aqueous solution
Method of Administration	IV Bolus, single administration
Dose mg/kg	10
Radionuclide	^{14}C
Specific Activity	5 μCi/mg
Assay	Liquid scintillation counting (LCS)

10 mg/kg

Excretion Route	**Plasma (% dose in sample)**	**Urine (% dose in sample)**	**Feces (% dose in sample)**	**Bile (% dose in sample)**	**Cage wash (% dose in sample)**	**Total recovery (%)**				
						P	**U**	**F**	**B**	**CW**
Time										
0–48 hrs		70.0	0.42	0.26	5.68	---	70.0	0.42	0.26	5.68
						Total recovery all samples 76.4%				

P= plasma, U- Urine, F= feces, B= bile, CW= cage wash.

2.6.5.12.D. 97-DDM-4331-001: Tissue Distribution of [^{14}C] GS-4331 in Bealge Dogs Following Oral Administration

Study Number: 97-DDM-4331-001

Species	Dog
Gender (M/F) No. of Animals	12 (6M, 6F)
Feeding Condition	Fasted
Vehicle Formulation	aqueous solution
Method of Administration	Oral, single administration
Dose mg/kg	10
Radionuclide	^{14}C
Specific Activity	2.45 μCi/mg
Assay	Liquid scintillation counting (LCS)

10 mg/kg

Excretion Route	**Plasma (% dose in sample)**	**Urine + cage wash (% dose in sample)**	**Feces (% dose in sample)**	**Bile (% dose in sample)**	**Others**		**Total recovery (%)**					
Time					**GI Contents**	**Tissues**	**P**	**U**	**F**	**B**	**O GI**	**O T**
0–8 hrs		15.8										
8–24 hrs		14.2										
0–24 hrs			37.5									
24 hr	0.0			0.0	9.8	16.9	0.0	26.3	37.5	0.0	9.8	16.9
							Total recovery all samples 90.5%					

P= plasma, U- Urine, F= feces, B= bile, O= other, GI= gastrointestinal, T= tissues.

2.6.5.13. Pharmacokinetics: Excretion into Bile

The excretion study results for RPV (TMC278) in rats are described in the Tabulated Summary 2.6.5.8.F.

For TFV and TDF, please refer to Tabulated Summaries 2.6.5.12.B, and 2.6.5.12.C, and 2.6.5.12.D where these studies have been included.

2.6.5.14. Pharmacokinetics: Drug Drug Interactions

2.6.5.14.A. TMC278–NC194: Inhibition of Metabolism of Interacting Drugs by TMC278

Study No. TMC278–NC194

Type of Study: Inhibition of metabolism by TMC278 of interacting drugs was investigated

Method

The interaction of TMC278 with the metabolism of interacting drugs was investigated in a pooled batch of human liver microsomes. The inhibitory potential of TMC278 on the overall metabolism and/or the formation of their major metabolites is shown. The IC_{50}-values represent the concentration in µM or µg-base-eq/mL of TMC278 inhibiting the metabolism by 50%.

	IC_{50} (95% confidence interval)		Positive Control		
Interacting Drugs	**µM**	**µg-base-eq/mL**	**µM**	**Inhibitor**	**% Inhibition**
S-mephenytoin[a]	1.3[a] (0.74 ─ 1.8)	0.46[a] (0.27 ─ 0.65)	1	**3-benzyl-**	81
Sildenafil	1.4 (-0.13 ─ 3.0)	0.53 (-0.047 ─ 1.1)	1	**phenobarbital**	125
Clarithromycin	2.0 (0.042 ─ 4.0)	0.74 (0.015 ─ 1.46)	1	**ketoconazole**	93
Norethindron	3.9 (2.6 ─ 5.3)	1.44 (0.93 ─ 1.95)	1	**ketoconazole**	84
Sertraline	5.2 (-3.1 ─ 14)	1.9 (-1.1 ─ 4.9)	10[3]	**ketoconazole**	167[b]
Paroxetine	6.6 (-1.2 ─ 14)	2.4 (-0.42 ─ 5.3)	3	**1-aminobenzotriazole**	91
17α-Ethinyloestradiol[c]	6.5[c] (4.2 ─ 8.7)	2.4[c] (1.5 ─ 3.2)	1	**quinidine**	56/59[d]
Omeprazole	12.0 (7.0 ─ 17)	4.4 (2.6 ─ 6.2)	1 / 1	**ketoconazole**	92
Abacavir[e,f]	>30[f]	>11[f]	600	**3-benzyl-phenobarbital /**	95
Chlorzoxazone[g]	>30[g]	>11[g]	100	**ketoconazole**	-184[h]
				4-methylpyrazole	
				diethyldithiocarbamate	

Additional Information

a) As determined by the formation of the 4-hydroxy metabolite only.
b) This inhibition is not significantly different from the boiled fraction.
c) As determined by the formation of a hydroxy metabolite.
d) 56 / 59 % inhibition of metabolism of unchanged drug and inhibition of formation of a hydroxy metabolite, respectively.
e) Tested in cytosol fractions, not in microsomes.
f) As determined by disappearance from the unchanged abacavir, as well as the formation of its carboxylic acid metabolite.
g) As determined by disappearance from the unchanged chlorzoxazone, as well as the formation of its 6-hydroxy metabolite.
h) No inhibition was observed.

2.6.5.14. Pharmacokinetics: Drug Drug Interactions

Test Article: Tenofovir or tenofovir disoproxil fumarate or tenofovir amidate

2.6.5.14.B. R2000096 substudy R2001008: Biodistribution Study to Determine if High Doses of Tenofovir DF (300 mg/kg) Altered the Plasma and/or Tissue Concentrations of Adefovir Dipivoxil (ADV)

Study Number: R2000096 substudy R2001008

Type of Study: Biodistribution study to determine if high doses of TDF (300 mg/kg) altered the plasma and/or tissue concentrations of adefovir dipivoxil (ADV).

Method: Male, Sprague-Dawley rats were gavaged with either 40 mg/kg/day ADV only or 40 mg/kg/day ADV coadministered with 300 mg/kg/day TDF. Animals were given either a single dose (group 0 ADV only, group 1 ADV + TDF) or 6 consecutive daily doses (group 3 ADV only, group 4 ADV + TDF). On the final dose, animals were dosed with 0.1 μCi/g body weight of 3H-ADV and plasma was harvested at 0.15, 0.5, 1, 2, 4, 8, and 24 h post-administration of ADV. The animals were euthanized after collection of the last plasma sample and sections of tissues were obtained. Liquid scintillation was used to determine radioactivity.

Tabulated Results:

Summary of Mean Concentrations (CPM/g tissue) of Radioactivity from Tissues of either ADV- or ADV and TDF-treated Rats

	Group 0		Group 1		Group 2		Group 3	
Tissue	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Liver	8583	2312	12495	1846	25570	19442	8118	2047
Kidney	32930	4348	36783	4122	34713	13032	24809	3570
Duodenum	73698	30879	93422	23258	67299	42609	5587*	2694
Spleen	2835	569	2845	219	3500	223	1723*	489
Thymus	2653	304	1882	152	2963	337	1820	631
Brain	1442	515	1364	333	2372	194	1783	514
Bone Marrow	307	373	82	164	199	73	87	89

* $p<0.05$ compared to corresponding values in Group 2

2.6.5.14. Pharmacokinetics: Drug Drug Interactions

Test Article: Tenofovir or tenofovir disoproxil fumarate or tenofovir amidate

Area-under-the-Curves (AUC) Values for Plasma Concentrations of Radioactive Material

Group	Plasma AUC[1]
0	61220
1	36770
2	55610
3	28190

1 Plasma AUC was calculated from 0.15 h to 24 h.

Conclusion: These data demonstrate that TDF reduced the plasma concentrations of ADV or adefovir after a single or six consecutive daily administrations. The tissue concentrations were not altered after a single co-administration of TDF with ADV. After six doses of TDF, the duodenum and spleen both had reduced concentrations of ADV. The results of this study suggested that TDF may reduce the toxicity of ADV by reducing it's intestinal uptake.

2.6.5.14. Pharmacokinetics: Drug Drug Interactions

Test Article: Tenofovir or tenofovir disoproxil fumarate or tenofovir amidate

2.6.5.14.C. R2000096, substudy R2001032: Biodistribution Study to Determine if Tenofovir DF or Adefovir Dipivoxil Altered the Plasma and/ or Tissue Concentrations of the Other Drug When Administered at Clinically Relevant Doses

Study Number: R2000096, substudy R2001032

Type of Study: Biodistribution study to determine if TDF or ADV altered the plasma and/ or tissue concentrations of the other drug when administered at clinically relevant doses.

Method: Male, Sprague-Dawley rats (n=4 per group) were gavaged with either 0.2 or 5 mg/kg ADV and/or 5 mg/kg TDF. Animals were dosed for six consecutive days as shown below

Treatment Groups

Group #	Test Articles	Dose (mg/kg)
0	ADV/TDF	0.2*/5
1	ADV	0.2*
2	ADV/TDF	5*/5
3	ADV	5*
4	ADV/TDF	5/5*
5	TDF	5*

* indicates calculated compound on final dose.

Animals were dosed with 0.08 μCi/g body weight. Plasma was harvested at 0.5, 1, 4, 8, and 24 h post-administration of calculated compound. The animals were euthanized after collection of the last plasma sample and sections of tissues obtained. Tissue sample weights were determined at time of collection. Liquid scintillation was used to determine radioactivity. Results were recorded as mean CPM/g tissue or mean CPM/mL plasma ± SD.

Tabulated Results:

Concentrations of Radioactive Material in Tissues

Group	Dose of ADV/TDF[1]	Liver[2]	Duodenum[2]	Kidney[2]
0	0.2*/5	12009 ± 1706	17802 ± 5547	32294 ± 5203
1	0.2*/0	9070 ± 2104	15092 ± 6597	24123 ± 2960
2	5*/5	11562 ± 5487	17174 ± 3025	21899 ± 3134
3	5*/0	7843 ± 1477	14612 ± 8337	27652 ± 6638
4	5/5*	31003 ± 15911	462954 ± 154686	116540 ± 16406
5	0/5*	39507 ± 17621	387139 ± 88954	134543 ± 40951

1 Dose is in mg/kg. 2 Data is mean CPM ± SD/g tissue * - indicates which compound was calculated on final dose

Study Number: R2000096, substudy R2001032

Tabulated Results (cont):

Area-under-the-Curves (AUC) Values for Plasma Concentrations of Radioactive Material

Group	Plasma AUC[1]
0	53540
1	58560
2	52990
3	55930
4	29190
5	37260

1 Plasma AUC was calculated from 0.5 h to 24 h.

Conclusion: The results of this study demonstrate that, at clinically relevant doses, the biodistribution of ADV was not altered by the co-administration of TDF, nor was the biodistribution of TDF altered by the coadministration of ADV.

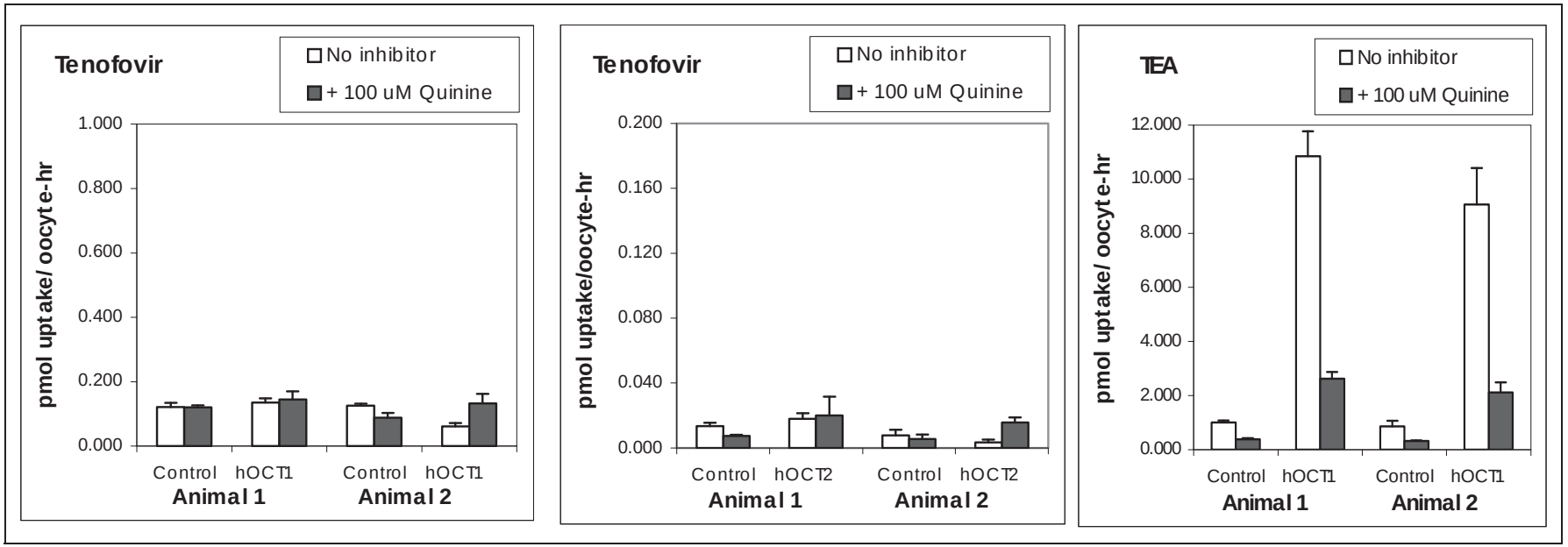
2.6.5.14.D. PC-103-2001: In Vitro Study to Evaluate the Interactions of Tenofovir (TVF) with Human Organic Anion Transporter hOAT3 and Human Cation Transporters hOCT1 and hOCT2

Study Number: PC-103-2001

Type of Study: In vitro study to evaluate the interactions of tenofovir (TVF) with human organic anion transporter hOAT3 and human cation transporters hOCT1 and hOCT2.

Method: The interaction of TFV with hOCT1, hOCT2 and hOAT3 were studied in a Xenopus oocyte expression system via radiolabeled uptake transport with [^{3}H]tenofovir (10 μM) and appropriate control substrates , [^{14}C]TEA (triethylamine, 100 μM) for hOCT1 and hOCT3 and [^{3}H]estrone sulphate (100 nM). Inhibition studies were also conducted for hOAT3 by measuring the uptake of [^{3}H]estrone sulphate (100 μM) in the presence TVF and the positive control inhibitor, probenicid, at concentrations from 50 – 1000 μM.

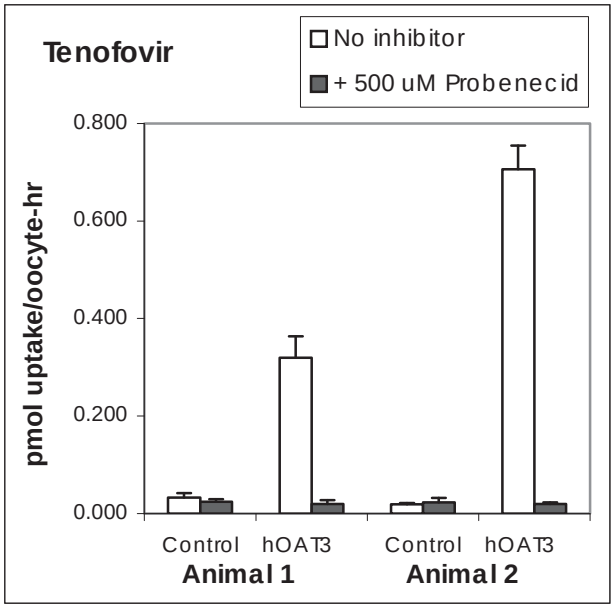
Results: The figures below shows the results of radiolabeled uptake studies of [^{3}H]tenofovir (10 μM) for hOCT1 and hOCT2 in comparison to a water control incubation in the presence and absence of the inhibitor quinine. The positive control substrate TEAshowed the expected high level of transport. Tenofovir was shown not to be a substrate for hOCT1 and hOCT2.

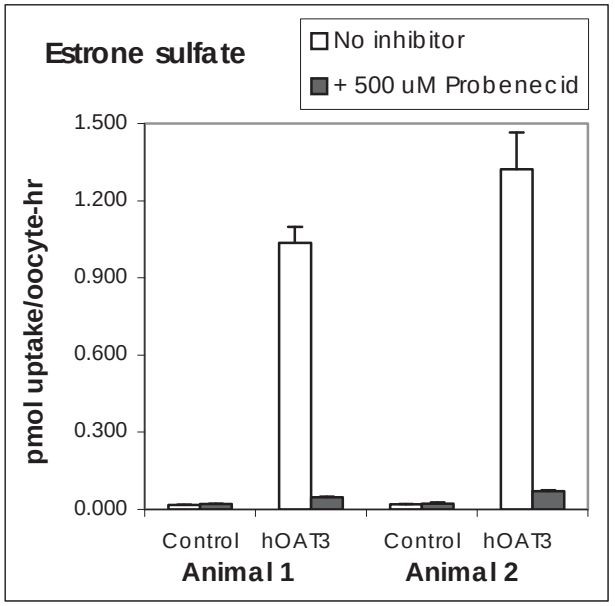


2.6.5.14. Pharmacokinetics: Drug Drug Interactions

Test Article: Tenofovir or tenofovir disoproxil fumarate or tenofovir amidate

The figures below displays the results of radiolabeled uptake studies of [^{3}H]tenofovir (10 μM) for hOAT3 in comparison to a water control incubation in the presence and absence of the control inhibitor quinine. The positive control substrate [^{3}H]estrone sulphate showed the expected high level of transport. Tenofovir was shown to be a substrate for hOAT3 transport.

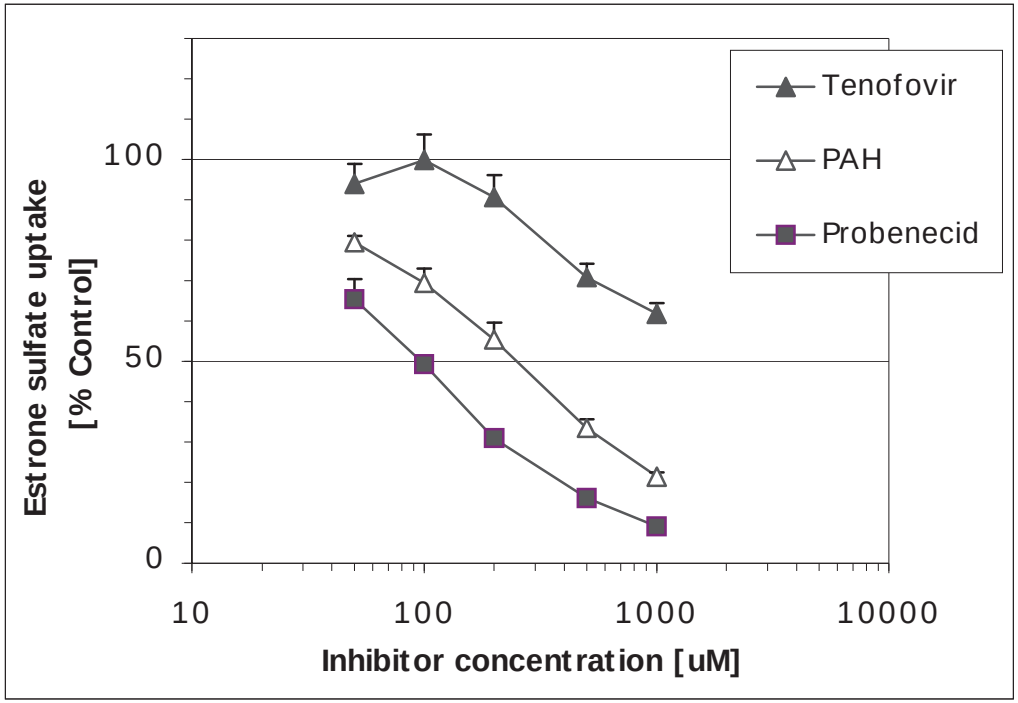




2.6.5.14. Pharmacokinetics: Drug Drug Interactions

Test Article: Tenofovir or tenofovir disoproxil fumarate or tenofovir amidate

The affinity of tenofovir for hOAT3 was assessed indirectly via the inhibition of transport of [^{3}H]estrone sulphate in comparison to that of probenicid and PAH. The results are shown in the figure below. No inhibition of hOAT3 was observed in the presence of up to 100 μM tenofovir indicating a low affinity interaction between tenofovir and hOAT3.



Conclusion: This study demonstrated that tenofovir (TVF) was not a substrate for human cation transporters hOCT1 and hOCT2. TVF was shown to be a low affinity substrate for hOAT3.

2.6.5.14. Pharmacokinetics: Drug Drug Interactions

Test Article: Tenofovir or tenofovir disoproxil fumarate or tenofovir amidate

2.6.5.14.E. AD-104-2001: In Vitro Study in Appropriate Cell Lines to Assess Whether Tenofovir (TVF) is a Substrate for Efflux Transport by Multidrug Resistance Related Proteins 2 and 4 (MRP2 and MRP4) and to Examine the Inhibition of Transport by the HIV Protease Inhibitors Atazanavir and Ritonavir

Study Number: AD-104-2001

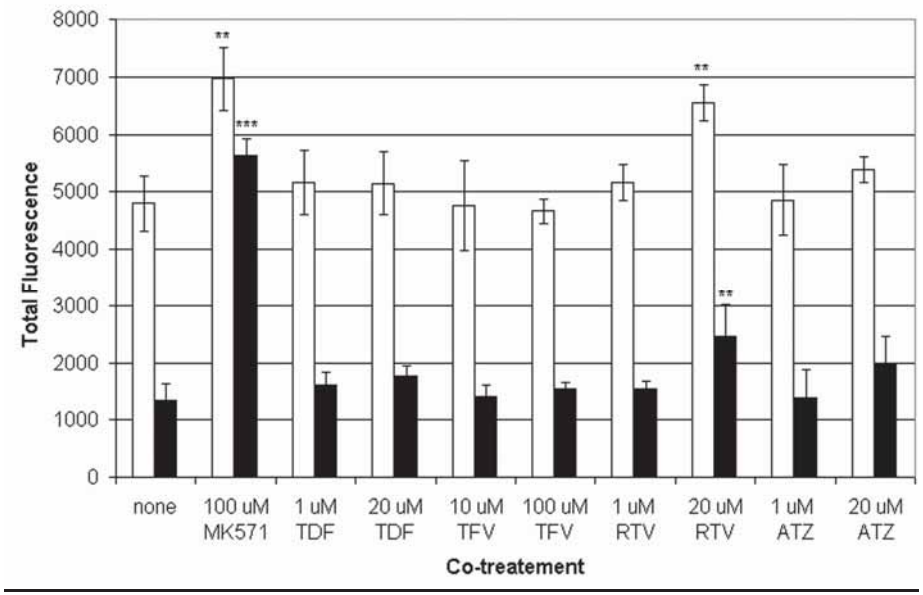
Type of Study: In vitro study in appropriate cell lines to assess whether tenofovir (TVF) is a substrate for efflux transport by multidrug resistance related proteins 2 and 4 (MRP2 and MRP4) and to examine the inhibition of transport by the HIV PIs atazanavir and ritonavir.

Method: In vitro inhibition studies of the accumulation of a model MRP2 and MRP1 substrate (calcein) by TFV were conducted following incubation of calcein AM in appropriate cell lines expressing MRP2 and MRP4. MRP2 interactions were studied in human ovarian carcinoma cell line 2008, while MRP4 interactions were studied in human T-leukemic CEM-R1 lymphoblast cell line. The effect of HIV protease inhibitors (PIs) on TFV transport was studied at 1 and 20 µM.

Results: The accumulation of calcein after incubation with calcein AM in MRP-2 over-expressing cells should increase in the presence of an MRP-2 substrate or inhibitor. The MRP inhibitor MK571 was capable of increasing the intracellular fluorescence from calcein in both parental and MRP-2 transfected cells (see figure below). An increase in fluorescence from calcein was noted in MRP-2 transfected cells (4.2-fold) relative to parental cells (1.45) for MK571. Neither TFV nor TDF were able to increase intracellular calcein fluorescence at any of the concentrations tested. Of the clinically relevant compounds for HIV therapy only 20 µM RTV was capable of significantly increasing the levels of calcein fluorescence in both parental and MRP-2 transfected cells. Unlike MK-571, the fold increase in both parental and MRP-2 transfected cells was similar (approximately 1.5-fold).

2.6.5.14. Pharmacokinetics: Drug Drug Interactions

Test Article: Tenofovir or tenofovir disoproxil fumarate or tenofovir amidate

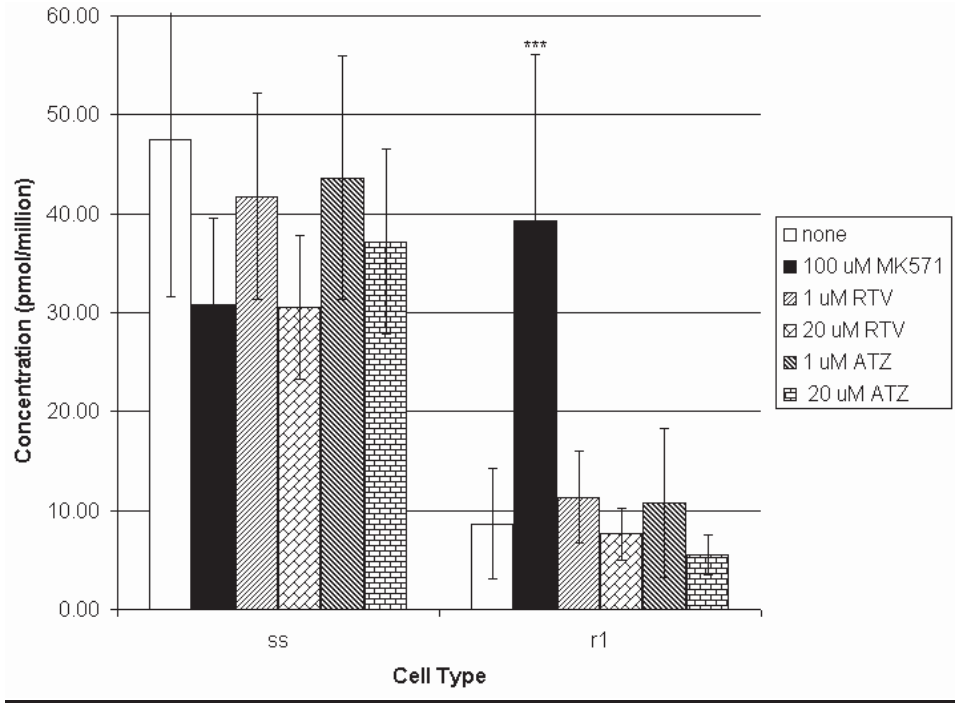


Statistical significance were assessed relative to no treatment in parental and MRP2 over-expressing cells by unpaired-two tailed student's t-test assuming equal variance (**P<0.01, ***P < 0.001).

The effects of MRP-4 over-expression and the HIV protease inhibitors RTV and ATZ on the accumulation of TFV following 1 hr incubation with 1 µM TDF in CEM-SS or –R1 cells is displayed in the figure below. It was found that >5-fold less TFV accumulated in CEM-R1cells (MRP4 over-expressing) relative to CEM-SS cells following TDF incubation. MK571 (100 µM, a potent inhibitor of MRPs) was able to increase TFV levels in CEM-R1 cells to concentrations similar to those in CEM-SS cells, but had no significant affect on TFV concentrations in CEM-SS cells. RTV and ATZ (1 and 20 µM) had no significant effect on TFV levels in CEM-SS or –R1 cells.

2.6.5.14. Pharmacokinetics: Drug Drug Interactions

Test Article: Tenofovir or tenofovir disoproxil fumarate or tenofovir amidate



Statistically significant change in intracellular TFV relative to no co-treatment in CEM-R1 cells (MRP4 over-expressing). Unpaired-two tailed student's t-test assuming equal variance (*P < 0.001).**

Conclusion: These findings indicate that MRP-4, but not MRP-2, as a transporter contributing to the active tubular secretion of TFV. The results of these studies also suggest that the HIV PIs ATZ and RTV, even when tested at supra-pharmacological concentrations, are either weak or not inhibitors of transport mediated by MRP-2 and -4. The finding reported here indicate that protease inhibitors will not decrease tenofovir transport at the apical side of the renal proximal tubule.

2.6.5.14. Pharmacokinetics: Drug Drug Interactions **Test Article:** Tenofovir or tenofovir disoproxil fumarate or tenofovir amidate

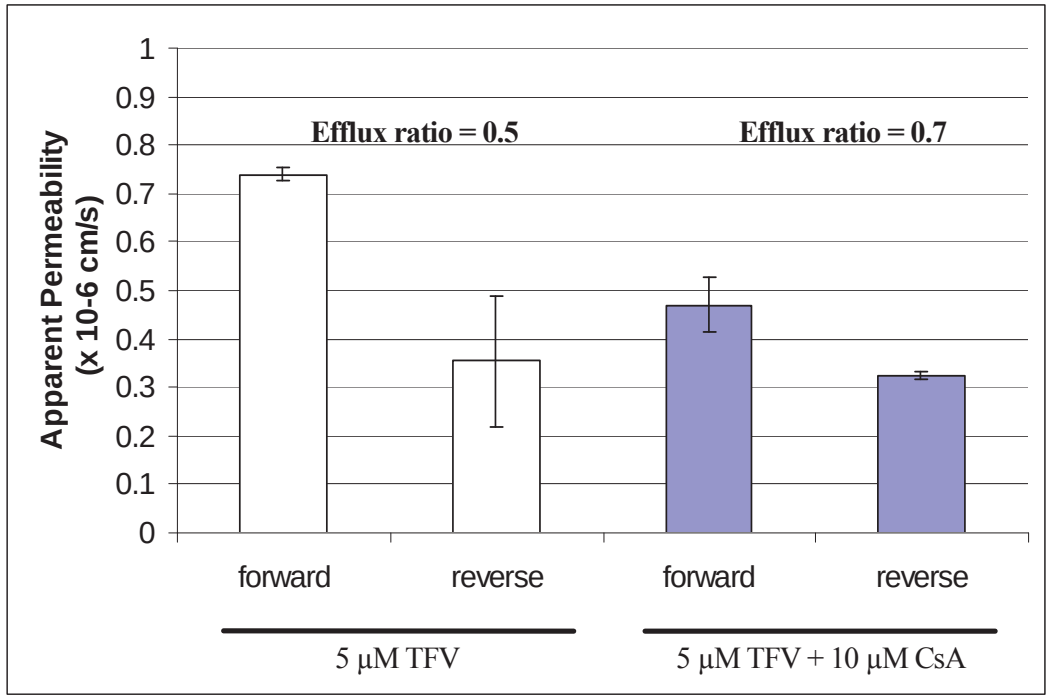
2.6.5.14.F. AD-104-2002: In Vitro Study in Appropriate Cell Lines to Assess Whether Tenofovir is a Substrate for Efflux by Pgp and to Elucidate the Potential of Pgp to Mediate Drug-Drug Interactions Between Tenofovir and Other Agents

Study Number: AD-104-2002

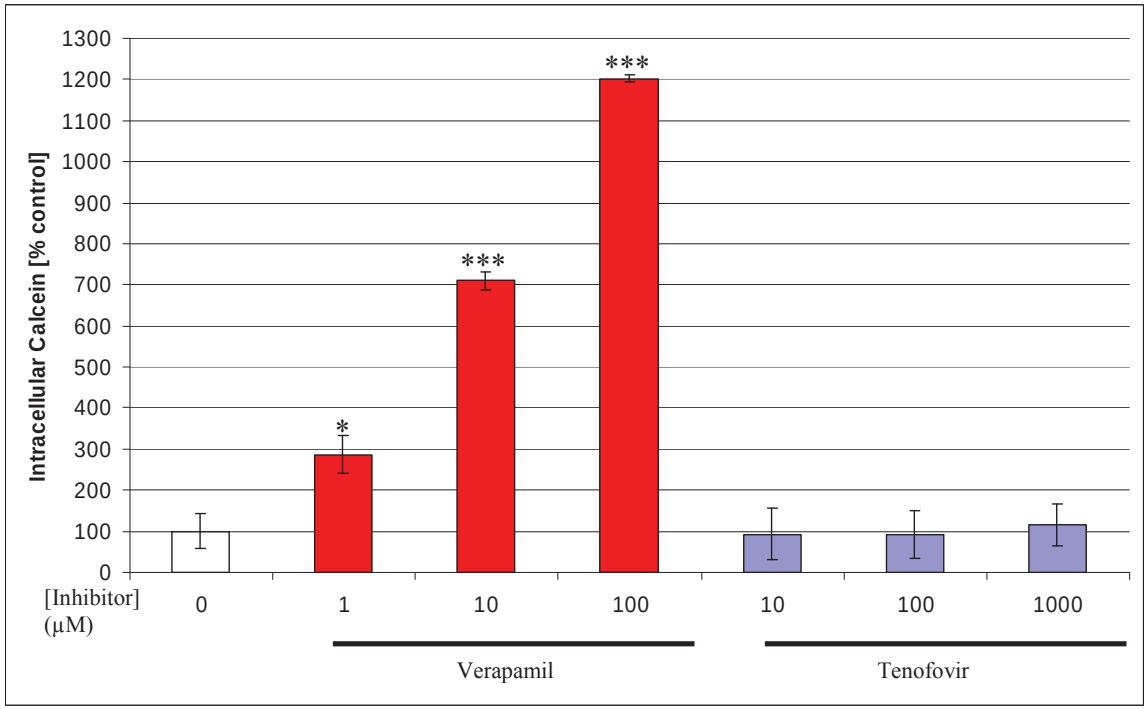
Type of Study: In vitro study in appropriate cell lines to assess whether tenofovir (TVF) is a substrate for efflux by Pgp and to elucidate the potential of Pgp to mediate drug-drug interactions between TVF and other agents.

Method: In vitro bidirectional permeability experiments with TFV (5 and 50 uM) in Caco-2 cell monolayers. In vitro inhibition studies of the accumulation of a model Pgp substrate (calcein) by TFV (up to 1000 µM) were conducted following incubation of a Madin-Darby canine kidney cell line (MDCKII), both parental and stably transfected with human Pgp, with calceinAM.

Results: The figure below shows the forward and reverse permeability of TVF through Caco-2 cell monolayers, and the calculated efflux ratio in the presence or absence of CsA. TFV had similar forward and reverse permeability that was unaffected by incubation with the Pgp inhibitor CsA (cyclosporine).



The figure below displays the effect of tenofovir and the control Pgp inhibitor verapamil on the accumulation of the fluorescent Pgp substrate calcein in MDCK cells transfected with human Pgp. Verapamil significantly inhibited Pgp and resulted in higher intracellular calcein levels with increasing concentration. Tenofovir at concentrations up to 1000 µM did not inhibit the Pgp efflux of calcein.



Conclusion: The observation that TFV has an efflux ratio close to 1, which is not affected by a Pgp inhibitor, in Caco-2 cell monolayers suggests that it is not a Pgp substrate. TFV did not inhibit the transport of a Pgp substrate when tested at suprapharmacological concentrations in MDCKII cells stably transfected with Pgp indicating that it is not a substrate or inhibitor of Pgp.

2.6.5.14. Pharmacokinetics: Drug Drug Interactions

Test Article: Tenofovir or tenofovir disoproxil fumarate or tenofovir amidate

2.6.5.14.G. PC-104-2010: In Vitro Study Conducted in Cell Lines Expressing Human Renal Organic Anion Transporter Type 1(hAOT1) to Examine the Effect of HIV Protease Inhibitors, and Other Therapeutics Frequently Administered with Tenofovir DF, on the Transport of Tenofovir by hOAT1

Study Number: PC-104-2010

Type of Study: In vitro study was conducted in appropriate cell lines expressing human renal organic anion transporter type 1(hAOT1) to examine the effect of HIV protease inhibitors, and other therapeutics frequently administered with TDF, on the transport of tenofovir (TVF) by hOAT1.

Method: The effect of protease inhibitors and other therapeutics on the hOAT1 mediated transport of TFV was studied in a stable cellular expression system (CHO-hOAT1 cells) via radiolabeled uptake transport with [^{3}H]tenofovir (1.2 μM). The test drugs were examined for their effect on TVF transport by hOAT1 at concentrations corresponding to their 3X, 1X and 0.33X their clinical Cmax values.

Results: A total of six individual approved HIV protease inhibitors and one frequently used combination were evaluated for their effect on hOAT1-mediated transport of TFV. At concentrations corresponding to 3x C_{max}, lopinavir and nelfinavir both reduced the transport of TFV by > 30%, whereas amprenavir and ritonavir showed less pronounced effect with 23 and 28% inhibition of TFV transport, respectively. At 3x C_{max}, saquinavir inhibited TFV transport by 12%, whereas atazanavir was devoid of any inhibitory effect. Combination of 3x lopinavir and low-dose ritonavir (co-formulated as Kaletra) was most potent among all tested PIs with a 45% inhibition of hOAT1-mediated transport of TFV. In order to directly test the effect of protein binding, the inhibition of hOAT1 by protease inhibitors was also evaluated in the presence of 50% human serum. Under these conditions, only nelfinavir at 3x C_{max} concentration exhibited minor (< 10%) inhibition of TFV transport via hOAT1. All the other protease inhibitors, including the combination of lopinavir/ritonavir, had no effect on TFV transport.

Tested drug	Concentration (fold C_{max})	Transport of TFV by hOAT1 [% control]	
		0% Human Serum	50% Human Serum[a]
None	-	100	100
Lopinavir	3x	62.5 ± 0.7	113.0 ± 19.8
	1x	87.5 ± 0.7	94.8 ± 1.8
	0.33x	96.0 ± 4.2	109.0 ± 7.1
Lopinavir/ritonavir	3x	55.0 ± 2.8	95.0 ± 5.7
	1x	86.5 ± 0.7	97.0 ± 5.7
	0.33x	109.5 ± 3.5	103.0 ± 0.0
Ritonavir	3x	73.0 ± 7.1	98.5 ± 6.4
	1x	89.5 ± 4.9	97.5 ± 7.8
	0.33x	95.5 ± 4.9	102.5 ± 13.4

Atazanavir	3x	104.0 ± 0.0	nd[b]
	1x	102.0 ± 2.8	nd
	0.33x	103.5 ± 3.5	nd
Saquinavir	3x	88.5 ± 9.2	102.5 ± 10.6
	1x	90.0 ± 8.5	96.0 ± 7.1
	0.33x	97.5 ± 2.1	98.0 ± 9.9
Nelfinavir	3x	63.5 ± 13.4	90.5 ± 6.4
	1x	78.5 ± 10.6	94.0 ± 1.4
	0.33x	95.0 ± 12.7	107.5 ± 0.7
Amprenavir	3x	78.5 ± 7.8	96.5 ± 12.0
	1x	85.0 ± 8.5	104 ± 9.9
	0.33x	98.0 ± 7.1	109 ± 8.5

The tested anti-infectives included antiviral drugs as well as antibiotics. Among these, only sulfamethoxazole at 3x C_{max} concentration reduced TFV transport by approximately 20%. None of the other tested anti-infectives had any substantial modulatory effects on the hOAT1-mediated transport of TFV. Furthermore, the inhibition by sulfamethoxazole was reduced to < 10% in the presence of human serum. The anti-inflammatory agents ibuprofen and acetaminophen were also evaluated for their potential to reduce the transport of TFV by hOAT1. At concentrations corresponding to 3x, 1x, and 0.33x C_{max}, ibuprofen inhibited the uptake of TFV by 93, 81, and 68%, respectively. By comparison, acetaminophen was a substantially less effective inhibitor of hOAT1. At 3x C_{max} concentration, acetaminophen reduced the transport of TFV by 20%. However, consistently with its high serum protein binding, the inhibitory effect of ibuprofen was completely eliminated in the presence of 50% human serum. Although serum protein binding of acetaminophen at its supertherapeutic levels is substantially less then that of ibuprofen (< 30%), the inhibition of TFV transport by therapeutic concentrations of acetaminophen was reduced to < 5% in the presence of 50% serum.

Tested Drug	**Concentration (fold C_{max})**	**Transport of TFV by hOAT1 [% control]**	
		0% Human Serum	**50% Human Serum[a]**
None	-	100	100
Acyclovir	3x	91 ± 2.8	nd [b]
	1x	94 ± 2.8	nd
	0.33x	101 ± 4.2	nd

Ganciclovir	3x	101 ± 0.0	nd
	1x	92.5 ± 7.8	nd
	0.33x	102.5 ± 0.7	nd
Oseltamivir carboxylate	3x	101 ± 2.8	nd
	1x	102 ± 1.4	nd
	0.33x	118 ± 2.8	nd
Trimethoprim	3x	103.5 ± 6.4	nd
	1x	98.5 ± 4.9	nd
	0.33x	101.5 ± 0.7	nd
Sulfamethoxazole	3x	79.5 ± 6.4	91.5 ± 0.7
	1x	93.5 ± 0.7	98.5 ± 0.7
	0.33x	102 ± 1.4	112 ± 4.2
Amoxicillin	3x	91 ± 5.7	nd
	1x	91.5 ± 3.5	nd
	0.33x	94 ± 8.5	nd
Ibuprofen	3x	7[c]	99.5 ± 2.1
	1x	19[c]	102.5 ± 0.7
	0.33x	32[c]	114.5 ±4.9
Acetaminophen	3x	78.5 ± 3.5	94.5 ± 3.5
	1x	88 ± 1.4	94.5 ± 9.2
	0.33x	97 ± 7.1	106.5 ± 3.5

Conclusion: When adjusted for plasma protein binding and tested at their clinical Cmax concentration, protease inhibitors and other therapeutic agents had no significant effect on TFV transport by hOAT1. Consequently, clinical drug interactions due to the inhibition of hOAT1 mediated transport of tenofovir by the coadmnistered drugs tested here are unlikely.

2.6.5.14. Pharmacokinetics: Drug Drug Interactions

Test Article: Tenofovir or tenofovir disoproxil fumarate or tenofovir amidate

2.6.5.14.H. PC-104-2011: In Vitro Study Conducted in Cell Lines Expressing hAOT3 to Define the Role of hOAT3 in Tenofovir Transport and to Assess the Effect of HIV Protease Inhibitors on the hOAT3 Transport of Tenofovir

Study Number: PC-104-2011

Type of Study: In vitro study was conducted in appropriate cell lines expressing hAOT3 to define the role of hOAT3 in tenofovir (TVF) transport and to assess the effect of HIV protease inhibitors on the hOAT3 transport of tenofovir.

Method: The effect of protease inhibitors on the hOAT3 mediated transport of TFV was studied in a stable cellular expression system (BHK-hOAT3 cells) via radiolabeled uptake transport with [^{3}H]tenofovir (1.2 µM). Protease inhibitors were tested for their effect on TVF transport by hOAT3 at concentrations corresponding to their 2X, 1X and 0.5X their clinical Cmax values. The kinetics of [^{3}H]tenofovir and [^{3}H]estrone sulphate transport was determined in BHK-hOAT3 cells.

Results: The transport kinetics diplayed in the Table below indicated that, in contrast to hOAT1, tenofovir was found to be a low-affinity substrate for hOAT3 with K_m value of 767 µM. Estrone sulfate displayed >250-fold higher affinity for hOAT3 than tenofovir and was transported with >20-fold higher efficiency.

Substrate	K_m [µM]	V_{max} [pmol/10^6 cells/min]	Transport efficiency (V_{max}/K_m)
Tenofovir (n=3)	767 ± 145	31.6 ± 8.0	0.043 ± 0.016
Estrone sulfate (n=4)	3.0 ± 1.2	2.9 ± 1.2	0.97 ± 0.15

A total of five individual HIV protease inhibitors and one frequently used combination were evaluated for their effect on hOAT3-mediated transport of tenofovir. The effect of HIV protease inhibitors on the transport of tenofovir by hOAT3, in the presence or absence of human serum protein, is shown in the table below. At concentrations corresponding to 2x Cmax, ritonavir, nelfinavir and the combination of lopinavir with low-dose ritonavir (co-formulated as Kaletra) reduced the transport of tenofovir by > 50%, whereas 2x amprenavir and atazanavir showed less pronounced effect with 21 and < 10% inhibition of tenofovir transport, respectively. At a concentration corresponding to 2x Cmax, saquinavir was devoid of any inhibitory effect. In order to directly test the effect of protein binding, the inhibition of hOAT3 by protease inhibitors was also evaluated in the presence of 40% human serum. Under these conditions, the effect of PIs on hOAT3 activity was markedly reduced. Atazanavir, nelfinavir, and saquinavir displayed no inhibition of tenofovir transport by hOAT3 at all concentrations tested. At 2x Cmax concentration, ritonavir, lopinavir + low-dose ritonavir, and amprenavir reduced the transport of tenofovir by 40, 22, and 14%, respectively. However, at 1x Cmax, only ritonavir showed > 10% inhibition of hOAT3 in the presence of 40% human serum. Since hOAT3 mediates the uptake of small organic anions from the peritubular capillary into proximal tubule cells, the presence of plasma proteins is likely to reduce the concentration of free drugs available for interacting with the transporter. Consequently, the inhibition assay adjusted for protein binding is the more relevant data for assessing the potential clinical interaction of TFV and protease inhibitors with respect to hOAT3 transport.

2.6.5.14. Pharmacokinetics: Drug Drug Interactions

Test Article: Tenofovir or tenofovir disoproxil fumarate or tenofovir amidate

Tested PI	Concentration [fold C_{max}]	Transport of TFV by hOAT3 [% control]	
		0% Human serum	40% Human serum[a]
No inhibitor	-	100	100
Ritonavir	0.5x	57.7 ± 5.1	87.1 ± 6.7
	1x	38.4 ± 5.7	65.9 ± 8.8
	2x	21.1 ± 3.2	59.4 ± 7.0
Lopinavir/ritonavir	0.5x	78.9 ± 2.6	93.3 ± 12.2
	1x	63.5 ± 8.7	92.1 ± 6.8
	2x	46.1 ± 5.1	78.5 ± 9.1
Atazanavir	0.5x	102.3 ± 1.6	103.8 ± 14.9
	1x	94.7 ± 7.8	98.5 ± 4.4
	2x	90.8 ± 2.4	97.1 ± 7.7
Nelfinavir	0.5x	84.6 ± 2.3	102.0 ± 10.2
	1x	73.1 ± 3.2	101.6 ± 11.2
	2x	47.7 ± 2.3	99.4 ± 9.8
Saquinavir	0.5x	98.3 ± 0.1	99.2 ± 8.7
	1x	95.1 ± 3.6	98.3 ± 3.7
	2x	98.8 ± 3.7	101.1 ± 13.2
Amprenavir	0.5x	87.8 ± 5.6	97.0 ± 2.7
	1x	81.4 ± 3.1	90.3 ± 5.2
	2x	78.7 ± 2.4	86.0 ± 1.5

Conclusion: TFVwas found to be a low affinity substrate for hAOT3 (Km of 767 uM). When adjusted for plasma protein binding and tested at their clinical Cmax concentration, protease inhibitors had no significant effect on TFV transport by hOAT3. Consequently, clinical drug interactions due to the inhibition of hOAT3 mediated transport of tenofovir by HIV protease inhibitors are unlikely.

2.6.5.14. Pharmacokinetics: Drug Drug Interactions

Test Article: Tenofovir or tenofovir disoproxil fumarate or tenofovir amidate

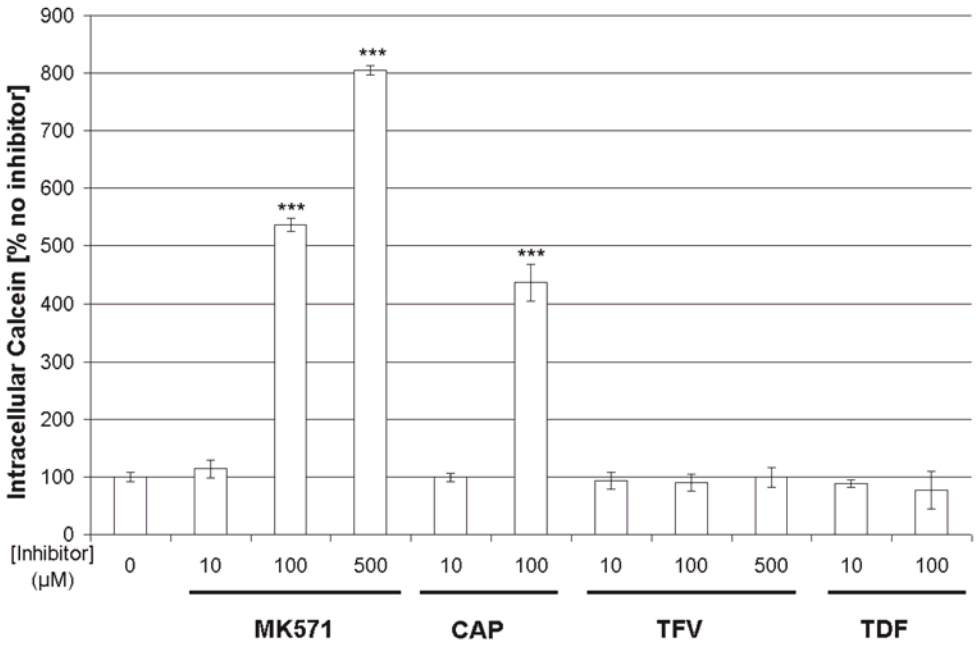
2.6.5.14.I. PC-104-2014: In Vitro Inhibition Studies of the Accumulation of a Model MRP1 Substrate (Calcein) by Tenofovir (up to 500 uM) Following Incubation of a Madin-Darby Canine Kidney Cell Line (MDCKII), Both Parental and Stably Transfected with Human MRP1, with CalceinAM

Study Number: PC-104-2014

Type of Study: In vitro cellular study to assess whether tenofovir (TVF) is a substrate for efflux by MRP1.

Method: In vitro inhibition studies of the accumulation of a model MRP1 substrate (calcein) by TFV (up to 500 uM) were conducted following incubation of a Madin-Darby canine kidney cell line (MDCKII), both parental and stably transfected with human MRP1, with calceinAM.

Results: The figure below displays the effect of tenofovir and the control inhibitors MK571 and CAP (caffeic acid phenethyl ester), on the accumulation of the fluorescent MRP2 substrate calcein in MDCK cells transfected with human MRP1.



Conclusion: TFV did not inhibit the transport of an MRP1 substrate at suprapharmacological concentrations in MDCKII cells stably transfected with MRP1.

2.6.5.14. Pharmacokinetics: Drug Drug Interactions **Test Article:** Tenofovir or tenofovir disoproxil fumarate or tenofovir amidate

2.6.5.14.J. AD-104-2010: In Vitro Study in Caco-2 Cell Monolayers, and Madin-Darby Canine Kidney (MDCKII) Cells Expressing Pgp, to Examine the Concentration Dependence of, and the Effect of Protease Inhibitors on the Forward Permeability of Tenofovir DF

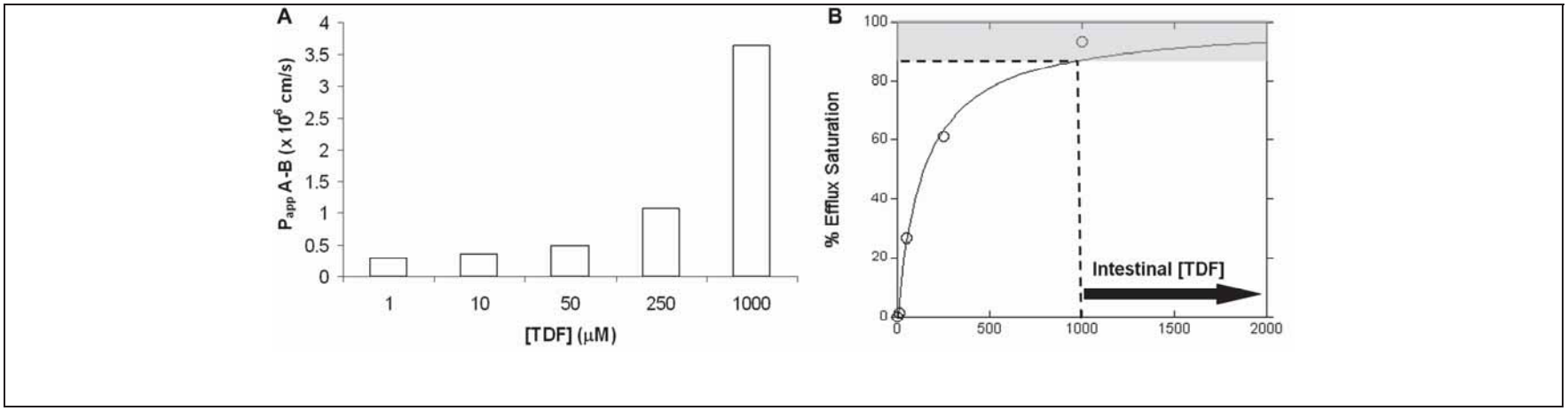
Study Number: AD-104-2010

Type of Study: In vitro study in Caco-2 cell monolayers, and Madin-Darby canine kidney (MDCKII) cells expressing Pgp, to examine the concentration dependence of, and the effect of protease inhibitors (PIs) on, the forward permeability of TDF. The inhibition of intestinal esterases and of Pgp by PIs was also determined in vitro.

Method: Bidirectional permeability experiments with TDF (1 - 1000 μM) were conducted in Caco-2 cell monolayers. The bidirectional permeability of TDF (10 μM) was determined following a 30 minute preincubation of MDCKII cells, both parental and stably transfected with human Pgp, with protease inhibitors (20μM).

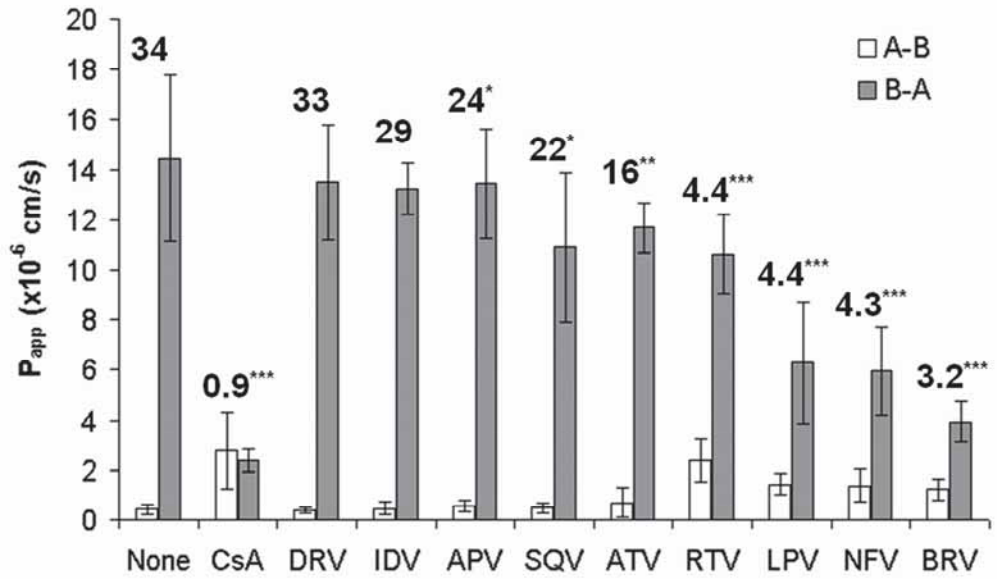
The inhibition of intestinal esterase-dependent degradation of TDF by PIs was also determined in vitro in intestinal S9 fractions and the inhibition Pgp transport by PIs was determined in vitro the measuring the accumulation of a model Pgp substrate (calcein) following incubation of MDCKII cells, both parental and stably transfected with human Pgp, with calceinAM.

Results: The efflux of TDF can be saturated and the forward permeability of TFV significantly increased in Caco-2 cell monolayers at concentrations of TFV that can be achieved in the intestinal lumen at a clinical dose (see figure below).



The effect of PIs on lower than pharmacologically relevant concentrations of TDF (10 μM) in the intestinal tract were used in MDCKII cell based permeability studies in order to increase efflux, facilitating the accurate assessment of the relative effects of PIs on TDF permeability. PIs and CsA decreased the Pgp-mediated efflux ratio (ER) of TDF. Results for PIs are organized left to right in order of increasing Pgp inhibition and the ER for each treatment condition is indicated above the respective bars. Values represent the mean ± standard deviation of at least 3 independent experiments done in duplicate.

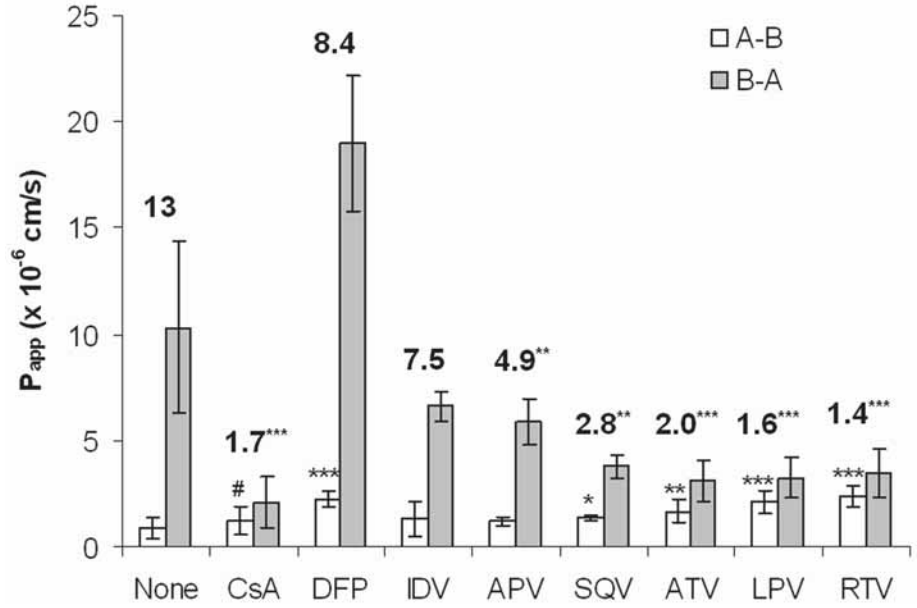
The PIs decreased the efflux of TDF in Pgp over-expressing cells (see figure below). The PIs BRV, NFV, LPV and RTV were the most potent inhibitors of TDF efflux while DRV, IDV and APV showed smaller effects.



The statistical significance of changes in the ER of TDF compared to the no coincubation control was assessed using Student's unpaired two-tailed t-tests assuming equal variance (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$)

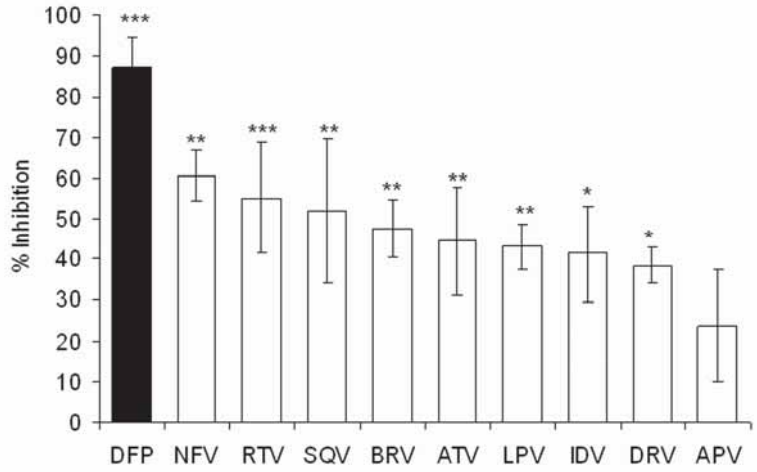
In order to study the effects of select PIs on the permeability of TDF in a more physiologically relevant model and concentration (50 μM) for gastrointestinal absorption, bi-directional permeability studies were done in Caco-2 cells. The effects of LPV, ATV, RTV, SQV, IDV and APV on the permeability of TDF were compared to that of the potent efflux inhibitor CsA and esterase inhibitor di-isopropyl fluorophosphates (DFP) (see figure below). PIs are organized left to right in order of increasing Pgp inhibition and the efflux ratio (ER) is indicated above the respective bars. Values represent the mean ± standard deviation of at least 3

independent experiments in duplicate. CsA and DFP increased the forward permeability of TDF by 1.4- and 2.5-fold, respectively. RTV, LPV, ATV and SQV caused greater increases in TDF P_{app} A-to-B than CsA. Comparison of permeability in both the A-to-B and B-to-A directions showed that CsA caused a near complete blockage of TDF efflux transport, while di-isopropyl fluorophosphate similarly increased the Papp of TDF in the A-to-B and B-to-A directions. Consistent with results from MDCKII-MDR1 cells, APV and IDV were among the weakest and LPV and RTV among the strongest inhibitors of TDF efflux in Caco-2 cells.



The statistical significance of changes in the ER of TDF compared to the no coincubation control were assessed using Student's unpaired two-tailed t-tests assuming equal variance (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$)

The potential for inhibition of TDF hydrolysis by PIs was assessed in a human intestinal subcellular fraction (S9). DFP and PIs were found to inhibit TDF degradation (see figure below). PIs were tested at concentrations near their solubility limits to model pharmacologically relevant concentrations potentially formed following dosing in the gastrointestinal tract (50 μM). While most PIs showed similar inhibition of TDF degradation, IDV and DRV showed less marked inhibition (≤ 40% inhibition). Results for APV did not reach statistical significance.



The statistical significance of changes in the rate of TDF degradation in the presence of inhibitors relative to no inhibitor were assessed by Student's unpaired two-tailed t-tests assuming equal variance (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).

The relative inhibition of Pgp by PIs was studied by monitoring the effects of co-incubation on the Pgp-dependent accumulation of the fluorescent compound calcein (metabolite of the Pgp substrate calcein-AM) in MDCKII-MDR1 cells. All PIs showed dose dependent inhibition of Pgp. The effects of PIs ranged from the most potent LPV to the least potent DRV with observed inhibition from 50 µM incubations of approximately 100% and 15%, respectively. The calculated IC_{50} values for the PIs and CsA are summarized in the table below. The rank-order of PIs was similar to that observed based on their effects on the bi-directional permeability of TDF through MDCKII-MDR1 cells. When tested at concentrations ranging between 0.78 and 50 µM, the most potent PIs LPV, NFV and BRV inhibited the efflux of calcein-AM by 50% (IC_{50}) at approximately 20 µM. APV, IDV and DRV were the weakest inhibitors (IC50 > 100 µM).

Inhibition of Pgp-dependent Efflux of Calcein-AM in MDCKII-MDR1 Cells with CsA or PIs	
Compound	**IC_{50} (μM)[a]**
Cyclosporine A (CsA)	9.34 ± 4.33
Lopinavir (LPV)	10.3 ± 4.4
Nelfinavir (NFV)	19.9 ± 21.1
Brecanavir (BRV)	24.1 ± 9.2
Ritonavir (RTV)	39.6 ± 19.4
Atazanavir (ATV)	67.8 ± 27.7
Saquinavir (SQV)	100 ± 68
Amprenavir (APV)	> 100[b]
Indinavir (IDV)	> 100
Darunavir (DRV)	> 100

a Values represent the mean ± standard deviation of between n = 3 and 10 independent assays each including 7 inhibitor concentrations done in duplicate.
b HIV PIs were tested at concentrations up to 50 μM in the absence of serum proteins. All PIs showed significant dose-dependent inhibition of Pgp during the assay although many did not attain ≥ 25% inhibition at 50 μM and their IC_{50} values are reported as > 100 μM.

Conclusion: We have shown that all PIs have the potential to modulate the intestinal absorption of TDF. The differential changes in circulating TFV levels observed clinically with different PIs is likely related to the relative ability of PIs to (i) inhibit TDF hydrolysis in intestinal tissue, (ii) inhibit Pgp-mediated efflux of TDF and (iii) induce Pgp expression. This study also clearly shows that the magnitude of perturbations in TDF absorption caused by concomitant agents should be limited by the significant saturation of the intestinal transport of TDF at the concentrations of TDF achieved in the gastrointestinal tract following oral administration of TD

Test Article: Emtricitabine, Rilpivirine, or Tenofovir disoproxil fumarate

2.6.5.15. Pharmacokinetics: Other

There are no additional studies to report under this heading.