SECTION 2.4
NONCLINICAL OVERVIEW

EMTRICITABINE/RILPIVIRINE/ TENOFOVIR DISOPROXIL FUMARATE FIXED-DOSE COMBINATION

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GLOSSARY OF ABBREVIATIONS AND DEFINITION OF TERMS

3TC	lamivudine
ABC	abacavir
ACTH	adrenocorticotropic hormone
ADV	adefovir dipivoxil (Hepsera®, Gilead)
ALP	alkaline phosphatase
ALT	alanine aminotransferase
API	active pharmaceutical ingredient
APTT	activated partial thromboplastin times
APV	amprenavir
AST	aspartate aminotransferase
ATPase	adenosine triphosphatase
ATV	atazanavir
AUC	area under the curve
AUC_{ss}	area under the plasma concentration curve at steady state
BID	twice daily
BMD	bone mineral density
CA	citric acid
CAC	Carcinogenicity Assessment Committee
Caco-2	colon carcinoma cell line
CHMP	Committee for Medicinal Products for Human Use
Cl_{bl}	blood clearance
C_{max}	maximum observed concentration of drug in serum, plasma, or peripheral blood mononuclear cells
[redacted]	[redacted]
CNS	central nervous system
COX II	cytochrome c oxidase II
CRF	corticotrophin releasing factor
CYP450, CYP	cytochrome P450
d4T	stavudine
dATP	deoxyadenosine triphosphate
dCTP	deoxycytidine triphosphate
ddC	zalcitabine
ddI	didanosine
DHEA	dehydroepiandrosterone
DLV	delavirdine
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DRV	darunavir
DXA	dual energy X-ray absorptiometry

GLOSSARY OF ABBREVIATIONS AND DEFINITION OF TERMS (CONTINUED)

EADs	early afterdepolarizations
EC_{50}	median effective concentration
ECG	electrocardiograph
EFV	efavirenz
EFV/FTC/TDF	efavirenz/emtricitabine/ tenofovir DF (Atripla®)
EMEA	European Medicines Agency
ENF	enfuvirtide
ETR	etravirine
F	female
F_0 generation	parents
F_1 generation	offspring
FDA	(US) Food and Drug Administration
FDC	fixed-dose combination
FTC	emtricitabine (Emtriva® Gilead)
FTC/TDF	emtricitabine/tenofovir DF (Truvada®)
FTC-TP	emtricitabine triphosphate
GD	gestation day
GI	gastrointestinal
GLP	Good Laboratory Practice
HBV	hepatitis B virus
HCl	hydrochloride
hERG	human ether-à-go-go
HIV-1	human immunodeficiency virus type 1
hOAT1, 2	human organic anion transporter type 1, 2
HPMC	hydroxypropyl-methyl-cellulose
IC_{50}	median concentration resulting in 50% of maximum inhibition
ICH	International Conference on Harmonization (of Technical Requirements for Registration of Pharmaceuticals for Human Use)
$I_{Ca,L}$	high threshold L-calcium current
IDV	indinavir
I_{K1}	inward rectifying potassium current
I_{Kr}	rapidly activating rectifying potassium current
I_{Ks}	slowly activating rectifying potassium current
I_{Na}	fast sodium current
I_{to}	transient outward potassium current
ITT	intent-to-treat
LC-MS/MS	liquid chromatography with tandem mass spectrometry
LD	lactation day
LH	luteinizing hormone

GLOSSARY OF ABBREVIATIONS AND DEFINITION OF TERMS (CONTINUED)

LOAEL	lowest observed adverse effect level
LPV	lopinavir
M	male
MAA	Marketing Authorization Application
MEL	minimal effect level
MDCK II	Madin-Darby canine kidney
MITT	modified intent-to-treat
MPS	mononuclear phagocytic system
MRP1, 2, or 4	multidrug resistance protein 1, 2, or 4
mtDNA	mitochondrial DNA
m/v	mass per volume
MVC	maraviroc
NFV	nelfinavir
NNRTI	nonnucleoside reverse transcriptase inhibitor
NOAEL	no observed adverse effect level
NOEL	no observed effect level
NRTI	nucleoside reverse transcriptase inhibitor
N(t)RTI	nucleoside or nucleotide reverse transcriptase inhibitor
NVP	nevirapine
OECD	Organization for Economic Cooperation and Development
PBMC	peripheral blood mononuclear cell
PFC	plaque forming cells
P-gp	P-glycoprotein
PEG400	polyethylene glycol 400
PI	protease inhibitor
PMPApp	tenofovir diphosphate
PSUR	periodic safety update report
PT	prothrombin time
QT	interval between the start of the Q wave and the end of the T wave on ECG
QTc	QT interval duration corrected for heart rate
QTcF	QT interval duration corrected for heart rate according to Fridericia
QWBA	quantitative whole body autoradiography
RAL	raltegravir
RAM	resistance associated mutation
RAP	resistance analysis population
RBC	red blood cell
RNA	ribonucleic acid
RPV, TMC278	rilpivirine (27.5 mg rilpivirine hydrochloride is equivalent to 25 mg rilpivirine)

GLOSSARY OF ABBREVIATIONS AND DEFINITION OF TERMS (CONTINUED)

RT	reverse transcriptase
RTV	ritonavir
S9	metabolic activation system
SDH	sorbitol dehydrogenase
SIV	simian immunodeficiency virus
SQV	saquinavir
T_3	triiodothyronine
T_4	thyroxine
TAM	thymidine analog mutation
TBG	thyroid binding globulin
TDF	tenofovir disoproxil fumarate, tenofovir DF, Viread®
TdP	Torsade de Pointes
TDR	transmural dispersion of repolarization
TFV	tenofovir
TMC	Tibotec Medicinal Compound
TPV	tipranavir
TSH	thyroid stimulating hormone
TTC	threshold of toxicological concern
TQT	thorough QT prolongation study according to ICH E14
UDPGT	uridine diphosphate glucuronosyltransferase
US	United States
Vd_{ss}	volume of distribution at steady state
WBC	white blood cell
WHV	Woodchuck hepatitis virus
ZDV, AZT	zidovudine

2.4. NONCLINICAL OVERVIEW

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). This FDC tablet is referred to as emtricitabine/rilpivirine/tenofovir disoproxil fumarate (FTC/RPV/TDF) throughout this document. Each FTC/RPV/TDF FDC tablet contains FTC, RPV, and TDF at the same dosages as recommended for the individual components in adults, i.e., 200 mg of FTC, 25 mg RPV (as hydrochloride; 27.5 mg rilpivirine hydrochloride is equivalent to 25 mg RPV), and 300 mg of TDF (equivalent to 245 mg of tenofovir disoproxil or 136 mg tenofovir [TFV]). The FDC FTC/RPV/TDF has demonstrated bioequivalence to the individual dosage forms (FTC, TDF, and RPV). Individual presentations of FTC (Emtriva®) and TDF (Viread®) are currently approved for the treatment of human immunodeficiency virus type 1 (HIV-1) in the United States (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, which is also available as an oral solution formulation, may be administered to children as young as 4 months of age. Emtricitabine and TDF are listed as preferred agents in United States (US) and international treatment guidelines {15207}, {12716}, {14065}. It is proposed that FTC/RPV/TDF FDC tablets be indicated for the treatment of HIV-1 infection in adults and taken orally once daily with a meal.

Emtricitabine is a nucleoside reverse transcriptase inhibitor (NRTI) and a synthetic analog of the naturally occurring nucleoside, 2′-deoxycytidine, a pyrimidine nucleoside. Intracellularly, FTC is phosphorylated by cellular enzymes to form emtricitabine triphosphate (FTC-TP), the active metabolite. Emtricitabine is the active ingredient in Emtriva hard capsules and oral solution. The Committee for Medicinal Products for Human Use (CHMP) approved Emtriva for the treatment of human immunodeficiency virus type 1 (HIV-1) infection on 24 October 2003 (EU/1/03/261/001-003).

Rilpivirine, developed by Tibotec BVBA, is an investigational agent of the nonnucleoside reverse transcriptase inhibitor (NNRTI) class which shows in vitro activity against wild-type HIV-1 and against HIV-1 strains harboring different mutations that result in resistance to the first-generation NNRTIs. Tibotec BVBA will submit their MAA application in accordance with Article 8.3 of Directive 20[redacted].

Tenofovir DF, the oral prodrug of TFV, is a nucleotide reverse transcriptase inhibitor (NtRTI). After absorption, TDF is rapidly converted to TFV, which is metabolized intracellularly to the active metabolite, tenofovir diphosphate. Tenofovir disoproxil (as fumarate) is the active ingredient in Viread 300-mg film-coated tablets. The CHMP approved Viread for the treatment of HIV-1 infection on 05 February 2002 (EU/1/01/200/001-2).

Truvada® is an FDC containing 200 mg of FTC and 300 mg of TDF. The CHMP approved Truvada for the treatment of HIV-1 infection on 21 February 2005 (EU/1/04/305/001).

Atripla® is an FDC containing 600 mg of efavirenz (EFV), an NNRTI, 200 mg of FTC, and 300 mg of TDF. The CHMP approved Atripla for the treatment of HIV-1 infection on 13 December 2007 (EU/1/07/430/001-2).

Comprehensive programs of nonclinical studies with FTC, RPV, and TDF have been conducted. 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).

This nonclinical overview provides all of the nonclinical information that is relevant to the assessment of the FDC of FTC, RPV, and TDF. In accordance with the advice received from the European Medicines Agency (EMA) at the strategy meeting held on [redacted] 20[redacted] (see Module 1.2.5.14, EMA strategy meeting minutes) and in accordance with the Committee for Proprietary Medicinal Products Guideline on Fixed Combination Medicinal Products (CPMP/EWP/240/95 Rev. 1, 21 February 2008), the present application for a marketing authorization is being submitted in accordance with Article 8.3 of Directive 2001/83/EEC, as amended. 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.

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 FTC, TDF, and FTC/TDF 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 Section 2.4.7. Nonclinical data for FTC, RPV, TDF, Truvada, and Atripla are provided in Module 2.6; and nonclinical study reports are provided in Module 4.

All of the definitive toxicology and toxicokinetic studies reported in this summary for FTC, RPV, TDF, and the FDC of FTC/TDF were conducted in accordance with guidelines issued by the International Conference on Harmonization (ICH) and with Good Laboratory Practice (GLP) or other applicable regulations promulgated by international health authorities. Pilot, exploratory, and mechanistic studies were not all conducted under strict GLP procedures, but were conducted using appropriate protocols and documentation to assure data integrity. Within this Module, and in the nonclinical written and tabulated summaries, RPV is referred to as TMC278 in the text and tables describing the nonclinical studies of rilpivirine alone.

The nonclinical data discussed within this document demonstrate an acceptable benefit/risk profile for the proposed use of FTC/RPV/TDF FDC tablets for the treatment of HIV-1 infection in adults. All information from nonclinical studies that is of relevance to the prescriber and patient has been included in the proposed Prescribing Information and Patient Leaflet.

2.4.1. OVERVIEW OF THE NONCLINICAL TESTING STRATEGY

This document provides an overview of the nonclinical information that is relevant to the assessment of the FDC of FTC, RPV, and TDF. The overview is structured as a logical overview of the studies in the various disciplines, including primary pharmacodynamics, secondary pharmacodynamics, safety pharmacology, pharmacokinetics, and toxicology. A critical assessment of the completeness and relevance of the nonclinical testing program and the key findings are included. An integrated safety assessment of FTC/RPV/TDF for the treatment of HIV-1 infected treatment-naive adults is included in the section, "Integrated Overview and Conclusions," of this document. Specific cross-disciplinary topics and proposals for the inclusion of nonclinical items in the product labeling are discussed throughout the text, as appropriate, and summarized at the end of the document.

2.4.1.1. Emtricitabine

Emtricitabine (5-fluoro-1-(2*R*,5*S*)-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine) is the (-) enantiomer of a thio analogue of cytidine, which differs from other cytidine analogues in that it has a fluorine in the 5-position. Emtricitabine is a nucleoside analogue that has activity against HIV and hepatitis B virus (HBV), and is indicated for use in combination with other antiretroviral agents in the treatment of HIV-1 infection.

A comprehensive nonclinical pharmacology/virology, pharmacokinetic, and toxicology program was undertaken in support of the registration of FTC. The results of this evaluation were presented in detail in the original licensing application and subsequent submissions for Emtriva.

2.4.1.2. Rilpivirine

Tibotec Medicinal Compound 278 (TMC278), a diarylpyrimidine derivative, is a potent NNRTI with in vitro activity against wild-type HIV-1 and HIV-1 NNRTI-resistant mutants. TMC278 has been developed for the treatment of HIV-1 infected antiretroviral treatment-naive subjects in combination with other antiretroviral agents.

The following convention is applied throughout this Module: reference is made to "TMC278 (or RPV)" when the hydrochloride (HCl) salt was administered, and to "TMC278 base (or RPV base)" when the base was administered. The dose or concentration is always given as base equivalent. The analyte in bioanalytical determinations is referred to as "TMC278." TMC278 base was used in the early phases of development and TMC278 in the later phases, after selection of the final chemical form.

The designs of the main carcinogenicity studies were according to the guidelines S1B: "Testing for Carcinogenicity of Pharmaceuticals" and S1C(R2): "Dose Selection for Carcinogenicity Studies of Pharmaceuticals" of the International Conference on Harmonisation (ICH) and in line with recommendations of the FDA Carcinogenicity Assessment Committee (CAC). The design and the conduct of several major nonclinical studies supporting clinical development of TMC278 in adult subjects and for a pediatric

indication were discussed with various Health Authorities worldwide and concurrence was obtained.

The nonclinical testing strategy for the TMC278 oral tablet indication is aimed at evaluating the primary and secondary pharmacodynamic effects of TMC278. Moreover, a variety of models and tests was applied to detect adverse effects of TMC278, its metabolites, as well as its impurities in the drug substance and drug product if relevant. The behavior of TMC278, in terms of absorption, kinetics, distribution, metabolism, and excretion, in the major models was studied. Considerable effort was made to elucidate the mechanism of action of the observed effects for a better understanding of their relevance and to allow an adequate safety assessment for man.

For in vitro studies, TMC278 base or TMC278 was usually dissolved in dimethyl sulfoxide (DMSO) and subsequently diluted with incubation medium. All in vivo studies were done by oral administration, with the exception of the sensitization and dermal irritation studies. For oral dosing, TMC278 base was dissolved in polyethylene glycol 400 (PEG400) usually with 100 mg/mL citric acid (CA) to improve exposure, with the exception of the rabbit studies in which TMC278 base was suspended in 0.5% (m/v) aqueous hydroxypropyl-methyl-cellulose (HPMC). TMC278 (hydrochloride) was suspended in 0.5% (m/v) aqueous HPMC in the mouse, rat, and dog studies. In the studies with cynomolgus monkeys, the vehicle was 1% (m/v) aqueous HPMC with 0.5% Tween 20.

2.4.1.3. Tenofovir DF

Tenofovir disoproxil fumarate, (9-[(R)-2-[[bis[[(isopropoxycarbonyl) oxy]methoxy]phosphinyl] methoxy]propyl] adenine fumarate(1:1)); GS-4331-05), is an oral prodrug (bisPOC-PMPA) of TFV (PMPA). Tenofovir is a nucleotide analogue that has activity against retroviruses and hepadnaviruses, and is indicated for use in combination with other antiretroviral agents in the treatment of HIV-1 infection.

A comprehensive nonclinical pharmacology/virology, pharmacokinetic, and toxicology program was undertaken in support of the registration of TDF. The results of this evaluation were presented in detail in the original licensing application and subsequent submissions for Viread.

2.4.1.4. Emtricitabine/Rilpivirine/Tenofovir DF

A comprehensive nonclinical pharmacology/virology, pharmacokinetic, and toxicology program was undertaken in support of the registration of FTC, RPV, and TDF. The FTC and TDF results of these evaluations were presented in detail in the original licensing application and subsequent submissions for Emtriva and Viread, respectively. Similarly for RPV, a complete nonclinical package is available. A small number of key studies were conducted using the combination of FTC and TDF, and FTC/RPV/TDF. The overall program, including the data from the combination and individual agent studies, is considered adequate to support the safety of the FTC/RPV/TDF combination tablets.

The absence of nonclinical safety studies with the combination is in accordance with the CHMP Guideline on the Non-Clinical Development of Fixed Combinations of Medicinal Products (EMEA/CHMP/SWP/258498/2005, January 2008). There are no anticipated clinically relevant pharmacokinetic or toxicological interactions expected for the FTC/RPV/TDF combination. The Scientific Advice Working Party agreed with the assessment that no further nonclinical studies are needed to support the FTC/RPV/TDF FDC based on the comprehensive nonclinical data set for RPV and the long-term safety from the Phase 2b trial TMC278-C204 (EMEA/CHMP/SAWP/670243/20[redacted] corrigendum, see Module 1.2, Annex 5.14). Further, extensive clinical safety data are available for the approved drugs FTC, TDF, and the FTC/TDF FDC product (Truvada). Additionally, data from the Phase 2b and Phase 3 trials with RPV and Truvada supports the use of these products (see Module 2.7). The clinical data, along with the lack of overlapping toxicity in animals support the safety of the new combination product.

2.4.2. PHARMACOLOGY/VIROLOGY

2.4.2.1. Primary Pharmacodynamics

Mechanism of Action

Intracellularly, FTC is converted through 3 phosphorylation reactions to its active phosphorylated anabolite FTC-TP {4527},{4535}. Emtricitabine triphosphate inhibits viral polymerases by direct binding competition with the natural deoxyribonucleotide substrate (deoxycytidine triphosphate; dCTP), and after incorporation into DNA, by DNA chain termination {4249}. Emtricitabine triphosphate is a very weak inhibitor of mammalian DNA polymerases α, β, and ε and mitochondrial DNA polymerase γ {4541},
(Tabulated Summary 2.6.3.1.1, Report TEZZ/93/0007).

TMC278 is an NNRTI that does not require modification for antiviral activity. The crystal structure of TMC278 bound to the HIV-1 reverse transcriptase (RT) complex revealed that TMC278 adapted to changes in the NNRTI-binding pocket, which could explain the increased genetic barrier to the development of resistance in vitro to this compound (Module 2.6.2, Pharmacology Written Summary, Section 2.6.2.2.6).

The antiviral activity of TMC278 in the presence of human serum proteins was comparable to that of efavirenz (EFV). TMC278 did not show antagonism when studied in combination with other antiretroviral agents. Rilpivirine showed additive to synergistic antiviral activity in combination with the N(t)RTIs abacavir (ABC), didanosine (ddI), FTC, lamivudine (3TC), stavudine (d4T), TFV, and zidovudine (ZDV); the PIs amprenavir (APV), atazanavir (ATV), darunavir (DRV), indinavir (IDV), lopinavir (LPV), nelfinavir (NFV), ritonavir (RTV), saquinavir (SQV), and tipranavir (TPV); the NNRTIs EFV, etravirine (ETR), and nevirapine (NVP); the fusion inhibitor enfuvirtide (ENF); the entry inhibitor maraviroc (MVC); and the integrase inhibitor raltegravir (RAL).

Tenofovir disoproxil fumarate is converted to TFV by serum esterases. Intracellularly, TFV is then converted through 2 phosphorylation reactions to its active phosphorylated anabolite,

TFV diphosphate (PMPApp) {1574}. Tenofovir diphosphate inhibits viral polymerases by direct binding competition with the natural deoxyribonucleotide substrate (deoxyadenosine triphosphate; dATP) and after incorporation into DNA, by DNA chain termination {1131}. Tenofovir diphosphate is a very weak inhibitor of mammalian DNA polymerases α, β, δ, ε, and mitochondrial DNA polymerase γ {1131},{2516}.

Tenofovir and FTC are analogues of 2 different nucleosides, adenosine and cytidine, respectively, and do not share a common intracellular metabolism pathway (see Section 2.4.3.5.1). Therefore, there should be no competition between TFV or FTC. In vitro analysis of intracellular phosphorylation demonstrated that activation of TFV to TFV diphosphate was not influenced by FTC, and the activation of FTC to FTC triphosphate was not affected by the presence of TFV (Tabulated Summary 2.6.3.1.4, PC-164-2001).

In Vitro Antiretroviral Activity

Emtricitabine, RPV, and TFV have antiviral activity against retroviruses and hepadnaviruses (see Section 2.4.2.2). The EC_{50} of FTC against laboratory adapted strains of HIV-1 ranged from 0.001 to 0.62 μM depending on cell type and virus strain used in the assay {4534}, {4541}, {4526}, (Tabulated Summary 2.6.3.1.1, Reports 462-v2 and 10498-v2). With clinical isolates of HIV-1, EC_{50} values range from 0.002 to 0.028 μM {4534}, (Tabulated Summary 2.6.3.1.1, Report 462-v2). Emtricitabine displays antiviral activity in vitro against HIV-1 subtypes A, B, C, D, E, F, and G, with EC_{50} values ranging from 0.007 to 0.075 μM (Tabulated Summary 2.6.3.1.1, Reports 10498-v2 and 11419-v2), and shows activity against HIV-2 (with an EC_{50} of 0.007 to 1.5 μM) {4534}, {4541}.

TMC278 showed subnanomolar EC_{50} values against wild-type HIV-1 group M isolates A, B, C, D, E, F, and G (0.07 to 1.01 nM or 0.03 to 0.37 ng/mL), HIV-1_{IIIB} (0.73 nM or 0.27 ng/mL), and nanomolar EC_{50} values against HIV-1 group O isolates (2.88 to 8.45 nM or 1.06 to 3.10 ng/mL) (see Module 2.6.2, Pharmacology Written Summary, Section 2.6.2.2.7). The TMC278 EC_{50} values observed in human monocyte-derived macrophages infected with HIV-1_{Ba-L} or HIV-1_{ADA} were comparable to those observed for HIV-1 group M isolates, showing the broad and robust spectrum of the antiviral activity exhibited by this NNRTI. TMC278 had antiviral activity in the micromolar range against HIV-2 and SIV, but no activity was observed against the non-HIV related viruses, including HBV, herpes simplex virus 2, human corona virus, influenza A virus, and vaccinia virus. A selectivity index of ± 8000 indicated that TMC278 was a potent and specific inhibitor of HIV-1. The in vitro cytotoxicity experiments performed in cell lines of various origins confirmed that the TMC278 selectivity index was robust.

The EC_{50} of TFV against wild-type HIV-1_{IIIB} is 1 to 6 μM in T lymphoblastoid cell lines and 0.2 to 0.4 μM in peripheral blood mononuclear cells (PBMCs) {1574}, {39}. With clinical isolates of HIV-1, EC_{50} values range from 0.4 to 2.2 μM {5044}. The mean EC_{50} values of TFV against HIV-1 subtypes A, C, D, E, F, G, and O in PBMCs were within 2-fold of subtype B (0.55 to 2.2 μM) {5044}. Tenofovir has an EC_{50} of 0.04 μM against HIV-1_{BaL} in primary monocyte/macrophage cells, and also is active against HIV-2 in vitro, with an EC_{50}

of 1.6 to 5.5 μM {39}, {625} (Tabulated Summary 2.6.3.1.3, Reports PC-104-2003 and PC-104-2013).

The anti-HIV-1 activity of 2-drug combinations of FTC, RPV, and TFV were found to be additive to synergistic in multiple in vitro assay systems, supporting the use of these agents in combination in HIV-1 infected patients (see Module 2.6.2, Pharmacology Written Summary, Section 2.6.2.5; Tabulated Summary 2.6.3.1.2, Report TMC278-IV1-AVMR; Tabulated Summary 2.6.3.1.4, Report PC-164-2001; Tabulated Summary 2.6.3.1.9, Report PC-164-2002; and Tabulated Summary 2.6.3.1.17, Report PC-264-2001). In addition, in vitro combination studies have shown that TFV, RPV, and FTC have additive to synergistic anti-HIV-1 activity with other approved NRTIs, NNRTIs, and PIs, as well as the integrase inhibitor elvitegravir (FTC and TFV) {1469}, (see Module 2.6.2, Section 2.6.2.5; Module 2.7.2, Section 2.7.2.3.1.9.1; and Reports PC-183-2004, 10804, 470, C1278-00005, and PC-264-2001).

The antiviral activity of the triple combination of FTC, RPV, and TFV in vitro demonstrates synergy for anti-HIV activity and no evidence of cytotoxicity (Tabulated Summary 2.6.3.1.20, Report PC-264-2002).

Resistance

In vitro, FTC has selected for an M184V or M184I mutation in RT. These mutations confer high-level (> 100-fold) resistance to FTC and 3TC {1794}, (Report PC-164-2003). Emtricitabine remains active in vitro against laboratory and clinical strains of HIV-1 containing mutations associated with reduced susceptibility to thymidine analogues (TAMs), didanosine (L74V, ddI), and NNRTIs (Tabulated Summary 2.6.3.1.1, Report 11148; and Report PC-264-2004). The K65R mutation results in 8- to 12-fold reductions in susceptibility to FTC in vitro (Tabulated Summary 2.6.3.1.1, Report 15883).

TMC278 retained antiviral activity against 63.0% (136 of 216) of the HIV-1_{HXB2} site-directed mutants carrying single, double, triple, and quadruple RT mutations; as compared to 57.7% for ETR, 45.8% for EFV, and 36.2% for NVP. This panel of HIV-1_{HXB2} site-directed mutants was created on the basis of resistance to ETR and the information on emerging RT mutations in patients failing TMC278 enrolled in the Phase 2b and Phase 3 trials. The analysis of this panel showed that TMC278 retained activity against 64 of 67 single HIV-1_{HXB2} site-directed mutants analyzed in vitro, and only the 3 HIV-1_{HXB2} site-directed mutants with K101P, Y181I, or with Y181V were resistant to TMC278. More than 1, and usually more than 2 NNRTI resistance-associated mutations (RAMs) were necessary to confer resistance to TMC278 in vitro. Resistance to TMC278 was mostly driven by the combination of specific NNRTI RAMs, rather than the total number of NNRTI RAMs. K103N in isolation was not associated with resistance to TMC278 or ETR. Furthermore, some of the NNRTI RAMs present in the site-directed mutants that showed resistance to TMC278 were uncommon in NNRTI treatment-experienced patients (see Module 2.6.2, Section 2.6.2.2.8.5).

In vitro selection of TMC278-resistant strains was performed at high and low virus inoculum using wild type HIV-1 of different origins and subtypes, as well as NNRTI resistant HIV-1.

At high virus inoculum, emergence of resistant strains from wild-type HIV-1 was prevented at concentrations equal or greater than 0.040 µM. The same was observed with resistant strains harboring the single NNRTI RAMs K103N or Y181C, or the NRTI RAM M184V. NNRTI RAMs that emerged in vitro under the selective pressure of TMC278 included the following: V90I, L100I, K101E, V106A, V106I, V108I, E138G, E138K, E138Q, E138R, V179F, V179I, Y181C, Y181I, G190E, H221Y, F227C, M230I, and M230L (Module 2.6.2, Sections 2.6.2.2.8.2, 2.6.2.2.8.3 and 2.6.2.2.8.4).

Analysis of the sensitivity to TMC278 of a panel of 4786 HIV-1 recombinant clinical isolates resistant to at least 1 of the first-generation NNRTIs (EFV or NVP) showed similar in vitro cross resistance that existed between EFV and NVP. This cross resistance was not shared to the same extent by TMC278 and ETR, with the majority of the isolates (62%) retaining sensitivity to these novel NNRTIs. Considerable in vitro cross resistance was observed between TMC278 and ETR (Module 2.6.2, Section 2.6.2.2.8.5).

Isolates of HIV-1 with the mutation K65R in RT have been selected in vitro with TFV and show 2- to 4-fold reduced susceptibility to TFV {2078} (Report 15883). Tenofovir shows reduced activity in vitro and in vivo against strains of HIV-1 with certain patterns of multiple thymidine analogue-associated mutations {5010},{5482}. Tenofovir shows slightly increased anti-HIV-1 activity against the M184V mutation selected by ABC, FTC, and 3TC {2078}, {1649}; and full activity against NNRTI-resistant clinical isolates expressing K103N, Y181C, and other NNRTI mutations (Module 2.6.2, Section 2.6.2.2.13.3; Reports P4331-00035 and PC-264-2004).

In the macaque model of SIV infection, TFV treatment selected for mutant SIV that contained the RT mutation K70E, which was present transiently and was replaced by the K65R mutation (Module 2.6.2, Section 2.6.2.2.14.4).

Mutant HIV-1 expressing both the K65R and M184V mutations has shown increased susceptibility to TFV compared to mutant HIV-1 expressing K65R in the absence of M184V, consistent with the effect of the M184V mutation on increasing TFV susceptibility {1649}. The range of susceptibilities to TFV for the K65R+M184V double mutant is from 1.1- to 2.0-fold of wild type in the various assay systems {2078}, {3852}, (Tabulated Summary 2.6.3.1.1, Report 15883; Tabulated Summary 2.6.3.1.3, Report PC-104-2004). HIV-1 expressing both the K65R and M184V mutations also showed further reductions in the replication capacity as compared to HIV-1 expressing either the K65R or M184V alone (Tabulated Summary 2.6.3.1.3, Report PC-104-2004).

Report PC-164-2005 describes the in vitro selection of HIV-1 with reduced susceptibility to the combination of TFV and FTC. Coselection with FTC and TFV yielded an M184I virus first, and then transitioned to a K65R virus at intermediate drug concentrations (8- to 16-fold EC_{50}). At higher FTC concentrations, a K65R+M184V double mutant was selected with high-level resistance to FTC, but lower resistance to TFV than K65R alone. These in vitro results demonstrate that the M184V/I mutation is the first mutation to develop for the combination of TFV and FTC, followed by the K65R mutation either on its own or in addition to the M184V/I mutation, depending on the concentration of FTC in the cell culture.

These results parallel the results obtained from resistance selection with the individual components, specifically a more rapid selection of M184V/I by FTC and delayed selection of resistance by TFV. Clinical trial data using the combination of TDF with 3TC or FTC support this conclusion as the M184V mutation was observed to develop more frequently than K65R (see below).

Based on in vitro analyses between FTC and TFV, no cross resistance to TFV has been demonstrated with the FTC-selected M184V/I mutation. In vitro cross resistance to FTC has been shown with the TFV-selected K65R mutation. However, in vitro and in vivo, the combination of FTC and TFV appears to select for the M184V/I mutation prior to selection of the K65R mutation. The much higher levels of resistance afforded by the M184V/I mutation and its greater ease of selection suggest that the M184V/I mutation may dominate the resistance profile for the combined use of FTC and TDF in vivo.

Clinical Resistance Analysis

In a pooled analysis for subjects receiving rilpivirine in combination with emtricitabine/tenofovir DF in clinical trials C209 and C215, there were 62 virologic failure subjects with resistance information available for 54 of those subjects (Report PC-264-2005). The amino acid substitutions associated with NNRTI resistance that developed most commonly in these subjects were: V90I, K101E, E138K/Q, Y181C, V189I, and H221Y. However, the presence of the substitutions V90I and V189I at baseline did not affect the viral response. The amino acid substitutions associated with NRTI resistance that developed in 3 or more subjects were: K65R, K70E, M184V/I, and K219E during the treatment period.

In Vivo Efficacy in Animal Models

The activity of FTC and TFV either alone or in combination has been investigated in numerous animal models of efficacy ({1133}, {2477}, {1576}, {1367}, {12759},{17},{35}, {7288}, {670}, {3873}, {11074}, {9457}). The primary animal model used for these studies was the Simian Immunodeficiency Virus (SIV)-infected macaque monkey.

No additional studies for the FTC/RPV/TDF combination are considered warranted in animal models in view of the extensive clinical experience with the use of FTC and TDF, both alone and in combination, and the potent efficacy results in Phase 3 trials with regimens containing FTC + RPV + TDF for the treatment of HIV-1 infection (see Module 2.7.3).

Emtricitabine/Rilpivirine/Tenofovir DF – Summary

Emtricitabine, RPV, and TFV are potent and selective inhibitors of HIV-1. All 3 drugs show potent antiretroviral activity against diverse subtypes of HIV-1 in vitro. Emtricitabine and TFV are phosphorylated intracellularly through nonoverlapping pathways, and in combination show no antagonism for the formation of their active metabolites. Rilpivirine does not require modification for activity. Dual-drug combinations of FTC, RPV, and TFV consistently show additive to synergistic anti-HIV-1 activity in vitro. The antiretroviral

activity of the triple combination of FTC, RPV, and TFV in vitro demonstrates synergy and no evidence of cytotoxicity (Tabulated Summary 2.6.3.1.20, Report PC-264-2002).

In vitro dose-escalation resistance selection with the combination of FTC, RPV, and TFV resulted in the selection of M184I. Resistance selection using fixed-dose breakthrough experiments with the combination of FTC, RPV, and TFV resulted in the selection of M184I at drug concentrations fixed at 1.7-fold the EC_{50} for each drug, and K65R at drug concentrations that were fixed at 3.3-fold the EC_{50} for each drug. At higher drug concentrations, no virus was able to replicate. No resistance to RPV was detected in either of the selection experiments with the triple combination. Clinical study data of subjects with virologic failure using the combination of FTC/TDF with RPV was associated with the emergence of NNRTI and nucleoside or nucleotide reverse transcriptase inhibitor (N[t]RTI) mutations, with the combination of E138K and M184I/V being the most common, and a loss of phenotypic susceptibility to TMC278 in approximately 50% of subjects (see Module 2.7.2)

2.4.2.2. Secondary Pharmacodynamics

In vitro and in vivo secondary pharmacodynamics data for FTC, RPV, TDF, and the FTC/TDF combination are presented in detail in the Pharmacology Written Summary, Module 2.6.2. The studies conducted are listed in Tabulated Summary 2.6.3.1.

TMC278 did not cause any inhibition in vitro of α- or β-adrenergic, dopaminergic, muscarinergic, serotonergic, opioid, interleukin, or chemokine receptors (up to 10 µM, 3.7 µg/mL); or human DNA polymerase α, β, or γ (up to 1000 µM, 366 µg/mL).

TMC278 showed no agonistic or antagonistic activity on histamine H_2 receptors in the isolated guinea pig right atrium and no inhibition of adenosine triphosphatase (ATPase) in isolated pig stomach. In vivo, TMC278 did not cause any significant inhibition of pentagastrin-induced gastric acidity in rats.

It is concluded that TMC278 is essentially devoid of any clinically relevant secondary pharmacodynamic effect at unbound concentrations that greatly exceed the median pooled total C_{max} of TMC278 of 0.13 µg/mL in clinical Phase 3 studies TMC278-C209 and TMC278-C215 following an oral dose of 25 mg once daily (Module 5.3.5.3., TMC278-TiDP6-C904-Anal-Eff-Viral, Display PK/PD.1). The total C_{max} value corresponds with 0.3% (unbound fraction TMC278 in human plasma protein binding study) of 0.13 µg/mL (i.e., 0.00039 µg/mL unbound TMC278; Pharmacokinetics Written Summary, Module 2.6.4, Section 4.2).

Anti-Hepatitis B Activity

Both FTC and TFV are inhibitors of human HBV. The in vitro anti-HBV activity of FTC was determined in HepG2 2.2.15 cells, with EC_{50} values ranging from 0.01 to 0.04 µM based on the quantitation of HBV DNA in the extracellular medium and an EC_{50} value of 0.16 µM determined by the quantitation of HBV DNA intracellularly {4533}, {4535}. HBV resistance

to FTC has been observed and is associated with mutations in the resistance analysis population motif of the HBV polymerase (M204V). This mutation is also selected by 3TC and confers resistance to both FTC and 3TC {1746}.

Tenofovir inhibits HBV production in HepG2 2.2.15 and HB611 cells with EC_{50} values of 1.1 and 2.5 μM, respectively, {21},{9266} (Report P4331-00038). Tenofovir was shown to be equally effective against both wild-type and 3TC-resistant HBV in a cell culture assay {2368}, {8381}.

The in vivo efficacy of TDF, adefovir dipivoxil (ADV), 3TC, and FTC, as well as the combinations of TDF or ADV each with 3TC or FTC, were evaluated by treating chronic woodchuck hepatitis virus (WHV)-infected woodchucks for 48 weeks (Report PC-174-2004). At Week 48, the treatment groups of TDF alone, TDF + 3TC, or TDF + FTC each had a mean serum viral load reduction of 2.9, 5.8, and 6.1 log10, respectively. Over the 48-week dosing period, there was no evidence of toxicity in woodchucks treated with any of the drugs or drug combinations.

No antiviral activity of TMC278 (EC_{50} values > 10 μM) was observed against human HBV (Module 2.6.2, Pharmacology Written Summary, Section 2.6.2.3.5).

Cellular Cytotoxicity in vitro

For FTC, no cytotoxicity was observed in vitro in human PBMC, MT-2, HepG2, CEM, MOLT-4, and Vero cells at concentrations up to 100 μM {4531}, {4534}. Emtricitabine was also found to be nontoxic to human bone marrow progenitor cells in vitro. Rilpivirine has shown a low potential for in vitro cytotoxicity in a variety of human cell types and shows a high selectivity index of approximately 8000 (Module 2.6.2, Section 2.6.2.3.6.1). For TFV in quiescent human PBMCs, no cytotoxic effect was detected at concentrations as high as 100 μM {1574}. Low in vitro cytotoxicity of TFV was also demonstrated in human liver cells (HepG2), proliferating human skeletal muscle cells, or quiescent renal tubular epithelial cells (Report P4331-00038). In addition, TFV showed no toxicity to myeloid and erythroid hematopoietic progenitor cells in vitro {4077}. Thus, FTC, RPV, and TFV have a low order of cytotoxicity and a large therapeutic ratio in vitro.

The combination of TFV and FTC was studied for cytotoxicity in MT-2 cells. No cytotoxicity was observed at the highest concentrations tested, up to 50 μM TFV and 5 μM FTC (Tabulated Summary 2.6.3.1.9, Report PC-164-2002). Cytotoxicity studies were also conducted on the combination of TFV and FTC in HepG2 cells as detailed below; no cytotoxicity was observed (Tabulated Summary 2.6.3.1.9, Report TX-104-2001).

Mitochondrial Toxicity

A variety of in vitro studies have been conducted to evaluate the ability of FTC, RPV, or TFV alone to exert mitochondrial toxicity. Results from these studies suggest that TFV, RPV, and FTC have limited capability to inhibit human DNA polymerases or to mediate cytotoxicity or mitochondrial damage. TMC278 had no effects on DNA synthesis by human polymerase α, β, or γ at concentrations up to 1000 μM as determined by PCR (Module 2.6.2, Pharmacology Written Summary, Section 2.6.2.2.6.2).

In vitro combination studies have also been conducted in HepG2 cells to further evaluate the potential mitochondrial toxicity of FTC and TFV (as well as other nucleosides; Tabulated Summary 2.6.3.1.9, Report TX-104-2001). HepG2 cells were exposed to FTC and TFV (as well as other nucleosides), either alone or in combination. The results from these combination studies are summarized below.

HepG2 cells were treated for up to 25 days with concentrations of NRTIs equal to 1 time and 10 times the maximal therapeutic plasma levels. Assay endpoints included cell growth; extracellular production of lactic acid; relative cellular content of mitochondrial DNA (mtDNA) and mtDNA-encoded cytochrome c oxidase II (COX II); and intracellular lipid accumulation.

Tenofovir and FTC alone or in combination with each other or other nucleosides generally had no time- or concentration-dependent effects on cytotoxicity (cell counts) or mitochondrial parameters in HepG2 liver cells. The dual combination of high-dose FTC + ZDV, with or without TFV, appeared to have greater cytotoxicity than the agents alone, but showed no increase in mitochondrial effects.

These studies confirmed that the potential of FTC and TFV to interfere with mitochondrial functions is low, whether administered alone or in combination with other NRTIs.

Specialized parameters such as serum lactate, assessment of mtDNA content, or ultrastructural analysis of target tissues were added to several nonclinical studies with FTC or TDF (including the woodchuck model, an established model for detecting mitochondrial toxicity of nucleosides [FTC Reports TOX 600 and TOX 627; TDF Reports R2000096, W2000042 and P2000078]). There was no evidence of mitochondrial injury in these studies.

Additive mitochondrial toxicity is not expected for FTC or TDF when administered together due to the lack of observed mitochondrial toxicity associated with the individual drugs or the combination of FTC and TDF. Based on these data and the clinical experience with an FTC/TDF regimen, the potential for mitochondrial toxicity is considered to be low. Further, as mitochondrial toxicity is generally less relevant for NNRTIs than NRTIs, and as RPV is not anticipated to significantly increase the exposure of FTC or TFV, the potential for exacerbating mitochondrial toxicity is low. No additional nonclinical studies are therefore considered warranted with the combination of FTC, RPV, and TDF.

2.4.2.3. Safety Pharmacology

In vitro and in vivo safety pharmacology data for FTC, RPV, and TDF are presented in detail in the Pharmacology Written Summary, Module 2.6.2. The studies conducted are listed in Tabulated Summary 2.6.3.1 and individual study details are given in Tabulated Summary 2.6.3.4.

Neither FTC nor TDF had significant unwanted pharmacologic activity as determined in a variety of in vitro and in vivo safety pharmacology studies (see Module 2.6.2, Pharmacology Written Summary, Section 2.6.2.4). Rilpivirine had no effects on the core battery of safety pharmacology tests, apart from inhibitory effects on some potassium currents and channels, and moderate QT prolongation in the rabbit ventricular wedge. While the study designs of these studies varied between the individual products, the major organ systems have been comprehensively evaluated. Given the lack of effects for FTC and TDF on the cardiovascular system, no additional studies on the cardiovascular system with the combination of FTC/RPV/TDF are considered warranted.

2.4.2.3.1. Emtricitabine

A comprehensive range of safety pharmacology studies revealed no treatment-related adverse effects on any organ system at systemic exposure levels much higher than those anticipated in patients at the recommended clinical dose (10- to more than 50-fold). No effects on the cardiovascular system have been reported in anaesthetized dogs given a cumulative dose of 38.5 mg/kg of FTC intravenously over a 1-hour period. In addition, there were no abnormalities reported on the electrocardiogram (ECG) data obtained from the repeated-dose toxicity studies in monkeys, where AUC exposures were up to 26-fold higher than in humans given the 200 mg dose.

2.4.2.3.2. Rilpivirine

Cardiovascular Safety

The standard battery of cardiovascular safety studies showed a concentration-dependent inhibition of TMC278 on the rapidly activating rectifying potassium current (I_{Kr}) from 33% at 0.3 µM (0.11 µg/mL) to 80% at 3 µM (1.1 µg/mL) (Module 2.6.2, Pharmacology Written Summary, Section 2.6.2.4.8.1.1). However, no relevant effects by TMC278 were noted on other cardiovascular or electrocardiographic parameters in vitro in the right atrium of the guinea pig; in vivo in anesthetized guinea pigs; in anesthetized dogs given a single intravenous dose; in conscious instrumented dogs; or in telemetered dogs given a single oral dose (Module 2.6.2, Pharmacology Written Summary, Sections 2.6.2.4.8.1.2, 2.6.2.4.8.1.3, 2.6.2.4.8.1.4, 2.6.2.4.8.1.5 and 2.6.2.4.8.1.6, respectively). The mean C_{max} in the conscious dog studies at the highest dose was 1.5 to 1.7 µg/mL (extrapolated from a single dose pharmacokinetic study in dogs; see Module 2.6.4, Pharmacokinetics Written Summary, Section 2.6.4.3.3).

Delayed-onset (after 11 days of treatment) prolongation of the QTcF interval was reported in the thorough QT (TQT) Study TMC278-C131 (Module 2.7.4, Summary of Clinical Safety, Section 2.7.4.5.4). To investigate the mechanism of the QTc prolongation and of the delayed onset, additional nonclinical studies were done. Moreover, the potential of TMC278 to induce proarrhythmic effects, in particular Torsade de Pointes (TdP), was evaluated.

In order to investigate the mechanism of the clinical QTc prolongation, additional nonclinical cardiovascular safety studies were done with the following objectives:

- To investigate the mechanism of action of the QT prolongation seen in the TQT study with a dose of 75 mg once daily and above
- To investigate the mechanism of action of the delayed onset of the QTc prolongation
- To assess the potential of TMC278 to induce proarrhythmic effects

The additional studies showed inhibition of the slowly activating rectifying potassium current (I_{Ks}) from 17% at 1 µM (0.37 µg/mL) up to 73% at 10 µM (3.7 µg/mL), with an IC_{50} of 3.1 µM (1.15 µg/mL). In addition, the transient outward potassium current (I_{to}) was reduced by 14% at 0.3 µM (0.11 µg/mL) and by 36% at 1 µM (0.37 µg/mL). However, no effects were observed on the inward rectifying potassium current (I_{K1}), the fast sodium current (I_{Na}) or the high threshold L-calcium current ($I_{Ca,L}$) (Module 2.6.2, Pharmacology Written Summary, Section 2.6.2.4.8.2.1).

In vitro, a concentration-dependent inhibition of trafficking of the hERG channel by TMC278 was observed from 1 µM (0.37 µg/mL) and above (Module 2.6.2, Pharmacology Written Summary, Section 2.6.2.4.8.2.2). However, no signs of trafficking, determined as delayed onset of QT prolongation, were noted in an in vivo model. In this model, telemetered guinea pigs, orally dosed for 16 days at 10 mg/kg/day, achieved maximum plasma concentrations of TMC278 ranging from 0.6 to 0.9 µg/mL (Module 2.6.2, Pharmacology Written Summary, Section 2.6.2.4.8.2.4). This concentration was similar to the steady state median C_{max} at TMC278 75 mg once daily, a dose that caused delayed-onset QTc prolongation in TQT Study TMC278-C131 (Module 2.7.4, Summary of Clinical Safety, Section 2.7.4.5.4).

TMC278 showed only a marginal potential to induce proarrhythmic effects in the rabbit arterially perfused left ventricular wedge model (Module 2.6.2, Pharmacology Written Summary, Section 2.6.2.4.8.2.3). Up to 10 µM (3.7 µg/mL), TMC278 caused maximally 9% QT prolongation, but showed no effects on the transmural dispersion of the repolarization (TDR) across the ventricular wall or any early afterdepolarizations (EADs); resulting in a TdP score of 0.5. Arrhythmogenic drugs have a TdP score of around 5 in this model.

In order to find a dose that would not cause QTc prolongation, additional clinical studies were conducted. Pilot Study TMC278-C151 with 25 mg TMC278 once daily for 11 days showed no QTcF prolongation. TQT Study TMC278-C152 with 25 mg TMC278 once daily

for 11 days confirmed the absence of a clinically relevant QTcF prolongation of 25 mg TMC278 once daily, the recommended dose (Module 5.3.4.1, TMC278-TiDP6-C151-CRR).

Discussion and Conclusions:

An independent cardiologist made an integrated assessment of the QT parameters of the nonclinical and clinical trials to investigate the potential of TMC278 to influence cardiac repolarization. This assessment is included as a 'White Paper' in Module 5.4, TMC278‑20100613-Expert-TQT.

The standard battery of cardiovascular safety studies (Tabulated Summaries 2.6.3.4.3 and 2.6.3.4.4) indicated that TMC278 has the potential to partially inhibit the hERG channel at unbound concentrations of 0.11 µg/mL and above. However, no correlates of this effect were found in subsequent studies with models that are considered more physiologically relevant. In the isolated guinea pig right atrium, no effects possibly associated with potassium current reduction were noted, although the maximum unbound concentration was 3.69 µg/mL. In the anesthetized guinea pigs and dogs, no associated effects were noted at maximum plasma concentrations of 9.2 and 2.6 µg/mL, respectively. It is to be noted that the unbound plasma concentrations must have been lower than the concentration in the hERG study due to the high plasma protein binding of TMC278 in these species (Tabulated Summary 2.6.5.6.B and Tabulated Summary 2.6.5.6.C).

No other cardiovascular or cardio-electrophysiological parameters were affected by TMC278. The relevance of the decreased vascular resistance and increased cardiac output noted in the anesthetized dog study is questionable as the vehicle PEG400 appeared to have a clear effect on these parameters, opposite to that of TMC278.

Given the results of the standard battery studies, the observed QTc prolongation in the TQT Study TMC278-C131 was unexpected (see Table 1; Module 2.7.4, Summary of Clinical Safety, Section 2.7.4.5.4). Study TMC278-C131 with 75 and 300 mg TMC278 once daily for 11 days showed dose-related clinically relevant QTcF prolongation, which became manifest after 11 days of treatment.

Mechanism of QT prolongation

The potential of TMC278 to inhibit potassium currents I_{Kr}, I_{Ks}, and I_{to} involved in the repolarization phase of the cardiac action potential is probably a contributing factor, but cannot explain some aspects of the QTcF prolongation seen in TQT Study TMC278-C131. The inhibition of these potassium currents in the cells expressing their channels is a direct concentration-related effect. For that reason, this effect cannot explain the delayed onset of the QTc prolongation in the TQT study. On Day 1, 300 mg TMC278 once daily did not elicit a clinically relevant QTc prolongation, whereas 75 mg once daily did on Day 11 (Module 2.7.4, Summary of Clinical Safety, Section Section 2.7.4.5.4).

The actual unbound concentrations of TMC278 at which the inhibition of the potassium currents occurred in the serum-free in vitro conditions, nominally 0.11 µg/mL and above,

was not determined, but was probably less than the nominal concentrations due to the adsorption of TMC278 to the in vitro equipment (Module 2.6.2, Pharmacology Written Summary, Section 2.6.2.4.8; Tabulated Summary 2.6.3.1.11). For this reason, the margin between the unbound concentrations inhibiting potassium currents and the unbound C_{max} values associated with QTcF prolongation in TQT Study TMC278-C131 (Table 1) cannot be accurately determined, but is probably significant.

Table 1. QTcF and C_{max} Data in Clinical QTc Prolongation Studies with TMC278

Study	TMC278 25 mg once daily						TMC278 75 mg once daily						TMC278 300 mg once daily					
	Day 1			Day 11			Day 1			Day 11			Day 1			Day 11		
	Δ	TC	UC	Δ	TC	UC	Δ	TC	UC	Δ	TC	UC	Δ	TC	UC	Δ	TC	UC
TQT C131							1.0	0.289	0.0009	10.4	0.636	0.0019	3.9	0.838	0.0025	23.8	1.665	0.005
Pilot C151				2.2	0.229	0.0007												
TQT C152				2.0	0.247	0.0007												

Δ = median increase from baseline of QT-interval corrected for heart rate according to Fridericia in ms; TC = total C_{max} of TMC278 in µg/mL; UC = unbound C_{max} of TMC278 = 0.3% of TC in µg/mL; TQT = thorough QTc prolongation study according to ICH E14; C131 = TMC278-C131; C151 = TMC278-C151; C152 = TMC278-C152

Source: For all 3 clinical studies see Module 2.7.4, RPV Summary of Clinical Safety. For exposure data of the 3 clinical studies, see respectively Module 5.3.4.1, TMC278-TiDP6-C131-CRR, Section 4.3.4.2; Module 5.3.4.1, TMC278-TiDP6-C151-CRR, Section 4.3.4; and Module 5.3.4.1, TMC278-TiDP6-C152-CRR, Section 4.3.4.1.

Mechanism of delayed onset

Several drugs (e.g., pentamidine, As_2O_3) are known to prolong the QT interval in man by interfering with hERG trafficking {15693}, and this can be demonstrated in repeat-dose animal models. The concentration-related inhibition of trafficking of the hERG channel seen in the in vitro test using TMC278 could contribute to the delayed onset of the QTcF prolongation seen in TQT Study TMC278-C131. However, it is to be noted that the in vitro inhibition of trafficking becomes manifest after hours, whereas the QTc prolongation by TMC278 needed days to become maximal in the TQT study. In addition, the repeat-dose study with telemetered guinea pigs failed to induce QT prolongation even after treatment for 16 days. The trafficking inhibition seen in vitro did not occur in vivo, whereas delayed-onset QTcF prolongation occurred in subjects treated with 75 mg TMC278 once daily in the TQT study at a median TMC278 C_{max} of 0.636 µg/mL (Module 5.3.4.1, TMC278-TiDP6-C131-CRR, Section 4.3.4.2). This value was similar to that in the repeat-dose study in guinea pigs (Tabulated Summary 2.6.3.4.4). Moreover, in a repeat-dose study with TMC278 in cynomolgus monkeys (Module 2.6.6, Toxicology Written Summary, Section 2.6.6.3.5.4) and in the repeat-dose general toxicity studies with TMC278 base in dogs (Module 2.6.6, Toxicology Written Summary, Sections 2.6.6.3.4.1 to 2.6.6.3.4.4), ECG recordings did not indicate any QT prolongation. The highest C_{max} value in the 12-month study in male dogs was 4.1 µg/mL, with a plasma protein binding of 99.3% (similar to that in man [99.7%]) (see Module 2.6.4, Pharmacokinetics Written Summary, Section 2.6.4.3.4.7 and Module 2.6.5, Pharmacokinetics Tabulated Summary, Section 2.6.5.6.B). This C_{max} value was approximately 6 times higher than that in man (0.636 µg/mL) at 75 mg TMC278 once daily in Study TMC278-C131, after 11 days of administration Table 1.

Noncardio-electrophysiological mechanisms to explain the delayed onset of the QTc prolongation comprise an increase over time of the exposure to TMC278 or to 1 or more of its metabolites, or an increase of the concentration of TMC278 or of 1 or more of its metabolites in the myocardium.

In healthy volunteers (Module 2.7.2, Summary of Clinical Pharmacology, Section 2.7.2.3.1.2.4) and HIV-1-infected subjects (Module 2.7.2, Summary of Clinical Pharmacology, Section 2.7.2.3.1.2.5), exposure to TMC278 increased over time until steady-state was reached by Day 7. However, in Study TMC278-C131, this increase in exposure from Day 1 to Day 11 at the dose of 75 mg once daily was less than the difference in exposure between 75 and 300 mg once daily on Day 1 (see Table 1). Therefore, it cannot explain the delayed onset of the QTcF prolongation noted at 300 mg once daily in TQT Study TMC278-C131, and likely also not that at 75 mg once daily. TMC278 is metabolized in man predominantly by oxidation to metabolites that are likely to have a shorter residence time than the parent compound. After a single radiolabelled dose, 76% of the radioactivity in plasma on the basis of C_{max} is unchanged drug, whereas it is 51% on the basis of AUC. There are no major human metabolites (> 10% of C_{max} of the parent compound), and man does not produce a unique metabolite compared to the nonclinical species (see Module 2.7.2 [Summary of Clinical Pharmacology) for human exposure data and Module 2.6.4, Pharmacokinetics Written Summary, Section 2.6.4.5.2 for metabolism data).

Single dose quantitative whole body autoradiography (QWBA) studies in rats showed heart to plasma ratios of radioactivity to be 2 or less during maximally 8 hours following dosing. The maximum levels of radioactivity in all tissues were reached 4 to 8 hours after dosing (see Module 2.6.4, Pharmacokinetics Written Summary, Section 2.6.4.4.2.5). The data do not indicate any particular affinity of TMC278 or its metabolites for heart tissue. It is to be noted that no QTcF prolongation occurred after 11 days of 25 mg TMC278 once daily in the pilot Study C151 or in the TQT Study C152.

Potential to induce TdP

The perfused rabbit ventricular wedge study indicated that TMC278 has at most a marginal potential to induce arrhythmogenic effects, like TdP. QTc prolongation as such does not lead necessarily to TdP. The dispersion of the repolarization of the cardiac action potential over the ventricular wall plays a role as important as QT prolongation, whereas the occurrence of EADs adds even more weight in the assessment of the potential to induce TdP {15697}. TMC278 did not induce EADs or any other proarrhythmic effects, and had no effect on TDR. Only the QT prolongation contributed to the TdP score of 0.5. Compounds with a history of TdP such as clarithromycin, erythromycin, and cisapride typically have a score of around 5 in this model {15697}. The margin between the nominal concentration of TMC278 that caused a marginal TdP score of 0.5 in the rabbit ventricular wedge model (3.7 µg/mL [Tabulated Summary 2.6.3.4.3]) and the unbound C_{max} of 0.0019 µg/mL at 75 mg TMC278 once daily after 11 days of treatment in TQT Study TMC278-C131 (Table 1) cannot be determined accurately due to the adsorption in the test system. However, it is likely that the margin is very high. The median unbound pooled median C_{max} values in HIV-1 infected treatment-naive subjects treated for 48 weeks with 25 mg once daily in clinical Phase 3 Studies TMC278-C209 and TMC278-C215 (0.3% of 0.13 µg/mL; see Module 5.3.5.3, TMC278-TiDP6-C904-Anal-Eff-Viral, Display PK/PD.1) is 0.0003 µg/mL; again 5 times lower.

Pulmonary Safety

TMC278 tested up to a maximum plasma concentration of 2.6 µg/mL in anesthetized dogs dosed with a single 1--hour intravenous infusion of 5 mg/kg or conscious instrumented dogs dosed with a single oral dose of 20 mg/kg showed no potential to affect respiratory parameters (Module 2.6.2, Pharmacology Written Summary, Section 2.6.2.4.9).

It is concluded that the margin of exposure for effects on the respiratory system by TMC278 at a clinical dose of 25 mg once daily is 20.

Nervous System Safety

At the level of the nervous system, TMC278 had no effects in conscious telemetered or instrumented dogs that received a single oral dose up to 160 mg/kg rendering a C_{max} of up to 1.5 µg/mL (Module 2.6.2, Pharmacology Written Summary, Section 2.6.2.4.7). In rats, incidental motor-affective and sensori-motor behavior parameters were affected and pupil size (autonomic parameter) was slightly reduced on the day of dosing, at an estimated

maximum plasma concentration of 10 µg/mL. The repeat-dose general toxicity studies have not indicated any effect of TMC278 on the nervous system (see Module 2.6.6, Toxicology Written Summary, Section 2.6.6.3).

It is concluded that the margin of exposure for effects of TMC278 on the nervous system at a clinical dose of 25 mg once daily is approximately 11.

2.4.2.3.3. Tenofovir DF

Tenofovir DF was evaluated in safety pharmacology studies of the central nervous system (CNS), cardiovascular system, gastrointestinal (GI) system, and renal system. There were no adverse effects detected in the CNS in rats dosed at 500 mg/kg, or of the cardiovascular system of dogs dosed at 30 mg/kg. There was reduced gastric emptying in rats dosed at 500 mg/kg, but not at 50 mg/kg. There was increased urinary electrolyte excretion and urine volume in rats dosed at 500 mg/kg, but not at 50 mg/kg (Module 2.6.2, Pharmacology Written Summary, Sections 2.6.2.4.10 to 2.6.2.4.14).

2.4.2.3.4. Emtricitabine/Rilpivirine/Tenofovir DF

A comprehensive safety pharmacology program has been conducted for the 3 individual agents. While the study designs for these studies varied between the agents, the major organ systems were comprehensively evaluated. Emtricitabine and TDF had little effect on vital organ systems in safety pharmacology studies. Rilpivirine has shown the potential for QT prolongation, an effect confirmed in the TQT study, but only a marginal potential to induce proarrhythmic effects. Minimal effects were observed with high-dose administration of FTC and TDF (decreased urinary output and reduced gastric emptying with high-dose TDF alone). At the 25 mg dose of RPV, the observed change in QTcF was not considered clinically relevant, and the combination product is not anticipated to exacerbate the small cardiovascular effect seen with RPV alone.

2.4.2.4. Pharmacodynamic Drug Interactions

In vitro interaction studies of FTC with NRTIs (ABC, 3TC, d4T, TFV, zalcitabine [ddC], and ZDV), NNRTIs (delavirdine [DLV], EFV, NVP, RPV), protease inhibitors (PIs; APV, NFV, RTV, SQV), and the integrase inhibitor elvitegravir showed additive to synergistic antiviral effects (Tabulated Summary 2.6.3.1.16, Reports 470 and 10804; Tabulated Summary 2.6.3.1.19, Report 14379; Tabulated Summary 2.6.3.1.18, Report PC-183-2004).

TMC278 did not show antagonism when studied in combination with other antiretroviral agents. Rilpivirine showed additive to synergistic antiviral activity in combination with the NRTIs ABC, ddI, FTC, 3TC, d4T, TFV, and ZDV; the PIs APV, ATV, DRV, IDV, LPV, NFV, RTV, SQV, and TPV; the NNRTIs EFV, ETR, and NVP; the integrase inhibitor RAL; the fusion inhibitor ENF; and the entry inhibitor MVC (see Module 2.6.2, Section 2.6.2.5.2).

In vitro interaction studies of TFV with NRTIs (ABC, FTC, 3TC, d4T, ddC, ZDV), NNRTIs (DLV, EFV, NVP, RPV), PIs (APV, IDV, NFV, RTV, SQV), and the integrase inhibitor

elvitegravir showed additive to synergistic antiviral effects {1469}, (Tabulated Summary 2.6.3.1.18, Reports C1278-00005 and PC-183-2004 and Reports 4379). No antagonistic interactions were observed for any of these 2 drug combinations. The 3 drug combinations of TFV, 3TC, and either ABC or ddI also showed no evidence of antagonistic interactions in PBMCs (Tabulated Summary 2.6.3.1.18, Reports PC-104-2005 and PC-104-2006).

The combination of TFV and FTC showed synergistic anti-HIV activity using either a laboratory-adapted strain of HIV-1 (Tabulated Summary 2.6.3.1.19, Report 14379) or a recombinant HIV-1 strain derived from a wild-type patient isolate (Tabulated Summary 2.6.3.1.9, Report PC-164-2002). Analyses of intracellular phosphorylation of FTC and TFV demonstrate increased phosphorylation to their active intracellular metabolites when both drugs are incubated together versus separately {8998}. These results suggest a possible mechanism for the observed antiviral synergy of these drugs in vitro (Tabulated Summary 2.6.3.1.5, Report PC-264-2003; {13981}).

The triple combination of FTC, RPV, and TFV demonstrated synergistic antiviral activity in vitro (Tabulated Summary 2.6.3.1.20, Report PC-264-2002).

2.4.2.5. Summary of Pharmacology

The HIV-1 NNRTI, RPV, and the NRTIs, FTC and TFV, have potent antiretroviral activity against wild-type and many drug-resistant strains of HIV-1 in vitro and in vivo. The combination of FTC, RPV, and TFV in 2-drug combination experiments showed additive to synergistic anti-HIV-1 activity, and synergistic anti-HIV-1 activity in 3-drug combination experiments. Additive antiretroviral activity would be expected given that the active metabolites of FTC and TFV compete with different natural substrates for incorporation into viral DNA during the reverse transcription step, and RPV would also be expected to be additive as it binds at a distinct binding pocket on RT to inhibit reverse transcription. The observation of synergistic anti-HIV-1 activity for the FTC/RPV/TFV combination suggests potentiation of the individual anti-HIV activities of these compounds within cells.

HIV-1 resistance to FTC develops readily in vitro with an M184V or I mutation in the RT. The M184V/I mutation results in high-level resistance to FTC and 3TC, but increased susceptibility to TFV. Nonnucleoside reverse transcriptase inhibitor mutations that emerged in HIV-1 under selective pressure of RPV included combinations of V90I, L100I, K101E, V106A/I, V108I, E138G/K/Q/R, V179F/I, Y181C/I, V189I, G190E, H221Y, F227C, and M230I/L, where E138R represented a newly identified NNRTI mutation. HIV-1 resistance to TFV is associated with the K65R mutation in the HIV RT that is slow to develop both in vitro and in vivo. The K65R mutation showed reduced susceptibility to FTC and 3TC; however, much higher fold resistance is associated with M184V/I for FTC, suggesting that this mutation is more clinically relevant for FTC.

In vitro resistance selection with the combination of TFV and FTC results first in the selection of M184V/I. These results predict that in the case of treatment failure in vivo, there would be step-wise development of resistance as is the case for most antiretroviral regimens,

with the M184V/I mutation developing more readily. In vitro resistance selection experiments performed with the combination of FTC + RPV + TFV resulted in HIV-1 with the M184I RT mutation by 47 days that was maintained with no additional mutations present by Day 74 using a dose escalation selection approach; and HIV-1 with the M184I RT or K65R mutation using a fixed-dose selection approach. No resistance mutations to RPV were observed in any of the FTC + RPV + TFV triple combination selections.

In a pooled analysis for subjects receiving rilpivirine in combination with emtricitabine/tenofovir DF in clinical trials C209 and C215 (Report PC-264-2005), there were 62 virologic failure subjects with resistance information available for 54 of those subjects. The amino acid substitutions associated with NNRTI resistance that developed most commonly in these subjects were: V90I, K101E, E138K/Q, Y181C, V189I, and H221Y. However, the presence of the substitutions V90I and V189I at baseline did not affect the viral response. The amino acid substitutions associated with NRTI resistance that developed in 3 or more subjects were: K65R, K70E, M184V/I, and K219E during the treatment period.

Nucleoside reverse transcriptase inhibitors carry a class labeling for mitochondrial toxicity; however, both FTC and TDF have shown a low potential for mitochondrial toxicity in long-term toxicity studies. The potential for mitochondrial toxicity of RPV was low by in vitro assessment of the inhibitory activity on human polymerase γ. However, as mitochondrial toxicity is generally less relevant for NNRTIs than NRTIs, and as RPV is not anticipated to significantly increase the exposure of FTC or TFV, the potential for exacerbating mitochondrial toxicity is low.

From in vitro data, pharmacokinetic studies in dogs (Module 2.6.4) and clinical experience (Module 2.7.2), there are no anticipated pharmacokinetic interactions between FTC, RPV, and TDF. Emtricitabine and TDF had little effect on vital organ systems in safety pharmacology studies. Rilpivirine has shown the potential for QT prolongation, an effect confirmed in a TQT study in healthy subjects. At the 25-mg dose of RPV, the observed change in QTcF was not considered clinically relevant, and the combination product is not anticipated to exacerbate the cardiovascular effect seen with RPV alone. No additional safety pharmacology studies are considered necessary with the FTC/RPV/TDF combination.

The absence of nonclinical safety studies with the combination is in accordance with the CHMP Guideline on the Non-Clinical Development of Fixed Combinations of Medicinal Products (EMEA/CHMP/SWP/258498/2005, January 2008). There are no anticipated clinically relevant pharmacokinetic or toxicological interactions expected in the FTC/RPV/TDF combination. The Scientific Advice Working Party agreed with the assessment that no further nonclinical studies are needed to support the FTC/RPV/TDF FDC based on the lack of relationship between the primary pharmacodynamics of RPV and the individual NRTIs (FTC and TDF); the toxicities of these compounds; the comprehensive nonclinical data set for FTC, RPV, and TDF; and the long-term safety from the Phase 2b Study TMC278-C204 (EMEA/CHMP/SAWP/670243/2009 corrigendum). Further, extensive clinical safety data are available for the approved drugs FTC, TDF, and the FTC/TDF FDC product Truvada. Additionally, data from the Phase 2b and Phase 3 studies with RPV and

Truvada support the use of these products (see Module 2.7). The clinical data, along with the lack of overlapping toxicity in animals, support the safety of the new combination product.

Overall, the pharmacodynamic and pharmacological assessment of FTC, RPV, and TDF supports the effective and safe use of these 3 agents together in combination therapy for HIV-1 disease.

2.4.3. PHARMACOKINETICS

The absorption, distribution, metabolism, and excretion of FTC, RPV, and TFV/TDF were evaluated in a variety of animal models. A summary overview of the relevant data for the individual products is provided in the sections that follow, and are listed in the pharmacokinetics Tabulated Summary 2.6.5.1.

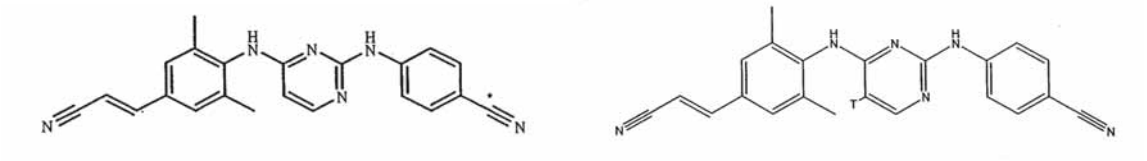
In addition, the nonclinical pharmacokinetics of TMC278 was investigated in a series of in vitro and in vivo studies and is described in detail in the Pharmacokinetics Written Summary (Module 2.6.4). In vivo studies were conducted in CD-1 and CB6F1-nonTgrasH2-transgenic mice, pigmented Long Evans and 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.

2.4.3.1. Analytical Methods

Each of the components of the FDC in biological, or in vitro samples, has been determined by LC/MS/MS and in some cases by HPLC as indicated in the study report. Each method was validated with regards to accuracy and precision, linearity, specificity, and limit of quantitation (Module 2.6.4, Pharmacokinetics Written Summary, Section 2.6.4.2).

TMC278 and/or its metabolites were determined in various in vitro and biological samples (plasma, urine, bile, and feces). Several studies were conducted with radiolabeled TMC278, which was labeled with ^{14}C or ^{3}H as outlined in Figure 1.

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



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

The tissue distribution of [^{14}C]TMC278 base was studied by QWBA. The total radioactivity in biological samples was measured by liquid scintillation counting. The metabolites were identified by cochromatography with authentic substances using different high performance

liquid chromatography methods and detection systems such as radiodetection and tandem mass spectrometry (LC-MS/MS).

A LC-MS/MS method was validated for the determination of TMC278 in mouse, rat, rabbit, dog, monkey ethylenediamine tetra-acetic acid anticoagulated plasma, and heparinized dog plasma.

2.4.3.2. Absorption and Pharmacokinetics

2.4.3.2.1. Emtricitabine

Single-dose pharmacokinetics of FTC have been studied in mice (Tabulated Summaries 2.6.5.3.A, 2.6.5.3.B and 2.6.5.3.C; Reports TEIN/93/0003, TEIN/93/0004, and IUW00101, respectively) rats {4570} and cynomolgus monkeys (Tabulated Summaries 2.6.5.3.D and 2.6.5.3.E; Reports TEZZ/93/0019 and IUW00301, respectively). In these species, FTC was rapidly and well absorbed with oral bioavailability ranging from 58% to 97% over the dose range of 10 to 600 mg/kg.

2.4.3.2.2. Rilpivirine

The transepithelial permeability of TMC278 was intermediate in human colon carcinoma-derived (Caco-2) cells. Passive transcellular diffusion is proposed as a mechanism for TMC278 intestinal absorption. TMC278 was not a substrate of P-glycoprotein (P-gp). TMC278 showed P-gp inhibitory properties with an apparent 50% inhibitory concentration (IC_{50}) of 9.2 µM (3.4 µg/mL). Therefore, inhibition of transepithelial permeation of P-gp substrates by TMC278 cannot be excluded (Module 2.6.4, Pharmacokinetics Written Summary, Section 2.6.4.3.2).

After oral administration of TMC278 base, the absolute oral bioavailability of TMC278 was 32%, 54%, 31%, and 24% to rats, rabbits, dogs and monkeys, respectively. Adding CA in the formulation administered to rats and dogs usually increased the exposure showing that the absorption of TMC278 is pH-dependent in these species as in humans (see Module 2.7.2 [Summary of Clinical Pharmacology Studies]).

After oral administration of TMC278 (base and HCl 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 and species including humans (see Module 2.7.2 [Summary of Clinical Pharmacology Studies] and Module 2.6.4 [Pharmacokinetics Written Summary], Sections 2.6.4.3.3 and 2.6.4.3.4), plasma concentrations of TMC278 increased dose-proportionally or more often less than dose-proportionally, due to low solubility. At very high dose levels in animals, no further increase in exposure was seen.

A summary of plasma pharmacokinetic parameters of TMC278 in animals following single or repeated oral administration of TMC278 or TMC278 base is presented in Table 2.

Table 2. Summary of Plasma Pharmacokinetic Parameters of TMC278 in Animals Following Single or Repeated Oral Administration of TMC278 or TMC278 Base

Species	TMC278 Formulation	Sampling Period	Sex/n	Dose (mg/kg/day)	C_{max} (µg/mL)	AUC[a] (µg.h/mL)
Mouse	TMC278 in HPMC (0.5% w/v)	Day 1	M/15	20	9.9	60
				60	23	239
				160	41	440
			F/15	20	13	61
				60	24	182
				160	38	345
		Week 28	M/9	20	9.8	76
				60	22	230
				160	36	505
			F/9	20	9.9	51
				60	29	278
				160	58	766
Rat	TMC278 base in PEG400/CA (10%)	Day 1	M/6	40	2.9	19
				120	6.4	53
				400	9.1	92
			F/6	40	6.5	32
				120	8.5	83
				400	17	160
		Day 175[b]	M/3	40	1.7	12
				120	3.0	35
				400	6.2	73
			F/5	40	6.6	50
			F/5	120	8.8	116
			F/6	400	16	244

CA = citric acid; HPMC = hydroxypropyl-methylcellulose; M = male; F = female; PEG400 = polyethylene glycol 400; NC = not calculated; ND = not determined; GD = day of gestation;

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

b Total dosing volume of 10 mL/kg was changed after Day 83 to 2 administrations of 5 mL/kg, with 1.5 hours between the 2 administrations

c AUC_{0-24h}

d After the first dosing

Table 2. Summary of Plasma Pharmacokinetic Parameters of TMC278 in Animals Following Single or Repeated Oral Administration of TMC278 or TMC278 Base (continued)

Species	TMC278 Formulation	Sampling Period	Sex/n	Dose (mg/kg/day)	C_{max} (µg/mL)	AUC[a] (µg.h/mL)
Rat	TMC278 in HPMC (0.5% w/v)	Day 1	M/9	40	1.6	19
				200	2.6	34
				500	4.5	45
				1500	6.1	58
			F/9	40	2.2	17
				200	4.2	40[c]
				500	6.5	63
				1500	7.0	81
		Week 39	M/9	40	0.82	6.3
				200	1.3	8.2
				500	1.8	14
				1500	2.2	18
			F/9	40	2.1	14
				200	4.7	41
			F/8	500	8.5	46
			F/9	1500	9.4	84
Pregnant rat	TMC278 base in PEG400/CA (10%)	Day 1 (GD 6)	F/6	40	4.9	33
				120	6.0	65
				400	14	182
		Day 11 (GD 16)	F/4	40	5.6	37
				120	7.2	63
			F/6	400	13	152
Juvenile rat (aged 25days)	TMC278 in HPMC (0.5% w/v)	Day 14	M/8	40	2.6	12
			M/7	120	3.7	34
			M/7	400	9.1	50
			F/8	40	5.8	18
			F/8	120	3.6	28
			F/7	400	7.3	53

CA = citric acid; HPMC = hydroxypropyl-methylcellulose; M = male; F = female; PEG400 = polyethylene glycol 400; NC = not calculated; ND = not determined; GD = day of gestation;

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

b Total dosing volume of 10 mL/kg was changed after Day 83 to 2 administrations of 5 mL/kg, with 1.5 hours between the 2 administrations

c AUC_{0-24h}

d After the first dosing

Table 2. Summary of Plasma Pharmacokinetic Parameters of TMC278 in Animals Following Single or Repeated Oral Administration of TMC278 or TMC278 Base (continued)

Species	TMC278 Formulation	Sampling Period	Sex/n	Dose (mg/kg/day)	C_{max} (µg/mL)	AUC[a] (µg.h/mL)
Pregnant rabbit	TMC278 base in HPMC (0.5% w/v)	Day 1 (GD6)	F/3	5	6.4	95[c]
				10	9.7	162[c]
				20	13	219[c]
		Day 14 (GD19)	F/3	5	6.7	105
				10	10	170
				20	15	232
Dog	TMC278 base in PEG400/CA (10%)	Day 1	M/4	5	0.70	11[c]
				10	0.90	15[c]
				40	2.4	37[c]
			F/4	5	0.75	9.7[c]
			F/5	10	1.2	15[c]
			F/4	40	2.5	41[c]
		Day 363	M/4	5	1.1	17
			M/2	10	1.3	24
			M/4	40	4.1	65
			F/4	5	1.5	19
				10	2.2	36
			F/3	40	5.5	61
Monkey	TMC278 in HPMC (1%)/Tween 20	Day 55	F/8	100 BID	0.14[d]	2.7
			F/7	250 BID	0.31[d]	4.6

CA = citric acid; HPMC = hydroxypropyl-methylcellulose; M = male; F = female; PEG400 = polyethylene glycol 400; NC = not calculated; ND = not determined; GD = day of gestation;

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

b Total dosing volume of 10 mL/kg was changed after Day 83 to 2 administrations of 5 mL/kg, with 1.5 hours between the 2 administrations

c AUC_{0-24h}

d After the first dosing

2.4.3.2.3. Tenofovir DF

Single-dose pharmacokinetics of TFV following oral administration of TDF have been examined in mice (Tabulated Summaries 2.6.5.4.Z and 2.6.5.3.N; Reports M990203-PK and 97-TOX-4331-008-PK, respectively), rats (Tabulated Summaries 2.6.5.4.BB, 2.6.5.4.CC, 2.6.5.4.DD and 2.6.5.4.EE; Reports 96-TOX-4331--003--PK; R2000036-PK; 97-TOX-4331-002-PK; and R990204, respectively), woodchucks (Tabulated Summary 2.6.5.3.O, Report W2000108), dogs (Tabulated Summaries 2.6.5.3.P, 2.6.5.4.GG, 2.6.5.4.HH and 2.6.5.4.II; Reports D2000076; 96-TOX-4331-004-PK; 98-TOX-4331-003-PK; and 97-TOX-4331-001-PK, respectively), and monkeys (Tabulated Summary 2.6.5.3.Q, Report P2000031). Following oral administration of TDF, absorption and conversion to TFV was rapid, with maximal concentrations of TFV in plasma reached between 0.25 to 1.5 hours

postdosing in all species, and declined in a biphasic manner. The observed terminal half life values were approximately 7, 9, and 60 hours in rats, monkeys, and dogs, respectively. The oral bioavailability of TFV following oral administration of TDF ranged from 20% to 46% in these species. No substantial gender differences were observed in the rate or extent of TFV appearance in plasma within any species.

2.4.3.2.4. Emtricitabine/Rilpivirine/Tenofovir DF

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, Report AD-264-2023, Module 2.6.4, Section 2.6.4.3.6). Previously, similar results were obtained in single-dose comparison of the exposure of FTC/RPV/TDF after oral administration of coformulated 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, Report AD-264-2001; Module 2.6.4, Section 2.6.4.3.6). No further nonclinical studies are considered necessary.

2.4.3.3. Repeat Dose Pharmacokinetics

2.4.3.3.1. Emtricitabine

The multiple-dose pharmacokinetic parameters for FTC were derived as part of the repeat-dose toxicity studies in mice (80 to 3000 mg/kg; Tabulated Summary 2.6.5.4.A [Report] TOX109 and Reports IUW01001, TOX599, and TOX628), rats (60 to 3000 mg/kg; Tabulated Summary 2.6.5.4.B [Report TOX108] and Report TOX097), and monkeys (40 to 2000 mg/kg; Tabulated Summaries 2.6.7.7.U, 2.6.7.7.V and 2.6.7.7.W; Reports TOX600, TOX627 and TOX032 respectively) for periods of 3 days to 104 weeks. In general, there were no significant differences 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.

2.4.3.3.2. Rilpivirine

The C_{max} and AUC values of TMC278 after repeated oral administration in various animal species used in the toxicology studies are summarized in Table 2. In general, after oral administration of RPV (base and HCl forms), plasma concentrations of TMC278 increased dose proportionally or more often less than dose proportionally, likely due to low solubility. At very high dose levels in animals, no further increase in exposures was seen.

2.4.3.3.3. Tenofovir DF

The multiple-dose pharmacokinetics of TFV were characterized in toxicokinetic studies following oral administration of TDF in mice (Tabulated Summary 2.6.5.4.Z, Report M990203-PK), rats (Tabulated Summaries 2.6.5.4.BB, 2.6.5.4.CC, 2.6.5.4.DD and

2.6.5.4.EE; Reports 96-TOX-4331-003-PK, R2000036-PK, 97-TOX-4331-002-PK, and R990204), dogs, (Tabulated Summaries 2.6.5.4.GG, 2.6.5.4.HH, 2.6.5.4.II and 2.6.5.4.FF; Reports 96-TOX-4331-004-PK, 98-TOX-4331-003-PK, 97-TOX-4331-001-PK, and 96-TOX-4331-001-PK), and monkeys (Tabulated Summary 2.6.5.4.JJ, Report P2000078-PK). No apparent changes in exposure were observed after repeat dosing in mice (100 to 1000 mg/kg), rats (30 to 1000 mg/kg), or monkeys (30 to 600 mg/kg) for periods of 56 days to 104 weeks. In contrast, dogs showed a 2- to 3-fold increase in exposure over time and generally reached steady-state by Day 28, with daily TDF doses of 10 or 30 mg/kg.

2.4.3.3.4. Emtricitabine/Rilpivirine/Tenofovir DF

No additional repeat-dose pharmacokinetic studies were considered warranted with the combination of FTC, RPV, and TDF in view of the single-dose data in dogs with the bilayer FTC/RPV/TDF formulation that showed comparable exposure to all 3 individual agents; the lack of anticipated pharmacokinetic interactions between the 3 agents; and the extensive clinical experience with the components in antiretroviral combination therapy for the treatment of HIV-1 infection.

2.4.3.4. Distribution

2.4.3.4.1 Protein Binding

The protein binding of FTC was very low (< 5%) in mouse, rabbit, monkey, and human plasma (Tabulated Summary 2.6.5.6.A, Report TBZZ/93/0025). Similarly, the protein binding of TFV was very low (< 10 %) in the plasma and serum of humans and all other species examined (Report P0540-00039).

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% (Tabulated Summary 2.6.5.6.B, Report TMC278-NC112). TMC278 was extensively bound to human albumin (99.5% at the physiological protein concentration of 4.3% and irrespective of the TMC278 concentration) and to lesser extent to α_1-acid glycoprotein (48.8% at the physiological protein 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. The distribution of TMC278 to red blood cells (RBCs) is limited in all species (Module 2.6.4, Section 2.6.4.4.1.2).

Emtricitabine and TFV exhibit very low protein binding, and the protein binding of RPV has been well characterized, so no further studies have been conducted with the FTC/RPV/TDF FDC combination.

2.4.3.4.2 Tissue Distribution

The tissue distribution of [^{14}C]FTC was characterized in rats and cynomolgus monkeys after a single oral dose of 200 mg/kg (Tabulated Summaries 2.6.5.5.A and 2.6.5.8.B;

Reports TOX092 and TOX063, respectively). Emtricitabine was widely distributed in the body, with measurable concentrations found in all tissues within 1 hour following oral administration. Tissue concentrations generally declined in parallel with plasma concentrations, with no indication of accumulation in any tissue examined. Virtually no radioactivity remained in the body at 72 hours after dosing. The highest concentrations of FTC were found in the kidneys and liver. Concentrations in CNS tissues were 2% to 10% of the concentration in plasma.

The tissue distribution of TMC278 in rats has been studied using QWBA following single oral administration of [^{14}C]TMC278 (40 mg/kg). Studies were performed in pigmented Long Evans rats and pregnant female Sprague Dawley rats. Distribution to placenta and fetuses was examined in the latter study (Module 2.6.4, Pharmacokinetics Written Summary, Section 2.6.4.4.3.3).

In rats, tissue distribution of [^{14}C]TMC278 base 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 base 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, suggesting that the placenta presents a partial barrier for TMC278 and/or its metabolites.

Following single intravenous administration of [^{14}C]TFV in male rats, the highest radioactivity concentrations were found in the kidney, liver, urine, and large intestine (Tabulated Summary 2.6.5.5.D, Report 95-DDM-1278-002). One hour following an oral dose of [^{14}C]TDF (10 mg/kg) in dogs, radioactivity was detected in all tissues, except brain. The majority of the radioactivity was present in the contents of the GI tract, jejunal tissue, and liver (> 66%). ^{14}C-radioactivity concentrations were highest in bile, kidney, liver, and jejunum. By 6 hours postdosing, radioactivity levels were significantly decreased in all tissues. At 24 hours postdosing, the last time point sampled, the highest concentrations of radioactivity were present in kidney, liver, and the intestinal contents (Tabulated Summary 2.6.5.5.E, Report 97-DDM-4331-001).

Tissue distribution studies have not been conducted with the FTC/TDF or FTC/RPV/TDF combinations as each of the drugs in the combination tablet has been evaluated thoroughly and a distribution interaction is unlikely.

2.4.3.5. Metabolism

The metabolism of FTC, RPV, and TFV has been well characterized. In addition, the potential for drug interactions based on in vitro intracellular metabolism and the effect on drug metabolizing enzymes has been well studied.

2.4.3.5.1. Intracellular Metabolism

Emtricitabine and TFV are analogues of 2 different nucleosides, cytosine and adenosine, respectively, and do not share a common intracellular metabolism pathway. In experiments where both drugs were incubated together at concentrations higher than achieved in the plasma (10 μM), the intracellular phosphorylation of FTC and TFV demonstrate increased phosphorylation to their active intracellular metabolites when both drugs are incubated together (Tabulated Summary 2.6.3.1.4, Report PC-164-2001).

2.4.3.5.2. In Vitro Metabolism

Emtricitabine and TFV were not subject to significant metabolism by cytochrome P450 (CYP450) isozymes. CYP3A4 was demonstrated to be the sole CYP450 isoenzyme responsible for the metabolism of FTC to form 1 minor metabolite (~1%). Microsomal incubations in the presence and absence of selective inhibitors of various CYP450 isoforms confirmed the low rate of FTC metabolism, and also suggested the possible involvement of FAD-containing monooxygenase enzymes in the metabolism of FTC (Report 15396 v1).

In vitro, TMC278 metabolism was studied in hepatocytes and liver subcellular fractions (microsomes and 12,000 g supernatant fractions) of mice, rats, guinea pigs, rabbits, dogs, monkeys, and humans. In each species, a large number of metabolites were detected. The main metabolic pathways proposed on the basis of in vitro studies were consistent with those observed in vivo. The minor pathways such as 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), were not observed in vitro in rodents, but were observed in vivo (Module 2.6.4, Pharmacokinetics Written Summary, Section 2.6.4.5.1).

The potential for TDF to induce rat hepatic CYP450 enzymes was evaluated in microsomes prepared from livers of rats necropsied at the end of a 28-day toxicity study (Tabulated Summary 2.6.5.11.E, Report R2001024). The rats were dosed with either vehicle control article or TDF at 40 or 400 mg/kg daily doses. No effect was observed on the 6β--hydroxylation of testosterone (CYP450 3A). A small enhancement (less than 2-fold) of CYP 1A1 mediated O-deethylation of phenacetin and CYP 2B mediated formation of androstenedione was observed at the high dose.

In vitro metabolism studies have not been conducted with FTC/RPV/TDF, as each of the drugs in the combination tablet have been evaluated thoroughly and any interactions based on in vitro metabolism are unlikely.

2.4.3.5.3. In Vitro Drug Interactions

Cytochrome P450 Interactions

Emtricitabine was not an inhibitor for CYP1A2, 2A6, 2B6, 2C9, 2C19, 2D6, 2E1, and 3A4/5 in human liver microsomes. Emtricitabine also did not show inhibition of 7-hydroxycoumarin glucuronidation (Report 15247). At concentrations substantially higher

(~ 300-fold) than those observed in vivo, TFV or TDF did not significantly inhibit CYP3A4, CYP2D6, CYP2C9, CYP2E1, or CYP1A in human liver microsomes (Tabulated Summary 2.6.5.11.D, Report V990172-104). The potential for CYP mediated interactions involving TFV and FTC with other coadministered medicinal products is low.

In vitro, the CYP3A4 isoenzyme played a major role in the biotransformation of TMC278. Therefore, some effects of drugs modulating CYP3A4 enzyme activity on plasma concentrations of TMC278 were expected in humans. Such effects were seen in drug-drug interaction trials with CYP3A4 inducers such as rifampicin and rifabutin, which both decreased the exposure of TMC278; and with CYP3A4 inhibitors such as ketoconazole and ritonavir-boosted protease inhibitors, which increased the exposure of TMC278 (Module 2.7.2, Summary of Clinical Pharmacology Studies, Sections 2.7.2.2.2.8.3, 2.7.2.2.2.8.4, 2.7.2.2.2.8.6, 2.7.2.2.2.8.7 and 2.7.2.2.2.8.8).

In vitro, TMC278 was an inhibitor of CYP3A4 (IC_{50} > 4.2 µM [1.5 µg/mL]) and CYP2C19 (IC_{50} < 0.06 µM [0.021 µg/mL]). Further in vitro data indicated a possible inhibitory effect of TMC278 on the metabolism of substrates of CYP3A4, e.g. clarithromycin (IC_{50} = 2 µM, 0.72 µg/mL), norethindrone (IC_{50} = 3.9 µM, 1.4 µg/mL), and sildenafil (IC_{50} = 1.4 µM, 0.5 µg/mL); and on the metabolism of omeprazole (IC_{50} = 12 µM, 4.3 µg/mL), a substrate of CYP3A4 and CYP2C19. However, no impact was expected in the clinic taking into account the mean C_{max} of 0.13 µg/mL (Week 4 to 8) obtained in HIV-infected treatment-naive patients at 25 mg once daily, the recommended dose. In vivo, some drug-drug interaction studies were performed using substrates of CYP3A4 (atorvastatin, omeprazole [omeprazole sulfone pathway], and norethindrone) and a substrate of CYP2C19 (omeprazole [hydroxyomeprazole pathway]). No increase in exposure of these 3 compounds with TMC278 was observed (Module 2.7.2, Summary of Clinical Pharmacology Studies, Sections 2.7.2.2.2.8.10, 2.7.2.2.2.8.12 and 2.7.2.2.2.8.14). In vitro, TMC278 is an inhibitor of CYP2C8 and CYP2C9 with K_i values of 10 µM (3.7 µg/mL) and 1.7 µM (0.62 µg/mL), respectively. Taking into account the mean C_{max} of 0.13 µg/mL obtained in HIV-infected subjects, inhibition of CYP2C8 and CYP2C9 by TMC278 is not expected.

For other CYPs (CYP1A1, CYP2A6, and CYP2D6), taking into account the mean C_{max} of 0.13 µg/mL, inhibition by TMC278 is unlikely. TMC278 might be a very weak inducer of CYP1A2 and CYP2B6.

In addition, in vitro, TMC278 was a moderate inducer of CYP3A4 and CYP2C19 activities. In the clinic, a modest decrease in exposure of atorvastatin, norethindrone, and CYP2C19-mediated metabolism of omeprazole was observed at the high dose of TMC278 (150 mg once daily; Module 2.7.2, Summary of Clinical Pharmacology Studies, Sections 2.7.2.2.2.8.10, 2.7.2.2.2.8.12 and 2.7.2.2.2.8.14). TMC278, at the recommended dose of 25 mg once daily, does not inhibit or induce CYP3A4 and is unlikely to result in a clinically relevant interaction for CYP2C19.

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 of the CYP4A family (up to 25-fold in mice and up to 4.7-fold in rats). Additionally, TMC278 induced uridine

diphosphate-glucuronosyltransferase activity in mice (up to 2.3-fold) and to a lesser extent in rats (up to 1.3-fold only at a high dose in males). In dogs, treatment with TMC278 did not result in any enzyme induction. The main objective to generate these data was to explain the toxicity findings in animals. The findings in carcinogenicity studies could be associated with the observed enzyme induction.

The clinical drug-drug interaction studies are described in detail in the Clinical Pharmacology summary (Module 2.7.2, Summary of Clinical Pharmacology Studies, Section 2.7.2.3.1.9).

Transporter Drug Interactions

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 the human organic anion transporter type 1 (hOAT1) and multidrug resistance protein type 4 (MRP4) acting in series as the major uptake and efflux transporters in proximal tubules, respectively (Tabulated Summaries 2.6.5.14.D, 2.6.5.14.E and 2.6.5.14.F; Reports PC-103-2001, AD-104-2001, AD-104-2002), {2520}, {7299}, {8418}, {9318}, {10260}, {11309}. Human organic anion transporter type 3 may play a secondary role in the tubular uptake of TFV. Neither P-gp nor multidrug resistance protein type 2 (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 (Tabulated Summaries 2.6.5.14.G and 2.6.5.14.H; Reports PC-104-2010 and PC-104-2011, respectively), {9863}. Furthermore, the PIs atazanavir, lopinavir, and ritonavir did not exhibit any effect on the active cellular elimination of TFV mediated by the 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 (Tabulated Summary 2.6.5.14.I, 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, tenofovir disoproxil, is limited by a combination of

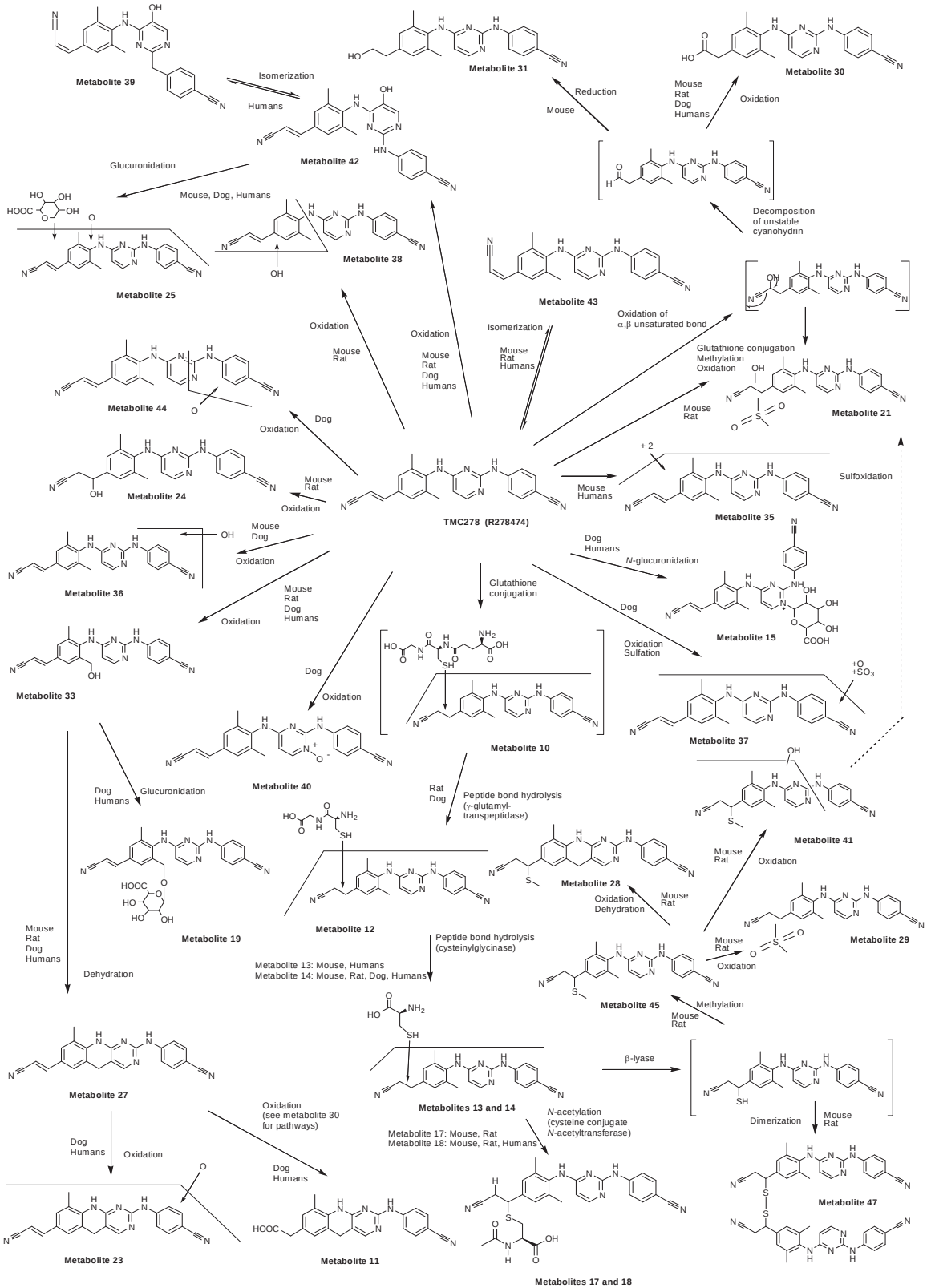
P-gp mediated efflux transport and esterase degradation {5939}. Further studies in human intestinal S9 fractions, the Caco-2 cell line, and Madin-Darby canine kidney (MDCKII) cells stably transfected with the human gene that encodes P-gp have suggested that the relative ability of PIs to inhibit esterase 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, Report AD-104-2010), {11255}.

2.4.3.5.4. In Vivo Metabolism

Emtricitabine was not extensively metabolized and is eliminated primarily as unchanged drug by renal excretion in mice, rats, and cynomolgus monkeys. Over 90% of the ^{14}C-radioactivity in mouse and rat urine and 64% of the radioactivity in monkey urine was unchanged drug. Only trace levels of metabolites were found in feces {4570}, {4251} (Tabulated Summaries 2.6.5.8.A, 2.6.5.8.C and 2.6.5.8.B; Reports TEIN/93/0015, TEIN/93/0016, and TOX063, respectively). In all 3 species, metabolism accounted for only a minor percentage of FTC elimination. Emtricitabine is subject to Phase I metabolism (oxidation to a diastereomeric sulfoxide) and to some direct conjugation (glucuronidation of hydroxymethyl group) as minor metabolic routes.

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 predominated, whereas in dogs and man, oxidation of TMC278 was predominant. No unique human metabolites were observed (Figure 2). In plasma of animals and human, unchanged TMC278 was more abundant than all metabolites combined.

Figure 2. In Vivo Metabolic Pathways of TMC278 in Animals and Humans



Tenofovir was primarily eliminated by renal excretion of unchanged parent drug and was not extensively metabolized in rats. Following oral administration of TDF in rats, tenofovir soproxil accounted for 37% of the radioactivity in the 0.5- to 2.2-hour bile fraction, but 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 (Tabulated Summaries 2.6.5.12.A and 2.6.5.12.B; Reports 96-DDM-1278-001 and 97-DDM-4331-003).

The available data indicate no significant potential for metabolic interaction among the individual components based on metabolism. Consequently, no additional studies with the combination product were conducted.

2.4.3.6. Excretion

The primary route of elimination of FTC was via renal excretion of parent drug after oral and intravenous administration in mice, rats, and cynomolgus monkeys {4570}, (Tabulated Summaries 2.6.5.8.A, 2.6.5.8.B, 2.6.5.8.C and 2.6.5.5.A; Reports TEIN/93/0015, TOX063, TEIN/93/0016, and TOX092, respectively). The majority of the FTC recovered in the feces after oral administration most likely represents unabsorbed drug, rather than biliary excretion. Although FTC is metabolized to only a minor extent, its metabolites are also excreted via the kidneys.

The routes and extent of TMC278 excretion were studied after single oral administration of [^{14}C]TMC278 base in CD-1 mice (20 and 320 mg/kg), Sprague-Dawley rats (40 mg/kg), dogs (5 mg/kg), and humans (150 mg). In all animal species, the predominant route of [^{14}C]TMC278 excretion was via feces and amounted to 87% to 96%, 93%, 95% and 85% of the administered radioactive dose in mice, rats, dogs and humans, respectively. [^{14}C]TMC278 was mainly excreted in feces as unchanged TMC278 in mice (33% to 34% at 320 mg/kg), in rats (43% to 47%), and in dogs (43%) by 48 hours after dosing. Only in mice at 20 mg/kg, 1 metabolite M42 was the most abundant in feces. Urinary excretion of radioactivity was less than 4.2% in mice, rats, and dogs, but was slightly higher (6.1%) in humans. In all species, including humans, the amount of unchanged TMC278 in urine was negligible. Therefore, the renal clearance of TMC278 is negligible. TMC278 was also excreted in the bile in rats (18% and 25% of the administered radioactivity in restrained and nonrestrained animals, respectively). The amount of unchanged TMC278 excreted in bile was negligible (about 0.2 % within 24 hours).

Renal excretion is the primary systemic route of elimination of TFV in all preclinical species tested. The majority of radioactivity was recovered in the urine in rats and dogs (Tabulated Summaries 2.6.5.12.A and 2.6.5.12.C; Reports 96-DDM-1278-001 and 97-DDM-4331-001, respectively) following intravenous administration of [^{14}C]TFV. Less than 0.3% of radioactivity was recovered in bile following intravenous administration of TFV to bile duct cannulated rats and dogs. The radioactivity recovered in the feces following oral administration of [^{14}C]TDF represents primarily unabsorbed drug.

Given that the excretion routes of the individual components have been well characterized and that FTC, RPV, and TDF have no clinical interaction with respect to renal elimination, no further preclinical excretion studies have been performed.

2.4.3.7. Distribution in Pregnant or Nursing Animals

2.4.3.7.1 Distribution in Pregnant Animals

Pharmacokinetic parameters for TDF and FTC in pregnant animals appeared to be generally similar to those reported for nonpregnant animals. Placental transfer studies were conducted for TFV (rhesus monkeys) and FTC (mice and rabbits). Both drugs are transferred across the placenta, but did not concentrate in fetal tissues. Fetal/maternal exposure ratios, determined on appropriate gestation days (GDs) by the concentrations of TFV in serum and FTC in plasma and umbilical cord blood, were ≤ 0.5 (see Section 2.4.3.2 and Tabulated Summaries 2.6.5.5.I, 2.6.5.7.A and 2.6.5.7.B; Reports 96-DDM-1278-005, TOX103; and TOX038; TOX038 [12/2001], respectively).

These studies demonstrated that the fetus was exposed to FTC and TFV in all species tested.

The tissue distribution of TMC278 in rats has been studied using QWBA following single oral administration of [^{14}C]TMC278 (40 mg/kg). Studies were performed in pigmented Long Evans rats and pregnant female Sprague Dawley rats. Distribution to placenta and fetuses was examined in the latter study (Module 2.6.4, Pharmacokinetics Written Summary, Section 2.6.4.4.3.3).

2.4.3.7.2 Excretion into Breast Milk

No studies have been conducted to assess directly the excretion of TMC278 into milk. However, in the QWBA study in pregnant Sprague Dawley rats (see Module 2.6.4, Pharmacokinetic Written Summary, 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 pre- and postnatal developmental study (Tabulated Summary 2.6.7.14.B, Report 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 (LD7), 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 LD7 was approximately 20- to 35-fold lower than in pups directly dosed by oral gavage on Day 25 of age (see Module 2.6.4, Pharmacokinetic Written Summary, Section 2.6.4.3.4.5).

Tenofovir was excreted into the breast milk of lactating rats and rhesus monkeys (Reports R990202-PK; P2000116). The TFV milk/plasma concentration percentages ranged from 11% to 23.5% in rats and 18.6% to 21.5% in rhesus monkeys. Excretion into milk has not been evaluated for FTC.

The proposed Prescribing Information for the combination tablet indicates that mothers must be instructed not to breastfeed if they are receiving the combination tablet (see Section 2.4.5.2).

2.4.3.8. Summary of Pharmacokinetics

A comprehensive nonclinical program defining the absorption, disposition, metabolism, and drug interaction potential of FTC, RPV, and TFV/TDF has been completed. The nonclinical pharmacokinetic and disposition studies discussed in this section provided an adequate basis for comparing and interpreting results from toxicology and clinical studies.

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.

Emtricitabine does not undergo extensive first-pass or systemic metabolism, and is eliminated primarily by renal excretion of unchanged drug. The total body clearance of FTC exceeds the glomerular filtration rate, suggesting the drug is actively secreted by renal tubules into the urine. In all animal species, the predominant route of [^{14}C]TMC278 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 indication that TMC278 was excreted in milk. Renal excretion is the primary systemic route of elimination of TFV in all preclinical species tested.

Single-dose pharmacokinetic studies in dogs demonstrate that comparable exposures for each component can be achieved through coformulation in a bilayer tablet relative to the clinical formulations administered separately. More compelling support for this assumption is derived from the human clinical data (see Module 2.7.2).

No additional pharmacokinetic studies are considered warranted with the combination of FTC, RPV, and TDF in view of the results of extensive nonclinical and clinical pharmacokinetic studies of the 3 components.

2.4.4. TOXICOLOGY

Comprehensive nonclinical programs with FTC, RPV, and TDF have been completed. These studies have characterized the acute toxicity, subchronic/chronic toxicity, mutagenicity, carcinogenicity, and reproductive toxicity of each the individual agents, and the FTC/TDF combination. These studies are described in detail in Module 2.6.6 (Toxicology Written Summary), and are listed in Tabulated Summary 2.6.7.1. The nonclinical toxicology studies

discussed in this section provide an adequate basis for comparing and interpreting results from clinical studies.

2.4.4.1. Acute Toxicity

Emtricitabine has demonstrated minimal acute toxicity in rodents (oral LD_{50} > 4000 mg/kg and intravenous LD_{50} > 200 mg/kg; Tabulated Summary 2.6.7.5.A, Reports TTEP/93/0020, TTEP/93/0023, TTEP/93/0021, and TTEP/93/0024).

No formal single-dose studies were conducted with TMC278 as single-dose evaluations were part of the initial oral dose range-finding studies or, in the case of mice, part of the bone marrow micronucleus test.

In mice, no relevant effects were noted following an oral single dose of up to 1600 mg/kg TMC278 base in PEG400 + CA, the maximum feasible dose in this vehicle for this species. Exposures at 1600 mg/kg were similar to those at 400 mg/kg, indicating saturation of absorption (Tabulated Summary 2.6.7.9.B, Report TMC278-Exp5538).

Rats dosed with an oral maximum feasible single dose of 800 mg/kg TMC278 base in PEG400 showed no treatment-related effects (Tabulated Summary 2.6.7.6.A).

Dogs that received an oral maximum feasible dose of 80 mg/kg TMC278 base in PEG400 or PEG400 + CA vomited more frequently and had softer stool than dogs treated with the vehicle. No other effects were noted (Tabulated Summary 2.6.7.6.A).

The single dose no observed adverse effect level (NOAEL) of TDF in rats was 1500 mg/kg (Tabulated Summary 2.6.7.5.C, Report R990200). The no observed effect level (NOEL) in dogs given a single dose of TDF was 30 mg/kg (treatment-related lesions in the kidneys at 90 and 270 mg/kg [Tabulated Summary 2.6.7.5.C, Report D990201]).

No single-dose studies have been performed with the combination of FTC, RPV, and TDF. Single-dose toxicity studies indicated that all 3 agents had low acute toxicity. With no overlapping toxicities, coadministration is unlikely to change the acute toxicity profile.

2.4.4.2. Subchronic and Chronic Toxicity

2.4.4.2.1. Emtricitabine

A series of Good Laboratory Practice (GLP) oral repeat-dose toxicity studies were conducted with FTC in mice (1 month [Tabulated Summary 2.6.7.7.B, Reports TOX599 and TOX599 addendum]; Report TOX118] and 6 months [Tabulated Summaries 2.6.7.7.C and 2.6.7.7.D, Reports TOX022 and TOX628, respectively]), rats (3 months [Tabulated Summary 2.6.7.7.G, Report TOX097]), and cynomolgus monkeys (1 month [Tabulated Summary 2.6.7.7.U, Reports TOX600 and TOX600 addendum], 3 months [Tabulated Summary 2.6.7.7.V, Report TOX627], and 1 year [Tabulated Summary 2.6.7.7.W, Report TOX032]). Effects associated with the administration of FTC in the toxicology studies were confined to high-dose groups. Changes in RBC parameters, interpreted as a mild, reversible anemia

occurred at the highest dose in several studies (i.e., 1- and 6-month mouse; 3-month rat; and 1-year monkey). The NOELs for the longest treatment period in each species were 500 mg/kg/day in mice (6 months), 600 mg/kg/day in rats (3 months), and 200 mg/kg/day in monkeys (1 year). The exposures based on plasma AUC values at the NOEL doses in the animals were approximately 30- to 50-fold (mice), 30-fold (rats), and 10-fold (monkeys) higher than the AUC in patients treated with FTC at 200 mg once daily. For an overview of all repeat-dose toxicity studies, see Tabulated Summary 2.6.7.1.

2.4.4.2.2. Rilpivirine

Systemic toxicity of TMC278 base or TMC278 after repeat dosing was studied in mice, rats, rabbits, dogs, and cynomolgus monkeys. Pivotal repeat-dose studies were conducted in mice (3 months), rats (1 and 6 months), dogs (1, 3, 6, and 12 months), and cynomolgus monkeys (8 weeks).

The mouse study served as dose range finder for the carcinogenicity study in that species. The 5-day rabbit study was a pilot for the rabbit embryo-fetal toxicity study. The studies in rats and dogs were designed to investigate the toxicity profile of TMC278 and to support clinical studies and marketing authorization. A further mechanistic juvenile toxicity study was done in immature female cynomolgus monkeys. The reversibility upon repeat dosing was investigated in rats and dogs.

All studies in rats and dogs were conducted with TMC278 base dissolved in PEG400, usually with CA. In the rabbit study, TMC278 base was suspended in 0.5% (m/v) HPMC in water, as rabbits do not tolerate PEG400. Following the selection of the HCl salt as the chemical form to be marketed, 1-month studies in rats and dogs compared the kinetics and toxicity of TMC278 base and TMC278. The studies in mice and the studies in cynomolgus monkeys were conducted with TMC278 suspended in aqueous HPMC. For an overview of all repeat-dose toxicity studies, see Tabulated Summary 2.6.7.1.

In this section only pivotal studies are summarized.

Mice

The doses of TMC278 in the 3-month oral gavage study with CD-1 mice were 20, 80, or 320 mg/kg/day (Tabulated Summary 2.6.7.7.E, Report TMC278-NC119). There were no mortalities. Effects occurred almost exclusively at 320 mg/kg/day. No clinical signs were noted, except abdominal distention from Week 6 of treatment onwards. Body weight and body weight gain were increased in line with higher food consumption throughout the dosing period. Red blood cell count, hemoglobin, and hematocrit were lower; in females, associated with an increase in reticulocyte count. In males, leukocyte count was decreased. Serum alkaline phosphatase (ALP) and alanine aminotransferase (ALT) activities, and calcium and inorganic phosphate concentrations were increased. Females showed an increase in serum concentrations of urea, cholesterol, total protein, and albumin. Liver weight was increased in a dose-related fashion and showed hepatocellular hypertrophy with a dose-related increase in incidence and severity at 80 and 320 mg/kg/day. At 320 mg/kg/day, this effect was

associated with hepatocellular vacuolization, single cell necrosis, and pigmentation and proliferation of Kupffer cells; all to a slight to moderate degree. Moreover, electron microscopy revealed peroxisome proliferation. In the kidney, female mice showed minimal to moderate nephropathy characterized by slight to marked multifocal tubular basophilia; minimal to slight glomerulopathy (atrophic glomeruli with thickened Bowman's capsule amidst basophilic tubules); minimal to moderate mononuclear cell infiltration; minimal to slight interstitial fibrosis; minimal tubular dilatation; and slight cortical mineralization. In adrenal glands, a marginally increased incidence of swollen cells and/or cells with dense cytoplasm in the zona fasciculata was noted in males, whereas females showed a marginal decrease of a clear X-zone with increased brown degeneration in that zone. Ovaries showed a marginal decrease of the number and generations of corpora lutea. Also, granulocyte infiltration in the endometrium was marginally decreased. It cannot be excluded that these gonadal effects indicate a reduced cyclic activity. Moreover, extramedullar hematopoiesis in liver (marginal) and spleen (slight to moderate); and slight to moderate increase of the myeloid/erythroid ratio in bone marrow was noted. These effects are probably associated with the effects on RBC parameters.

Based on the liver findings in animals administered 80 mg/kg/day, the NOAEL was considered to be 20 mg/kg/day, associated with AUC values of 80 and 61 µg.h/mL for males and females, respectively.

Rats

TMC278 base formulated in PEG400 + CA was administered once daily, by oral gavage, for 1 month to Sprague Dawley rats at 0 (water, negative control), 0 (vehicle), 10, 40, or 160 mg/kg/day in 10 mL/kg, with satellite animals in each group for the plaque forming cell (PFC) assay with sheep RBCs (Tabulated Summary 2.6.7.7.H, Report TMC278-Exp5692). There were no mortalities associated with TMC278, no relevant clinical signs, no effects on body weight or food consumption, no treatment-related ophthalmic effects, and no effects on the PFC assay. Higher thyroid gland and liver weights compared to the vehicle group were recorded in the groups dosed with 40 and 160 mg/kg/day. The increase in thyroid gland weight was associated with minimal follicular hypertrophy. The weight of the pituitary gland was slightly increased in animals dosed with 160 mg/kg/day.

The NOAEL was established at 10 mg/kg/day, associated with AUC values of 7.2 and 14 µg.h/mL for males and females, respectively. Moreover, the absence of effects in the PFC assay indicated that TMC278 has no immunotoxic potential.

In the 6-month study, TMC278 base formulated in PEG400 + CA was administered once daily, by oral gavage, to Sprague Dawley rats at 0 (vehicle), 40, 120, or 400 mg/kg/day in 10 mL/kg (Tabulated Summary 2.6.7.7.I, Report TMC278-NC101). Increasing difficulties with the daily administration by gavage, morbidity, and deaths started to occur after the first 2 months of dosing, with comparable incidences in all groups, including controls. For this reason, the dosing regimen was changed from once daily to twice daily (BID) dosing from Day 84 onwards. There were no relevant effects on ophthalmic examinations, body weight, or food consumption.

Hematology changes included increases in activated partial thromboplastin time (APTT) and prothrombin time (PT) in males of all groups, without a dose-related trend, at all sampling times, including the end of the 1-month recovery period. Red blood cell count, hemoglobin, and hematocrit of males at 400 mg/kg/day were reduced slightly. The eosinophil count in females of all groups was decreased, without a clear dose-related trend. Red blood cell parameters and eosinophil counts showed complete recovery after termination of dosing.

Serum chemistry showed increases in total protein (females at 400 mg/kg/day), albumin (both sexes at 120 and 400 mg/kg/day), inorganic phosphate (females of all groups and males at 120 and 400 mg/kg/day), urea (males at 120 and 400 mg/kg/day), and creatinine (males of all groups). Alkaline phosphatase activity was increased in males at 120 and 400 mg/kg/day. Decreases in triglycerides and total bilirubin were recorded in all groups. All serum chemistry changes showed complete reversibility at the end of the recovery period. Urinalysis showed no effects.

Serum thyroid stimulating hormone (TSH) concentrations were increased in all dose groups, associated with a decrease of serum thyroxine (T_4) concentrations. In contrast, triiodothyronine (T_3) concentrations showed lesser and equivocal effects; decreased in males at 40 mg/kg/day and increased in males at 400 mg/kg/day. At the end of the postdosing period, all parameters, except T_4, showed recovery. Hormone concentrations showed an overall trend indicating a decrease of corticosterone levels and an increase in adrenocorticotropic hormone (ACTH) and progesterone concentrations at 120 and 400 mg/kg/day. No trend of any effect was observed at the end of the recovery period.

At necropsy, increased liver weight was associated with hepatocellular hypertrophy at 120 and 400 mg/kg/day. Thyroid gland weight was increased in all groups associated with a dose-related increase of diffuse follicular hypertrophy. Reversibility of the effect in females was not complete at 1 month after dosing. In the pituitary gland of males from all groups, the number of swollen/vacuolated cells in the pars distalis was increased. These cells are known to produce TSH. This effect was not noted in animals killed at the end of the postdosing period, indicating complete recovery. In animals treated with 400 mg/kg/day, the macrophages that spontaneously form aggregates in the mesenteric lymph nodes had a swollen-vacuolated appearance. This effect showed no recovery.

In view of the effects on coagulation parameters and the thyroid and pituitary glands observed at the lowest dose of 40 mg/kg/day (associated with AUC values of 12 and 50 µg.h/mL in males and females, respectively), a NOAEL could not be established in this study.

Dogs

TMC278 base formulated in PEG400 + CA was administered once daily, by oral gavage, for 1 month to beagle dogs at 0 (water, negative control), 0 (vehicle), 5, 10, or 40 mg/kg/day in 1 mL/kg (Tabulated Summary 2.6.7.7.N, Report TMC278-Exp5650). Reversibility of the effects was evaluated in a 1-month postdosing period. No mortalities occurred in this study.

At 40 mg/kg/day, body weight loss and reduced body weight gain were noted and were associated with a reduced food intake.

Red blood cell count, hemoglobin, and hematocrit were lower and white blood cell (WBC) count was higher at 40 mg/kg/day. Albumin, total protein, and triglyceride concentrations at 10 and 40 mg/kg/day were lower. The concentrations of cholesterol and total bilirubin, as well as the activities of ALP and ALT, at 40 mg/kg/day were higher. Progesterone concentrations were increased in a more or less dose-related fashion in all groups. The AUCs of ACTH were increased at the end of the dosing period. The AUCs of cortisol showed a tendency to decrease at 10 and 40 mg/kg/day.

In the adrenal cortex, the number of swollen cells with dense cytoplasm and reduced Oil red O-staining was increased at 10 and 40 mg/kg/day. Weight of ovaries was increased in all groups in a dose-related way. The female genital tract and mammary glands showed increased activation at 10 and 40 mg/kg/day. In the ovaries, corpora lutea were detected in 2 animals at 10 mg/kg/day and in 1 animal at 40 mg/kg/day. More prominent tertiary follicles were noted in all test article-treated animals that had not ovulated at the end of dosing period. In mammary glands, increased alveolar development was observed. In liver, a minimal to moderate centrilobular perivascular inflammatory reaction was observed in males. Minimal increase in the number of multifocally dispersed centrilobular hepatocytes with a clear appearance was noted at 10 and 40 mg/kg/day. Moreover, a slight to moderate increase of mononuclear phagocytic system (MPS)-aggregates; slight to minimal centrilobular hepatocellular single cell necrosis and multifocal centrilobular perivascular fibrosis; and minimal multifocal bile duct proliferation occurred at 10 and 40 mg/kg/day. All adverse effects were completely reversible within a 1-month recovery period, except for the changes in the liver and the increased level of ALP in the serum.

Given the dose-related trend in endocrinology results and ovary weights already evident in the group treated with the low dose of 5 mg/kg/day (associated with AUC values of 27 and 37 µg.h/mL in males and females, respectively) a NOEL was not established.

In the 6-month study, TMC278 base formulated in PEG400 + CA was administered once daily, by oral gavage, to beagle dogs at 0 (vehicle), 5, 10, or 40 mg/kg/day in 1 mL/kg (Tabulated Summary 2.6.7.7.O, Report TMC278-NC115). One third of the animals were killed for an interim evaluation after 3 months. No mortalities occurred in this study. Animals at 40 mg/kg/day lost body weight associated with a reduction of food consumption. There were no relevant effects on heart rate, ECG, ophthalmology, hematology, or urinalysis.

At 40 mg/kg/day, serum chemistry showed increases in cholesterol and total bilirubin (also in females at 10 mg/kg/day) concentrations and ALP activity (also in females at 5 mg/kg/day, and in males and females at 10 mg/kg/day).

The cortisol precursor 17α-hydroxyprogesterone was increased in all groups. The AUCs of cortisol showed a dose-related decrease in males of all groups. The AUCs of ACTH showed clear increases in males of all groups. Females showed similar effects as males for cortisol and ACTH, but to a lower extent.

Histopathology showed effects on the female genital tract and adrenal glands similar to those seen in the 1-month study. In addition, testes, liver, and gall bladder were affected by TMC278. In liver, a minimal number of macrophages laden with presumably lipogenic (Perl's negative) pigment was noted perivascularly in some animals at 10 and 40 mg/kg/day. Minimal brown pigmentation of the gall bladder epithelium was noted at 40 mg/kg/day, and incidentally at 10 mg/kg/day. In testes, minimal to slight Leydig cell hypertrophy occurred in 1 animal dosed with 10 mg/kg/day and in the 2 animals dosed with 40 mg/kg/day after 3 months of treatment, and in 2 animals (minimal) treated for 6 months with 40 mg/kg/day. Ovarian weight was increased in all groups after 3 months, and at 10 and 40 mg/kg/day after 6 months of dosing. After 6 months at 40 mg/kg/day, the ovaries, uterus, and vagina had a swollen aspect. Histopathology showed a slight increase in the number of atretic follicles at 10 and 40 mg/kg/day and of regressive corpora lutea at 40 mg/kg/day, whereas the number of tertiary follicles was increased in all groups.

Given the changes seen in adrenals and ovaries at the low dose of 5 mg/kg/day (associated with AUC values of 21 and 17 µg.h/mL in males and females, respectively) no NOAEL was established.

For a 12-month toxicity evaluation, TMC278 base formulated in PEG400 + CA was administered once daily, by oral gavage, to beagle dogs at 0 (vehicle), 5, 10, or 40 mg/kg in 1 mL/kg (Tabulated Summary 2.6.7.7.P, Report TMC278-NC107). No test article-related mortalities occurred. A reduction in body weight gain was noted in all groups.

Hematology, serum chemistry, and urinalysis parameters were affected only at 40 mg/kg/day. Red blood cell count, hemoglobin, and hematocrit in males were lower. Serum calcium and total bilirubin concentrations were decreased, and those of inorganic phosphate in females and of creatinine in males were increased. Alkaline phosphatase activity in serum was increased.

Hormone analyses showed basically the same results as in the 6-month study. The additional hormones determined, LH and testosterone, showed no treatment-related effects. Estradiol concentrations in males were undetectable and in females were highly variable due to the estrous cycle.

Post-mortem evaluations showed basically the same effects as in the 6-month study, with exceptions for liver, gall bladder, testes, and kidneys. In liver, yellow pigmentation in hepatocytes and canaliculi was noted at 40 mg/kg/day, and incidentally at 10 mg/kg/day. Prominent brown pigment in the epithelium of the gall bladder was noted at 40 mg/kg/day. In testes, minimal hypertrophy of the Leydig cells was recorded in 2 males given 40 mg/kg/day. However, this effect had no impact on Sertoli cell functioning or spermatogenesis. In kidney at 40 mg/kg/day, acute interstitial nephritis in 2 males and minimal to slight corticomedullary mineralization in all terminally killed females were noted.

As a consequence of the body weight and adrenal changes at the low dose group of 5 mg/kg/day, associated with AUC values of 17 and 19 µg.h/mL in males and females, respectively, no NOAEL was established.

Monkeys

TMC278 suspended in 1% m/v aqueous HPMC with 0.5% Tween 20 was administered for 8 weeks to immature female cynomolgus monkeys at 0 (vehicle), 200, or 500 mg/kg/day. Animals were dosed 0, 100, or 250 mg/kg BID, with a 6-hour interval, at a volume of 5 mL/kg (Tabulated Summary 2.6.7.7.X, Report TMC278-NC248). No mortalities occurred in this study. No adverse or relevant effects were observed on body weight, clinical pathology, organ weights, or gross lesions.

In (trough level) samples taken prior to the daily dosing, increased levels of 17α-hydroxyprogesterone and progesterone were noted. Reduced concentrations of androstenedione were evident. Decreased dehydroepiandrosterone (DHEA) concentrations were noted only in the group dosed with 500 mg/kg/day. Upon ACTH challenge, serum concentrations of 17α-hydroxyprogesterone showed a strong dose-related increase. A similar pattern was visible for progesterone upon challenge. Androstenedione and DHEA levels in the vehicle group showed only limited response to challenge. The C_{max} values of androstenedione and DHEA at 500 mg/kg/day were lower. Cortisol C_{max} values at 500 mg/kg/day were decreased at the end of the dosing period.

Vaginal swabs were not indicative of menses. Moreover, no follicular or ovulatory effects were noted on serum levels of progesterone, estradiol, or LH. Microscopic evaluation of ovaries did not show any indication of activation. Minimal follicular cell hypertrophy in the thyroid gland was scored in 1 control animal, 3 animals dosed with 200 mg/kg/day, and 4 animals dosed with 500 mg/kg/day.

Since at the lowest dose of 200 mg/kg/day, associated with an AUC value of 2.7 µg.h/mL, several hormonal effects were evident, a NOAEL was not established.

2.4.4.2.3. Tenofovir DF

A series of GLP oral repeat-dose studies were conducted in mice (13 week; Report M990203), rats (28 day and 13/42 week; Reports 96-TOX-4331-003; 97-TOX-4331-002), dogs (28 day and 13/42 week; Reports 96-TOX-4331-004; 97-TOX-4331-001) and monkeys (56 day; Report P2000078). For an overview of all repeat dose toxicity studies, see Tabulated Summary 2.6.7.1.

Test article-related renal karyomegaly was observed in all species at most dose levels. This finding was considered a morphologic change without pathological or toxicological consequences.

Treatment-related findings in the 13-week mouse study (doses from 100 to 1000/600 mg/kg/day) included tubular karyomegaly in the kidneys and epithelial hypertrophy in the duodenum. The NOAEL for this study was 100 mg/kg/day (Tabulated Summary 2.6.7.7.F, Report M990203), a dose equivalent to approximately 5-fold the human exposure (Tabulated Summary 2.6.7.7.F, Report M990203-PK).

In a 13/42 week rat study (doses ranging from 30 to 1000 mg/kg/day), reversible changes in serum chemistry parameters included dose-related decreases in cholesterol and triglycerides; and increases in ALT, aspartate aminotransferase (AST), and creatinine (Tabulated Summary 2.6.7.7.L, Report 97-TOX-4331-002). Duodenal mucosal hyperplasia was observed in the ≥ 300 mg/kg/day groups, and duodenal epithelial hypertrophy occurred at doses ≥ 100 mg/kg/day. Renal tubular epithelial karyomegaly and pigment accumulation occurred at doses ≥ 30 mg/kg/day. Decreases in bone mineral content and bone mineral density were observed at 1000 mg/kg/day after 13 or 42 weeks of treatment. There were no test article-related effects on bone parameters at the 30 or 100 mg/kg/day dose levels, and no gross or histopathological changes were observed in the bone at any dose. The renal, gastrointestinal, and bone changes were partially reversible. The NOAEL was considered to be 30 mg/kg/day. Exposure at this dose is approximately equivalent to human exposure (Tabulated Summary 2.6.7.7.L, Report 97-TOX-4331-002-PK).

In a 13/42 week dog study (doses of 3, 10, and 30 mg/kg/day), changes in clinical biochemistry parameters were slight to moderate, dose-related, and reversible (Tabulated Summary 2.6.7.7.S, Report 97-TOX-4331-001). Bone resorption markers generally were increased in the 30 mg/kg/day dose group at all time points evaluated. Dose-related histopathological changes included slight to mild renal tubular dilatation or degeneration/regeneration and interstitial nephritis in animals in the 10- and/or 30-mg/kg/day dose groups. Slight to moderate renal tubular karyomegaly was observed in the 3-, 10-, and 30-mg/kg/day dose groups. Slight decreases in bone mineral content and bone mineral density of the distal femur of treated animals were observed at 30 mg/kg/day. There was no consistent evidence of recovery in bone parameters at Week 55. The NOAEL for this study was considered to be 3 mg/kg/day, a dose with exposure approximately equivalent to human exposure (Report P4331-00006).

The toxicity profile in rhesus monkeys administered doses of 30, 250, or 600 mg/kg/day TDF for 56 days was similar to that reported in other animal models (Tabulated Summary 2.6.7.17.C, Report P2000078). Renal toxicity was dose limiting (cell degeneration observed at ≥ 250 mg/kg) and there were some indications of secondary alterations in the liver and thymus in the 600-mg/kg/day group. A dose-dependent reduction in mean serum phosphorus concentrations was observed through the first 4 weeks of study. Following oral phosphate supplementation (beginning Day 29), the serum phosphate concentrations normalized. There were no consistent changes in biochemical markers of bone remodeling and no alterations in bone morphology at any dose. Biochemical analysis (cytochrome c oxidase and citrate synthase activities, mitochondrial DNA content), serum lactate levels, and electron microscopic examination of sections of the kidney, liver, cardiac muscle, and skeletal muscle in animals in the TDF 30 and 250 mg/kg/day groups showed no evidence of drug-related mitochondrial injury after 56 days of treatment. Due to serum phosphate changes noted at 30 mg/kg/day, a NOAEL was not established for this study.

2.4.4.2.4. Emtricitabine/Tenofovir DF

A 14-day toxicity study was conducted with FTC and TDF in rats (Tabulated Summary 2.6.7.7.M, Report TX-164-2001). Rats were given formulations prepared from crushed tablets that were experimentally degraded by humidity and high temperatures or formulations prepared from crushed tablets that were not degraded. The doses (mg/kg/day FTC/TDF) were 0/0, 20/30, 67/100 and 200/300 mg/kg/day. The only treatment-related clinical sign was salivation. This finding was observed in earlier studies with TDF alone. There were no effects on body weights, food consumption, hematology, serum biochemistry, or urinalysis parameters. Adrenal weights were increased in treated groups, but there were no macro- or microscopic findings in the adrenal glands. Duodenal hyperplasia, noted in previous TDF studies, was observed in the high-dose FTC/TDF (200/300) groups. The NOAEL for this study was considered to be 67/100 mg/kg/day. It was concluded that no new toxicities or exacerbation of previously defined toxicity occurred in rats given a combination of FTC and TDF. In addition, by testing 3 dose levels of the drug combination using test material that was intentionally degraded, this documented a substantial margin of safety for the impurities and degradants potentially found in the FTC/TDF combination.

A 4-week toxicity study was conducted with FTC and TDF in dogs to examine the possible exacerbation of renal toxicity with combination treatment and to assess possible effects on the immune system (Tabulated Summary 2.6.7.7.T, Report TX-164-2004). Male dogs were treated with vehicle, FTC alone (20 mg/kg/day), TDF alone (30 mg/kg/day), or a low dose (2/3 mg/kg/day) or high dose (20/30 mg/kg/day) of the combination. No adverse effects were observed in the FTC alone group or the low dose combination group. No remarkable changes were observed for immunophenotyping or natural killer cell assay values for any treatment group. Tenofovir DF at 30 mg/kg alone or in combination with 20 mg/kg FTC caused minimally increased APTT and creatinine. Minimal tubular epithelial necrosis and slight to moderate tubular epithelial regeneration were seen in animals given TDF at 30 mg/kg alone or in combination with 20 mg/kg FTC. There were no overall differences in the incidences and mean severities of the renal findings between the 2 groups. Renal findings were reversible after a 4-week recovery period (examined for combination only). Systemic exposure (AUC) was not altered with combination dosing when compared to the agents dosed individually. The NOAEL for the combination of FTC/TDF is 2/3 mg/kg/day in dogs.

2.4.4.2.5. Emtricitabine/Rilpivirine/Tenofovir DF

Administration of FTC/TDF in combination is unlikely to exacerbate known toxicities of the individual agents. This was confirmed by the absence of any new or more marked toxicities in a 14-day rat toxicology study of the FTC/TDF tablet in comparison with studies of a similar duration with the individual agents and a 4-week dog study with the combination (Tabulated Summaries 2.6.7.7M and 2.6.7.7.T, Reports TX-164-2001 and TX-164-2004, respectively). In addition, coadministration of TDF and FTC to SIV-infected pig-tailed monkeys at dose levels many times higher than the intended clinical dosages for up to 6 months had no obviously deleterious effects on the animals (Tabulated Summary 2.6.3.1.4, {5477}).

The repeat-dose toxicity studies with RPV (TMC278) have identified a series of targets of toxicity: RBC in mouse, rat, and dog; coagulation in rat; liver in rat and dog; kidneys in mouse and dog; thyroid gland with secondary effects on the pituitary gland in rat; adrenal glands in mouse, rat, dog, and cynomolgus monkey; testes in dog; ovaries in dog; and in immature females, secondary effects on other parts of the genital tract and on mammary glands compared to immature control animals. The majority of these effects appeared to be completely reversible after a recovery period. The effects on thyroid gland and coagulation in rats showed signs of recovery but this was not complete at the end of the 1-month postdosing period. For mice and rats, a NOAEL could be established. However, dogs and cynomolgus monkeys showed effects at the level of the adrenal glands at the low dose tested. Moreover, dogs also showed effects on ovaries in almost all pivotal studies at the low dose given. These effects prevented establishment of NOAELs in these species.

The 3 drugs, FTC, RPV and TDF, exhibit different patterns of target organ toxicity. Specifically, the only significant effect of FTC identified at dose levels constituting large clinical multiples was a minor anemia. In contrast, extensive nonclinical investigations of the toxicity of TDF have shown that the bone marrow is not a target for this agent, and that the target organs for TDF are distinctly different (GI, bone, and kidney). Given that kidney effects have been observed with RPV in mice and dogs only at high dose levels and exposures, and that the routes of excretion differ for RPV and TDF, renal toxicity is not anticipated to be an issue with the FTC/RPV/TDF combination product. From in vitro data, pharmacokinetic studies in dogs (Module 2.6.4), and clinical experience (Module 2.7.2), there are no anticipated pharmacokinetic interactions between FTC, RPV, and TDF. The ample nonclinical safety database on these drugs strongly indicates that little potential for drug interaction exists and further toxicological investigations are unlikely to yield new data relevant to humans.

Further studies of longer duration with the combinations seem unwarranted given the lack of pharmacokinetic interactions and significant overlapping toxicities, and the use of additional animals that would be required to obtain such information.

2.4.4.3. Genotoxicity

2.4.4.3.1. Emtricitabine

Emtricitabine was not genotoxic in the reverse mutation bacterial test (Ames test) (Tabulated Summaries 2.6.7.8.A, 2.6.7.8.B and 2.6.7.8.C, Reports 18637-0-409R, MUT203, and K01-3154, respectively), mouse lymphoma (Tabulated Summary 2.6.7.8.D, Report TOX012), or mouse micronucleus assays (Tabulated Summary 2.6.7.9.A, Report TOX011).

2.4.4.3.2. Rilpivirine

Genotoxicity testing of TMC278 base or TMC278 comprised 4 nonmammalian (bacterial) reverse gene mutation (Ames) tests, 2 mammalian (mouse lymphoma) forward mutation assays, and 1 in vivo mouse bone marrow micronucleus assay.

TMC278 was tested up to the maximum concentration that allowed scoring due to precipitation. No increased mutation frequency (Tabulated Summaries 2.6.7.8.E, 2.6.7.8.F, and 2.6.7.8.G) or increased frequency of structural chromosomal aberrations (Tabulated Summaries 2.6.7.8.I and 2.6.7.8.J) was noted. In the Ames test with human S9 (Tabulated Summary 2.6.7.8H), metabolites M30/M31, that are formed following Michael addition at the [redacted] moiety of TMC278 (Module 2.6.4, Pharmacokinetic Written Summary, Section 2.6.4.5.1), were present at the same level as determined in human feces (Module 2.7.2, Clinical Pharmacology Summary, Section 2.7.2.3.1.2.3). Also this Ames test did not show an increased mutation frequency.

In the in vivo mouse bone marrow micronucleus test, the maximum feasible dose of 1600 mg/kg TMC278 base did not induce an increase of micronuclei (C1h was 60 and 58 µg/mL and AUC0-6h was 307 and 287 µg.h/mL for males and females, respectively; Tabulated Summary 2.6.7.9.B, Report TMC278-Exp5538).

2.4.4.3.3. Tenofovir DF

Tenofovir DF was mutagenic in the in vitro mouse lymphoma assay (Tabulated Summary 2.6.7.8.N, Report 97-TOX-4331-007), weakly positive in an unscheduled DNA synthesis test (Tabulated Summary 2.6.7.9.D, Report 23291-0-494OECD), and generally negative in in vitro bacterial mutagenicity tests (Ames test) (Tabulated Summaries 2.6.7.8.K, 2.6.7.8.L and 2.6.7.8.M, Reports 96-TOX-4331-005, 97-TOX-1278-003, and K01-3037, respectively). In an in vivo mouse micronucleus assay, TDF was negative when administered to male mice (Tabulated Summary 2.6.7.9.C, Report 97-TOX-4331-008).

2.4.4.3.4. Emtricitabine/Tenofovir DF

No exacerbation of mutagenicity was apparent in either the bacterial reverse mutation assay (Ames assay) or the in vitro mammalian cell gene mutation assay (L5178Y/TK$^{+/-}$ mouse lymphoma assay) when FTC and TDF were administered together compared with each agent alone (Tabulated Summaries 2.6.7.8.O and 2.6.7.8.P, Reports TX-164-2002 and TX-164-2003, respectively).

2.4.4.3.5. Emtricitabine/Rilpivirine/Tenofovir DF

Of the 3 compounds, only TDF had positive findings in genotoxicity studies (mouse lymphoma cell assay and UDS assay). The combination of FTC and TDF in a mouse lymphoma cell assay did not worsen the genotoxic potential of TDF. The combination of the 3 components is not expected to have an altered genotoxicity profile as compared with that of the individual agents.

2.4.4.4. Carcinogenicity

2.4.4.4.1. Emtricitabine

In long-term carcinogenicity studies of FTC, no drug-related increases in tumor incidence were found in mice at doses up to 750 mg/kg/day (26-fold the human systemic exposure at the therapeutic dose of 200 mg/day) (Tabulated Summary 2.6.7.10.A, Report TOX109) or in rats at doses up to 600 mg/day/day (31-fold the human systemic exposure at the therapeutic dose) (Tabulated Summary 2.6.7.10.B, Report TOX108)

2.4.4.4.2. Rilpivirine

In the mouse carcinogenicity study, TMC278 was administered once daily, by oral gavage, for 24 months. Groups of 60 male and 60 female CD-1 mice were given 0 (vehicle), 20, 60, or 160 mg/kg/day at a dose volume of 10 mL/kg (Tabulated Summary 2.6.7.10.C, Report TMC278-NC120; Module 2.6.6, Toxicology Written Summary, Section 2.6.6.5.3). The trend test for mortality with dose was statistically significant for male mice when all treated groups were included. For females, the trend test for mortality with dose was not statistically significant. In male and female mice dosed with 160 mg/kg/day, hepatic tumors caused death more frequently than in males and females of the control group. In the liver, a statistically significant dose-related increase in total hepatocellular tumors (adenomas and carcinomas combined) was seen in males dosed with TMC278 from 20 mg/kg/day and above. The incidences of carcinomas and of adenomas for all groups treated with TMC278 and for the groups dosed with 60 and 160 mg/kg/day, respectively, were above the ranges expected from background data of the testing facility. In female mice treated with 60 and 160 mg/kg/day, the incidences of liver adenomas and of adenomas and carcinomas combined were statistically significantly increased and above the background data range.

It was concluded that oral administration of TMC278 to CD-1 mice produced a dose-related increase in total hepatocellular tumors (adenoma and carcinoma) in males dosed with 20 mg/kg/day and in males and females that received 60 and 160 mg/kg/day. For that reason, a NOAEL could not be determined. The associated systemic exposure expressed as AUC values determined in Week 28 in animals dosed with 20 mg/kg/day was 76 and 51 µg.h/mL in males and females, respectively.

In the rat carcinogenicity study, TMC278 was administered once daily by oral gavage for 24 months. Groups of 65 male and 65 female Sprague Dawley rats were given 0 (vehicle), 40, 200, 500, or 1500 mg/kg/day at a dose volume of 10 mL/kg (Tabulated Summary 2.6.7.10.D, Report TMC278-NC123; Module 2.6.6, Toxicology Written Summary, Section 2.6.6.5.4). There was no effect of treatment on mortality. No treatment-related pathologies contributing to death and no adverse clinical signs were observed. Hematology did not show treatment-related effects. Serum chemistry showed liver-associated effects mainly at 500 and 1500 mg/kg/day.

In liver, an increase in hepatocellular adenomas was seen in animals given 40, 200, 500, or 1500 mg/kg/day. There was no apparent dose-related trend and the differences from control

values did not reach statistical significance. When compared with the historical background data, the incidence of tumors in females was above the background level in all groups treated with TMC278; whereas in the males the incidence of tumors was equal to the maximum historical background incidence recorded.

In thyroid gland, a statistically significant increase of the number of follicular cell adenomas and of adenomas and carcinomas combined was seen in all groups, with only a marginally apparent dose-related trend. The incidence of follicular cell adenomas was above background levels at 200, 500, or 1500 mg/kg/day. The incidence of the carcinomas was above background levels in males only at 200 and 1500 mg/kg/day. None of the liver or thyroid gland tumors were considered contributory to death in any rat.

It is concluded that the oral administration of TMC278 to Sprague Dawley rats at doses of 40, 200, 500, or 1500 mg/kg/day produced neoplastic changes in liver and thyroid gland. For that reason, a NOAEL could not be determined. The associated systemic exposures expressed as AUC values determined in Week 39 in animals dosed with 40 mg/kg/day were 6.3 and 14 µg.h/mL in males and females, respectively.

2.4.4.4.3. Tenofovir DF

Long-term oral carcinogenicity studies of TDF in mice and rats were carried out at exposures up to approximately 16 times (mice) and 5 times (rats) those observed in humans at the therapeutic dose for HIV infection (Tabulated Summaries 2.6.7.10.E and 2.6.7.10.F, Reports M990205 and R990204). Mice showed a low incidence of duodenal tumors, considered likely related to high local concentrations in the GI tract at the highest dose of 600 mg/kg/day. Rats did not show any carcinogenic potential in the long-term study.

2.4.4.4.4. Emtricitabine/Rilpivirine/Tenofovir DF

Emtricitabine and TDF have both demonstrated low carcinogenic potential in conventional 2-year bioassays at exposures that exceeded (TDF) or far exceeded (FTC) human exposures at the therapeutic dose. Carcinogenicity studies with RPV demonstrated an increased incidence of hepatocellular and thyroid tumors that are not considered relevant for humans (discussed in Section 2.4.4.10). It is considered unlikely that combination dosing would change this profile as no exposure difference would be expected and no exacerbation of toxicity/genotoxicity is expected. Given the difficulty of extrapolating rodent results to humans and the large number of animals required to carry out these studies, the conduct of carcinogenicity studies with the FTC/RPV/TDF combination seems unjustifiable.

2.4.4.5. Reproductive Toxicity

2.4.4.5.1. Emtricitabine

The incidence of fetal variations and malformations was not increased in embryo-fetal toxicity studies performed with FTC in mice at exposures (AUC) approximately 60-fold higher and in rabbits at exposures approximately 120-fold higher than human exposures

(Tabulated Summaries 2.6.7.13.A and 2.6.7.13.B, Reports TOX037 and TOX038, respectively). Emtricitabine did not affect fertility in male rats at approximately 140-fold or in male and female mice at approximately 60-fold higher exposures (AUC) than in humans given the recommended 200 mg daily dose (Tabulated Summaries 2.6.7.12.B and 2.6.7.12.A, Reports TTEP/95/0028 and TOX036, respectively). Fertility was normal in the offspring of mice exposed daily from before birth (in utero) through sexual maturity at daily exposures (AUC) of approximately 60-fold higher than human exposures at the recommended 200 mg daily dose (Tabulated Summary 2.6.7.14.A, Report TOX039).

2.4.4.5.2. Rilpivirine

Fertility

For a male fertility study, TMC278 was administered once daily, by oral gavage, to Sprague Dawley rats at 0 (vehicle), 100, 400, or 1600 mg/kg/day in a dose volume of 10 mL/kg (Tabulated Summary 2.6.7.12.C, Report TMC278-NC124). Treatment started 10 weeks prior to mating, during mating, and for 3 to 4 weeks after the mating period. The males were paired 1:1 for mating with untreated females. There were no mortalities associated with TMC278. There were no relevant clinical signs and no relevant effects on body weight; food consumption; gross or histopathological lesions; weights of epididymides or testes; and no adverse effects on the motility, concentration, or morphology of the sperm. There was no effect on fertility up to 1600 mg/kg/day. Weights of liver and thyroid gland showed a dose-related increase in all groups receiving TMC278. The NOAEL for male fertility was at least 1600 mg/kg/day.

Female Sprague Dawley rats were dosed with TMC278 once daily, by oral gavage, at 0 (vehicle), 40, 120, or 400 mg/kg/day in a dose volume of 10 mL/kg (Tabulated Summary 2.6.7.12.D, Report TMC278-NC125). Treatment started 2 weeks prior to mating, during mating, and until Day 7 of pregnancy. The females were paired 1:1 for mating with untreated males. No mortalities were noted. Moreover, there were no relevant clinical signs and no relevant effects on body weight, food consumption, gross pathology, estrous cycle, mating or pregnancy rate, the number of corpora lutea, implantations or live fetuses, or early embryonic development indices. It was concluded that there was no effect on female fertility, fecundity, or early embryonic development up to 400 mg/kg. Therefore, the NOAEL for female fertility, fecundity, and early embryonic development was at least 400 mg/kg/day.

Embryo-Fetal Development

TMC278 base was administered once daily by oral gavage from GD 6 to GD 17 (day of sperm detection is GD 0) to pregnant Sprague Dawley rats at 0 (vehicle), 40, 120, or 400 mg/kg/day at a dose volume of 10 mL/kg (Tabulated Summary 2.6.7.13.C, Report TMC278-NC105). The dams were necropsied on GD 21. There were no mortalities associated with TMC278. Reduced body weight gain and food consumption were noted in dams given 120 and 400 mg/kg/day. Weight of thyroid gland showed a dose-related increase at 120 and 400 mg/kg/day. Visceral examinations showed a slight increase in a minor variation, dilated renal pelvis, in 5 out of 149 and 7 out of 149 fetuses from the groups treated

with 120 and 400 mg/kg/day, respectively. The maternal and embryo fetal NOAEL was 40 mg/kg/day based on the changes on the body weight, food consumption, and the increase of dilated renal pelvis seen at higher doses. Systemic exposure expressed as AUC value determined at the end of the dosing period in animals dosed with the NOAEL was 37 µg.h/mL.

TMC278 base was administered once daily by oral gavage from GD 6 to 19 to pregnant New Zealand white rabbits at 0 (vehicle), 5, 10, or 20 mg/kg/day in a dose volume of 5 mL/kg (Tabulated Summary 2.6.7.13.D, Report TMC278-NC130). The females were killed on GD 28 and a necropsy was performed. There was a slight increase in numbers of fetuses exhibiting changes commonly seen in rabbits that are considered to have little or no biological significance. The maternal NOAEL was at least 20 mg/kg/day and the fetal NOAEL was 10 mg/kg/day. Systemic exposure of the dams expressed as AUC values determined at the end of the dosing period at the fetal NOAEL was 170 µg.h/mL.

Peri- and Postnatal Development

TMC278 was administered once daily, by oral gavage, to time-mated female Sprague Dawley rats from GD 6 to LD 20 at 0 (vehicle), 40, 120, or 400 mg/kg/day in a dose volume of 10 mL/kg (Tabulated Summary 2.6.7.14.C, Report TMC278-NC131). Developmental landmarks of the pups were recorded on LDs 3, 5, 15, and 21. At necropsy of the dams after weaning of their litters, the number of implantation scars in the uterus was determined. From the offspring (F_1 generation), 20 males and 20 females per group were kept and their growth, post-weaning development, and behavior and reproductive performance were assessed. There were no effects on any of the determined parameters. The NOAEL for both the F_0 and F_1 generation was at least 400 mg/kg/day.

2.4.4.5.3. Tenofovir DF

Embryo-fetal toxicity studies have been performed in rats (Tabulated Summary 2.6.7.13.E, Report 97-TOX-4331-004) and rabbits (Tabulated Summary 2.6.7.13.F, Report 98-TOX-4331-005) at doses up to 14 and 19 times the human dose based on body surface area comparisons and revealed no evidence of harm to the fetus due to TDF. There were no effects on fertility, mating performance or early embryonic development when TDF was administered to male rats at a dose equivalent to 10 times the human dose based on body surface area comparisons for 28 days prior to mating and to female rats for 15 days prior to mating through GD 7 (Tabulated Summary 2.6.7.12.E, Report 98-TOX-4331-006). There was, however an alteration of the estrous cycle in female rats. In a perinatal study in rats (Tabulated Summary 2.6.7.14.D, Report R990202), the NOEL for behavioral, reproductive, and development toxicity was 150 mg/kg/day (exposure approximately 4-fold the human exposure). Maternally toxic doses (≥ 450 mg/kg/day) had effects on pup survival, pup body weights, and sexual maturation.

2.4.4.5.4. Emtricitabine/Rilpivirine/Tenofovir DF

Fertility and Early Embryonic Development

The reproductive and developmental NOELs for the individual agents were at exposure levels well above human exposures. With no expected pharmacokinetic or toxicologic interactions with the FTC/RPV/TDF combination, further studies with FTC/RPV/TDF are not considered necessary.

Embryo-Fetal Development

There were no effects on embryo-fetal development in rats or rabbits when FTC, RPV, and TDF were tested individually. No cause for concern has been identified and studies with the FTC/RPV/TDF combination are unlikely to show new effects.

Peri- and Postnatal Development

Slightly longer estrous cycles were observed in F_1 generation rats after exposure to high doses of FTC and a delay in sexual maturation was observed in F_1 generation rats after exposure to high (maternally toxic) doses of TDF. No significant effects were noted for RPV. For all 3 individual agents, NOELs were at exposures above human exposures. As with other reproductive toxicity tests, a repeat of this test with the FTC/RPV/TDF combination is unlikely to add any new information.

2.4.4.6. Juvenile Toxicity

2.4.4.6.1. Emtricitabine

Repeat-dose studies with FTC have not shown effects in developing organ systems, and reproductive and developmental NOELs for FTC were at exposure levels well above human exposures. Emtricitabine is approved for use in infants (aged 4 months of age or older), children, adolescents, and adults. No specific juvenile toxicity studies are considered warranted with FTC.

2.4.4.6.2. Rilpivirine

TMC278 was administered once daily, by oral gavage, to time-mated female Sprague Dawley rats from GD 6 to LD 7 (day of delivery is LD 0) at 0 (vehicle, 2 groups), 40, 120, and 400 mg/kg/day in a dose volume of 10 mL/kg (Tabulated Summary 2.6.7.14.B, Report TMC278-NC168). On LD 7, 8 male and 8 female pups of each group were selected for oral dosing by gavage with the same dose and at the same dose volume as their mothers. The selected pups from the second vehicle group were to be dosed with 400 mg/kg/day. The selected pups were dosed from LD 12 up to and including LD 25. Blood samples for toxicokinetics were taken on LD 25. Necropsy was performed after the last sampling. Two pups from the group treated with 400 mg/kg/day were killed for humane reasons. The cause of death could not be established. There were no effects on the pups dosed by gavage with TMC278 during the dosing period or at necropsy. Systemic exposures for control pups dosed

with 400 mg/kg/day and pups from dams treated with 400 mg/kg/day and subsequently dosed with the same dose expressed as AUC values determined at the end of the dosing period were 40 to 50 µg.h/mL in male and female pups, respectively.

Dogs

No specific dog juvenile toxicity studies were done. However, in all dog studies, the animals were 6 to 8 months old at the start of dosing, i.e. immature. In the 1-month dog study, the negative control female animals were still immature (no signs of ovarian maturation or ovulation) at necropsy. TMC278 induced more prominent tertiary follicles in the ovaries of animals that did not ovulate and induced ovulation in 3 animals. The effects by TMC278 in the dogs that ovulated in the 1-month study were basically similar to those observed in mature dogs at the end of the 6- and 12-month dog studies (see Module 2.6.6, Toxicology Written Summary, Section 2.6.6.3.4).

Cynomolgus Monkeys

In the 8-week immature female cynomolgus monkey study (see Section 2.4.4.2.2), TMC278 caused only effects indicative of inhibition of adrenal CYP21 and CYP17. These effects comprised increased serum concentrations of progesterone and 17-hydroxyprogesterone, and decreased serum concentrations of androstenedione and DHEA. TMC278 did not induce any effects on ovaries such as those observed in immature dogs.

2.4.4.6.3. Tenofovir DF

Although no specific juvenile toxicity studies have been conducted with TDF, data are available from more than 125 rhesus monkeys across 5 studies that have been administered TFV or TDF with treatment initiated from 1 day to 7.5 years of age (Tabulated Summary 2.6.7.17.C). This age range covers the human equivalent of infant, juvenile, and adolescent phases of growth. The duration of treatment has ranged from 12 weeks to 13 years. In these studies, bone abnormalities were reported in the juvenile macaque/simian immunodeficiency virus (SIV) model at subcutaneous doses of 30 mg/kg/day (with systemic exposure approximately 40-fold higher than adolescent or adult exposure). The abnormalities included reduced bone mineral density (BMD), joint swellings, and bone fractures. Elevated ALP activity, decreased serum phosphorus concentration, glucosuria, and proteinuria were also observed in the macaques with bone lesions; serum calcium values were normal. Discontinuation of treatment or reduction of the dose to 10 mg/kg/day (6-fold adolescent exposure) was associated with resolution of the bone abnormalities and biochemical changes. Subcutaneous administration of TFV at 10 mg/kg/day to 3 newborn rhesus monkeys for more than 5 years had no observed adverse effect on their bone density or growth {7311}. Nonclinical studies that investigated potential mechanisms for direct effects of TFV on bone provided no evidence of in vitro and in vivo cytotoxicity to bone. The mechanism underlying this bone effect is unknown.

2.4.4.6.4. Emtricitabine/Rilpivirine/Tenofovir DF

No specific studies were conducted with the combination. Use of the FTC/RPV/TDF tablet in young children is not anticipated at this time. Guidance is provided in the prescribing information that the FTC/RPV/TDF tablet should not be administered to children or adolescents, however a Paediatric Investigation Plan has been agreed with the EMA Paediatric Development Committee (EMEA-000774-PIP01-09, the EMA Opinion is located in MAA Module 1.10) to conduct a study in adolescents and a study in children with the FTC/RPV/TDF FDC, once the pharmacokinetics, safety, and efficacy of RPV and TDF have been established. (The pharmacokinetics, safety and efficacy of FTC have been investigated in HIV-1 infected children, and in general, the pharmacokinetics of FTC in pediatric subjects are similar to those seen in adults.)

2.4.4.7. Local Tolerance

TMC278 tested negative for the potential to cause phototoxicity in vitro and skin irritation in rabbits, and delayed-type hypersensitivity in the mouse local lymph node assay. TMC278 was classified as a moderate eye irritant in an in vitro test (Module 2.6.6, Toxicology Written Summary, Section 2.6.6.7).

Tenofovir DF is considered to be a very severe irritant to rabbit ocular tissue (Tabulated Summary 2.6.7.16.B, Report B990265), and a slight irritant to rabbit skin (Tabulated Summary 2.6.7.16.B, Report B990166).

The FTC/RPV/TDF tablet is intended for oral use. No local tolerance studies were conducted for the FTC/RPV/TDF combination.

2.4.4.8. Other Toxicity

2.4.4.8.1. Immunotoxicity

The immunotoxicity of FTC was evaluated in a 28-day study in CD rats at doses up to 1000 mg/kg/day (Tabulated Summary 2.6.7.17.A, Report TOX146). There were no adverse effects of FTC during the dosing period and FTC did not affect the IgM antibody titers to sheep RBCs at any of the doses administered. It was concluded that 1000 mg/kg/day was a NOEL for the type of immunotoxicity investigated.

The lack of effects on the PFC-assay in the 1-month rat study (Tabulated Summary 2.6.7.7.H, Report TMC278-TOX5692) indicated that TMC278 had no immunotoxic potential.

In a 4-week study in dogs where animals received FTC, TDF, or the combination of FTC and TDF, no remarkable changes were observed for immunophenotyping or natural killer cell assay values (Tabulated Summary 2.6.7.7.T, Report TX-164-2004).

With no evidence of immunotoxicity induced by individual agents or the combination of FTC and TDF, additional studies with the FTC/RPV/TDF combination are not considered necessary.

2.4.4.8.2. Toxicological Findings of Emtricitabine/Tenofovir in SIV Efficacy Studies

Tenofovir (20 to 30 mg/kg/day) was given subcutaneously in combination with FTC (50 mg/kg/day) to pig-tailed macaques infected with SIV for approximately 6 months {5477}. Although the number of animals was small and the study was not intended to reveal the safety of the combination therapy, it is noteworthy that the animals did not show any adverse effects of the treatment with the large clinical multiples that were given over the approximately 6-month period of administration.

Tenofovir administered to newborn or infant rhesus monkeys at doses of 4 to 30 mg/kg did not cause adverse effects in short term studies (up to 12 weeks). However, prolonged TFV treatment (generally more than 4 months of daily treatment at 30 mg/kg/day administered subcutaneously) resulted in a Fanconi-like syndrome with glucosuria, aminoaciduria, hypophosphatemia, growth restriction, and bone pathology (osteomalacia) {7311}, (Tabulated Summary 2.6.7.17.C, Report T1278-00034). The exposure (AUC_{ss}) in rhesus monkeys at this dosage is more than 30-fold that in humans following the oral administration of 300 mg/day of TDF. Clinical, biochemical, and radiographic resolution/improvement occurred with dose reduction (from 30 to ≤ 10 mg/kg/day) or discontinuation of treatment.

Three animals (1 SIV-infected) were dosed chronically, beginning as neonates, with 10 mg/kg/day TFV administered subcutaneously. After more than 5 years of treatment, there are no clinical, radiographic, or DXA scan {7311} findings of an adverse effect on bone. The AUC_{ss} associated with this dosage are 3- to 18-fold greater than the human AUC_{ss} following a 300 mg/day dose of TDF.

2.4.4.8.3. Mitochondrial Toxicity

An overview of investigations into the potential of FTC, RPV, and TDF to mediate mitochondrial toxicity is provided in Section 2.4.2.1.

2.4.4.8.4. Impurities/Degradation Products

Emtricitabine

The process impurities and degradation products of FTC have been qualified in animal studies. The major degradation product, 不純物FF*, was qualified in 2 genotoxicity studies (Tabulated Summary 2.6.7.17.A, Reports TOX151 and TOX152) using a batch of FTC that contained 1% (w/w) of the 不純物FF*degradant. Both studies were negative for genotoxicity. In addition, there was no toxicity in a 28-day mouse study at doses (FTC 不純物FF*) of 50/1 mg/kg/day, 150/3 mg/kg/day, and 450/9 mg/kg/day (Tabulated Summary 2.6.7.17.A, Report TOX153). The degradation product 不純物FF* has been found to undergo additional

degradation to form 2 new degradation products, GS-9237 and GS-492127, which are 2 [redacted] of 不純物FF'* . Both of these impurities were present in degraded crushed tablets that contained a combination of TDF and FTC (Tabulated Summary 2.6.7.7.M, Report TX-164-2001).

A 28-day mouse bridging study (Tabulated Summary 2.6.7.17.A, Report TX-162-2001) was performed to qualify impurities in FTC synthesized by the [redacted] chemistry process (specifically 不純物FC*). There was no toxicity of FTC at doses of 50, 150, and 450 mg/kg/day, giving a 136-fold safety margin on a mg/kg basis.

Rilpivirine

The TMC278 drug substance contains 3 impurities that needed to be qualified according to Guideline Q3A of the International Conference on Harmonization (ICH), entitled: "Impurities in New Drug Substances." Two of these, 不純物B* and 不純物C* , have been evaluated upon spiking into the drug substance at a level of 4%. Qualification comprised a bacterial reverse mutation Ames test, a mouse lymphoma assay, and a 1-month oral rat study. The presence of the impurities at 4% did not modify the effects of TMC278 in any of the tests. The third impurity, 不純物A* , is the [redacted]-isomer of TMC278. This isomer was present in all drug substance batches involved in pivotal nonclinical studies at the level of minimally 0.61%. In view of the close structural relationship with TMC278 and the overage between the lowest nonclinical dose (5 mg/kg/day) and the recommended dose of 25 mg once daily, separate qualification of 不純物A* is not considered relevant (Module 2.6.6, Toxicology Written Summary, Section 2.6.6.8.6 and Tabulated Summary 2.6.7.17.B). The impurities qualified at the level of 4% according to ICH Q3A (Module 2.6.6, Toxicology Written Summary, Section 2.6.6.8.6) occurred in representative TMC278 drug substance batches manufactured according to the proposed commercial synthesis method for less than 0.1% for 不純物C* , less than 0.25% for 不純物B* , and less than 0.25% for the non-qualified [redacted]-isomer of TMC278, 不純物A* .

Three further trace impurities, 不純物D* , 不純物E* , and 不純物F* , that contain moieties with a mutagenic alert are present in the drug substance at levels that do not warrant qualification according to ICH Q3A. The mutagenic potential of the HCl salt of 不純物D* , namely 不純物D'* , and of 不純物E* and 不純物F* was tested in an Ames test. Only 不純物D* , tested as its HCl salt 不純物D'* , showed a mutagenic potential. It is therefore concluded that 不純物D* is a genotoxic impurity, whereas the potential impurities 不純物E* and 不純物F* with structural analogy to 不純物D* did not induce an increased mutation frequency in the Ames test (Module 2.6.6, Toxicology Written Summary, Section 2.6.6.8.6; Tabulated Summary 2.6.7.17B). Probably, the [redacted] moiety of 不純物D* caused the genotoxic effect. The maximum allowable level of genotoxic impurity 不純物D* (Module 2.6.6, Toxicology Written Summary, Section 2.6.6.8.6) in a daily dose of 25 mg TMC278 was calculated to be 60 ppm on the basis of the Threshold of Toxicological Concern (TTC) approach (CHMP. Guideline on the limits of genotoxic impurities. CPMP/SWP/5199/02. EMEA/CHMP/QWP/251344/2006 28 June 2006) with daily treatment for longer than 12 months. The levels of 不純物D* in representative TMC278 drug substance batches manufactured according to the proposed commercial synthesis method is less than

5 ppm (Module 3, Quality, Section 3.2.S.3.2, Impurities [Rilpivirine]). This impurity is controlled at less than 5 ppm.

Tenofovir DF

At least 17 impurities, which are related substances to TDF, have been identified in batches of the active pharmaceutical ingredient (API) produced under both GLP and GMP conditions. All of the impurities derived from the chemical synthesis or formed as degradation products were present in the test material used in nonclinical toxicity studies; hence their toxicity was adequately assessed.

Five organic volatile impurities are potentially present in TDF. [redacted], [redacted], and [redacted] are present in quantities below the ICH limits and pose no toxicity risk. According to literature references, the residual solvent [redacted] has a low order of toxicity and is not genotoxic or teratogenic {2615}, {2619}, {2618}. [redacted] ([redacted]), which is present in the API, has been tested in a 28-day rat study and in 2 mutagenicity assays. While [redacted] was shown to be mutagenic in both in vitro assays (Reports 1432/022 and 1432/023), it was shown to have a low order of toxicity in the 28-day oral dosing study in rats. The NOEL in this rat study was 15 mg/kg/day (Reports 1432/009 and 1432/021), representing a 1000-fold safety margin for toxicity compared to anticipated human exposure. Although the toxicity of [redacted] has not been studied extensively, the 1000-fold margin of subchronic toxicity in rats treated with the neat compound and the extended duration of exposure to the compound in the 13/42-week rat study (Tabulated Summary 2.6.7.7.L, Report 97-TOX-4331-002) is considered adequate to qualify the proposed [redacted] limit.

A 14-day study was conducted to determine if there were unexpected toxicologic effects of degraded products for TDF tablets that had been degraded under accelerated conditions (Tabulated Summary 2.6.7.17.C, Report R2000081). The study was conducted in Sprague-Dawley rats dosed with either nondegraded, unformulated TDF or degraded TDF tablets, via oral gavage, for 14 consecutive days. No treatment-related findings were observed following 14 days of oral gavage dosing of either TDF or degraded TDF tablets at doses up to 300 mg/kg/day to rats. No differences between treatment with either TDF or degraded TDF were noted.

Emtricitabine/Tenofovir DF

Based on their impurity profiles, the multiple GLP batches of TDF and FTC tested in the toxicology programs are considered, in composite, to be representative of the GMP materials; hence, the nonclinical studies support the specified limits of impurities proposed for commercial production.

As described in Section 2.4.4.2, rats were dosed for 14 days with crushed tablets, both experimentally degraded and undegraded, that contained a combination of TDF and FTC (Tabulated Summary 2.6.7.7.M, Report TX-164-2001). Two new degradation products were observed in degraded product (Adduct 1 and Adduct 2). There was no previous unobserved

toxicity, nor did toxicity occur at lower dose levels in this study as compared to results of toxicology studies with TDF and FTC tested independently.

Emtricitabine/Rilpivirine/Tenofovir DF

The impurities and degradation products present in FTC, RPV, TDF, and FTC/TDF tablets have been qualified through toxicology studies which employed drug substance from normal productions batches, laboratory scale batches with enhanced levels of impurities, and samples subjected to forced degradation conditions (high heat and humidity). There are no unique impurities or degradation products present in the FTC/RPV/TDF tablets that require qualification (Module 3.2.P.5.5, Characterization of Impurities *[FTC/RPV/TDF Tablets]* and Module 3.2.P.5.6, *Justification of Specifications [FTC/RPV/TDF Tablets]*).

2.4.4.9. Summary of Toxicology and Target Organ Effects

2.4.4.9.1. Rilpivirine Mortality

No TMC278-related mortality occurred in any of the single- or repeat-dose studies with rats, rabbits, dogs, and cynomolgus monkeys. Incidental mortalities occurred or humane killings were necessary as result of gavage incidents notably in studies with the PEG400 + CA vehicle, due to its viscosity.

In the pilot 2-week CD-1 mouse study, doses of 2000 mg/kg/day by oral gavage and 5000 mg/kg/day via diet were not tolerated. In the 4-week study with transgenic CB6F1-nonTgrasH2 mice, 2 TMC278-related mortalities occurred at 320 mg/kg/day. The cause of these deaths could not be established (Tabulated Summary 2.6.7.6.A).

In the mouse carcinogenicity study, the trend test for mortality with dose was statistically significant for male mice when all treated groups were included. For females, the trend test for mortality with dose was not statistically significant. In male and female mice dosed with 160 mg/kg/day, hepatic tumors caused death more frequently than in males and females of the control group (Module 2.6.6, Toxicology Written Summary, Section 2.6.6.5.3). In the rat carcinogenicity study, there was no trend for mortality with dose and none of the tumors contributed to the deaths of the animals (Module 2.6.6, Toxicology Written Summary, Section 2.6.6.5.4).

In the 6-month rat study, increasing difficulties with the daily administration by gavage, morbidity, and deaths started to occur after the first 2 months of dosing with comparable incidences in all groups including controls. For this reason, the dosing regimen was changed from once daily to BID dosing (5 mL/kg with an interval of 1.5 hour) from Day 84 onwards. The cause of death (reaching 50% in some of the groups) was concluded to be associated with the relatively high volume and viscosity of the formulation, necessitating a long gavage time during which the animals physically opposed the manual restraint. This led to gavage errors, regurgitation, and restraint trauma. In the 1-month postdosing period, no further mortalities occurred (see Module 2.6.6, Toxicology Written Summary, Section 2.6.6.3.2 and Tabulated Summary 2.6.7.7.I, Report TMC278-NC101).

2.4.4.9.2. Safety Margins

Emtricitabine and Tenofovir DF

Cross-species comparisons of exposure (expressed based on area under the plasma concentration curve at steady state [AUC_{ss}] levels) for the major target organs are shown in Table 3. The margin relative to the human AUC_{ss} is given for the NOEL and the minimal-effect-level (MEL) for TDF, where the MEL was available from the same study in which the NOEL was determined (Table 3).

For FTC, the NOELs obtained in the toxicity studies represent systemic exposures in animals well in excess of those expected in humans given the daily recommended dose of 200 mg.

Although the GI toxicity in the rat following TDF administration appears to have a very low margin of safety based on the systemic exposure levels, this effect is not related to systemic exposure, but to local GI exposure, which is 6- to 20-fold higher than the human exposure on a direct dosage basis.

Dogs were the most sensitive species to renal effects of TDF. The NOEL was based on morphologic lesions that were not associated with renal functional or biochemical changes. The NOEL for renal effects in monkeys is likely above 30 mg/kg/day, but no interim doses were tested between 30 and 250 mg/kg/day, where mild effects were noted. Monkeys are quite sensitive to the TDF effects related to decreased serum phosphorus. In the study on phosphate changes in monkeys (Tabulated Summary 2.6.7.17.C, Report P2000078), there was not a significant dose response between 30 and 250 mg/kg/day, and a NOEL for this effect has not been established. All species tested have shown some loss of bone mineral density at relatively high doses ($\geq 6 \times$ human exposure); however, clinically evident osteomalacic lesions occurred only in juvenile monkeys (treated subcutaneously daily with TFV) whose TFV AUC_{ss} levels were approximately $> 30 \times$ those of humans receiving the 300 mg/day dose. Therefore, the effect on serum phosphate levels at lower doses appears to have no toxicologic consequence.

Table 3. Estimated Safety Margins of Emtricitabine and Tenofovir DF Based on AUCss When Comparing Animal No-Effect-Level (NOEL) or Minimal-Effect-Level (MEL)

Target Organ Effect	Species	Study Duration	NOEL/MEL (mg/kg/day)	AUC_{ss} (µg·h/mL) NOEL/MEL	Margin Relative to Human AUC_{ss}
			FTC		
Anemia	Mouse	6 months	500	350	34 X
	Rat	3 months	600	346	33 X
	Monkey	1 year	200	98	10 X
			TDF		
GI Toxicity	Rat	42 weeks	30/100	4/8	1/3 X
	Dog	42 weeks	30	30	10 X
	Monkey	56 days	250	20	7 X
Renal Toxicity	Rat	42 weeks	300/1000	18/65	6/20 X
	Dog	42 weeks	3/10	2/7	1/2 X
	Monkey	56 days	30	4	1 X
Reduced Serum Phosphate	Rat	42 weeks	1000	65	20 X
	Dog	42 weeks	30	30	10 X
	Monkey	56 days	< 30	4	< 1 X
Bone Mineral Loss	Rat	42 weeks	100/300	8/18	3/6 X
	Dog	42 weeks	10/30	7/30	2/10 X
	Monkey	56 days	ND	ND	ND

Human AUC_{ss} (~ 10 µg·h/mL) following a 200 mg/day dose of FTC.

Human AUC_{ss} (~ 3 µg·h/mL) following a 300 mg/day dose of TDF.

Rilpivirine

The safety margins of RPV are expressed as the ratio of the AUC value at the NOAEL or Lowest Observed Adverse Effect Level (LOAEL) in animals and that in man at the recommended dose of 25 mg TMC278 once daily. At this dose, the C_{max} of TMC278 in patients was 0.13 µg/mL and the $AUC_{0\text{-}24h}$ was 2.4 µg.h/mL. The AUC values and the NOAELs and LOAELs are presented in Table 4.

Table 4. Ratio Animal/Man Exposures at NOAEL or LOAEL

Species	Type study	NOAEL (mg/kg/day)		LOAEL (mg/kg/day)		AUC_{0-24h} (µg.h/Ml)		Ratio	
		M	F	M	F	M	F	M	F
Mouse	3-mo Tox	20	20			80	61	33	25
	Carcinogenicity			*20*	*20*	*76*	*51*	*32*	*21*
Rat	1-mo Tox	10	10			7.2	14	3	6
	6-mo Tox			*40*	*40*	*12*	*50*	*5*	*21*
	Teratogenicity		40 [b]				37		15
	Carcinogenicity			*40*	*40*	*6.3*	*14*	*3*	*6*
Rabbit	Teratogenicity		20 [b]				232		97
Dog	12-mo Tox			5	5	17	19	7	8
Monkey	8-wk Tox				200		2.7		1.1
Man	CPIII 25 mg Once Daily [a]					2.4			

NOAEL: No Observed Adverse Effect Level; LOAEL: Lowest Observed Adverse Effect Level; AUC: area under the time vs concentration curve; Ratio: AUC animal/AUC man; M: male; F: female; mo: month; wk: week; CPIII: clinical phase III; *Italic*: effect observed not relevant for man

a Pooled data C215 C209 Module 5.3.5.3, TMC278-TiDP6-C904-Anal-Eff-Viral, Display PK/PD.1

b Fetal NOAEL

In the dog and the cynomolgus monkey, a NOAEL could not be established as effects pertaining to inhibition of key enzymes of adrenal steroidogenesis were still present at the lowest dose tested. However, upon close monitoring in man, no indications of interference of TMC278 with adrenal steroidogenesis could be found in long term safety data of TMC278-C204 and in the Phase 3 studies, C209 and C215 (see Module 2.7.4 [RPV Summary of Clinical Safety]) with a dose of up to 150 mg once daily. In the carcinogenicity studies, a NOAEL could not be established as neoplastic lesions occurred in the lowest doses tested. The results of the carcinogenicity studies are evaluated in Section 2.4.4.4.2 and the relevance for man is assessed in Section 2.4.4.10.2. It is concluded that RPV induced tumors in liver of mice and rats and in thyroid gland of rats by an epigenetic mechanism involving liver enzyme induction. This mechanism bears little or no relevance for man.

2.4.4.10. Target Organ Effects

2.4.4.10.1. Emtricitabine and Tenofovir DF

Emtricitabine and TDF exhibit different patterns of target organ toxicity. Specifically, the only significant effect of FTC identified at dose levels constituting large clinical multiples was a minor anemia. In contrast, extensive nonclinical investigations of the toxicity of TDF have shown that the bone marrow is not a target for this agent, and that the target organs for

TDF are distinctly different (GI, bone, and kidney). While the toxicity profile for TDF is more diverse than that of FTC, the ample nonclinical safety database on both drugs strongly indicates that no potential for drug interaction exists and further toxicological investigations are unlikely to yield new data relevant to humans.

In a variety of in vitro and in vivo studies, FTC and TDF have shown a low potential for mitochondrial toxicity. In vitro studies to examine the potential mitochondrial effects of the combination of TDF and FTC showed no significant findings.

Further studies of longer duration with the FTC/TDF combination seem unwarranted given the lack of effects in toxicity studies of up to 4 weeks, the lack of overlapping toxicities, and the extensive clinical experience with the individual agents and the combination product, Truvada, within antiviral combination therapy for the treatment of HIV-1 infection.

Bone Toxicity, Renal Toxicity, and Gastrointestinal Tract

Nonclinical studies of TDF conducted in rats (Tabulated Summary 2.6.7.17.C, Report P2000078; Tabulated Summary 2.6.7.7.L, Report 97-TOX-4331-002), dogs (Tabulated Summary 2.6.7.7.S, Report 97-TOX-4331-001), and monkeys {7311} (Tabulated Summary 2.6.7.17.C, Report T1278-00034) revealed target organ effects in the GI tract (rodents only), kidney, bone, and a decrease in serum phosphate concentration. Bone toxicity was diagnosed as osteomalacia in monkeys and reduced BMD in rats and dogs. Findings in the rat and monkey studies indicated that there was a substance-related decrease in intestinal absorption of phosphate with potential secondary reduction in BMD. The mechanisms of these toxicities are not completely understood.

Genotoxicity & Carcinogenicity

No specific cause for concern was identified for FTC and TDF. Relevant information is presented in Sections 2.4.4.3.1 and 2.4.4.4.1.

Reproductive and Developmental Toxicity

No specific cause for concern was identified for FTC and TDF. Relevant information is presented in Sections 2.4.4.5.1 and 2.4.4.5.3.

2.4.4.10.2. Rilpivirine

The targets of toxicity of RPV identified in the repeat-dose studies were the following: RBCs (mouse, rat, and dog), coagulation (rat), liver (rat and dog), kidneys (mouse and dog), thyroid gland with secondary effects on the pituitary gland (rat), adrenal glands (mouse, rat, dog, and cynomolgus monkey), testes (dog), and ovaries (dog, in immature females with secondary effects on other tissues of the genital tract and on mammary glands). The majority of the induced effects appeared to be completely reversible after a 1-month postdosing period. The effects on thyroid gland and coagulation in rats, and on liver and serum ALP in dogs showed signs of recovery, but this was not complete at the end of the 1-month postdosing period. A

number of targets were affected at the low dose tested in dogs and cynomolgus monkeys preventing establishment of a NOAEL in these species.

Liver

Liver effects in mice and rats (Module 2.6.6, Toxicology Written Summary, Sections 2.6.6.3.1 and 2.6.6.3.2) differed qualitatively from those in dogs. In the rodent species, predominantly hepatocellular hypertrophy occurred accompanied by effects on liver-associated serum parameters. Liver enzyme activities (serum ALT and ALP) were more consistently increased in the mouse studies than in the studies in rats. Moreover, serum total protein concentrations mainly due to serum albumin were increased. However, serum triglycerides and total bilirubin (rats, only) concentrations were reduced.

Mice showed some additional or different effects compared to rats. These differences may be associated with the much higher exposures in mice than in rats. The hepatocellular hypertrophy in mice was accompanied by an increase in vacuolization and single cell necrosis. Electron microscopy indicated that this hypertrophy was associated with peroxisome proliferation. By contrast, the incidence of these additional effects was lower in rats. In addition, mice showed pigmentation and proliferation of Kupffer cells and increased serum concentrations of cholesterol, calcium, and inorganic phosphorus. The interrelationship of the serum chemistry effects is doubtful and an association with the Kupffer cell effects is not very likely.

In dogs (Module 2.6.6, Toxicology Written Summary, Section 2.6.6.3.4), the liver effects changed with the duration of treatment. In the 1-month study, perivascular inflammatory reactions together with multifocal perivascular fibrosis and single cell necrosis were noted in the central part of the lobules. Moreover, increased MPS aggregates occurred and multifocal bile duct proliferation. These effects seem to be associated with increased serum concentrations of cholesterol and total bilirubin, and increased activities of ALP and ALT. In the 6--month study, pigment-laden perivascular macrophages were noted and in males only, prominent brown pigment in the gall bladder epithelium. The pigment in the macrophages was not stainable with Perl's stain indicating that it was probably of lipogenic origin. This pigmentation was the only liver effect observed after 3 months of dosing (in high dose males only), indicating the relatively slow development of this effect, similar to development of the pigmentation in the gall bladder. In the 12-month dog study, pigmentation of hepatocytes and canaliculi was noted together with prominent brown pigment in gall bladder epithelium, both in males and females. The histopathological effects were associated with an increased serum concentration of total bilirubin and with an increase in serum ALP activity.

The nature and degree of the observed effects in serum chemistry, liver histopathology, and liver enzyme induction, as well as the absence of significant bioaccumulation, generation of reactive metabolites, and immune-related hepatic effects support the conclusion that TMC278 has a low potential for inducing hepatotoxicity (Draft Non-Clinical Guideline for Drug-Induced Hepatotoxicity. European Medicines Agency. CHMP. Doc. Ref. EMEA/CHMP/SWP/150115/2006. London, 24 January 2008).

In the studied species, liver effects did not occur at the lowest dose tested indicating that the exposure in animal studies without liver effects is at least 3-fold higher than the exposure at the intended human dose of 25 mg TMC278 once daily (Table 3). Long-term safety data of TMC278-C204 do not indicate an adverse effect of TMC278 on liver (see Module 2.7.4 [RPV Summary of Clinical Safety]).

Thyroid Gland

Effects on the thyroid gland in rats were characterized by increased organ weight, hypertrophy of follicular epithelium, and reduced serum concentrations of T_4 (Module 2.6.6, Toxicology Written Summary, Section 2.6.6.3.1). The effects occurred similarly in males and females and were noticed after 7 days of treatment. Upon longer duration of treatment, increased serum concentrations of TSH were recorded and also a differential effect on T_3. The serum concentrations of this thyroid hormone were less reduced than those of T_4 and in some studies not affected or even increased. The effects on the thyroid gland are thought to be caused by an increased clearance of T_4. A well-known and frequent cause of the enhanced thyroxine clearance in rats is induction of T_4-uridine diphosphate glucuronosyltransferase (UDPGT) in liver. Ex vivo enzyme induction determinations in liver homogenate from rats treated with TMC278 for 6 months showed only a 20% higher UDPGT activity of the high-dose group dosed with 400 mg/kg/day compared to controls. There are no indications that TMC278 has a direct effect on thyroid gland or a particular affinity to thyroidal tissues. The NOAEL for the thyroid gland effects is 10 mg/kg/day associated with AUC levels of 7.2 and 14 µg.h/mL in male and female rats, respectively, and at least 3-fold higher than the exposure at the intended clinical dose of 25 mg TMC278 once daily (Tabulated Summary 2.6.7.7.H; Report TMC278-Exp5692).

The minimal follicular cell hypertrophy that was noted in the thyroid gland of immature female cynomolgus monkeys in the 8-week study is considered associated with the young age of these animals at the start of the study. In this study, no levels of thyroid hormones or TSH were determined. Microscopic evaluation of other organs did not show any cause for the thyroidal effect.

The majority of circulating T_4 in rodents is unbound to plasma proteins due to low expression of thyroid binding globulin (TBG). Primarily, the unbound fraction of T_4 is metabolized by UDPGT. Consequently, even a small increase in metabolic clearance has a significant impact on the plasma concentrations of T_4 in rodents. In contrast, T_4 in man is almost completely bound primarily to TBG and to a lesser extent to transthyretin and albumin. Should UDPGT induction occur in man, this will have only a small effect on the total T_4 concentration in plasma. For this reason, the thyroid effect seen in rats bears no relevance for man. Long-term safety data of TMC278-C204 do not indicate any effect of TMC278 on plasma T_4 concentrations (see Module 2.7.4 [RPV Summary of Clinical Safety]).

Pituitary Gland

Effects on the pituitary gland occurred only in rats and were characterized by an increase of swollen and vacuolated cells in the pars distalis. These effects are considered secondary to

the effects on T_4 clearance. The indicated cells produce TSH. The low concentration of T_4 in the circulation is the feedback signal for the pituitary gland to produce more TSH. The NOAEL for effects on the pituitary gland or serum concentrations of TSH is 10 mg/kg/day associated with AUC levels of 7.2 and 14 µg.h/mL in male and female rats, respectively (Tabulated Summary 2.6.7.7.H; Report TMC278-Exp5692). As the effects on the pituitary gland and TSH are secondary to the effects of TMC278 on rodent-specific T_4 clearance, they bear no relevance for man. Long-term safety data of TMC278-C204 do not indicate any effect of TMC278 on plasma T_4 concentrations (see Module 2.7.4 [RPV Summary of Clinical Safety]).

Kidneys

Transgenic mice treated with 320 mg/kg/day, and a few treated with 80 mg/kg/day, and wild type mice at 320 mg/kg/day showed minimal to moderate degenerative or necrotic nephropathy (Module 2.6.6, Toxicology Written Summary, Section 2.6.6.3.1). In the carcinogenicity study with CD-1 mice (Module 2.6.6, Toxicology Written Summary, Section 2.6.6.5), no increased incidence of nephropathy, mortality associated with renal effects, or renal neoplasia was noted. The NOEL for this effect is 20 mg/kg/day. At this dose, the exposure is at least 25 times higher than the exposure at the recommended dose of 25 mg TMC278 once daily in man (Table 4).

Dogs treated for 12 months with 40 mg/kg/day showed nephritis in males and mineralization in the corticomedullary region in females (Module 2.6.6, Toxicology Written Summary, Section 2.6.6.3.4). The NOEL for these effects is 10 mg/kg/day. At this dose, the exposure is at least 10 times higher than the exposure at the recommended dose of 25 mg TMC278 once daily in man (Table 4).

The mechanism of these kidney effects in mice and dogs is not clear taking into account the low percentage of the dose excreted via urine (see Module 2.6.4, Pharmacokinetic Written Summary, Section 2.6.4.6.1). Long-term safety data of TMC278-C204, and from Phase 3 studies C209 and C215, indicate a small, but consistent increase of the serum creatinine concentration (see Module 2.7.4 [RPV Summary of Clinical Safety]). The kidney effects seen in mice and dogs are not indicative of an effect on glomerular filtration or proximal tubular resorption. For these reasons and in view of the large safety margins (25-fold and 10-fold in mice and dogs, respectively), the nonclinical kidney effects are considered of unknown relevance for man.

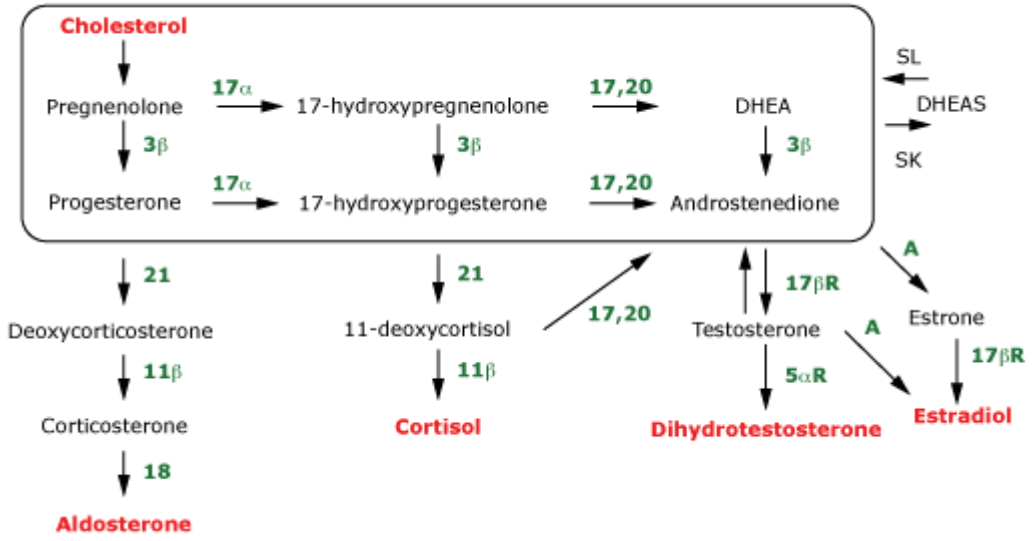
Adrenal Glands

In all species involved in nonclinical safety studies with TMC278, except rabbits, an effect on adrenal gland has been demonstrated (Module 2.6.6, Toxicology Written Summary, Section 2.6.6.3). The levels of adrenal hormones or their precursors and ACTH in serum of rats and dogs, and the results of the in vitro studies (Module 2.6.6, Toxicology Written Summary, Section 2.6.6.8.3.1) with guinea pig primary cell cultures and homogenate of adrenal gland cortex of dog indicate that TMC278 partially inhibits 21-hydroxylase, also known as CYP450 isozyme 21 (CYP21) (see Figure 3). CYP21 catalyzes the conversion

from progesterone and 17α-hydroxyprogesterone to 11-deoxycorticosterone and 11-deoxycortisol, respectively; the direct precursors of corticosterone and cortisol, respectively. A partial block of this pathway will lead to a reduced production of downstream hormones, of which cortisol and corticosterone are the most important. Moreover, an accumulation of the substrates of CYP21, progesterone and 17α-hydroxyprogesterone, and more upstream hormones may occur. In vivo, the reduced serum levels of cortisol and corticosterone due to inhibition of CYP21 will trigger an ACTH response from the pituitary. The increased stimulus of the adrenal cortex by ACTH will partially counteract the reduction of the cortisol and cortico-sterone synthesis, but at the same time will increase the accumulation of the CYP21 substrates.

An additional effect of the CYP21 inhibition, notably in species that have a significant androgenic adrenal pathway, is that the increased ACTH stimulus and subsequent accumulation of progesterone and 17α-hydroxyprogesterone may lead to an increased androgen production. 17α-hydroxypregnenolone and 17α-hydroxyprogesterone are converted to DHEA and androstenedione, respectively (catalyzed by 17, 20 lyase [CYP17]). CYP17 in the zona fasciculata catalyzes the hydroxylation of pregnenolone and progesterone and is also known as 17-hydroxylase. To investigate the impact of the stimulation of the androgenic pathway, a study was conducted in cynomolgus monkeys. This monkey strain and all nonhuman primates share with man a significant androgenic adrenal pathway. The 8-week study in cynomolgus monkeys (Tabulated Summary 2.6.7.7.X, Report TMC278-NC248) showed that TMC278 inhibits both CYP21 and CYP17. This dual effect led to a clear accumulation of progesterone and 17α-hydroxyprogesterone, both being substrates for both CYP21 and CYP17, and a reduction of the unchallenged serum levels of androstenedione and DHEA and of the response of these androgens to ACTH challenge in this species.

Figure 3. Synthetic Pathways for Adrenal Steroid Synthesis in Nonhuman Primates and Man



The first step in adrenal steroid synthesis is the combination of acetyl CoA and squalene to form cholesterol, which is then converted into pregnenolone. The enclosed area contains the core steroidogenic pathway utilized by the adrenal glands and gonads.
17α: 17α-hydroxylase (CYP17, P450c17); 17,20: 17,20 lyase (also mediated by CYP17); 3β: 3β-hydroxysteroid dehydrogenase; 21: 21-hydroxylase (CYP21A2, P450c21); 11β: 11β-hydroxylase; (CYP11B1, P450c11); 18 refers to the 2-step process of aldosterone synthase (CYP11B2, P450c11as), resulting in the addition of an hydroxyl group that is then oxidized to an aldehyde group at the 18-carbon position; 17βR: 17β-reductase; 5αR: 5α-reductase; DHEA: dehydroepiandrostenedione; DHEAS: DHEA sulfate; A: aromatase (CYP19).

The effects of TMC278 on adrenal gland and its hormonal biosynthetic pathways are more prominent than the effects on gonads and their pathways (see below in more detail). This may be explained by the affinity of TMC278 for adrenal gland in repeat-dose studies in dogs and of radiolabel in the rat single-dose QWBA studies with [^{14}C]TMC278 (see Module 2.6.4, Pharmacokinetic Written Summary, Section 2.6.4.4.2). In the QWBA studies, no particular affinity for gonads was noted.

Effects on adrenal hormones were noted in all repeat-dose dog studies with treatment duration of at least 7 days. Apparently, the effect needs some time to become manifest. This may explain why no effects were noted in the single oral dose dog study (Tabulated Summary 2.6.7.17B) with a corticotrophin releasing factor (CRF) challenge 24 hours after dosing.

The fact that effects on adrenal pathways were already detected at the lowest dose levels in the dog and cynomolgus studies prevented establishment of a NOAEL in these species. Long-term safety data from TMC278-C204 and from Phase 3 studies C209 and C215 showed no clinically relevant effects of inhibition of CYP21 and CYP17 in adults (Module 2.7.4, Summary of Clinical Safety, Section 2.7.4.4.3 and Module 2.7.4, RPV Summary of Clinical Safety).

Ovaries

The effects in ovaries of dogs after at least 1 month of treatment with TMC278 were characterized by increased numbers of tertiary follicles, (cystic) luteinized follicles, and in some dogs by corpora lutea (Module 2.6.6, Toxicology Written Summary, Section 2.6.6.3.4). These effects did not intensify over time after 3 months of dosing. The age of the dogs at the start of dosing in the 1-month studies was between 6.5 and 8 months. In the 1-month studies, the ovarian effects were associated with changes in the other parts of the female genital tract and with the prominent activation of the mammary glands. It is conceivable that the mechanism of action of the effects on the ovaries in dogs is associated with the inhibition of CYP21 and CYP17 by TMC278. But the exact mechanism of action of the observed early maturation (1-month studies) or activation of ovaries in the dog could not be evaluated from the general toxicity studies. Effects of TMC278 at the level of serum concentrations of progesterone and estradiol were difficult to evaluate due to the estrous cycle of the dogs.

The absence of ovarian effects in rats and immature cynomolgus monkeys provided no contribution to the establishment of the mechanism of the ovarian effect in dogs. The absence of the ovarian effects in the cynomolgus monkeys cannot be explained by lack of sensitivity of that species for the endocrine effects of TMC278. The compound had a clear effect on adrenal CYP21 and CYP17 in that species as shown by the increases in serum progesterone and 17α-hydroxyprogesterone and from the reduction of DHEA and androstenedione. The more early stage of development of the monkeys (approximately 20 months of age at necropsy, at least 1 year prior to the onset of puberty) compared to the dogs (approximately 7 months of age at necropsy, a few months prior to the onset of puberty) may have contributed to the difference in response. However, a dog-specific effect cannot be excluded.

A NOAEL could not be established as the ovarian effects occurred also in the lowest dose tested in dogs. However, long-term safety data from TMC278-C204 and from Phase 3 studies C209 and C215 showed no clinically relevant effects on the estrous cycle (Module 2.7.4, Summary of Clinical Safety, Section 2.7.4.4.3.3 and Module 2.7.4, RPV Summary of Clinical Safety).

Testes

The Leydig cell changes seen in dogs only were characterized by bilateral diffuse swelling or hypertrophy noted at the end of 1 month and 12 months of treatment, and hyperplasia and hypertrophy after 3 and 6 months of treatment (Module 2.6.6, Toxicology Written Summary, Section 2.6.6.3.4). The effects were minimal in the 1-month study, most prominent after 3 months of treatment, and again minimal after 6 and 12 months of treatment. It cannot be excluded that the minimal Leydig cell hypertrophy is associated with the effects of TMC278 on CYP21 and CYP17. However, the exact mechanism of action could not be evaluated in these general toxicity studies in which there were no effects on serum testosterone or luteinizing hormone (LH). Moreover, long-term safety data from TMC278-C204 and from Phase 3 studies C209 and C215 showed no clinically relevant effects (Module 2.7.4, Summary of Clinical Safety, Section 2.7.4.4.3.3 and Module 2.7.4, RPV Summary of Clinical Safety).

In addition to the Leydig cell effects, marginal findings were noted in the seminiferous tubules and secondarily in the epididymides. The marginally increased focal unilateral atrophic tubules seen at the high dose after 3 and 6 months of treatment were associated with marginal cellular debris in the epididymides in the mid- and the high-dose groups. Reviewing all the data on testes after completion of the development program, this effect and the reduced spermiogenesis leading to reduced numbers of spermatozoa in the epididymides of a single animal in the mid- and high-dose groups after 6 months of treatment are not considered of toxicological relevance. The main reasons for this conclusion are the similarity with background data and the lack of effects on spermiogenesis in the 12-month study upon staging of the spermatogenic cycles.

Red Blood Cells

In mice, rats, and dogs, TMC278 caused a small (never exceeding 10%) reversible reduction of RBC parameters in combination with regenerative signs. The effects usually occurred only in the high-dose groups in each of the studies with the indicated species. There were no clinical signs or symptoms of anemia noted in any of the species. Since exposures at the high doses in the various species differed considerably, it seems that the effect on RBCs is not directly associated with exposure. Regeneration was shown by extramedullar hematopoiesis leading to increases in reticulocyte counts. The mechanism of action of the effect on RBC parameters could not be established in any species. However, there were no signs indicative of bone marrow suppression. The increased cellularity in bone marrow of female mice at the high dose is likely due to an increase of erythroid elements compensatory to the decrease of RBCs in the circulation. The increased myeloid/erythroid ratio in bone marrow of male mice is assumed due to an increase in myeloid precursor cells compensating the decrease of lymphocytes in the circulation. Moreover, in the mouse and rat carcinogenicity studies, no effects were noted on bone marrow and hematopoietic cells.

The NOEL for this effect with the lowest exposure in terms of AUC was established in rats at 120 mg/kg/day (Tabulated Summary 2.6.7.7.I, Report TMC278-NC101). The associated AUC value of 35 µg.h/mL is approximately 15 times higher than the exposure in man at the recommended dose of 25 mg TMC278 once daily. For this reason, it is not likely that the small effect on RBCs in the nonclinical species is associated with the decrease of RBC count in clinical Phase 3 studies TMC278-C209 and TMC278-C215 (Module 2.7.4, [RPV Summary of Clinical Safety]).

Coagulation

In male rats, increased values for APTT and PT were noted in the 2-week pilot study with TMC278 at doses of 1500 mg/kg/day and higher and in the 6-month study at all doses of TMC278 from 40 up to 400 mg/kg/day. Increased values of intrinsic (APTT) and extrinsic (PT) coagulation times indicate a reduced efficacy of these major coagulation pathways. The relevance of this limited effect (less than 30% increase) is questionable since it was not associated with any bleeding, did not demonstrate a dose effect relationship, and occurred in males only.

The effect did not occur in the lowest dose tested, 10 mg/kg/day, associated with an AUC value in males that was 3 times higher than the exposure at the recommended dose of 25 mg TMC278 once daily (Table 5). Moreover, long-term safety data from TMC278-C204 showed no clinically relevant effects on coagulation (Module 2.7.4, [RPV Summary of Clinical Safety]).

Genotoxicity and Carcinogenicity

Genotoxicity tests, in vitro and in vivo, have shown TMC278 to be free of a genotoxic potential (Module 2.6.6, Toxicology Written Summary, Section 2.6.6.4). Nevertheless, the carcinogenicity studies with TMC278 in mice and rats induced hepatocellular adenomas and carcinomas and in rats follicular adenomas and carcinomas in the thyroid (Module 2.6.6, Toxicology Written Summary, Section 2.6.6.5).

Hepatocellular adenomas and carcinomas are common spontaneous liver neoplasms in rodents {14837}. In general, hepatocarcinogens are divided into genotoxic and nongenotoxic agents {15694}. Since TMC278 is not genotoxic in a battery of in vitro and in vivo assays, the neoplastic lesions in liver observed in the mouse and rat carcinogenicity studies are considered a consequence of a nongenotoxic mechanism of the action of TMC278, rather than an expression of a direct carcinogenic potential of the compound. For nongenotoxic carcinogens, several mechanisms of action have been reported for rodent liver neoplasm development including phenobarbital-like CYP450 induction {15695}. The increased liver weight associated with signs of enzyme induction caused by TMC278 was already evident in the repeat-dose general toxicity studies (Module 2.6.6, Toxicology Written Summary, Section 2.6.6.3). Ex vivo hepatic enzyme activity evaluated in the 3-month mouse study and the 6--month rat study showed that TMC278 caused strong induction of the CYP4A family in male and female mice and male rats, and induction of the CYP3A family in female rats (Module 2.6.4, Pharmacokinetics Written Summary, Section 2.6.4.5.1.2). Electron microscopy of mouse liver noted peroxisome proliferation, a lesion commonly associated with CYP4A induction {15696}.This pattern of liver enzyme induction correlates well with the incidence of hepatocellular adenomas and carcinomas {15696}.

The repeat-dose toxicity studies in rats (Module 2.6.6, Toxicology Written Summary, Section 2.6.6.3.2) have demonstrated, without exception, effects on the thyroid gland considered due to increased clearance of T_4 by UDPGT induction. UDPGT induction is a well-known cause of increased thyroid hormone clearance and, if it occurs life-long, such induction is associated with the development of follicular adenomas and carcinomas {15703}.

No neoplastic lesions were observed in adrenal glands in spite of the high affinity of TMC278 and/or its metabolites for this tissue and the observed indications of inhibition of CYP21. The latter caused a reduced output of cortisol and corticosterone in several species including the rat. The decreased serum levels of these corticosteroids lead to increased stimulation of the adrenal gland by ACTH to compensate for the reduced output. The absence of neoplasia in adrenal tissue for which TMC278 has a high affinity indicates the absence of a direct genotoxic potential of the compound.

Taking into account the results of the genotoxicity and the carcinogenicity studies, it is concluded that TMC278 has no potential to induce direct DNA-related effects.

The epigenetic carcinogenic effects on mouse and rat liver, and rat thyroid gland, are associated with induction of liver enzymes CYP3A, CYP4A, and UDPGT. A similar association between liver enzyme induction and carcinogenesis does not exist for man {15694}, {15695}.

For these reasons, it is concluded that the epigenetic carcinogenic effects of TMC278 in mouse and rat bear no relevance for man.

Reproductive and Developmental Toxicity

The reproductive and developmental toxicity studies did not demonstrate any effects on male or female fertility or fecundity (Module 2.6.6, Toxicology Written Summary, Section 2.6.6.6.1). The NOAEL in the male fertility study was at least 1600 mg/kg/day, associated with an $AUC_{0\text{-}24h}$ of approximately 85 µg.h/mL obtained from 2-week oral rat studies with TMC278 (see Tabulated Summary 2.6.7.12.C, Report TMC278-NC124). The NOAEL in the female fertility study is at least 400 mg/kg/day, associated with an $AUC_{0\text{-}24h}$ of approximately 100 µg.h/mL obtained from 2-week oral rat studies with TMC278 (see Tabulated Summary 2.6.7.12.D, Report TMC278-NC125). The exposures at NOAEL in rats are at least 35 times higher than the exposure at the recommended dose of 25 mg TMC278 once daily in man.

The results of the embryo-fetal developmental studies in rats and rabbits with the highest feasible exposures to TMC278 demonstrated the absence of a potential for teratogenicity (Module 2.6.6, Toxicology Written Summary, Section 2.6.6.6.2). The maternal and fetal NOAELs in rats were established at 40 mg/kg/day, associated with a maternal $AUC_{0\text{-}24h}$ of 37 µg.h/mL. In rabbits, the fetal NOAEL was established at 10 mg/kg/day, associated with a maternal $AUC_{0\text{-}24h}$ of 170 µg.h/mL. The exposures at the NOAEL for embryo-fetal toxicity are at least 15 times higher than the exposure at the recommended dose of 25 mg TMC278 once daily in man. The safety of TMC278 has not been assessed in pregnant women in well controlled clinical trials. Therefore, women of child bearing potential should only use TMC278 if the potential benefit justifies the potential risk.

In the peri- and postnatal developmental study in rats, no effects were observed on maternal behavior after parturition and during weaning, or on development of offspring from dams treated with TMC278 during pregnancy and lactation (Module 2.6.6, Toxicology Written Summary, Section 2.6.6.6.3). The NOAEL in the peri- and postnatal developmental study is at least 400 mg/kg/day, associated with a maternal $AUC_{0\text{-}24h}$ of approximately 100 µg.h/mL obtained from 2-week oral rat studies with TMC278 (see Tabulated Summary 2.6.7.14). This exposure is at least 40 times higher than the exposure at the recommended dose of 25 mg TMC278 once daily in man. In animals, no studies have been conducted to assess directly the excretion of TMC278 into the milk. In humans it is not known if TMC278 is excreted in milk. Because of the potential for HIV transmission to nursing infant, mothers should be instructed not to breastfeed if they are receiving TMC278.

Juvenile Toxicity

In the rat juvenile toxicity study with pups from TMC278-treated mothers dosed by gavage from LDs 12 to 25, no effects were noted (Tabulated Summary 2.6.7.14B, Report TMC278-NC168). The exposure of these pups was similar to that of adult rats dosed with the same dose of TMC278. Studies with immature dogs and cynomolgus monkeys showed no effects different from those in adult animals in the case of dogs, and no effects apart from those associated with inhibition of adrenal CYP21 and CYP17 in the immature female cynomolgus monkeys. These adrenal effects in cynomolgus monkeys are considered independent of the age of development and are similar (with respect to CYP21) to those seen in immature and adult dogs and adult rats. The ovarian effects in immature dogs did not occur in immature cynomolgus monkeys and are considered dog-specific and not relevant for man (Module 2.6.6, Toxicology Written Summary, Section 2.6.6.3). Therefore, it is concluded that TMC278 will not induce different effects in children and adolescents from those it has in adults.

2.4.5. INTEGRATED DISCUSSION AND CONCLUSIONS

2.4.5.1. Correlation of Nonclinical and Clinical Findings

The correlation of key nonclinical findings with clinical findings is addressed below in *Justification for Text in Labeling*.

2.4.5.2. Justification for Text in Labeling

The proposed Prescribing Information for the FTC/RPVTDF FDC includes all relevant nonclinical safety findings.

Based on findings in the nonclinical studies, the key safety points for consideration that are related to FTC, RPV, or TDF include: (1) potential for bone loss upon chronic dosing due to TDF, (2) potential for renal toxicity due to TDF, especially related to use with other drugs that have been shown to cause renal toxicity and in patients with renal impairment, (3) use in patients with hepatic impairment (4) use during pregnancy and lactation, (5) potential for mitochondrial toxicity, (6) potential for carcinogenicity, and (7) potential for QT prolongation due to RPV. For RPV, several additional points should be considered for inclusion in the appropriate sections of the product label: (1) RPV is a substrate of CYP3A and as a result potent inhibitors or inducers of this family of isozymes may alter plasma concentrations of RPV and its therapeutic effects, (2) RPV is bound more than 99% to plasma proteins, primarily to albumin, and (3) the main route of excretion is by feces (urinary elimination in man is less than 1% of the dose).

In regard to these possible concerns, the following should be considered:

1. A reduction in bone mineral density has been observed in nonclinical and clinical studies with TDF. It is therefore considered prudent to include a warning within the 'Warnings and Precautions' section of the proposed Prescribing Information to highlight the small decreases in bone mineral density that have been observed in clinical studies. A statement has also been included in the section on 'Preclinical data' to highlight that preclinical studies of TDF revealed effects on bone and that the mechanisms are not completely understood.

2. As nephrotoxicity has been seen nonclinically and there have been postmarketing reports of renal toxicity with TDF, warnings regarding these reports and appropriate monitoring guidance is included on the proposed Prescribing Information.

3. The potential for hepatotoxicity appears to be low. Emtricitabine and TFV are not metabolized, do not interact significantly with P450 enzymes and are not excreted to any significant extent by the liver. In addition, there was no substantive hepatotoxicity identified in the nonclinical studies with these agents. Rilpivirine has a low potential for inducing hepatotoxicity. Results from a pharmacokinetic study in patients with hepatic impairment demonstrated that TDF 300 mg once daily maybe administered without regard to hepatic function. No dose adjustment is required for RPV in patients with mild

or moderate hepatic impairment. As has been previously observed for other antiretroviral agents with anti-hepatitis B activity (e.g., 3TC, adefovir dipivoxil), hepatitis flares, or possible signs and symptoms of hepatitis flares, have been observed following withdrawal of treatment with TDF or FTC in patients co-infected with HBV or HCV. Consequently, a warning statement regarding posttreatment hepatic flares in patients co-infected with hepatitis B is included in the labeling.

4. Animal data indicate that neither FTC, nor RPV or TDF, causes reproductive or fetal toxicity. However, TFV has been shown to cross the placenta in monkeys and to be excreted in milk. Emtricitabine has also been shown to cross the placenta and the ratio of FTC concentrations in plasma in pregnant mice and rabbits as compared to their fetuses was approximately 0.4. It is not known if RPV is excreted in milk. Therefore, caution is appropriate until additional data are gathered in pregnant human patients.

5. Emtricitabine, RPV, and TDF appear to have a low potential for mitochondrial toxicity, as demonstrated by enzyme and cell analyses in vitro and by markers of mitochondrial injury in vivo. Ongoing assessment of clinical safety data from company-sponsored clinical studies and postmarketing experience has shown that the risk of mitochondrial toxicity with FTC and TDF is low. Nonetheless, since the risk cannot be excluded, a warning regarding lactic acidosis/severe hepatomegaly with steatosis is included in the proposed Prescribing Information. In addition, a statement has been inserted regarding the potential for mitochondrial dysfunction in HIV-negative infants exposed to NRTIs in utero.

6. In long-term carcinogenicity studies of FTC, no drug-related increases in tumor incidence were found in mice or in rats. Rilpivirine is not genotoxic. In long-term carcinogenicity studies, RPV caused liver tumors in mice and rats and thyroid gland tumors in rats by an epigenetic mechanism associated with liver enzyme induction and rat-specific enhanced clearance of thyroid hormones. These findings are considered rodent-specific, associated with liver enzyme induction, and are of limited relevance to humans. Tenofovir DF was negative in the rat carcinogenicity assay, but weakly positive at the highest dose in the mouse carcinogenicity assay (duodenal tumors). While the mechanism of this tumor formation is uncertain, the findings are unlikely to be of relevance to humans. Appropriate information regarding the results of the carcinogenicity studies is included in the proposed Prescribing Information.

7. Rilpivirine inhibits potassium channels involved in the repolarization of the cardiac action potential, trafficking of the hERG channel, and induces QT prolongation in the rabbit wedge model with a low potential to induce TdP. At the recommended dose of 25 mg once daily, RPV is not associated with a clinically relevant effect on QTc. In a study of healthy subjects, supratherapeutic doses of rilpivirine (75 mg once daily and 300 mg once daily) have been shown to prolong the QTc interval. Appropriate information regarding coadministration with drugs with a known risk of Torsade de Pointes is included in the proposed Prescribing Information.

In addition to the items addressed above, which are product specific, other appropriate warnings have been included in the proposed Prescribing Information. These include warnings or precautions regarding use in patients with HIV and HBV co-infection, lipodystrophy and metabolic abnormalities, and immune reactivation syndrome.

The toxicities of potential concern outlined above are adequately highlighted and addressed in the current Prescribing Information for the individual agents and the proposed Prescribing Information for the combination tablet. The proposed dose of the combination tablet for administration to adults is justified from a safety perspective based on the nonclinical data presented in this dossier.

2.4.5.3. Overall Conclusions

The pharmacologic basis to recommend the FTC/RPV/TDF FDC tablet for the treatment of HIV infection is scientifically sound given the nonclinical in vitro and in vivo efficacy data for the individual components and the combination of the agents presented in this dossier.

The pharmacokinetic and toxicologic profiles of FTC, RPV, and TFV are well characterized in multiple animal species and the findings are applicable for consideration of the use of these agents in combination. Data from controlled clinical studies of combination regimens of FTC+RPV+TDF demonstrated acceptable tolerability and safety profiles to support use in the adult HIV-1 infected population. Further, extensive postmarketing experience with the FTC and TDF individual components of the combination, and also the Truvada combination tablet, supports the proposed use of the FTC/RPV/TDF FDC tablet for the treatment of HIV-1 infection in adults.

A comprehensive nonclinical pharmacology/virology, pharmacokinetic, and toxicology program was undertaken in support of the registration of FTC and TDF. The results of these evaluations were presented in detail in the original licensing application and subsequent submissions for Emtriva and Viread, respectively. A small number of key studies were conducted using the combination of FTC and TDF. Similarly for RPV, all nonclinical studies required to support chronic use have been performed as part of the safety assessment of this novel antiretroviral agent. These included the following: a comprehensive set of primary and secondary pharmacodynamics studies; complete core safety pharmacology; complete pharmacokinetic evaluation; chronic toxicity studies; mechanistic studies to elucidate the most important effects; genotoxicity; carcinogenicity; assessment of fertility; early embryonic development; pre- and postnatal development and juvenile toxicity; evaluation of the antigenicity, immunotoxicity, phototoxicity, and skin and eye irritation; and qualification of impurities. The overall program including the data from the combination and individual agent studies is considered adequate to support the efficacy and safety of the FTC/RPV/TDF FDC combination tablets based on the following considerations.

The HIV-1 NNRTI, RPV, and the NRTIs, FTC and TFV, have potent antiretroviral activity against wild type and many drug-resistant strains of HIV-1 in vitro and in vivo. The combination of FTC, RPV, and TFV in 2-drug combination experiments showed additive to synergistic anti-HIV-1 activity, and synergistic anti-HIV-1 activity in 3-drug combination

experiments. Additive antiretroviral activity would be expected given that the active metabolites of FTC and TFV compete with different natural substrates for incorporation into viral DNA during the reverse transcription step, and RPV would also be expected to be additive when it binds at a distinct binding pocket on RT to inhibit reverse transcription. The observation of moderate synergistic anti-HIV-1 activity for the FTC/RPV/TFV combination suggests clear potentiation of the individual anti-HIV activities of these compounds within cells.

NRTIs carry a class labeling for mitochondrial toxicity; however, both FTC and TDF have shown a low potential for mitochondrial toxicity in long-term toxicity studies. The potential for mitochondrial toxicity of RPV was low by in vitro assessment of the inhibitory activity on human polymerase γ. However, as mitochondrial toxicity is generally less relevant for NNRTIs than NRTIs, and as RPV is not anticipated to significantly increase the exposure of FTC or TFV, the potential for exacerbating mitochondrial toxicity is low.

From in vitro data, pharmacokinetics studies in dogs (Module 2.6.4), and clinical experience (Module 2.7.2), there are no anticipated pharmacokinetic interactions between FTC, RPV, and TDF. Emtricitabine and TDF had little effect on vital organ systems in safety pharmacology studies. Rilpivirine has shown the potential for QT prolongation, an effect confirmed in a thorough QT study in healthy subjects. At the 25-mg dose of RPV, the observed change in QTcF was not considered clinically relevant, and the combination product is not anticipated to exacerbate the cardiovascular effect seen with RPV alone. No additional safety pharmacology studies are considered necessary with the FTC/RPV/TDF combination.

The toxicity profiles of the 3 agents differ substantially. The only toxicity observed in chronic animal studies with FTC was mild, reversible anemia in mice and minor decreases in erythrocyte counts/increases in MCH in monkeys at large multiples of clinical exposure (168-fold in mice; 26-fold in monkeys); therefore, these hematological findings are not considered relevant to clinical use. The principal target organs of toxicity following administration of RPV were the adrenal glands and associated steroid biosynthesis (mouse, rat, dog, and cynomolgus monkey), liver (mouse, rat, and dog), thyroid and pituitary glands (rat), kidney (mouse, dog), male and female reproductive organs (dog), the hematopoietic system (mouse, rat, and dog), and the coagulation system (rat). In general, effects were reversible. Effects on the liver, kidney, hematopoietic system and coagulation system were mild and occurred at higher doses and exposures. The principal target organs of toxicity following oral administration of TDF were the kidney (karyomegaly, tubular degeneration), bone, and GI tract (in rodents). Given that kidney effects with RPV have been observed in mice and dogs only at high doses, and that the routes of excretion differ for RPV and TDF, renal toxicity is not anticipated to be an issue with the combination product. Given that pharmacokinetic interactions are unlikely and that the target organ profiles are different, administration of the combination product is unlikely to exacerbate known toxicities of the individual agents.

Of the 3 compounds, only TDF had positive findings in genotoxicity studies (mouse lymphoma cell assay and UDS assay). The combination of FTC and TDF in a mouse

lymphoma cell assay did not worsen the genotoxic potential of TDF. Tenofovir DF did not have significant effects in the long-term carcinogenicity studies. Rilpivirine does not show any potential for direct DNA-related effects, and the relevance of the epigenetic carcinogenic effects on mouse and rat liver and rat thyroid are not considered relevant for humans.

Emtricitabine, RPV, and TDF have not shown significant adverse effects in reproductive and developmental toxicity studies, and the combination of the 3 components is not expected to have an altered reproductive toxicity profile compared with that of the individual agents.

The FTC/RPV/TDF combination is not anticipated to produce any new metabolites. All impurities present in the individual drug substances and in Truvada (FTC/TDF) have been qualified. There are no unique impurities or degradation products present in the FTC/RPV/TDF tablets that require qualification.

The combination of FTV, RPV, and TDF is not anticipated to exacerbate known toxicities or lead to new toxicities. Emtricitabine has an established clinical safety profile with no significant toxicities observed. Potential toxicities related to RPV that were observed preclinically, but have not been observed in clinical studies include effects on the adrenal system and associated changes in 17-OH progesterone and cortisol levels, and liver and thyroid toxicity. The known clinical toxicities for TDF are renal and bone toxicity.

The absence of nonclinical safety studies with the combination is in accordance with the CHMP Guideline on the Non-Clinical Development of Fixed Combinations of Medicinal Products (EMEA/CHMP/SWP/258498/2005, January 2008). There are no anticipated clinically relevant pharmacokinetic or toxicological interactions expected in the FTC/RPV/TDF combination. The Scientific Advice Working Party agreed with the assessment that no further nonclinical studies are needed to support the FTC/RPV/TDF FDC based on the comprehensive nonclinical data set for RPV and the long-term safety from the Phase 2b Trial TMC278-C204 (EMEA/CHMP/SAWP/670243/2009 corrigendum). Further, extensive clinical safety data are available for the approved drugs FTC, TDF, and the FTC/TDF FDC product Truvada. Additionally, data from the Phase 2b and Phase 3 trials with RPV and Truvada supports the use of these products (see Module 2.7). The clinical data and the pharmacodynamic, pharmacological, and toxicological assessment of FTC, RPV, and TDF, along with the lack of overlapping toxicity in animals, support the safety of the new combination product for HIV-1 disease.

2.4.6. REFERENCES

2.4.6.1. Literature References

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2.4.7. LIST OF GILEAD NONCLINICAL REPORTS

Table 5 provides a list of all nonclinical studies conducted for FTC, TDF, FTC/TDF, and EFV/FTC/TDF. All nonclinical studies considered pivotal to support this MAA are provided within Module 4 as outlined within the table.

This table indicates the nonclinical studies that are considered pivotal to support the FDC, which are included within Module 4, and studies that are not provided but are available on request within 48 hours (as they are not considered pivotal to the FDC and have previously been submitted and reviewed within the context of the MAAs for Emtriva (EMEA/H/C/533), Viread (EMEA/H/C/419), Truvada (EMEA/H/C/594), and Atripla (EMEA/H/C/797). All studies relating to RPV and the FDC are not included within this table, but can be found within Module 4 as these are all considered pivotal to the FDC.

Table 5. Gilead Nonclinical Reports

Nonclinical Report Number	Study Title	Cross-Reference to Study Report					
		Product	Location within FDC MAA	EU Procedure (& number)	Date Submitted	Dossier Number/Type	Volume
TESF/91/0014	Phosphorylation of 523W91 and 524W91 by calf thymus deoxycytidine kinase	Truvada	Available upon request	Original MAA (EMEA/H/C/594)	[redacted]20[redacted]	Module 4	Volume 3
TESF/92/0002	Kinetic constants for 523W91 and 524W91 with calf thymus deoxycytidine kinase	Truvada	Available upon request	Original MAA (EMEA/H/C/594)	[redacted]20[redacted]	Module 4	Volume 3
TEIT/92/0005	Phosphates of 523W91 and 524W91: results with dCPMP kinase	Truvada	Available upon request	Original MAA (EMEA/H/C/594)	[redacted]20[redacted]	Module 4	Volume 3
TGZZ/93/0025	Phosphorylation of the 5' monophosphate of 524W91 to the 5'-Di-and 5'triphosphates by cellular enzymes	Truvada	Available upon request	Original MAA (EMEA/H/C/594)	[redacted]20[redacted]	Module 4	Volume 3
TPI 15883	Activity of emtricitabine on the HIV-1 reverse transcriptase mutant K65R; biochemical and phenotypic analysis	Truvada	Available upon request	Original MAA (EMEA/H/C/594)	[redacted]20[redacted]	Module 4	Volume 1
TEZA/92/0062	Anabolism of (-) 3'Thia-2',3'-dideoxy-5-[6-^{3}H]fluorocytidine (524W91,(-) FTC) and (+)3'-Thia-2',3'-dideoxy-5-[6-^{3}H]fluorocytidine (524W91, (+)FTC) in Hep G_2 2.2.15 (P5A) Cells	Truvada	Available upon request	Original MAA (EMEA/H/C/594)	[redacted]20[redacted]	Module 4	Volume 3
TEZA/92/0103	Anabolism of (-) 3'-Thia-2',3'-dideoxy-5-[6-^{3}H]fluorocytidine (524W91, (-) FTC) in CEM T-lymphoblast cells	Truvada	Available upon request	Original MAA (EMEA/H/C/594)	[redacted]20[redacted]	Module 4	Volume 3

Nonclinical Report Number	Study Title	Cross-Reference to Study Report					
		Product	Location within FDC MAA	EU Procedure (& number)	Date Submitted	Dossier Number/Type	Volume
TEZA/92/0111	Anabolism of (-) 3'-Thia-2',3'-dideoxy-5-[6-^{3}H]fluorocytidine (524W91, (-) FTC) in Hep G_2 (human hepatocellular carcinoma) cells	Truvada	Available upon request	Original MAA (EMEA/H/C/594)	[redacted]20[redacted]	Module 4	Volume 3
TEZZ/93/0007	Inhibition of HeLa DNA polymerases α, β, γ and ε and HIV-1 reverse transcriptase by the triphosphates of ddC (16Y82), (+) FTC (523W91), (-) FTC (524W91), (+) 3TC (1960U90) and (-)3TC (1961U90)	Emtriva	Avaliable upon request	Original MAA (EMEA/H/C/533)	[redacted]20[redacted]	Part III	Volume 21
TPI 11985	Effect of orally administered emtricitabine [(-)-FTC] and lamivudine [3TC] in the HuPBMC-SCID mouse model of HIV-1 infection	Truvada	Available upon request	Original MAA (EMEA/H/C/594)	[redacted]20[redacted]	Module 4	Volume 3
TPI 462 v2	Antiviral activity of FTC, (2R-cis)-4-amino-5-fluoro-1-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-2(1H)-pyrimidinone against HIV-1	Truvada	Available upon request	Original MAA (EMEA/H/C/594)	[redacted]20[redacted]	Module 4	Volume 1
TPI 10498 v2	Evaluation of the antiviral activity of emtricitabine against HIV-1 (Group M and Subtype O) and HIV-2	Truvada	Available upon request	Original MAA (EMEA/H/C/594)	[redacted]20[redacted]	Module 4	Volume 1

Nonclinical Report Number	Study Title	Cross-Reference to Study Report					
		Product	Location within FDC MAA	EU Procedure (& number)	Date Submitted	Dossier Number/Type	Volume
10518-v2	MAGI-LU assay validation 1: Inhibitory effect of FTC on HIV-1 xxLAI viral infection is independent of multiplicity of infection (MOI) of the infecting virus	Truvada	Available upon request	Original MAA (EMEA/H/C/594)	[redacted]20[redacted]	Module 4	Volume 3
11773	Effect of multiplicity of infection on inhibition of HIV-1 replication by FTC	Truvada	Available upon request	Original MAA (EMEA/H/C/594)	[redacted]20[redacted]	Module 4	Volume 3
10247	DXG, FTC, and AZT: time of addition	Truvada	Available upon request	Original MAA (EMEA/H/C/594)	[redacted]20[redacted]	Module 4	Volume 3
463	Effect of human serum on the anti-HIV-1 activity of FTC, (2R-cis)-4-amino-5-fluoro-1-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-2(1H)-pyrimidinone	Truvada	Available upon request	Original MAA (EMEA/H/C/594)	[redacted]20[redacted]	Module 4	Volume 3
TPI 11419 v2	Evaluation of the antiviral activity of emtricitabine against HIV-1 (group M and subtype O) and HIV-2 using the MAGI-LU assay in cMAGI cells	Truvada	Available upon request	Original MAA (EMEA/H/C/594)	[redacted]20[redacted]	Module 4	Volume 1
TPI 11148	Phenotypic evaluation of FTC, DXG and MKC442 on recombinant clinical isolates of HIV-1	Truvada	Available upon request	Original MAA (EMEA/H/C/594)	[redacted]20[redacted]	Module 4	Volume 2
TPI 9501	Inhibition of human mitochondrial DNA polymerase by (+) FTC, (-) FTC, and DXG triphosphates	Truvada	Available upon request	Original MAA (EMEA/H/C/594)	[redacted]20[redacted]	Module 4	Volume 5

Nonclinical Report Number	Study Title	Cross-Reference to Study Report					
		Product	Location within FDC MAA	EU Procedure (& number)	Date Submitted	Dossier Number/Type	Volume
P4331-00035	In vitro drug sensitivity of nucleoside and non-nucleoside reverse transcriptase inhibitor resistant clinical HIV-1 isolates to tenofovir	Viread	Module 4.2.1.1	Original MAA (EMEA/H/C/ 419)	[redacted]20	Part III	Volume 24
PC-104-2003	In vitro activity of tenofovir against HIV-2	Truvada	Module 4.2.1.1	Original MAA (EMEA/H/C/594)	[redacted]20	Module 4	Volume 1
PC-104-2004	Effects of the K65R mutation on HIV-1 replication capacity	Truvada	Module 4.2.1.1	Original MAA (EMEA/H/C/594)	[redacted]20	Module 4	Volume 1
PC-104-2008	In vitro phosphorylation of tenofovir and abacavir	Viread	Available upon request	Provided in 3rd Annual Risk Benefit (EMEA/H/C/419/S/ 48)	[redacted]20	3rd Annual Risk Benefit	Volume 5
PC-104-2013	Antiviral activity vs. HIV-1 & HIV-2	Viread	Module 4.2.1.1	Previously not submitted.			
PC-104-2017	Susceptibility to tenofovir and tenofovir disoproxil fumarate (tenofovir DF) of virologic failure isolates from study GS-01-934	Viread	Module 4.2.1.1	Previously not submitted.			
PC-164-2007	Week 144 virology report of study GS-01-934	Truvada	Available upon request	Provided in Type II variation (EMEA/H/C/594 II/036)	[redacted]20	Module 5	Volume 1

Nonclinical Report Number	Study Title	Cross-Reference to Study Report					
		Product	Location within FDC MAA	EU Procedure (& number)	Date Submitted	Dossier Number/Type	Volume
P0393-00025	Tenofovir, adefovir and zidovudine susceptibilities of human immunodeficiency virus type I isolates with non-B subtypes or nucleoside resistance	Viread	Available upon request	Original MAA (EMEA/H/C/419)	[redacted] 20[redacted]	Part III	Volume 23
PC-164-2001	In vitro phosphorylation of tenofovir and emtricitabine	Truvada	Module 4.2.1.1	Original MAA (EMEA/H/C/594)	[redacted] 20[redacted]	Module 4	Volume 2
PC-164-2005	In vitro resistance selection with tenofovir and emtricitabine	Truvada	Available upon request	Submitted in PSUR [redacted] 20[redacted] to [redacted] 20[redacted])	[redacted] 20[redacted]	PSUR	Volume 2
233	Data from clonogenic assays CFU-GM and BFU-E and mitochondrial assays for TP0001 and TP0004 as compared to AZT	Truvada	Available upon request	Original MAA (EMEA/H/C/594)	[redacted] 20[redacted]	Module 4	Volume 4
TPI 11963	An in vitro evaluation of the effects on cell growth and mitochondrial functions in the MT2 cell line after long term exposure to antiviral xenobiotics	Truvada	Available upon request	Original MAA (EMEA/H/C/594)	[redacted] 20[redacted]	Module 4	Volume 4
TGZZ/93/0016	Effect of antiviral nucleoside analogues on mitochondrial DNA synthesis in Molt-4 cells	Truvada	Module 4.2.2.4	Original MAA (EMEA/H/C/594)	[redacted] 20[redacted]	Module 4	Volume 5
TGZZ/93/0023	Effect of 524W91 on mitochondrial DNA synthesis on Molt-4 cells	Truvada	Module 4.2.3.1	Original MAA (EMEA/H/C/594)	[redacted] 20[redacted]	Module 4	Volume 5
P4331-00038	Activity of tenofovir and tenofovir disoproxil fumarate against hepatitis B virus in cell culture	Viread	Module 4.2.1.2	Original MAA (EMEA/H/C/419)	[redacted] 20[redacted]	Part III	Volume 24

Nonclinical Report Number	Study Title	Cross-Reference to Study Report					
		Product	Location within FDC MAA	EU Procedure (& number)	Date Submitted	Dossier Number/Type	Volume
PC-104-2012	Antiviral activity against hepatitis B virus with the rtA194T mutation	Viread	Module 4.2.1.2	Provided in Type II variation (EMEA/H/C/419 /II/75)	[redacted]20[redacted]	Module 5	Volume 1
PC-174-2003	In vitro susceptibility of HBV rtA194T mutants to tenofovir	Viread	Module 4.2.1.2	Provided in Type II variation (EMEA/H/C/419 /II/75)	[redacted]20[redacted]	Module 5	Volume 1
C4331-00013 (P4331-00037)	In vitro cytotoxicity of tenofovir in various human cell types - comparison with other NRTIs	Viread	Available upon request	Original MAA (EMEA/H/C/419)	[redacted]20[redacted]	Part III	Volume 24
P1278-00042	In vitro assessment of tenofovir mitochondrial toxicity – comparison with approved NRTIs	Truvada	Module 4.2.1.2	Original MAA (EMEA/H/C/594)	[redacted]20[redacted]	Module 4	Volume 4
TX-104-2001	Mitochondrial toxicity of combinations of nucleoside and nucleotide analogue reverse-transcriptase inhibitors in HepG2 cells	Viread	Module 4.2.1.2	Provided in response to CHMP request EMEA/CPMP /5836/03	[redacted]20[redacted]	Response document	Volume 1
PC-174-2004	A 48-week oral dosing study of adefovir dipivoxil (ADV), tenofovir disoproxil fumarate (TDF), emtricitabine (FTC), and lamivudine 93TC) alone and in combination using the woodchuck model of hepatitis B virus infection	Viread	Module 4.2.1.2	Provided in Type II variation (EMEA/H/C/419/ II/75)	[redacted]20[redacted]	Type II variation	Volume 1

Nonclinical Report Number	Study Title	Cross-Reference to Study Report					
		Product	Location within FDC MAA	EU Procedure (& number)	Date Submitted	Dossier Number/Type	Volume
PC-164-2004	In vitro combination testing of tenofovir and emtricitabine against hepatitis B virus	Viread	Available upon request	Provided in Type II variation (EMEA/H/C/419/ II/75)	[redacted]20[redacted]	Module 5	Volume 1
TPZZ/93/0002	In vitro receptor binding potencies of 524W91	Truvada	Module 4.2.1.3	Original MAA (EMEA/H/C/594)	[redacted]20[redacted]	Module 4	Volume 6
TPZZ/92/0055	In vitro autonomic pharmacology of 524W91 and its effects on peripheral autonomic receptors	Truvada	Module 4.2.1.3	Original MAA (EMEA/H/C/594)	[redacted]20[redacted]	Module 4	Volume 6
TPZZ/92/0056	Effects of 524W91 on isolated cardiac muscle of rat, guinea-pig and cat	Truvada	Module 4.2.1.3	Original MAA (EMEA/H/C/594)	[redacted]20[redacted]	Module 4	Volume 6
477	General high dose testing results for FTC	Truvada	Module 4.2.1.3	Original MAA (EMEA/H/C/594)	[redacted]20[redacted]	Module 4	Volume 6
TPZZ/93/0001	General pharmacology of 524W91 over an extended dose range in mice and rats	Truvada	Module 4.2.1.3	Original MAA (EMEA/H/C/594)	[redacted]20[redacted]	Module 4	Volume 6
TPZZ/93/0119	Effects of 524W91 on conditioned avoidance responce in rats	Truvada	Available upon request	Original MAA (EMEA/H/C/594)	[redacted]20[redacted]	Module 4	Volume 6
TPZZ/92/0057	Effects of 524W91 on systolic blood pressure and heart rate of conscious normotensive rats	Truvada	Module 4.2.1.3	Original MAA (EMEA/H/C/594)	[redacted]20[redacted]	Module 4	Volume 6
TPZZ/92/0076	Effects of intravenous injection of 524W91 on cardiovascular, respiratory and autonomic function in anaesthetized dogs	Truvada	Module 4.2.1.3	Original MAA (EMEA/H/C/594)	[redacted]20[redacted]	Module 4	Volume 6

Nonclinical Report Number	Study Title	Cross-Reference to Study Report					
		Product	Location within FDC MAA	EU Procedure (& number)	Date Submitted	Dossier Number/Type	Volume
V2000020	Spectrum screen of GS-4331-05 and GS-1278	Truvada	Module 4.2.1.3	Original MAA (EMEA/H/C/594)	[redacted]20[redacted]	Module 4	Volume 5
V2000009	Guinea pig ileum contractile response	Truvada	Module 4.2.1.3	Original MAA (EMEA/H/C/594)	[redacted]20[redacted]	Module 4	Volume 5
R990152	A pharmacological safety assessment of the effect of tenofovir DF (GS-4331-05) on the central nervous system of the rat	Truvada	Module 4.2.1.3	Original MAA (EMEA/H/C/594)	[redacted]20[redacted]	Module 4	Volume 5
R990153	A pharmacological assessment of the effect of tenofovir DF (GS-4331-05) on gastrointestinal motility in the rat	Truvada	Module 4.2.1.3	Original MAA (EMEA/H/C/594)	[redacted]20[redacted]	Module 4	Volume 6
R990154	A pharmacological assessment of the effect of tenofovir DF (GS-4331-05) on the renal system of the rat	Truvada	Module 4.2.1.3	Original MAA (EMEA/H/C/594)	[redacted]20[redacted]	Module 4	Volume 6
D990155	Cardiovascular profile study following a single oral administration of tenofovir DF in the unrestrained conscious beagle dog	Truvada	Module 4.2.1.3	Original MAA (EMEA/H/C/594)	[redacted]20[redacted]	Module 4	Volume 6
470	In vitro synergy studies of FTC in combination with MKC 442, AZT, nelfinavir (NELF) and nevirapine (NEV) against HIV	Truvada	Module 4.2.1.4	Original MAA (EMEA/H/C/ 594)	[redacted]20[redacted]	Module 4	Volume 2
10804	In vitro synergy studies with FTC and other anti-HIV compounds	Emtriva	Module 4.2.1.4	Included in response to CPMP Day 120 List of Questions (EMEA/H/C/533)	[redacted]20[redacted]	Response to Questions	Volume 3

Nonclinical Report Number	Study Title	Cross-Reference to Study Report					
		Product	Location within FDC MAA	EU Procedure (& number)	Date Submitted	Dossier Number/Type	Volume
12207	Synergy of emtricitabine (FTC) and lamivudine (3TC) in combination with stavudine (d4T) and nevirapine (NVP) against HIV	Truvada	Module 4.2.1.4	Original MAA (EMEA/H/C/594)	[redacted]20[redacted]	Module 4	Volume 6
C1278-00005	In vitro synergy of tenofovir combinations against HIV-1	Viread	Module 4.2.1.4	Original MAA (EMEA/H/C/419)	[redacted]20[redacted]	Part III	Volume 23
PC-104-2005	Tenofovir, abacavir and lamivudine; evaluation of the in vitro anti-HIV activity of the combination in PBMC	Truvada	Module 4.2.1.4	Original MAA (EMEA/H/C/594)	[redacted]20[redacted]	Module 4	Volume 2
PC-104-2006	Tenofovir, didanosine and lamivudine; evaluation of the in vitro anti-HIV activity of the combination in PBMC	Truvada	Module 4.2.1.4	Original MAA (EMEA/H/C/594)	[redacted]20[redacted]	Module 4	Volume 2
PC-104-2007	In vitro combination studies of tenofovir and other nucleoside analogs with ribavirin against HIV-1	Truvada	Module 4.2.1.4	Original MAA (EMEA/H/C/594)	[redacted]20[redacted]	Module 4	Volume 6
PC-183-2004	Antiviral activity in combination with other antiretroviral drugs	Viread	Module 4.2.1.4	Previously not submitted			
14379	In vitro synergy studies with emtricitabine and tenofovir	Emtriva	Module 4.2.1.4	Included in Response to CPMP Day 120 List of Questions (EMEA/H/C/533)	[redacted]20[redacted]	Follow-up Measure	Volume 3
PC-164-2002	In vitro anti-HIV synergy studies of tenofovir and emtricitabine	Truvada	Module 4.2.1.4	Original MAA (EMEA/H/C/594)	[redacted]20[redacted]	Module 4	Volume 2

Nonclinical Report Number	Study Title	Cross-Reference to Study Report					
		Product	Location within FDC MAA	EU Procedure (& number)	Date Submitted	Dossier Number/Type	Volume
1010	Determination of TP-0006/96 in human, mouse and monkey plasma and human urine by HPLC-MS (SIM)	Truvada	Available upon request	Original MAA (EMEA/H/C/594)	[redacted]20[redacted]	Module 4	Volume 8
6159v1	Determination of emtricitabine in mouse, monkey and rabbit plasma by LC/MS/MS	Truvada	Available upon request	Original MAA (EMEA/H/C/594)	[redacted]20[redacted]	Module 4	Volume 8
7582v1	Determination of emtricitabine in human or monkey urine by LC/MS/MS	Truvada	Available upon request	Original MAA (EMEA/H/C/594)	[redacted]20[redacted]	Module 4	Volume 8
6447v5	Determination of emtricitabine in human and rat plasma using LC/MS/MS	Truvada	Available upon request	Original MAA (EMEA/H/C/594)	[redacted]20[redacted]	Module 4	Volume 8
P4331-00009	Validation of a high performance liquid chromatographic method for the determination of GS-4331-05 in dose formulations	Truvada	Available upon request	Original MAA (EMEA/H/C/594)	[redacted]20[redacted]	Module 4	Volume 7
P4331-00008: 97-TOX-4331-008	Cross-validation of an HPLC method for the quantitation of GS-1278 (PMPA) in mouse plasma and determination of PMPA in mouse plasma samples	Truvada	Available upon request	Original MAA (EMEA/H/C/594)	[redacted]20[redacted]	Module 4	Volume 7
P1278-00001 (OLI-VRA-144.1)	Validation of an HPLC assay for the quantitation of GS-1278 (PMPA) in rat plasma and cross-validation in cynomolgus monkey plasma	Truvada	Available upon request	Original MAA (EMEA/H/C/594)	[redacted]20[redacted]	Module 4	Volume 7

Nonclinical Report Number	Study Title	Cross-Reference to Study Report					
		Product	Location within FDC MAA	EU Procedure (& number)	Date Submitted	Dossier Number/Type	Volume
P1278-00028 (001097/NDK)	Validation of a high performance liquid chromatographic mass spectrometric method for the determination of GS-1278 (R-PMPA) in rat plasma (sodium citrate) - cross validation of GS-1278 (R-PMPA) in rat plasma (heparin) - cross validation of GS-1278 (R-PMPA) in mouse plasma (sodium heparin)	Truvada	Available upon request	Original MAA (EMEA/H/C/594)	[redacted]20[redacted]	Module 4	Volume 8
P1278-00034: Project No. 003105OU1	Validation of a high performance liquid chromatographic mass spectrometric method for the determination of GS-1278 in rat milk	Truvada	Available upon request	Original MAA (EMEA/H/C/594)	[redacted]20[redacted]	Module 4	Volume 8
P4331-035-3 (Oread No: OLI-RE748-9901-DNS-1)	Cross validation of an HPLC method for the quantitation of GS-1278 (PMPA) in rabbit plasma and determination of PMPA in rabbit samples (from study 98-TOX-4331-005)	Truvada	Available upon request	Original MAA (EMEA/H/C/594)	[redacted]20[redacted]	Module 4	Volume 8
P1278-00017	Cross-validation of an HPLC assay for the quantitation of GS-1278 (PMPA) in dog plasma	Truvada	Available upon request	Original MAA (EMEA/H/C/594)	[redacted]20[redacted]	Module 4	Volume 8

Nonclinical Report Number	Study Title	Cross-Reference to Study Report					
		Product	Location within FDC MAA	EU Procedure (& number)	Date Submitted	Dossier Number/Type	Volume
P4331-0037 (MDS Project No. 003296OTN)	Mini-validation of a high performance liquid chromatographic mass spectrometric method for the determination of GS-1278 (R-PMPA) in dog plasma (EDTA)	Truvada	Available upon request	Original MAA (EMEA/H/C/594)	[redacted]20[redacted]	Module 4	Volume 8
P1278-00029 (MDSPS Project No: 002092OFH)	Validation of a high performance liquid chromatographic mass spectrometric method for the determination of GS-1278 (R-PMPA) in monkey plasma (EDTA)	Truvada	Available upon request	Original MAA (EMEA/H/C/594)	[redacted]20[redacted]	Module 4	Volume 8
TEIN/93/0003	Pharmacokinetics of 524W91 in male CD-1 mice following oral and intravenous administration	Emtriva	Module 4.2.2.2	Original MAA (EMEA/H/C/533)	[redacted]20[redacted]	Part III	Volume 22
TEIN/93/0004	Pharmacokinetics of 100 mg/kg oral and intravenous 524W91 in male CD-1 mice	Emtriva	Module 4.2.2.2	Original MAA (EMEA/H/C/533)	[redacted]20[redacted]	Part III	Volume 22
IUW00101	Pharmacokinetic study in male mice following single oral and intravenous administration of L-(-)-2'3'-dideoxy-5-fluoro-3'-thiacytidine	Truvada	Module 4.2.2.2	Original MAA (EMEA/H/C/594)	[redacted]20[redacted]	Module 4	Volume 10
TEZZ/93/0019	A pharmacokinetic study of 524W91 in cynomolgus monkeys following oral and intravenous administration	Emtriva	Module 4.2.2.2	Original MAA (EMEA/H/C/533)	[redacted]20[redacted]	Part III	Volume 22

Nonclinical Report Number	Study Title	Cross-Reference to Study Report					
		Product	Location within FDC MAA	EU Procedure (& number)	Date Submitted	Dossier Number/Type	Volume
IUW00301	Pharmacokinetic study in cynomolgus monkeys following single oral and intravenous administration of L-(-)-2',3'-dideoxy-5-fluoro-3'-thiacytidine	Emtriva	Module 4.2.2.2	Original MAA (EMEA/H/C/533)	[redacted] 20[redacted]	Part III	Volume 22
W2000108	Single dose oral bioavailability of tenofovir DF in woodchucks	Truvada	Module 4.2.2.2	Original MAA (EMEA/H/C/594)	[redacted] 20[redacted]	Module 4	Volume 3
D2000076	Single dose oral bioavailability of tenofovir DF in beagle dogs	Viread	Module 4.2.2.2	Original MAA (EMEA/H/C/ 419)	[redacted] 20[redacted]	Part III	Volume 29
P2000031	A single dose oral bioavailability study of tenofovir DF in rhesus monkeys	Viread	Module 4.2.2.2	Original MAA (EMEA/H/C/ 419)	[redacted] 20[redacted]	Part III	Volume 30
PC-174-2006	Anti-HBV activity of in vitro combinations of tenofovir with nucleoside analogs	Viread	Module 4.2.2.2	Provided in Type II variation (EMEA/H/C/419/ II/75)	[redacted] 20[redacted]	Module 5	Volume 1
TBZZ/93/0025	Protein binding of 524W91 in human, monkey, mouse and rabbit plasma (PDM-037)	Emtriva	Module 4.2.2.3	Original MAA (EMEA/H/C/533)	[redacted] 20[redacted]	Part III	Volume 25
TOX103	Toxicokinetic study to determine fetal exposures in CD-1 mice given TP-0006 orally	Emtriva	Module 4.2.2.3	Original MAA (EMEA/H/C/533)	[redacted] 20[redacted]	Part III	Volume 25
TOX103 [Addendum]	Addendum to TOX103: toxicokinetic study to determine fetal exposures in CD-1 mice given TP-0006 orally	Truvada	Module 4.2.2.3	Original MAA (EMEA/H/C/594)	[redacted] 20[redacted]	Module 4	Volume 11

Nonclinical Report Number	Study Title	Cross-Reference to Study Report					
		Product	Location within FDC MAA	EU Procedure (& number)	Date Submitted	Dossier Number/Type	Volume
TOX092	[^{14}C]TP-0006: A tissue distribution and excretion study in rats	Emtriva	Module 4.2.2.3	Original MAA (EMEA/H/C/533)	[redacted] 20[redacted]	Part III	Volume 24
P0504-00039.1	Protein binding of cidofovir, cyclic HPMPC, PMEA and PMPA in human plasma and serum	Viread	Module 4.2.2.3	Original MAA (EMEA/H/C/419)	[redacted] 20[redacted]	Part III	Volume 35
95-DDM-1278-002	Determination of distribution of [14C]-PMPA in male rats following single administration using whole body autoradiography	Truvada	Module 4.2.2.3	Original MAA (EMEA/H/C/594)	[redacted] 20[redacted]	Module 4	Volume 10
97-DDM-4331-001	Tissue distribution of [14C] GS-4331 in beagle dogs following oral administration	Viread	Module 4.2.2.3	Original MAA (EMEA/H/C/419)	[redacted] 20[redacted]	Part III	Volume 35
96-DDM-1278-005	Placental transfer and pharmacokinetics of PMPA (GS-1278) in infant rhesus monkeys	Viread	Module 4.2.2.3	Original MAA (EMEA/H/C/ 419)	[redacted] 20[redacted]	Part III	Volume 35
P2000116	Pharmacokinetics of tenofovir in healthy adult female lactating rhesus monkeys following a single 30 mg/kg subcutaneous dose of tenofovir	Viread	Module 4.2.2.3	Original MAA (EMEA/H/C/419)	[redacted] 20[redacted]	Part III	Volume 35
Doc #15247	In vitro evaluation of emtricitabine (FTC) as an inhibitor of human cytochrome P-450 enzymes and 5'-uridine diphosphate glucuronosyl transferase (UGT) (CTBR Project No. 48171)	Truvada	Module 4.2.2.4	Original MAA (EMEA/H/C/594)	[redacted] 20[redacted]	Module 4	Volume 3

Nonclinical Report Number	Study Title	Cross-Reference to Study Report					
		Product	Location within FDC MAA	EU Procedure (& number)	Date Submitted	Dossier Number/Type	Volume
15396 v1	Identification of the principal human cytochrome P-450 isoenzyme(s) and potential glucuronidation responsible for the metabolism of emtricitabine (FTC) using pooled human liver microsomes and bactosomes containing cDNA-expressed human cytochrome P-450 (CYP) isoenzymes	Emtriva	Module 4.2.2.4	Included in response to CPMP Day 120 List of Questions (EMEA/H/C/533)	[redacted]20	Response to Questions	Volume 2
TEIN/93/0015	Metabolic disposition and balance studies in male CD-1 mice following oral administration of 120 mg/kg [6-^{3}H]524W91 (EXT020)	Emtriva	Module 4.2.2.4	Original MAA (EMEA/H/C/533)	[redacted]20	Part III	Volume 24
TOX063	Metabolism and excretion of [^{14}C]TP-0006 following oral administration to male cynomolgus monkeys	Emtriva	Module 4.2.2.4	Original MAA (EMEA/H/C/533)	[redacted]20	Part III	Volumes 22--23
TEIN/93/0016	Metabolic disposition of 80 mg/kg orally administered [6-^{3}H]524W91 in cynomolgus monkeys	Emtriva	Module 4.2.2.4	Original MAA (EMEA/H/C/533)	[redacted]20	Part III	Volume 25
P1278-00008; 96-DDM-1278-003	In vitro metabolism of ^{14}C-PMPA in human and animal tissues	Truvada	Available upon request	Original MAA (EMEA/H/C/594)	[redacted]20	Module 4	Volume 12
P4331-00003; 97-VIT-1278-001	In vitro stability of bis-POC PMPA (GS-4331) in biological fluids	Truvada	Available upon request	Original MAA (EMEA/H/C/594)	[redacted]20	Module 4	Volume 12

Nonclinical Report Number	Study Title	Cross-Reference to Study Report					
		Product	Location within FDC MAA	EU Procedure (& number)	Date Submitted	Dossier Number/Type	Volume
P4331-00015.1; 98-VIT-4331-001	Epithelial transport and metabolism of tenofovir disoproxil (Bis-POC PMPA; GS-4331) in caco-2 cell monolayers	Truvada	Available upon request	Original MAA (EMEA/H/C/594)	[redacted]20[redacted]	Module 4	Volume 12
V990172-104	The effect of tenofovir and tenofovir DF on the activities of the cytochrome P-450 isoforms in human hepatic microsomes	Truvada	Module 4.2.2.4	Original MAA (EMEA/H/C/594)	[redacted]20[redacted]	Module 4	Volume 3
R2001024	GLP *ex-vivo* rat cytochrome P-450 induction study following treatment with tenofovir DF	Truvada	Module 4.2.2.4	Original MAA (EMEA/H/C/594)	[redacted]20[redacted]	Module 4	Volume 12
96-DDM-1278-001	Effect of dose on the recovery of [^{14}C]-PMPA following intravenous administration to Sprague-Dawley rats	Viread	Module 4.2.2.5	Original MAA (EMEA/H/C/ 419)	[redacted]20[redacted]	Part III	Volume 36
97-DDM-4331-003/4; P4331-00014	Determination of tenofovir disoproxil and metabolite concentrations in bile and gastrointestinal tract, following oral administration of tenofovir disoproxil to rats (Gilead Study Nos. 97-DDM-4331-003 and 97-DDM-4331-004)	Viread	Module 4.2.2.5	Original MAA (EMEA/H/C/419)	[redacted]20[redacted]	Part III	Volume 36
P1278-00011; 96-DDM-1278-002	A pilot study of biliary excretion of ^{14}C-PMPA in the beagle dog	Truvada	Module 4.2.2.3	Original MAA (EMEA/H/C/594)	[redacted]20[redacted]	Module 4	Volume 14

Nonclinical Report Number	Study Title	Cross-Reference to Study Report					
		Product	Location within FDC MAA	EU Procedure (& number)	Date Submitted	Dossier Number/Type	Volume
PC-104-2018	Effect of HIV protease inhibitors on MRP4-mediated efflux of tenofovir	Viread	Module 4.2.2.5	Submitted in response to Follow-up Measure (EMEA/H/C/419)	[redacted]20[redacted]	Follow-up Measure	Volume 1
PC-104-2019	In vitro effect of HIV protease inhibitors on the accumulation of tenofovir in fresh human kidney tissue	Viread	Module 4.2.2.5	Submitted in response to Follow-up Measure (EMEA/H/C/419)	[redacted]20[redacted]	Follow-up Measure	Volume 1
PC-103-2001	In vitro interactions of acyclic nucleoside phosphonate analogs with human organic cation and anion transporters	Viread	Module 4.2.2.6	Submitted in response to Follow-up Measure (EMEA/H/C/419)	[redacted]20[redacted]	Follow-up Measure	Volume 1
AD-104-2001	Effect of HIV protease inhibitors on the transport of tenofovir by the multidrug resistance related proteins 2 and 4	Viread	Available upon request	Submitted in Response to Follow-Up Measure (EMEA/H/C/419)	[redacted]20[redacted]	Follow-up Measure	Volume 1
AD-104-2002	Lack of contribution from P-glycoprotein (Pgp) in the active tubular secretion of tenofovir	Viread	Module 4.2.2.5	Submitted in Response to Follow-up Measure (EMEA/H/C/ 419)	[redacted]20[redacted]	Follow-up Measure	Volume 1
PC-104-2010	Effect of HIV protease inhibitors and other therapeutics on the transport of tenofovir by human renal organic anion transporter type I (hOAT1)	Viread	Module 4.2.2.6	Provided in Type II Variation (EMEA/H/C/419/ II/29)	[redacted]20[redacted]	Type II variation	Volume 1

Nonclinical Report Number	Study Title	Cross-Reference to Study Report					
		Product	Location within FDC MAA	EU Procedure (& number)	Date Submitted	Dossier Number/Type	Volume
PC-104-2011	Effect of HIV protease inhibitors on the transport of tenofovir by human renal organic anion transporter type 3 (hOAT3)	Viread	Module 4.2.2.6	Provided in Type II Variation (EMEA/H/C/419/ II29)	[redacted] 20[redacted]	Type II variation	Volume 1
PC-104-2014	Lack of contribution from MRP1 in tubular re-absorption of tenofovir	Viread	Module 4.2.2.6	Submitted in Response to Follow-up Measure (EMEA/H/C/419)	[redacted] 20[redacted]	Follow-up Measure	Volume 1
AD-104-2010	Effect of HIV protease inhibitors on the intestinal absorption of tenofovir disoproxil fumarate in vitro	Viread	Module 4.2.2.5	Submitted in Response to Follow-up Measure (EMEA/H/C/419)	[redacted] 20[redacted]	Follow-up Measure	Volume 1
PC-177-2001	The triple combination of tenofovir, emtricitabine and efavirenz shows synergistic anti-HIV-1 activity in vitro: a mechanism of action study	Atripla	Module 4.2.2.6	Submitted in PSUR [redacted] 20[redacted] to [redacted] 20[redacted])	[redacted] 20[redacted]	Module 5	Volume 1
PC-180-2018	Effect of increasing multiplicity of infection on the EC50 of GS-9131 and GS-9148	Viread	Module 4.2.2.6	Previously Not Submitted			
R2000075	Single-dose iv PK of tenofovir at two doses in Sprague-Dawley rats	Truvada	Module 4.2.2.7	Original MAA (EMEA/H/C/594)	[redacted] 20[redacted]	Module 4	Volume 14
P2001025	Intracellular kinetics of ^{14}C – PMPA in rhesus monkeys	Truvada	Available upon request	Original MAA (EMEA/H/C/594)	[redacted] 20[redacted]	Module 4	Volume 14
P2000117	Pharmacokinetics of tenofovir in healthy and infected rhesus monkeys administered chronic subcutaneous doses of tenofovir	Truvada	Available upon request	Original MAA (EMEA/H/C/594)	[redacted] 20[redacted]	Module 4	Volume 15

Nonclinical Report Number	Study Title	Cross-Reference to Study Report					
		Product	Location within FDC MAA	EU Procedure (& number)	Date Submitted	Dossier Number/Type	Volume
R2000065	Comparison of plasma pharmacokinetics in rats of tenofovir following oral administration of GS-7340-02 or tenofovir DF as either a suspension in CMC or a solution in citric acid	Truvada	Available upon request	Original MAA (EMEA/H/C/594)	[redacted] 20[redacted]	Module 4	Volume 15
TTEP/93/0020	An acute oral toxicity study in the mouse with 524W91	Emtriva	Module 4.2.3.1	Original MAA (EMEA/H/C/533)	[redacted] 20[redacted]	Part III	Volume 1
TTEP/93/0023	An acute intravenous toxicity study in the mouse with 524W91	Emtriva	Module 4.2.3.1	Original MAA (EMEA/H/C/533)	[redacted] 20[redacted]	Part III	Volume 1
TTEP/93/0021	An acute oral toxicity study in the rat with 524W91	Emtriva	Module 4.2.3.1	Original MAA (EMEA/H/C/533)	[redacted] 20[redacted]	Part III	Volume 1
TTEP/93/0024	An acute intravenous toxicity study in the rat with 524W91	Emtriva	Module 4.2.3.1	Original MAA (EMEA/H/C/533)	[redacted] 20[redacted]	Part III	Volume 1
R990200	An acute oral gavage toxicity study of tenofovir DF (GS-4331-05) in the albino rat followed by a 14-day observation period ([redacted] Ltd Report No. 89285)	Viread	Module 4.2.3.1	Original MAA (EMEA/H/C/ 419)	[redacted] 20[redacted]	Part III	Volume 1
D990201	An acute oral gavage toxicity study of tenofovir DF (GS-4331-05) in the beagle dog followed by a 14-day observation period ([redacted] Ltd Report No. 89286)	Viread	Module 4.2.3.1	Original MAA (EMEA/H/C/419)	[redacted] 20[redacted]	Part III	Volume 1
IUW00701	Fourteen-day oral (Gavage) toxicity study in mice given FTC	Truvada	Module 4.2.3.2	Original MAA (EMEA/H/C/594)	[redacted] 20[redacted]	Module 4	Volume 30

Nonclinical Report Number	Study Title	Cross-Reference to Study Report					
		Product	Location within FDC MAA	EU Procedure (& number)	Date Submitted	Dossier Number/Type	Volume
TOX599	A 30-day oral toxicity study in mice given 524W91	Emtriva	Module 4.2.3.2	Original MAA (EMEA/H/C/533)	[redacted]20	Part III	Volumes 3-4
TOX599 [Addendum]	Addendum - A 30-day oral toxicity study in mice given 524W91	Emtriva	Module 4.2.3.2	Original MAA (EMEA/H/C/533)	[redacted]20	Part III	Volume 4
TOX022 (IUW01001)	Toxicokinetic report for a 6-month oral (gavage) toxicity study in mice given FTC with a 3-month interim kill	Emtriva	Module 4.2.3.2	Original MAA (EMEA/H/C/533)	[redacted]20	Part III	Volumes 5-6
TOX022 [Addendum]	Toxicokinetic report for a 6-month oral (gavage) toxicity study in mice given FTC with a 3-month interim kill	Truvada	Module 4.2.3.2	Original MAA (EMEA/H/C/594)	[redacted]20[redacted]	Module 4	Volume 32
TOX628	A 6-month oral toxicity study (with a 3-month interim sacrifice) in mice given 524W91	Emtriva	Module 4.2.3.2	Original MAA (EMEA/H/C/533)	[redacted]20	Part III	Volumes 7-9
TOX097	A 3-month oral gavage study for bioassay dose selection in CD rats	Emtriva	Module 4.2.3.2	Original MAA (EMEA/H/C/533)	[redacted]20	Part III	Volumes 9-10
TOX600	A 30-day oral toxicity study in cynomolgus monkeys given 524W91	Emtriva	Module 4.2.3.2	Original MAA (EMEA/H/C/533)	[redacted]20	Part III	Volumes 11-12
TOX600	Addendum - A 30-day oral toxicity study in cynomolgus monkeys given 524W91	Emtriva	Module 4.2.3.2	Original MAA (EMEA/H/C/533)	[redacted]20	Part III	Volume 12
TOX627	A 3-month oral toxicity study in cynomolgus monkeys given 524W91	Emtriva	Module 4.2.3.2	Original MAA (EMEA/H/C/533)	[redacted]20	Part III	Volumes 12-13
TOX032	52-week oral toxicity study with TP-0006 in cynomolgus monkeys with a 4-week recovery period	Emtriva	Module 4.2.3.2	Original MAA (EMEA/H/C/533)	[redacted]20	Part III	Volumes 13-14

Nonclinical Report Number	Study Title	Cross-Reference to Study Report					
		Product	Location within FDC MAA	EU Procedure (& number)	Date Submitted	Dossier Number/Type	Volume
M990191	A 14-day repeat dose oral toxicity study of tenofovir DF in ICR CD-1® mice	Truvada	Module 4.2.3.2	Original MAA (EMEA/H/C/594)	[redacted]20[redacted]	Module 4	Volume 17
M990203 (T4331-00017.1)	A 13-week oral gavage toxicity study of tenofovir disoproxil fumarate (Tenofovir DF) in the albino mouse	Viread	Module 4.2.3.2	Original MAA (EMEA/H/C/419)	[redacted]20[redacted]	Part III	Volumes 2-3
M990203-PK	A 13-week oral gavage toxicity study of tenofovir disoproxil fumarate (tenofovir DF) in the albino mouse (Gilead Study #M990203)	Viread	Module 4.2.3.2	Original MAA (EMEA/H/C/ 419)	[redacted]20[redacted]	Part III	Volume 30
96-TOX-4331-002	5-day repeated dose oral toxicity study of GS-4331-02 in male Sprague-Dawley rats	Truvada	Available upon request	Original MAA (EMEA/H/C/594)	[redacted]20[redacted]	Module 4	Volume 18
98-TOX-4331-004	A 14-day oral gavage toxicity study of bis-POC PMPA fumarate (GS-4331-05; PMPA prodrug) in the albino rat	Truvada	Module 4.2.3.2	Original MAA (EMEA/H/C/594)	[redacted]20[redacted]	Module 4	Volume 18
98-TOX-4331-004-PK	Toxicokinetic report for a 14-day oral gavage toxicity study of bis-POC PMPA fumarate [GS-4331-05; PMPA prodrug] in the albino rat (98-TOX-4331-004)	Truvada	Module 4.2.3.2	Original MAA (EMEA/H/C/594)	[redacted]20[redacted]	Module 4	Volume 18
96-TOX-4331-003 (T4331-00003.2)	A 28-day oral gavage toxicity study of GS-4331-05 in the albino rat	Viread	Module 4.2.3.2	Original MAA (EMEA/H/C/ 419)	[redacted]20[redacted]	Part III	Volume 7

Nonclinical Report Number	Study Title	Cross-Reference to Study Report					
		Product	Location within FDC MAA	EU Procedure (& number)	Date Submitted	Dossier Number/Type	Volume
96-TOX-4331-003-PK (P4331-00004)	Pharmacokinetics of tenofovir following oral gavage of tenofovir DF in a 28-day toxicity study in albino rats (A report on the analysis of plasma concentration data from Toxicity Study #96-TOX-4331-003)	Viread	Module 4.2.3.2	Original MAA (EMEA/H/C/ 419)	[redacted]20[redacted]	Part III	Volume 32
97-TOX-4331-002	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	Viread	Module 4.2.3.2	Original MAA (EMEA/H/C/ 419)	[redacted]20[redacted]	Part III	Volumes 8-12
97-TOX-4331-002-PK	Pharmacokinetics of tenofovir in a 13 and 42-week oral gavage Toxicity study (with a 13-week recovery period) of tenofovir DF (GS-4331-05) in rats (A report on the analysis of plasma concentration in data from toxicity study #97-TOX-4331-002)	Viread	Module 4.2.3.2	Original MAA (EMEA/H/C/419)	[redacted]20[redacted]	Part III	Volume 33
96-TOX-4331-001	5-day repeated oral dose toxicity study with GS-4331 in dogs	Truvada	Available upon request	Original MAA (EMEA/H/C/594)	[redacted]20[redacted]	Module 4	Volume 25
96-TOX-4331-001-PK	Pharmacokinetics of tenofovir in a pilot five day oral toxicity study of tenofovir DC (GS-4331-02) in beagle dogs (Toxicity Study #96-TOX-4331-001)	Viread	Module 4.2.3.2	Original MAA (EMEA/H/C/ 419)	[redacted]20[redacted]	Part III	Volume 33
96-TOX-4331-004 (T4331-00004.2)	A 28-day oral gavage toxicity study of GS-4331-05 in the beagle dog	Viread	Module 4.2.3.2	Original MAA (EMEA/H/C/419)	[redacted]20[redacted]	Part III	Volume 14

Nonclinical Report Number	Study Title	Cross-Reference to Study Report					
		Product	Location within FDC MAA	EU Procedure (& number)	Date Submitted	Dossier Number/Type	Volume
96-TOX-4331-004-PK (P4331-00005)	Pharmacokinetics of tenofovir following oral gavage of tenofovir DF in a 28-day repeat dose toxicity study in beagle dogs (Gilead Sciences Study #96-TOX-4331-004-PK)	Viread	Module 4.2.3.2	Original MAA (EMEA/H/C/ 419)	[redacted]20[redacted]	Part III	Volume 33
98-TOX-4331-003	A 28-day oral gavage toxicity study of GS-4331-05 (bis-POC PMPA; PMPA Prodrug) in the beagle dog	Truvada	Available upon request	Original MAA (EMEA/H/C/594)	[redacted]20[redacted]	Module 4	Volume 26
98-TOX-4331-003-PK	Pharmacokinetics of tenofovir in a 28-day repeated dose oral gavage toxicity study of tenofovir DF in beagle dogs (A report on analysis of data from toxicity study 98-TOX-4331-003)	Viread	Module 4.2.3.2	Original MAA (EMEA/H/C/ 419)	[redacted]20[redacted]	Part III	Volume 33
97-TOX-4331-001	A 13- and 42-week oral gavage toxicity study (with a 13-week recovery period) of Bis-POC PMPA (GS-4331-05) in the beagle dog	Viread	Module 4.2.3.2	Original MAA (EMEA/H/C/ 419)	[redacted]20[redacted]	Part III	Volumes 15-17
97-TOX-4331-001-PK (P4331-00006)	Pharmacokinetics of tenofovir following oral gavage of tenofovir DF in a 13- and 42-week repeat dose toxicity study in beagle dogs (A report on analysis of plasma concentration data from toxicity study #97-TOX-4331-001)	Viread	Module 4.2.3.2	Original MAA (EMEA/H/C/ 419)	[redacted]20[redacted]	Part III	Volume 34

Nonclinical Report Number	Study Title	Cross-Reference to Study Report					
		Product	Location within FDC MAA	EU Procedure (& number)	Date Submitted	Dossier Number/Type	Volume
TX-164-2001	A 14-day oral gavage toxicity study comparing non-degraded and degraded TDF/FTC in Sprague-Dawley rats	Truvada	Module 4.2.3.2	Original MAA (EMEA/H/C/594)	[redacted] 20[redacted]	Module 4	Volumes 4-5
TX-164-2004	4-week oral gavage toxicity and toxicokinetic study with emtricitabine/tenofovir disoproxil fumarate (FTC/TDF) in male dogs with a 4-week recovery period	Truvada	Module 4.2.3.2	Provided in Type II Variation (EMEA/H/C/594 /II/010)	[redacted] 20[redacted]	Module 4	Volumes 1-2
18637-0-409R	Mutagenicity test with FTC in the Salmonella - Escherichia coli/mammalian-microsome reverse mutation assay with a confirmatory assay	Emtriva	Module 4.2.3.3.1	Original MAA (EMEA/H/C/533)	[redacted] 20[redacted]	Part III	Volume 24
MUT203	Salmonella/mammalian-microsome assays with 524W91	Emtriva	Module 4.2.3.3.1	Original MAA (EMEA/H/C/533)	[redacted] 20[redacted]	Part III	Volume 20
K01-3154	Mutagenicity test of FTC using microorganisms	Truvada	Module 4.2.3.3.1	Submitted in PSUR [redacted] 20[redacted] to [redacted] 20[redacted])	[redacted] 20[redacted]	PSUR	Volume 2
TOX012	In vitro mammalian cell gene mutation test (mouse lymphoma assay)	Emtriva	Module 4.2.3.3.1	Original MAA (EMEA/H/C/533)	[redacted] 20[redacted]	Part III	Volume 20
96-TOX-4331-005	Mutagenicity test with GS-4331-05 in the Salmonella- escherichia coli mammalian microsome reverse mutation assay	Viread	Module 4.2.3.3.1	Original MAA (EMEA/H/C/ 419)	[redacted] 20[redacted]	Part III	Volume 23

Nonclinical Report Number	Study Title	Cross-Reference to Study Report					
		Product	Location within FDC MAA	EU Procedure (& number)	Date Submitted	Dossier Number/Type	Volume
97-TOX-1278-003	Mutagenicity test with GS-1278 (PMPA) Lot no. [redacted], GS-1278 (PMPA) Lot No. [redacted], GS-4331-05 (PMPA prodrug, bis-POC PMPA) Lot no. [redacted] ([redacted]) in the Salmonella mammalian reverse mutation assay	Viread	Module 4.2.3.3.1	Original MAA (EMEA/H/C/ 419)	[redacted]20[redacted]	Part III	Volume 23
K01-3037	Mutagenicity test of tenofovir using microorganisms	Viread	Available upon request	Provided in 3rd Annual Risk Benefit (EMEA/H/C/419/S/ 48)	[redacted]20[redacted]	3rd Annual Risk Benefit	Volume 2
97-TOX-4331-007	Mutagenicity test on GS-4331-05 in the L5178Y/TK+/- mouse lymphoma forward mutation assay	Viread	Module 4.2.3.3.1	Original MAA (EMEA/H/C/ 419)	[redacted]20[redacted]	Part III	Volume 23
TX-164-2002	Bacterial reverse mutation assay with emtricitabine/tenofovir disoproxil fumarate	Truvada	Available upon request	Submitted in Response to Follow-up Measure (EMEA/H/C/594)	[redacted]20[redacted]	Follow-up Measure	Volume 1
TX-164-2003	In vitro mammalian cell gene mutation test (L5178Y/TK+/- mouse lymphoma assay) with emtricitabine/tenofovir disoproxil fumarate	Truvada	Module 4.2.3.3.1	Submitted in Response to Follow-up Measure (EMEA/H/C/594)	[redacted]20[redacted]	Follow-up Measure	Volume 1
TOX011	In vivo mammalian erythrocyte micronucleus assay	Emtriva	Module 4.2.3.3.2	Original MAA (EMEA/H/C/533)	[redacted]20[redacted]	Part III	Volume 20

Nonclinical Report Number	Study Title	Cross-Reference to Study Report					
		Product	Location within FDC MAA	EU Procedure (& number)	Date Submitted	Dossier Number/Type	Volume
97-TOX-4331-008	Mutagenicity test on GS-4331-05 in the in vivo mouse micronucleus assay	Viread	Module 4.2.2.1 & Module 4.2.3.3.2	Original MAA (EMEA/H/C/419)	[redacted]20	Part III	Volume 23
97-TOX-4331-008-PK	Tenofovir levels in mouse plasma: pharmacokinetics from a single dose micronucleus study in mice (97-TOX-4331-008)	Viread	Module 4.2.3.3.2	Original MAA (EMEA/H/C/419)	[redacted]20	Part III	Volume 28
23291-0-494OECD	In vivo/in vitro unscheduled DNA synthesis in rat primary hepatocyte cultures at two time points	Viread	Module 4.2.3.3.2	Submitted in Response to Specific Obligation (EMEA/H/C/ 419)	[redacted]20	Specific Obligation	Volume 1
TOX109	Two-year oral oncogenicity study in CD-1 mice	Emtriva	Module 4.2.3.4.1	Original MAA (EMEA/H/C/533)	[redacted]20	Part III	Volume 20
TOX108	TP-0006: two-year oral oncogenicity study in CD rats	Emtriva	Module 4.2.3.4.1	Original MAA (EMEA/H/C/533)	[redacted]20	Part III	Volume 20
M990205	An oral carcinogenicity study of tenofovir disoproxil fumarate (tenofovir DF) in the albino mouse	Viread	Module 4.2.3.4.1	Provided in Type II Variation (EMEA/H/C/419 /II/32	[redacted]20	Type II variation	Volumes 1-10
R990204	An oral carcinogenicity study of tenofovir disoproxil fumarate (tenofovir DF) in the albino rat	Viread	Module 4.2.3.4.1	Submitted in Response to Specific Obligation (EMEA/H/C/ 419)	[redacted]20	Specific Obligation	Volumes 1-8

Nonclinical Report Number	Study Title	Cross-Reference to Study Report					
		Product	Location within FDC MAA	EU Procedure (& number)	Date Submitted	Dossier Number/Type	Volume
TOX036	Study of fertility and early embryonic development of TP-0006 administered by gavage to CD-1 mice (segment 1)	Emtriva	Module 4.2.3.5.1	Original MAA (EMEA/H/C/533)	[redacted] 20[redacted]	Part III	Volume 15
TTEP/95/0028	A fertility study in male rats given 524W91 by gavage	Emtriva	Module 4.2.3.5.1	Original MAA (EMEA/H/C/533)	[redacted] 20[redacted]	Part III	Volume 15
98-TOX-4331-006	Oral (gavage) fertility and general reproduction toxicity study of GS-4331-05 (bis-POC PMPA) in Sprague-Dawley rats	Viread	Module 4.2.3.5.1	Original MAA (EMEA/H/C/ 419)	[redacted] 20[redacted]	Part III	Volume 19
TOX033	A dose range-finding study of the effects of TP-0006 on embryo/fetal development in mice	Truvada	Module 4.2.3.5.2	Original MAA (EMEA/H/C/594)	[redacted] 20[redacted]	Module 4	Volume 85
TOX037	A study of the effects of TP-0006 (FTC) on embryo/fetal development in mice	Emtriva	Module 4.2.3.5.2	Original MAA (EMEA/H/C/533)	[redacted] 20[redacted]	Part III	Volumes 16-17
TOX037	Addendum to document No: TOX037	Truvada	Module 4.2.3.5.2	Original MAA (EMEA/H/C/594)	[redacted] 20[redacted]	Module 4	Volume 85
TOX034	A dose range-finding study of the effects of TP-0006 on embryo/fetal development in rabbits	Truvada	Module 4.2.3.5.2	Original MAA (EMEA/H/C/594)	[redacted] 20[redacted]	Module 4	Volume 85
TOX038	A study of the effects of TP-0006 on embryo/fetal development in rabbits	Emtriva	Module 4.2.3.5.2	Original MAA (EMEA/H/C/533)	[redacted] 20[redacted]	Part III	Volumes 18-19
TOX038 [Addendum]	Addendum to TOX038: A study of the effects of TP-0006 on embryo/fetal development in rabbits	Truvada	Module 4.2.3.5.2	Original MAA (EMEA/H/C/594)	[redacted] 20[redacted]	Module 4	Volume 86

Nonclinical Report Number	Study Title	Cross-Reference to Study Report					
		Product	Location within FDC MAA	EU Procedure (& number)	Date Submitted	Dossier Number/Type	Volume
97-TOX-4331-004	Oral (gavage) developmental toxicity study of GS-4331-005 in rats	Viread	Module 4.2.3.5.2	Original MAA (EMEA/H/C/ 419)	[redacted]20[redacted]	Part III	Volume 20
98-TOX-4331-005	Oral (stomach tube) developmental toxicity study of GS-4331-05 in rabbits	Viread	Module 4.2.3.5.2	Original MAA (EMEA/H/C/ 419)	[redacted]20[redacted]	Part III	Volume 22
98-TOX-4331-005-PK	Pharmacokinetics of tenofovir in an oral (stomach tube) developmental toxicity study of GS-4331-05 (bis-POC PMPA) in rabbits (A report on analysis of data from toxicity study 98-TOX-4331-005)	Truvada	Module 4.2.3.5.2	Original MAA (EMEA/H/C/594)	[redacted]20[redacted]	Module 4	Volume 85
TOX039	Pre- and postnatal development study in CD-1 mice given TP-0006 by gavage (segment III)	Emtriva	Module 4.2.3.5.3	Original MAA (EMEA/H/C/533)	[redacted]20[redacted]	Part III	Volume 19
R990202	Oral (gavage) developmental and perinatal/postnatal reproduction toxicity study of GS-4331-05 (bis-POC PMPA) in rats including a postnatal behavioural / functional evaluation	Viread	Module 4.2.3.5.3	Provided in Type II Variation (EMEA/H/C/419/ II/10)	[redacted]20[redacted]	Type II variation	Volumes 1-2
R990202-PK	Tenofovir (GS-1278) plasma toxicokinetics from a developmental and perinatal/postnatal reproduction toxicity study of tenofovir DF in female rats	Viread	Module 4.2.3.5.3	Provided in Type II Variation (EMEA/H/C/419 /II/10)	[redacted]20[redacted]	Type II variation	Volume 2

Nonclinical Report Number	Study Title	Cross-Reference to Study Report					
		Product	Location within FDC MAA	EU Procedure (& number)	Date Submitted	Dossier Number/Type	Volume
B990165	A primary eye irritation study in rabbits with tenofovir DF (GS-4331-05)	Truvada	Module 4.2.3.6	Original MAA (EMEA/H/C/594)	[redacted]20[redacted]	Module 4	Volume 89
B990166	A primary skin irritation study in rabbits with tenofovir DF (GS-4331-05)	Truvada	Module 4.2.3.6	Original MAA (EMEA/H/C/594)	[redacted]20[redacted]	Module 4	Volume 89
G990167	A dermal sensitization study in guinea pigs with tenofovir DF (GS-4331-05) - modified Buehler design	Truvada	Module 4.2.3.7.1	Original MAA (EMEA/H/C/594)	[redacted]20[redacted]	Module 4	Volume 89
TOX146	TP-0006 28-day oral (gavage) immunotoxicity study in rats.	Truvada	Module 4.2.3.7.2	Original MAA (EMEA/H/C/594)	[redacted]20[redacted]	Module 4	Volume 27
R2000096	A 28-day oral repeat dose study to evaluate the toxicity of adefovir dipivoxil (GS-0840), tenofovir DF (GS-4331-05), or adefovir dipivoxil in combination with tenofovir DF in male Sprague-Dawley rats	Viread	Module 4.2.3.7.3	Submitted in Response to Specific Obligation (EMEA/H/C/419)	[redacted]20[redacted]	Specific Obligation	Volume 1
W2000042	A 90-day repeat dose oral toxicity study of tenofovir DF in woodchucks	Viread	Module 4.2.3.7.3	Submitted in Response to Specific Obligation (EMEA/H/C/ 419)	[redacted]20[redacted]	Specific Obligation	Volume 4
W2000042-PK	Toxicokinetics of tenofovir following daily oral administration of tenofovir DF to male and female woodchucks for 90 days	Truvada	Module 4.2.3.7.3	Original MAA (EMEA/H/C/594)	[redacted]20[redacted]	Module 4	Volume 91

Nonclinical Report Number	Study Title	Cross-Reference to Study Report					
		Product	Location within FDC MAA	EU Procedure (& number)	Date Submitted	Dossier Number/Type	Volume
V2000122	Human osteoblast calcium deposition in vitro	Truvada	Module 4.2.3.7.3	Original MAA (EMEA/H/C/594)	[redacted]20[redacted]	Module 4	Volume 89
R2000095	A 3-day oral repeat dose study to evaluate serum and urine phosphorus levels in tenofovir DF (GS-4331-05)-treated male Sprague-Dawley rats supplemented, following the final dose, with intraperitoneal or oral phosphate	Truvada	Module 4.2.3.7.3	Original MAA (EMEA/H/C/594)	[redacted]20[redacted]	Module 4	Volume 89
R2000099	A 3-day oral or intravenous repeat dose study to evaluate serum and urine phosphate concentrations in male Sprague-Dawley rats treated with tenofovir (GS-1278) or tenofovir DF (GS-4331-05) and supplemented with oral phosphate	Truvada	Module 4.2.3.7.3	Original MAA (EMEA/H/C/594)	[redacted]20[redacted]	Module 4	Volume 89
R2000043	A 6-day repeat dose oral exploratory study of tenofovir DF in male Sprague-Dawley rats	Truvada	Module 4.2.3.7.3	Original MAA (EMEA/H/C/594)	[redacted]20[redacted]	Module 4	Volume 89
R2000036	A 28-day study to evaluate the effects of tenofovir disoproxil fumarate (tenofovir DF) on bone following daily administration by gavage in the Sprague-Dawley rat	Truvada	Module 4.2.3.7.3	Original MAA (EMEA/H/C/594)	[redacted]20[redacted]	Module 4	Volume 90

Nonclinical Report Number	Study Title	Cross-Reference to Study Report					
		Product	Location within FDC MAA	EU Procedure (& number)	Date Submitted	Dossier Number/Type	Volume
R2000036-PK	A 28-day study to evaluate the effect of tenofovir disoproxil fumarate (tenofovir DF) on bone following daily administration by gavage in the Sprague-Dawley rat	Viread	Module 4.2.3.7.3	Original MAA (EMEA/H/C/419)	[redacted]20[redacted]	Part III	Volume 32
P2000078	A 56-day study of tenofovir DF administered orally and of PMPA administered by subcutaneous injection to rhesus monkeys	Viread	Module 4.2.3.7.3	Submitted in Response to Specific Obligation (EMEA/H/C/ 419)	[redacted]20[redacted]	Specific Obligation	Volume 2
P2000078-PK	Toxicokinetics of tenofovir DF administered orally and tenofovir administered by subcutaneous injection to rhesus monkeys for 56-days	Viread	Module 4.2.3.7.3	Provided in Type II Variation (EMEA/H/C/419/ II/10)	[redacted]20[redacted]	Type II variation	Volume 1
TOX 153	A 1-month mouse oral qualification study of TP-0006 and TP-0296 (degradant)	Truvada	Module 4.2.3.7.6	Original MAA (EMEA/H/C/594)	[redacted]20[redacted]	Module 4	Volumes 95-96
TX-162-2001	A 1-month oral qualification study of TP-0006 produced by the [redacted] process in CD-1 mice	Truvada	Module 4.2.3.7.6	Original MAA (EMEA/H/C/594)	[redacted]20[redacted]	Module 4	Volumes 97-98
TOX151	Bacterial reverse mutation assay of TP-0006 to qualify degradant TP-0296	Truvada	Module 4.2.3.7.6	Original MAA (EMEA/H/C/594)	[redacted]20[redacted]	Module 4	Volume 95
TOX152	In vitro mammalian chromosome aberration study of TP-0006 to qualify degradant TP-0296	Truvada	Module 4.2.3.7.6	Original MAA (EMEA/H/C/594)	[redacted]20[redacted]	Module 4	Volume 95

Nonclinical Report Number	Study Title	Cross-Reference to Study Report					
		Product	Location within FDC MAA	EU Procedure (& number)	Date Submitted	Dossier Number/Type	Volume
R2000081	14-day oral toxicity study comparing tenofovir DF and degraded tenofovir DF in Sprague-Dawley rats	Truvada	Module 4.2.3.7.6	Original MAA (EMEA/H/C/594)	[redacted] 20[redacted]	Module 4	Volume 93
R2000081-PK	14-day oral toxicity study comparing tenofovir DF and degraded tenofovir DF in Sprague-Dawley rats	Truvada	Module 4.2.3.7.6	Original MAA (EMEA/H/C/594)	[redacted] 20[redacted]	Module 4	Volumes 30-94
[redacted] 1432/009	28-day repeated dose oral (gavage) toxicity study in the rat	Truvada	Available Upon Request	Original MAA (EMEA/H/C/594)	[redacted] 20[redacted]	Module 4	Volume 99
[redacted] 1432/021	28-day repeated dose oral (gavage) toxicity study in the rat	Truvada	Available Upon Request	Original MAA (EMEA/H/C/594)	[redacted] 20[redacted]	Module 4	Volume 99
[redacted] 1432/022	[redacted]: Reverse mutation assay "Ames Test" using Salmonella typhimurium and Escherichia coli	Truvada	Available Upon Request	Original MAA (EMEA/H/C/594)	[redacted] 20[redacted]	Module 4	Volume 98
[redacted] 1432/023	[redacted]: L5178Y/TK+/- mutation assay	Truvada	Available Upon Request	Original MAA (EMEA/H/C/594)	[redacted] 20[redacted]	Module 4	Volume 99
95-TOX-1278-006	Mutagenicity test with GS-1278 (PMPA) lot #[redacted] in the Salmonella-Escherrichia coli mammalian-microsome reverse mutation assay	Truvada	Module 4.2.3.7.7	Original MAA (EMEA/H/C/594)	[redacted] 20[redacted]	Module 4	Volume 98
95-TOX-1278-007	Mutagenicity test on GS-1278 (PMPA) in the L5178Y/TK+/- mouse lymphoma forward mutation assay	Truvada	Module 4.2.3.7.7	Original MAA (EMEA/H/C/594)	[redacted] 20[redacted]	Module 4	Volume 98

Nonclinical Report Number	Study Title	Cross-Reference to Study Report					
		Product	Location within FDC MAA	EU Procedure (& number)	Date Submitted	Dossier Number/Type	Volume
T1278-00034	PMPA in SIV-infected and uninfected rhesus macaques: studies from [redacted].	Viread	Available upon request	Original MAA (EMEA/H/C/419)	[redacted] 20[redacted]	Part III	Volume 26
T1278-00030	The effects of PMPA treatment on cortical bone strength in rhesus monkeys (Macaca mulatta). [redacted]	Truvada	Module 4.2.3.7.7	Original MAA (EMEA/H/C/594)	[redacted] 20[redacted]	Module 4	Volume 98