2.4 NONCLINICAL OVERVIEW

EMTRICITABINE/RILPIVIRINE/TENOFOVIR ALAFENAMIDE FIXED-DOSE COMBINATION (FTC/RPV/TAF [F/R/TAF] FDC)

Gilead Sciences

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CONFIDENTIAL AND PROPRIETARY INFORMATION

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LIST OF ABBREVIATIONS

AhR	aryl hydrocarbon receptor (AHR gene product)
ACTH	adrenocorticotropic hormone
ALP	alkaline phosphatase
ALT	alanine aminotransferase
APTT	activated partial thromboplastin times
ARV	antiretroviral
ATP	adenosine triphosphate
ATV	atazanavir (Reyataz®, Bristol-Myers Squibb)
AUC	area under the curve
AUC_{ss}	area under the plasma concentration curve at steady state
AUC_{tau}	the area under the plasma concentration-time curve from time zero to time tau over a dosing interval at steady state ($AUC_{0\text{-}tau}$), where tau is the length of the dosing interval.
BCRP	breast cancer resistance protein (ABCG2)
BDC	bile-duct cannulated
BID	twice daily
BSEP	bile salt excretory pump
CA	citric acid
Caco-2	human colon carcinoma cell line
CatA	cathepsin A
CC_{50}	drug concentration that results in a 50% reduction in cell viability
CD4	cluster determinant 4
cDNA	complimentary deoxyribonucleic acid
CHMP	Committee for Medicinal Products for Human Use
C_{max}	maximum observed concentration of drug in serum, plasma, or peripheral blood mononuclear cells
$C_{max,u}$	unbound concentration of drug at C_{max}
CNS	central nervous system
COBI	cobicistat (GS-9350, [Tybost®, Gilead])
COX II	cytochrome c oxidase II
CRF	corticotrophin releasing factor
CsA	cyclosporine A
CYP	cytochrome P450
dATP	deoxyadenosine triphosphate
dCTP	deoxycytidine triphosphate
DHEA	dehydroepiandrosterone
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DRV	darunavir (Prezista®, Janssen)
EADs	early afterdepolarizations

LIST OF ABBREVIATIONS (CONTINUED)

EC_{50}	concentration of a compound inhibiting virus replication by 50%
EC_{95}	concentration of a compound inhibiting virus replication by 95%
E/C/F/TAF	elvitegravir/cobicistat/emtricitabine/tenofovir alafenamide (coformulated)
ECG	electrocardiograph, electrocardiogram
EFV	efavirenz (Sustiva®, Bristol-Myers Squibb)
EMA	European Medicines Agency
EVG	elvitegravir (Vitekta®, Gilead)
EVG/COBI/FTC/TDF	elvitegravir/cobicistat/emtricitabine/tenofovir DF (coformulated); STB
EU	European Union
F_0 generation	parents
F_1 generation	offspring
FDA	Food and Drug Administration
FDC	fixed-dose combination
FMO	flavin monooxygenase
FTC, F	emtricitabine (Emtriva®, Gilead)
FTC/TDF	emtricitabine/tenofovir DF, TVD (Truvada®, Gilead)
FTC/EFV/TDF	emtricitabine/efavirenz/ tenofovir DF (Atripla®, Gilead)
FTC/RPV/TDF	emtricitabine/rilpivirine/tenofovir DF (Eviplera®/Complera®, Gilead)
EVG/COBI/FTC/TDF	elvitegravir/cobicistat/emtricitabine/tenofovir DF, E/C/F/TAF (Stribild®, Gilead)
FTC-TP	emtricitabine triphosphate
GD	gestation day
GI	gastrointestinal
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
GS-7340	tenofovir alafenamide (TAF) free base
GS-7340-02	tenofovir alafenamide (TAF) monofumarate
GS-7340-03	tenofovir alafenamide (TAF) hemifumarate
GSI	Gilead Sciences, Inc. (Gilead)
HBV	hepatitis B virus
HEK	human embryonic kidney (cell line)
hERG	human ether-à-go-go related gene
HIV-1	human immunodeficiency virus type 1
HPMC	hydroxypropylmethylcellulose
$[I]_1$	inhibitor concentration corresponding to steady state C_{max}
$[I]_2$	inhibitor concentration corresponding to theoretical maximum concentration in the intestinal lumen
IC_{50}	concentration resulting in 50% of maximum inhibition
ICH	International Conference on Harmonization (of Technical Requirements for Registration of Pharmaceuticals for Human Use)

LIST OF ABBREVIATIONS (CONTINUED)

$I_{Ca,L}$	high threshold L-calcium current
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
IV	intravenous
k_{inact}	theoretical maximum enzyme inactivation rate
LC-MS/MS	liquid chromatography with tandem mass spectrometry
LD	lactation day
LD_{50}	the estimated dose that results in lethality in 50 percent of a group
LH	luteinizing hormone
LPV	lopinavir
MATE1	multidrug and toxin extrusion protein 1 (SLC47A1)
MATE2-K	multidrug and toxin extrusion protein 2-K (SLC47A2)
MPS	mononuclear phagocytic system
MRP1, 2, or 4	multidrug resistance related protein 1, 2, or 4
MTD	maximal tolerated dose
mtDNA	mitochondrial DNA
m/v	mass per volume
NC	not calculated
ND	not determined
NDA	new drug application
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
OAT1	organic anion transporter 1 (SLC22A6)
OAT3	organic anion transporter 3 (SLC22A8)
OATP1B1	organic anion transporting polypeptide 1B1 (SLCO1B1)
OATP1B3	organic anion transporting polypeptide 1B3 (SLCO1B3)
OCT1	organic cation transporter 1
OCT2	organic cation transporter 2 (SLC22A2)
OCTN1	organic cation transporter novel, type 1 (SLC22A4)
PBMC	peripheral blood mononuclear cell
PEG	polyethylene glycol
PFC	plaque forming cell
P-gp	permeability glycoprotein

LIST OF ABBREVIATIONS (CONTINUED)

PI	protease inhibitor
PND	postnatal day
PR	PR interval is the period, measured in milliseconds, that extends from the beginning of the P wave until the beginning of the QRS complex
PT	prothrombin time
PXR	pregnane X receptor
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
RBC	red blood cell
RNA	ribonucleic acid
RPTECs	renal proximal tubule epithelial cells
RPV, R	rilpivirine, TMC278 (Edurant®, Tibotec)
RT	reverse transcriptase
RTV	ritonavir (Norvir®, Abbvie)
S9	tissue post-mitochondrial (9,000 x g) supernatant (metabolic activation system)
SI	selectivity index
SIV	simian immunodeficiency virus
STB	E/C/F/TAF (Stribild®, Gilead)
T_3	triiodothyronine
T_4	thyroxine
TAF	tenofovir alafenamide (GS-7340)
TBG	thyroid binding globulin
TDF	tenofovir disoproxil fumarate, tenofovir DF (Viread®, Gilead)
TdP	Torsade de Pointes
TDR	transmural dispersion of repolarization
TFV	tenofovir, PMPA, GS-1278
TFV-DP	tenofovir diphosphate
TMC	Tibotec Medicinal Compound
TQT	thorough QT prolongation study according to ICH E14
TSH	thyroid stimulating hormone
TTC	threshold of toxicological concern
TVD	emtricitabine/tenofovir DF, FTC/TDF (Truvada®, Gilead)
UDP-GT	uridine diphosphate glucuronosyltransferase
US	United States
WBC	white blood cell
ZDV	zidovudine, AZT (Retrovir®, GlaxoSmithKline)

1. NONCLINICAL OVERVIEW

This nonclinical overview is being submitted for a fixed-dose combination (FDC) that contains the nucleoside reverse transcriptase inhibitor (NRTI) emtricitabine (F, FTC, Emtriva®), the nonnucleoside reverse transcriptase inhibitor (NNRTI) rilpivirine (R, RPV, TMC278, Edurant®) and the nucleotide reverse transcriptase inhibitor (NtRTI) tenofovir alafenamide (TAF, formerly GS-7340); the FTC/RPV/TAF (F/R/TAF, 200/25/25 mg) tablet for the treatment of human immunodeficiency virus (HIV)-1 infection.

Tenofovir disoproxil fumarate (TDF, Viread®) is the first generation prodrug of tenofovir (TFV). Tenofovir alafenamide is a prodrug of TFV that, compared to TDF, has a longer plasma half-life and achieves higher levels of TFV into target cells at lower exposure of circulating TFV. TAF has a unique metabolism that provides enhanced delivery of TFV to HIV-target cells including lymphocytes and macrophages, resulting in higher intracellular levels of the active phosphorylated metabolite tenofovir-diphosphate (TFV-DP), and, yields approximately 90% lower circulating levels of TFV relative to TDF {7415}, {13119}, {22029}. These features translate into less risk of nephrotoxicity and less decrease (or improvements) in bone mineral density, both of which are known risks with TDF administration {21762}, {22031}, {30895}.

The FTC/RPV/TAF FDC contains the same dose of FTC (200 mg) that is currently approved within Emtriva®, FTC/TDF (Truvada®), FTC/efavirenz/TDF (Atripla®), FTC/RPV/TDF (Complera®/Eviplera®) and elvitegravir/cobicistat/FTC/TDF (Stribild®) and the same dose of RPV (25 mg) currently approved within Edurant® and Complera®/Eviplera®. Comprehensive nonclinical pharmacology/virology, pharmacokinetic, and toxicology programs were conducted to support of the registration of FTC, RPV and TAF. Information from nonclinical studies with FTC, TAF, TDF or TFV, and FTC/TDF should be considered in the context of their clinical data and post marketing clinical experience within antiretroviral (ARV) combination therapy for the treatment of HIV-1 infection.

To facilitate the evaluation of the FTC/RPV/TAF application, nonclinical virology studies of FTC, RPV, and TFV, TAF, and TDF are described in detail in the virology summary contained in m2.7.2, Section 4.1, together with the clinical virology data in Section 4.2.

The nonclinical data discussed within this document support the favorable benefit/risk profile for the proposed use of FTC/RPV/TAF for the treatment of HIV-1 infection. All information from nonclinical studies that is relevant to the prescriber and patient has been included in the proposed Prescribing Information and Patient Labeling.

1.1. Overview of the Nonclinical Testing Strategy

This document provides an overview of the nonclinical information that is relevant to the assessment of the FTC/RPV/TAF FDC. This document is structured as an 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/TAF for the treatment of

HIV-1 infection is included in Section 5, "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.

All of the definitive safety pharmacology, toxicology, and toxicokinetic studies reported in this summary for FTC, RPV, TAF, 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 either conducted in full compliance with GLP procedures or were conducted using appropriate protocols and documentation to assure data integrity.

1.1.1. FTC

Emtricitabine is an NRTI and the active ingredient in Emtriva 200 mg capsules and 10 mg/mL oral solution that have been approved in the US, the EU, and other countries worldwide for use in combination with other ARV products for the treatment of HIV-1 infection.

Emtricitabine 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. Intracellularly, FTC is phosphorylated by enzymes to form emtricitabine triphosphate (FTC-TP), the active metabolite. Emtricitabine is an NRTI that has activity against HIV and hepatitis B virus (HBV).

All nonclinical studies required to support chronic use have been performed as part of the safety assessment. The results of this evaluation were presented in detail in the original marketing application and subsequent submissions for Emtriva. The nonclinical toxicity studies demonstrate that there was no adverse effect of FTC for up to 26 weeks in the mouse and up to 52 weeks in the monkey at doses producing systemic exposure levels in animals 10- to 34-fold greater than those in patients treated with the recommended clinical dose.

1.1.2. RPV

Rilpivirine (TMC278) is an NNRTI active against wild type and NNRTI-resistant HIV-1 that is marketed as a once-daily tablet (Edurant® 25 mg) and as part of the FTC/RPV/TDF FDC (Complera®/Eviplera®) for the treatment of HIV-1 infection. The following convention is applied throughout this module: reference is made to "RPV" or "TMC278" when the hydrochloride (HCl) salt was administered and to "TMC278 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 "RPV" or "TMC278."

The nonclinical testing strategy was aimed at evaluating the primary and secondary pharmacodynamic effects of RPV. Moreover, a variety of models and tests was applied to detect adverse effects of RPV, its metabolites, as well as its impurities in the drug substance and drug product if relevant. The behavior of RPV, 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 RPV 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.

1.1.3. TAF

Comprehensive nonclinical pharmacology/virology, pharmacokinetic, and toxicology programs were undertaken in support of the registration of TAF. TAF is a prodrug of TFV, an NtRTI. After absorption, TAF is converted to TFV intracellularly, which is phosphorylated to the active metabolite, tenofovir diphosphate (TFV-DP) {1574}, that competes with natural 2'-deoxyadenosine triphosphate (dATP) for incorporation by the HIV-1 reverse transcriptase (RT) or HBV DNA polymerase and, once incorporated, results in chain-termination {21}, {1131}. Tenofovir diphosphate is a very weak inhibitor of mammalian DNA polymerases α, β, δ, ε, and mitochondrial DNA polymerase γ.

Tenofovir alafenamide is metabolized by cellular enzymes including carboxyl esterase 1 and cathepsin A (CatA) and has minimal interaction with typical xenobiotic metabolizing enzymes. Unlike tenofovir disoproxil fumarate (TDF, Viread), TAF is relatively stable in human plasma ($t_{½}$ ~90 minutes in vitro {7415} and 25-40 minutes in clinical studies [m2.7.2]). Because TAF is more stable in plasma than TDF, higher levels are achieved in HIV-target cells including lymphocytes and macrophages, providing enhanced delivery of TFV. During clinical studies with the FDCs, administration of TAF resulted in subsequent formation of > 4-fold (3-7-fold at 90% confidence interval) higher intracellular levels of TFV-DP in peripheral blood mononucleated cells (PBMCs) and 90% lower circulating levels of TFV relative to TDF {7415}, {13119}, {22029}. These features translate into less risk of nephrotoxicity and less decrease (or improvements) in bone mineral density, both of which are known risks with TDF administration {21762}, {22031}, {30895}.

Tenofovir alafenamide is well absorbed, generating sufficient exposure in animal species chosen for toxicity assessment. Tenofovir alafenamide was evaluated in mouse, rat, dog, and monkey repeat-dose toxicity studies up to 39 weeks in duration. In vitro and in vivo genotoxicity studies were conducted. The mouse was used for the in vivo genetic toxicity study and local lymph node assay. The rat was used for fertility and developmental toxicity studies and the rabbit was used for developmental and reproductive toxicity studies and local irritation. All in vivo studies utilized oral administration, the clinical route of administration, with the exception of the sensitization and dermal irritation studies. The rat and dog were demonstrated to have similar in vitro and in vivo metabolic profiles to humans. The vehicles for toxicity studies used were 1) 25 mM CA or 2) 0.5% polysorbate 20, 0.5% carboxymethylcellulose, 0.9% benzyl alcohol or 3) 0.1% (v/v) Tween 20 and 0.1% (v/v) HPMC.

Per separate agreements with the Food and Drug Administration (FDA) and Committee for Medicinal Products for Human Use (CHMP), [redacted]

The nonclinical toxicity studies demonstrate that there was no adverse effect of TAF for up to 26 weeks in the rat, up to 39 weeks in the dog, and 1 month in the monkey at doses producing TFV systemic exposure levels in animals 13-, 4- and > 20-fold greater, respectively, than those in patients treated with the recommended clinical dose of the elvitegravir (EVG,E)/cobicistat (COBI, C)/ FTC/TAF FDC (E/C/F/TAF).

1.1.4. FTC/RPV/TAF

Comprehensive nonclinical pharmacology/virology, pharmacokinetic, and toxicology programs were conducted with FTC, RPV, and TAF. The overall program, including the data from the combination and individual agent studies, is considered sufficient to support the safety of the FTC/RPV/TAF FDC tablet.

The proposed FDC is based on the complimentary pharmacology of FTC, RPV, and TAF and the body of clinical experience with NNRTIs and N[t]RTIs in HIV-infected patients. Combinations of these agents in cell-based in vitro assays show favorable anti-HIV activity and no evidence for antagonism. Because the toxicity profiles of the 3 agents differ substantially with no clinically significant overlapping toxicity and because there is no evidence of genotoxicity, carcinogenicity, or reproductive toxicity, administration of the FTC/RPV/TAF combination product is unlikely to introduce new toxicities or to exacerbate known toxicities of the individual agents. The ample nonclinical safety databases on these drugs strongly indicate further toxicological investigations are unlikely to yield new data relevant to humans. Additionally, the extensive clinical safety data available from the clinical trials with FTC/RPV/TDF, other FTC/TDF containing regimens, and FTC/TAF containing regimens support the safety of the new combination product for the treatment of HIV-1 infection.

The absence of nonclinical safety studies with the FTC/RPV/TAF combination is in accordance with the FDA Guidance for Industry, Nonclinical Safety Evaluation of Drug or Biologic Combinations, [redacted] and the CHMP Guideline on the Non-Clinical Development of Fixed Combinations of Medicinal Products ([redacted]).

2. PHARMACOLOGY/VIROLOGY

2.1. Primary Pharmacodynamics

Nonclinical virology studies of FTC, RPV, and TAF, and the drug combinations FTC+TFV and FTC+RPV+TFV are described in detail in m2.7.2, Section 4.1, together with the clinical virology data in m2.7.2, Section 4.2.

Mechanism of Action

Emtricitabine, an NRTI, is a synthetic analogue of the naturally occurring pyrimidine nucleoside, 2′-deoxycytidine. Intracellularly, FTC is converted through 3 phosphorylation reactions to its active tri-phosphorylated anabolite FTC-TP {4527}, {4535}. Emtricitabine triphosphate inhibits viral polymerases, including HIV-1 reverse transcriptase (RT) by direct binding competition with the natural deoxyribonucleotide substrate (deoxycytidine triphosphate; dCTP), and after incorporation into DNA, by DNA chain termination {4249}. 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}. With clinical isolates of HIV-1, EC_{50} values ranged from 0.002 to 0.028 μM {4534}.

Rilpivirine is a nonnucleoside reverse transcriptase inhibitor (NNRTI) that does not require modification for antiviral activity. Crystal structures of the binding site between RPV and the HIV-1 RT complex revealed that RPV, similarly to other members of the diarylpyrimidine family of inhibitors, binds to HIV-1 RT and adapts to the conformational changes in the NNRTI-binding pocket {15849}, {15850}, {15866}, {15854}. RPV shows 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), HIV-1IIIB (0.73 nM), and nanomolar EC_{50} values against HIV-1 group O isolates (2.88 to 8.45 nM) (TMC278-IV1-AVMR, TMC278-IV2-AVMR).

Following intracellular metabolism of TAF, TFV is phosphorylated to the active metabolite, TFV-DP. Tenofovir diphosphate inhibits HIV-1, HIV-2 and HBV polymerases, competing with deoxyadenosine triphosphate (dATP) for incorporation into nascent DNA and terminating the elongation of the viral DNA chain during the process of retroviral reverse transcription, thereby effectively blocking the replication and spread of infectious HIV {1131}. The kinetic inhibition (K_i) constant for TFV-DP against HIV-1 reverse transcriptase (ribonucleic acid [RNA]-directed DNA synthesis) is 0.02 μM, more than 200-fold lower than its K_i for human DNA polymerase α, and more than 3000-fold lower than its K_i values for human DNA polymerases β and γ {1131}.

Unlike TDF, TAF is relatively stable in human plasma ($t_{½}$ ~90 minutes in vitro {7415} and 25-40 minutes in clinical studies [m2.7.2]), but rapidly converts to TFV inside cells {7415}. Assessment of the intracellular metabolism of TAF in various types of immune cells including cluster determinant 4 (CD4)+ T-cells, lymphocytes, and monocytes showed efficient conversion of the prodrug to the active metabolite TFV-DP {20795}.

TAF exhibits potent anti-HIV activity in lymphoid T-cells, primary human PBMCs, and macrophages with EC_{50} values ranging from 3 to 14 nM. The in vitro activity of TAF against HIV-1 is 100- to 600-fold greater than TFV and 4- to 6-fold greater than TDF {1574}. In MT-2 cells, TAF shows low cytotoxicity with a selectivity index (SI) of > 10,000). Based on data generated with the parent nucleotide TFV, TAF is expected to be active against a wide range of HIV-1 subtypes and also against HIV-2 {1574}, {1649}, {39}. The in vitro HIV-1 resistance profile of TAF is defined by the resistance profile of the parent nucleotide TFV.

Additive to synergistic effects were observed in in vitro interaction studies of TFV, the active metabolite of TAF, with NRTIs (abacavir, FTC, lamivudine, stavudine, zalcitabine, zidovudine [ZDV]), NNRTIs (delavirdine, efavirenz [EFV], nevirapine), protease inhibitors (PIs) (amprenavir, indinavir, nelfinavir, ritonavir [RTV], saquinavir), and the integrase inhibitor EVG {1649}. No antagonistic interactions were observed for any of these 2-drug combinations in a T lymphoblastoid cell line. Data show similar results for the in vitro interactions of TAF with several commonly coadministered ARVs (PC-120-2002).

In Vivo Efficacy in Animal Models

The activity of FTC and TFV either alone or in combination has been shown 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/TAF FDC are warranted in animal models in view of the extensive clinical experience with the use of FTC, RPV, and TDF, FTC/TDF containing regimens, and the EVG/COBI/FTC/TAF FDC for the treatment of HIV-1 infection.

In summary, FTC, RPV, and TAF/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 TAF/TFV are phosphorylated intracellularly through nonoverlapping pathways, and in combination show no antagonism for the formation of their active metabolites. Additive to synergistic effects were observed in in vitro interaction studies of TFV, the active metabolite of TAF, with NRTIs, NNRTIs, PIs and the integrase inhibitor EVG {1649}.

2.2. Secondary Pharmacodynamics

2.2.1. In Vitro Cytotoxicity

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. The selectivity index of approximately 8,000 indicates that RPV is a potent and selective inhibitor of HIV-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 (P4331-00037). 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.

Tenofovir alafenamide showed low cytotoxicity in resting and in dividing PBMCs, in T-lymphoblastoid cells, and in hepatic cells, and providing ≥ 1997-fold increased selectivity relative to antiviral activity in T-lymphoblastoid cell lines. Tenofovir alafenamide also showed little to no effect on erythroid and myeloid progenitor proliferation in vitro.

In resting and activated human PBMCs, and in an established T-lymphocyte cell line, TFV exhibited low cytotoxicity, with CC_{50} values > 1 mM. Similar findings for TFV were observed in HepG2 cells, skeletal muscle cells of human origin, and in human renal proximal tubule epithelial cells. Similarly, TFV has shown no effect on hematopoietic progenitor cells in vitro.

Unlike TFV, TAF did not interact with the renal organic anion transporters 1 or 3 (OAT1 or OAT3), and TAF exhibited no OAT-dependent cytotoxicity in human epithelial kidney cells transiently expressing these transporters. In addition, the SI (considering CC_{50} in renal HEK293 cells expressing OAT1 or OAT3 relative to EC_{50} in primary CD4+ T lymphocytes) for TAF (29,000 and 4270, respectively) was much higher than for TFV (14 and 82, respectively). Therefore, TAF is unlikely to accumulate in renal proximal tubules in an OAT-dependent manner, supporting the potential for an improved renal safety profile.

When primary osteoblasts and PBMCs were treated with TAF doses consistent with human therapeutic exposure, comparable TFV-DP levels were achieved. At these therapeutically relevant doses of TAF, there were no in vitro effects on cell viability observed for primary osteoblasts or PBMCs.

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 (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 (TX-104-2001).

The cytotoxicity and antiviral activity of two TAF metabolites, M18 (GS-645552) and M28 (GS-652829), was evaluated in two T-lymphoblastoid cell lines (MT-2 and MT-4) following 5 days of compound exposure (PC-120-2021). These metabolites are also degradants and the testing supported manufacturing activities. Both TAF metabolites had no cytotoxicity up to the highest tested concentration (57 μM). Both metabolites/degradants showed weak inhibition of HIV-1 replication with 1723 to 2630-fold lower inhibitory potency relative to TAF (EC_{50} values of 7.41 to 21.04 μM) for metabolite M28 and 121 to 130-fold lower inhibitor potency relative to TAF (EC_{50} values of 0.56 to 0.97 μM) for metabolite M18. Collectively, these data demonstrate that FTC, TFV, and TAF have low cytotoxicity and a large SI in vitro.

2.2.2. Mitochondrial Toxicity

A variety of clinical symptoms observed in HIV-infected patients treated with prolonged NRTI therapy appear to be linked to mitochondrial toxicity {2522}. Several representatives of this class of HIV drugs inhibit mitochondrial DNA polymerase γ, by direct binding and competition with the natural deoxyribonucleotide substrate, incorporation into DNA, leading to DNA chain termination. A variety of in vitro studies have been conducted to evaluate the potential of FTC, TFV and TAF to exert mitochondrial toxicity. Results from these studies suggest that FTC and TFV have limited capability to inhibit human DNA polymerases or to mediate cytotoxicity or mitochondrial damage ({4541}, {6053}, {1131}, {2516}). 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, TX-104-2001). Additionally, RPV had no effects on DNA synthesis by human polymerase α, β, or γ at concentrations up to 1000 μM as determined by PCR (TMC278-1646_0005343).

HepG2 cells were exposed to FTC and TFV (as well as other nucleosides), either alone or in combination. Assay endpoints included cell growth; extracellular production of lactic acid; relative cellular content of 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.

Tenofovir alafenamide did not cause a specific depletion of mtDNA in HepG2 cells at concentrations as high as 1.0 μM, a level exceeding the maximum clinical systemic exposure of the 25 mg dose of TAF by more than 2-fold (C_{max} = 0.48 μM; GS-US-120-0104). Thus, TAF has a low potential for inhibiting mtDNA synthesis and inducing NRTI-related mitochondrial toxicities at the anticipated human exposure.

No effect of TFV was seen on the synthesis of mtDNA or lactic acid production in HepG2 human liver cells or in normal human skeletal muscle cells. The results of these studies indicate a low potential for TFV to interfere with mitochondrial functions.

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. Further, because administration of TAF results in lower exposure to TFV compared to TDF, the potential for mitochondrial toxicity is also low with the FTC/RPV/TAF FDC. No additional nonclinical studies are therefore warranted with the combination of FTC, RPV and TAF.

2.2.3. Off Target Activity

Emtricitabine had no pharmacologically significant binding affinity at 19 different receptors (TPZZ/93/0002), showed little or no direct effect on various isolated muscle preparations (cholinergic, adrenergic, histaminergic, and serotonergic), and had no major inhibitory effects on the contractile responses to acetylcholine, norepinephrine, serotonin, isoproterenol, arachidonic acid, histamine, bradykinin, and angiotensin II (TPZZ/92/0055).

Rilpivirine showed no significant interaction with 19 receptors in tissue or cellular preparation including α- or β-adrenergic, dopaminergic, muscarinic, serotonergic, opioid, interleukin, or chemokine receptors (TMC278-870219). The testing on a variety of human stomach ATPase, and gastric acidity did not reveal any significant effects secondary to the primary anti-HIV effect of RPV (TMC278-NC204).

Tenofovir and TDF showed no significant inhibition of or increased binding to a series of 111 protein targets (neuroreceptors, ion channels, transporters, and nuclear receptors) (V2000020).

2.3. Safety Pharmacology

2.3.1. FTC

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) (477, TPZZ/92/0056; TPZZ/93/0001; TPZZ/93/0119; TPZZ/92/0057). No effects on the cardiovascular system were reported in anesthetized dogs administered a cumulative dose of 38.5 mg/kg of FTC intravenously over a 1-hour period (TPZZ/92/0076). In addition, there were no abnormalities reported on the ECG data obtained from the repeated-dose toxicity studies in monkeys, where AUC exposures were up to 26-fold higher than in humans administered the 200 mg dose (TOX600; TOX627; TOX032).

2.3.2. RPV

A comprehensive range of safety pharmacology studies were conducted with RPV. The neurofunctional integrity of rats was not affected at 40 or 120 mg/kg, and there were no signs of neurotoxic effects recorded up to Day 7 after dosing (TMC278-Exp5560). At a dose of 400 mg/kg, occasionally animals showed a single slightly abnormal behavioral parameter 8 hours after dosing, and all animals showed a slightly reduced pupil size 2 hours after dosing. These parameters returned to normal 24 hours after dosing. No compound-related neurological changes or delayed neurotoxicity were noted. No change in behavior (TMC278-CPF654) or locomotor activity at cage side observations (TMC278-Exp5555) were observed in dog safety pharmacology studies.

No respiratory or arterial blood parameters were affected during or for 3 hours after a 1-hour intravenous infusion to anesthetized beagle dogs (TMC278-CPF648). No change in respiratory rate and tidal volume occurred in 4 telemetered conscious male beagle dogs after single oral escalating doses of 0 (vehicle), 20, 80 and 160 mg TMC278base/kg (TMC278-Exp5555). The mean plasma concentration of RPV at the end of the infusion was 2.62 µg/mL, and 3 hours after cessation of the infusion decreased to 0.484 µg/mL.

The standard battery of cardiovascular safety studies showed a concentration-dependent inhibition of RPV 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) (TMC278-CPF730). However, no relevant effects by RPV were noted on other cardiovascular or electrocardiographic parameters in vitro in

the right atrium of the guinea pig (TMC278-N168576), in vivo in anesthetized guinea pigs (TMC278-CPF643), in anesthetized dogs given a single intravenous dose (TMC278-CPF648), in conscious instrumented dogs (TMC278-CPF654), or in telemetered dogs given a single oral dose (TMC278-Exp5555). 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 [FK4244]).

Delayed-onset (after 11 days of treatment) prolongation of the QTcF interval was reported in the thorough QT (TQT) Study TMC278-TiDP6-C131. To investigate the mechanism of the QTc prolongation and of the delayed onset, additional nonclinical studies were done. The potential of RPV to induce proarrhythmic effects, in particular Torsade de Pointes (TdP), was also evaluated. The studies had 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) (TMC278-NC342). 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 ($I_{Ca,L}$).

In vitro, a concentration-dependent inhibition of trafficking of the hERG channel by RPV was observed from 1 µM (0.37 µg/mL) and above (TMC278-NC330). 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 RPV ranging from 0.6 to 0.9 µg/mL (TMC278-NC327). This concentration was similar to the steady state median C_{max} at RPV 75 mg once daily, a dose that caused delayed-onset QTc prolongation in TQT Study TMC278-C131.

Rilpivirine showed only a marginal potential to induce proarrhythmic effects in the rabbit arterially perfused left ventricular wedge model (TMC278-NC341). Up to 10 µM (3.7 µg/mL), RPV 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-TiDP6-C151 with 25 mg RPV once daily for 11 days showed no QTcF prolongation. TQT Study TMC278-TiDP6-C152 with 25 mg RPV once daily for 11 days confirmed the absence of a clinically relevant QTcF prolongation of 25 mg RPV once daily, the recommended dose.

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 RPV to influence cardiac repolarization. This assessment is included as a 'White Paper' (TMC278-20100613-Expert-TQT).

The standard battery of cardiovascular safety studies indicated that RPV 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 RPV in these species.

No other cardiovascular or cardio-electrophysiological parameters were affected by RPV. 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 RPV.

Given the results of the standard battery studies, the observed QTc prolongation in the TQT Study TMC278-C131 was unexpected. 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 RPV 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-TiDP6-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 RPV once daily did not elicit a clinically relevant QTc prolongation, whereas 75 mg once daily did on Day 11.

The actual unbound concentrations of RPV 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 RPV to the in vitro equipment. 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 RPV

	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		
Study	**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 exposure data of the 3 clinical studies, see respectively TMC278-TiDP6-C131-CRR, TMC278-TiDP6-C151-CRR, TMC278-TiDP6-C152-CRR.

2.3.3. TAF

Tenofovir alafenamide was evaluated in safety pharmacology studies of the rat central nervous, renal, GI, and cardiovascular systems. In vivo safety pharmacology experiments were conducted using TAF as the monofumarate form (GS-7340-02) in 50 mM CA. In the in vitro hERG assay, TAF as GS-7340-03 was dissolved in DMSO and diluted with HEPES-buffered physiological saline to a final concentration of 0.3% DMSO.

The IC_{50} for the inhibitory effect of TAF on hERG potassium current was estimated to be greater than 10 μM, far above human exposure (PC-120-2005).There were no adverse effects detected in the CNS in rats dosed at 1000 mg/kg (R990188), in the renal system in rats administered 1000 mg/kg (R990186) or in the cardiovascular system of dogs dosed at 100 mg/kg (80 mg free base equivalents/kg) (D2000006). There was reduced gastric emptying in rats dosed at 1000 mg/kg, but not at 100 mg/kg (R990187).

2.3.4. FTC/RPV/TAF

A comprehensive safety pharmacology program has been conducted for all individual agents. While the study designs for these studies varied between the agents, the major organ systems were thoroughly evaluated. Emtricitabine and TAF 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 (TMC278-TiDP6-C131). 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. Although TAF showed some potential to prolong the PR interval in the 39-week dog study at 18/12 mg/kg/day, the slight change was considered secondary to decreased weight gain, bone and renal toxicity, and significant decreases in triiodothyronine (T_3) {29101}, {29104}. No PR prolongation or any change in ECG results occurred in the safety pharmacology study that evaluated a TAF dose up to 100 mg/kg (D2000006) or in the thorough QT study (GS-US-120-0107). Overall, the pharmacological assessment of FTC, RPV, and TAF supports the effective use of these agents in combination therapy for the treatment of HIV-1 infection. Additional safety pharmacology studies on the FTC/RPV/TAF FDC are considered unwarranted.

2.4. Pharmacodynamic Drug Interactions

The potential for pharmacodynamic drug interactions for FTC, RPV, TAF, FTC+TFV, and FTC+RPV+TFV are presented in detail in the nonclinical virology summary contained in m2.7.2, Section 4.1.

2.5. Summary of Pharmacology

Emtricitabine, RPV, and TFV, have potent antiretroviral activity against wild-type and many drug-resistant strains of HIV-1 in vitro and in vivo. The nonclinical virology studies of FTC, RPV, TAF, FTC+TFV, and FTC+RPV+TFV are summarized and described in detail in the virology summary contained in m2.7.2, Section 4.1, together with the clinical virology data in m.2.7.2, Section 4.2.

Emtricitabine, RPV and TDF have a high selectivity for HIV RT. Although inhibition of human DNA polymerase γ (Pol γ) is one of the proposed mechanisms for nucleoside analogue-derived toxicity, FTC, TAF and TFV are very weak inhibitors of mammalian DNA polymerases α, β, δ, ε, and mitochondrial DNA polymerase γ. Rilpivirine also had no effects on DNA synthesis by human polymerases. 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 with the FTC/RPV/TAF combination is low. Emtricitabine, RPV, and TDF have no pharmacologically significant off-target binding affinity to the receptors tested. Emtricitabine, RPV and TAF have low in vitro cytotoxicity in a variety of human cell types.

Emtricitabine and TAF 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 (TQT) study in healthy subjects (TMC278-TiDP6-C131). 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. Although TAF showed some potential to prolong the PR interval in the 39-week dog study at 18/12 mg/kg/day, the slight change was considered secondary to decreased weight gain, bone and renal toxicity, and significant decreases in T3 {29101}, {29104}. No PR prolongation or any change in ECG results occurred in the safety pharmacology study that evaluated a TAF dose up to 100 mg/kg (D2000006) or in the thorough QT study (GS-US-120-0107).

Overall, the pharmacodynamic and pharmacological safety assessment of FTC, RPV, and TAF supports the effective and safe use of these agents in combination for the treatment of HIV-1 infection.

3. PHARMACOKINETICS

The absorption, distribution, metabolism, and excretion of FTC, RPV, and TFV/TAF were evaluated in vitro and in a variety of animal models in vivo. In addition, the drug-drug interaction profile was also evaluated. The pharmacokinetics of the FTC/RPV/TAF FDC is discussed based on the results of nonclinical studies completed with the individual agents.

A summary overview of the relevant data for the individual products is provided in the sections that follow.

3.1. Analytical Methods

For all agents and their metabolites, where appropriate, bioanalytical methods for toxicokinetic analysis supporting GLP safety studies were validated. Some of these methods for early nonclinical studies did not strictly conform to GLP guidelines but were evaluated for appropriate selectivity, sensitivity, linearity, as well as intra-assay accuracy and precision. All bioanalytical methods were conducted using appropriate protocols and documentation to assure data integrity.

3.2. In Vitro Absorption and Single Dose Pharmacokinetics

3.2.1. FTC

Single-dose pharmacokinetics of FTC have been studied in mice (TEIN/93/0003, TEIN/93/0004; IUW00101), rats {4570}, and cynomolgus monkeys (IUW00301, TEZZ/93/0019). 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.

3.2.2. RPV

The transepithelial permeability of RPV was intermediate in human colon carcinoma-derived (Caco-2) cells (TMC278-TiDP6-JRF [FK4155], TMC278-NC104). Passive transcellular diffusion is proposed as a mechanism for RPV intestinal absorption. Rilpivirine was not a substrate of P-glycoprotein (P-gp). Rilpivirine 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 RPV cannot be excluded.

After oral administration of TMC278 base, the absolute oral bioavailability of RPV 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 RPV is pH-dependent in these species as in humans.

After oral administration of RPV (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, plasma concentrations of RPV 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 RPV in animals following single or repeated oral administration of RPV or TMC278 base is presented in Table 2.

Table 2. Summary of Plasma Pharmacokinetic Parameters of RPV 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

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
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

Species	TMC278 Formulation	Sampling Period	Sex/n	Dose (mg/kg/day)	C_{max} (µg/mL)	AUC[a] (µg.h/mL)
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

3.2.3. TAF

In Caco-2 cell monolayers, TAF showed a dose-dependent increase in forward permeability and a decrease in efflux ratio indicating saturable efflux transport. Addition of the efflux transport inhibitor, cyclosporin A (CsA) diminished the efflux ratio and increased the forward permeability (AD-120-2037).

Single-dose plasma pharmacokinetics of TFV and/or TAF were evaluated following administration of TAF by dosing either GS-7340-02 or GS-7340-03 to male CD-1 mice or GS-7340-03 to both male and female 001178-W mice via oral gavage (AD-120-2014, AD-120-2016), to rats via oral gavage (AD-120-2015, R990130 and R2000065, to dogs via intravenous (IV) bolus of GS-7340-02 or oral administration of TAF as free base, its diastereomer GS-7339, the mixture GS-7171, or GS-7340-02 under fasted and under fed conditions (99-DDM-1278-001-PK, AD-120-2034). Tenofovir alafenamide was not detected in any of the rat studies. Additionally, the plasma PK profiles for TAF and TFV and TFV concentrations in PBMCs were determined in rhesus monkeys following a single oral dose of GS-7340-02 (P2000087). Tenofovir alafenamide is generated at sufficient exposures in nonclinical species chosen for assessment of toxicology. Consistent with dose-dependent permeability observed in vitro, the oral bioavailability of TAF increased with increasing dose in

dogs and the observed oral bioavailability was 14.3% at the 10 mg/kg dose {23907}. Following an oral dose of [^{14}C]TAF to a bile-duct cannulated (BDC) dog, the fraction absorbed was at least 41% based on excretion in urine and bile. Since 41% of the total dose was absorbed and 14.3% was found in systemic circulation, approximately 65% of the absorbed drug was hepatically extracted. This was consistent with hepatic extraction estimated from the in vitro stability study in dog hepatic tissue post-mitochondrial (9,000 x g) supernatant (S9) fractions (60.5%).

3.2.4. FTC/RPV/TAF

With respect to potential drug interactions within the combination that could affect absorption, FTC and RPV show high passive permeability and absorption is unlikely to be affected when administered with TAF. While TAF is an efflux substrate in the intestine, absorption is unlikely to be affected by FTC as intestinal efflux transport is not inhibited by FTC. Inhibition of P-gp by RPV observed in vitro is unlikely to be clinically relevant as RPV did not affect plasma exposure to P-gp substrates including digoxin and TAF in clinical drug interaction studies (TMC278IFD1001 and GS-US-120-1554, respectively). Although formal nonclinical studies of the absorption kinetics of the FTC/RPV/TAF FDC have not been conducted, clinical studies on the combination have been performed (m2.7.2, Section 1.2).

3.3. Repeat Dose Pharmacokinetics

3.3.1. FTC

The multiple-dose pharmacokinetic parameters for FTC were derived as part of the repeat-dose toxicity studies in mice (80 to 3000 mg/kg/day; TOX109; IUW00701; TOX599; TOX628), rats (60 to 3000 mg/kg/day; TOX108; TOX097), and monkeys (40 to 2000 mg/kg/day; TOX600; TOX627; TOX032) dosed 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.

3.3.2. RPV

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.

3.3.3. TAF

The multiple-dose pharmacokinetics of TFV were characterized in a pharmacokinetic study in dogs orally administered TAF (AD-120-2033) and in toxicokinetic studies following oral administration of TAF in mice (TX-120-2007), rats (R990182, TOX-120-001), dogs, (D990175-PK, TOX-120-002 and monkeys (P2000114-PK). After repeat dosing in mice or monkeys for up to 13 weeks or 4 weeks, respectively, no accumulation of TFV occurred; slight accumulation (up to ~3-fold) of TFV occurred in rats and dogs dosed for up to 26 and 39 weeks, respectively.

3.3.4. FTC/RPV/TAF

No additional repeat-dose pharmacokinetic studies were considered warranted with the combination of FTC, RPV, and TAF With respect to potential drug interactions within the combination that could affect absorption, FTC and RPV shows high passive permeability and absorption is unlikely to be affected when administered with TAF. While TAF is an efflux substrate in the intestine, absorption is unlikely to be affected by FTC as intestinal efflux transport is not inhibited by FTC. Inhibition of P-gp by RPV observed in vitro is unlikely to be clinically relevant as RPV did not affect plasma exposure to P-gp substrates including digoxin and TAF in clinical drug interaction studies (TMC278IFD1001 and GS-US-120-1554, respectively). Although formal nonclinical studies of the absorption kinetics of the FTC/RPV/TAF FDC have not been conducted, clinical studies on the combination have been performed (m2.7.2, Section 1.2).

3.4. Distribution

3.4.1. Protein Binding

3.4.1.1. FTC

The protein binding of FTC was very low (< 5%) in mouse, rabbit, monkey, and human plasma (TBZZ/93/0025).

3.4.1.2. RPV

Rilpivirine was extensively bound to plasma proteins in all species and the plasma protein binding was found to be concentration independent. Plasma protein binding values ranged between 99.08% and 99.97% (TMC278-NC112). Rilpivirine was extensively bound to human albumin (99.5% at the physiological protein concentration of 4.3% and irrespective of the RPV 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 RPV to red blood cells (RBCs) is limited in all species.

3.4.1.3. TAF and TFV

Since TAF is highly unstable in rodent plasma due to hydrolytic cleavage by plasma esterases, the extent of TAF binding to plasma was determined in dog and human plasma in vitro (AD-120-2026). In vitro protein binding of TAF was moderate in dog and human plasma with the percent unbound values of 48.0% and 46.8%, respectively. These in vitro values were higher than those observed in multiple human ex vivo studies with the mean percent unbound TAF ranging from 14% to 23% in all subjects (GS-US-120-0108 and GS-US-120-0114). Since the ex vivo results should be more clinically relevant than the in vitro values, percent unbound TAF of 20% was used for the assessments for potential drug interactions.

The protein binding of TFV was very low (< 10%) in the plasma and serum of humans and all other species examined (P0504-00039).

3.4.2. Tissue Distribution

3.4.2.1. FTC

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

3.4.2.2. RPV

The tissue distribution of RPV 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.

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 RPV and its metabolites to melanin.

3.4.2.3. TAF and TFV

Following oral administration of [^{14}C]TAF to mouse (AD-120-2011), rat (AD-120-2020), and dog (AD-120-2009, D990173-BP), [^{14}C]TAF-derived radioactivity was widely distributed to most of the tissues in all species. Consistent with high hepatic extraction, high levels of radioactivity were observed in the liver; high radioactivity was also measured in the kidney. Low levels of radioactivity were observed in brain and testis in mouse. No melanin binding was observed in rats. Distribution trends in the pigmented uveal tract of the eye and pigmented skin suggested that [^{14}C]TAF-related radioactivity was not selectively associated with melanin-containing tissues in the pigmented mouse. Distribution studies in dogs showed 5.7 to 15-fold higher ^{14}C-radioactivity in lymphoid tissues (iliac, axillary, inguinal and mesenteric lymph nodes, and spleen) 24 hours following administration of an equivalent dose of [^{14}C]-TAF relative to [^{14}C]-TDF {7415}. The concentration of TAF in dogs was relatively high also in lungs, thyroid, spleen, skeletal muscle, bone marrow, and some other tissues relative to TDF. Since the clinical TAF dose is >10-fold less than TDF, accumulation of TAF and/or its metabolites in these tissues should be similar (or less) to that with TDF.

Following single intravenous administration of [^{14}C]TFV in male rats, the highest concentrations of radioactivity were found in the kidney, liver, urine, and large intestine and trace amounts were observed in bone or bone marrow (95-DDM-1278-002).

3.4.3. Distribution in Pregnant Animals

Pharmacokinetic parameters for FTC in pregnant animals were similar to those reported for nonpregnant animals. While accumulation of TFV was observed after multiple dosing of TAF as GS-7340-02 up to 200 mg/kg/day in pregnant rats in the range-finding study (TX-120-2001), no accumulation of TAF and TFV was observed up to 250 mg/kg/day in the embryo-fetal development study (TX-120-2002). No accumulation occurred in pregnant rabbits in the range-finding study (TX-120-2004) or the embryo-fetal development study (TX-120-2005).

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 RPV and/or its metabolites.

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 (96-DDM-1278-005, TOX103, TOX038).

3.4.4. FTC/RPV/TAF Distribution

Drug interactions between FTC, RPV, and TAF that affect distribution would not be expected from the data available. While plasma protein binding is high for RPV and moderate for TAF, the binding was very low for FTC and TFV. Therefore, interactions through binding displacement would not be anticipated.

3.5. Metabolism

3.5.1. Intracellular Metabolism

Tenofovir alafenamide is subject to intracellular metabolism to TFV, which is further phosphorylated to the anabolites, TFV-MP and TFV-DP with TFV-DP being the pharmacologically active form. Intracellular metabolic activation of TAF in PBMCs or HIV-target cells including lymphocytes involves conversion to TFV by cathepsin A (CatA) {10427}, {13119}. In contrast to PBMCs, TAF is primarily hydrolyzed by carboxylesterase 1 in primary hepatocytes. Of the HIV PIs (DRV, ATV, LPV, and RTV), the boosting agent COBI, and HCV PIs (telaprevir, boceprevir, TMC-435, BI-201355, MK-5172, GS-9256, and GS-9451), the HCV PIs telaprevir and boceprevir, which are known to inhibit CatA, were the only ones that changed the antiretroviral effect of TAF in primary CD4+ T lymphocytes (reduced 23-fold and 3-fold, respectively). These data support the co-administration of the tested therapeutic PIs, with the exception of telaprevir or boceprevir, in combination with TAF, without negatively affecting its clinical pharmacology and intracellular conversion to TFV.

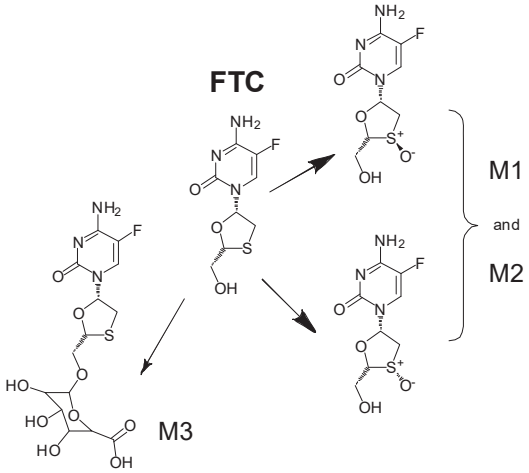
Emtricitabine and TFV are analogues of 2 different nucleosides, cytosine and adenosine, respectively, and do not share a common intracellular metabolism pathway. In experiments where both FTC and TFV were incubated together at concentrations higher than achieved in the plasma (10 μM), the intracellular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

3.5.2. Routes of Metabolism

3.5.2.1. FTC

Emtricitabine is not subject to significant metabolism by CYP enzymes. Generation of a minor (~1%) sulfoxide metabolite (M1 and/or M2) was catalyzed by CYP3A4, and inhibitor studies suggested that at least one other enzyme, possibly flavin-containing monooxygenase, may play a role (15396 v1). A minor direct glucuronide metabolite, M3, was also detected (Figure 1) {4570}.

Figure 1. Oxidative Metabolism of FTC



3.5.2.2. RPV

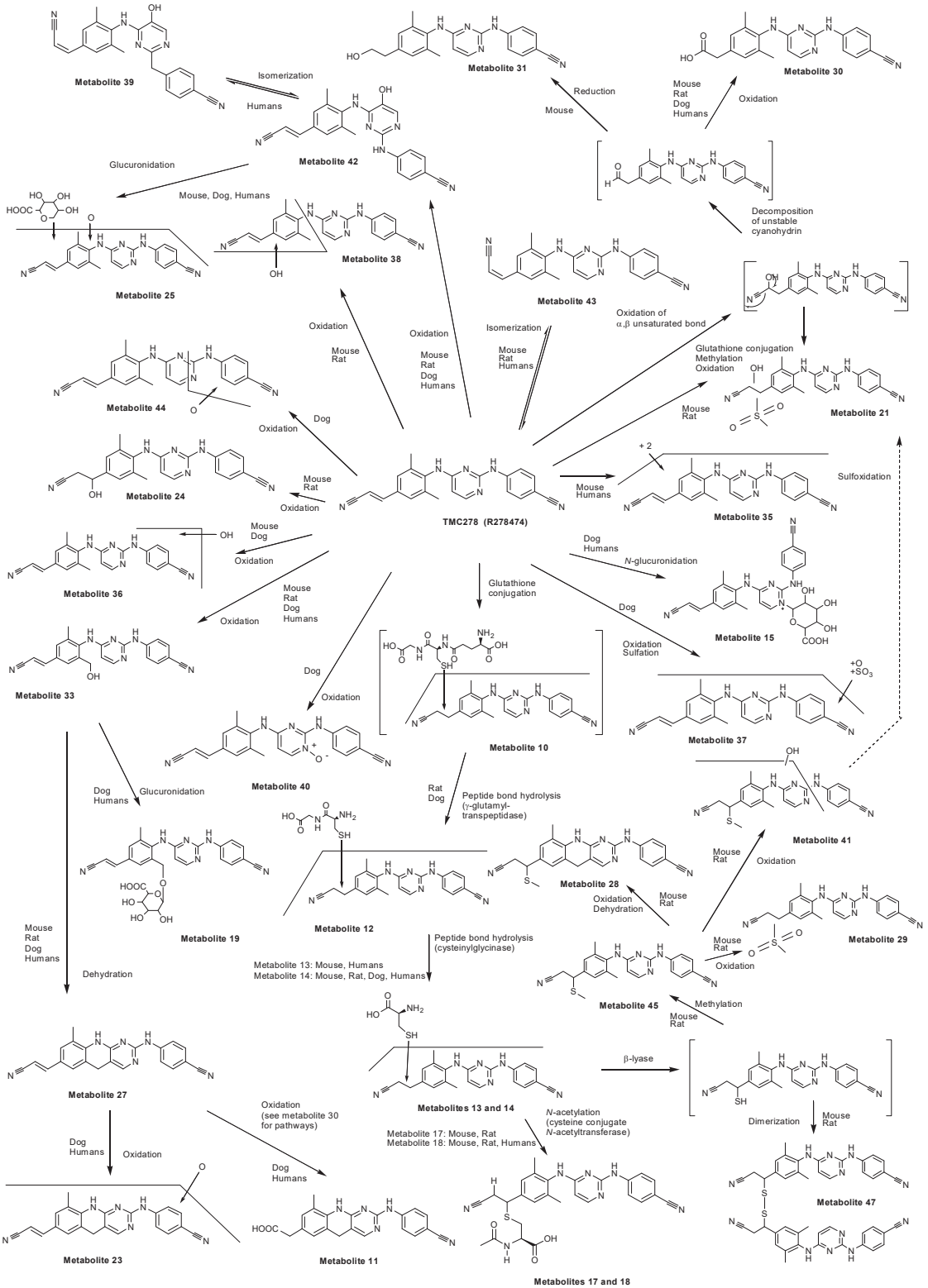
A number of RPV metabolites were identified in the in vivo studies in mice, rats, dogs, and humans (TMC278-NC190, TMC278-NC113, TMC278-NC114, and TMC278-NC157). The structures of these metabolites and the in vivo metabolic pathways are represented in Figure 2. Rilpivirine is metabolized via Phase I and Phase II reactions and the most important pathways are hydroxylation and glutathione conjugation. The contribution of the different metabolic pathways to the overall disposition of RPV is represented in Table 3.

Table 3. Total Percentage of the Administered Dose Metabolized per Major Pathways in Man and its Corresponding Percentages in Mice, Rats, and Dogs After Oral Administration of [^{14}C]RPV

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

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

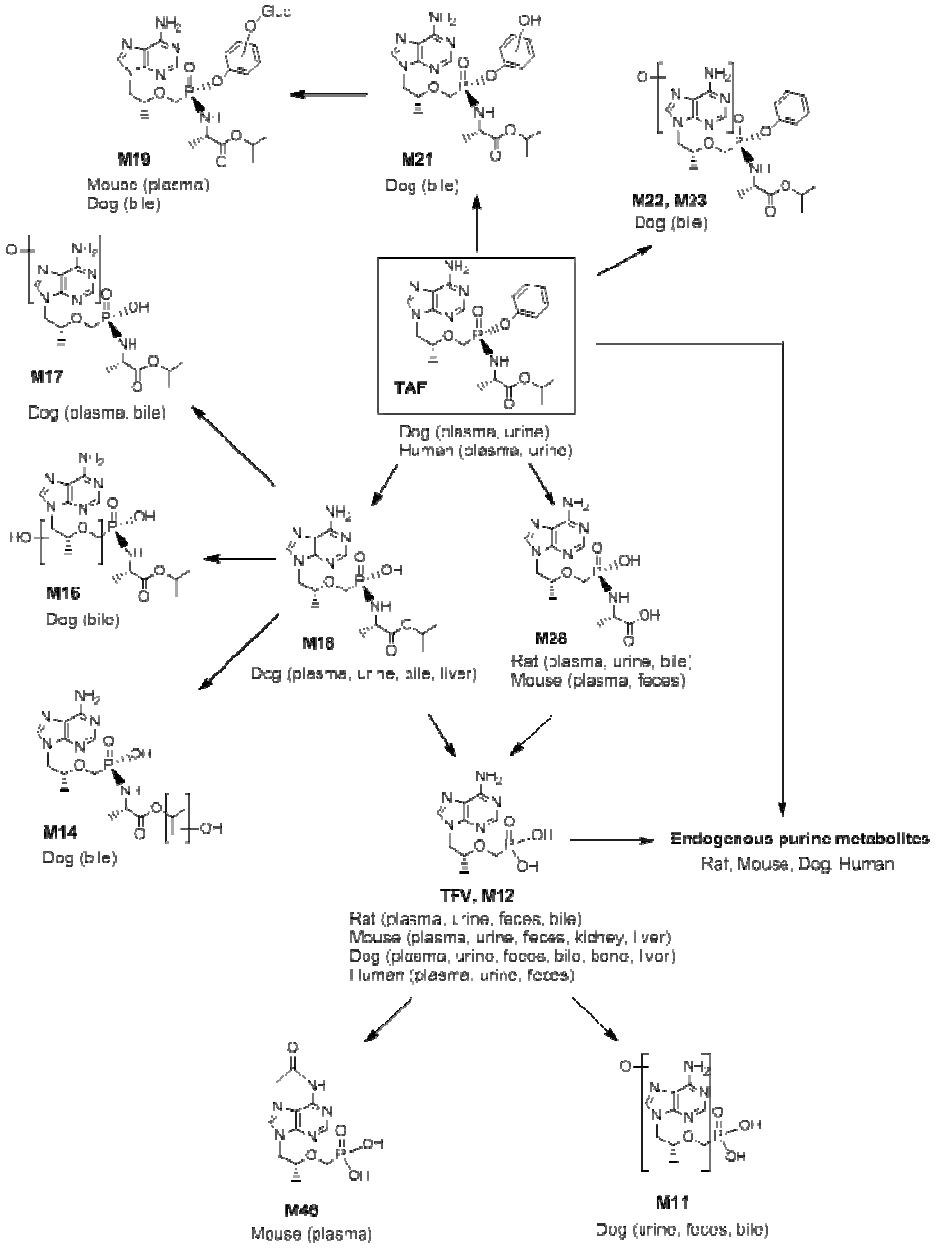
Figure 2. In Vivo Metabolic Pathways of RPV in Animals and Humans (Excluding Rat Bile)



3.5.2.3. TAF

The metabolic profiles of TAF were determined in plasma, urine, feces, kidney, liver, and nasal turbinate from mice (AD-120-2012), in plasma, urine, bile, and feces from rats (AD-120-2021), and in plasma, urine, bile, feces, bone, and liver from dogs (AD-120-2008). The metabolite profiles were also determined in human plasma, urine, and feces following administration of a single oral dose of [^{14}C]TAF (GS-US-120-0109). Based on the results from mouse, rat, dog, and human, a proposed biotransformation pathway is summarized (Figure 3). Tenofovir alafenamide is also subject to intracellular metabolism to TFV, which is further phosphorylated to the anabolites, TFV-MP and TFV-DP, with TFV-DP being the pharmacologically active form.

Figure 3. Metabolites of TAF



3.5.3. In Vitro Metabolism

3.5.3.1. FTC

An in vitro metabolism study was performed to identify the potential human CYP enzyme(s) responsible for the metabolism of FTC using human liver microsomes and Bactosomes containing cDNA-expressed human CYP enzymes (15396v1). The results showed that FTC was relatively stable in the incubation medium. One minor metabolite (~1%) was detected only in incubations with cDNA-expressed CYP3A4 incubations. It was not formed by CYP1A2, 2A6, 2B6, 2D6, 2E1, 2C8, 2C9, or 2C19. Human hepatic microsomal incubations in the presence and absence of selective inhibitors of various CYPs confirmed the low rate of FTC metabolism, and due to incomplete inhibition by the CYP3A-selective inhibitor, ketoconazole, also suggested the possible involvement of flavin monooxygenases (FMOs) in the metabolism of FTC. In vitro glucuronidation of FTC was not detected.

3.5.3.2. RPV

In vitro, the CYP3A4 isoenzyme plays a major role in the biotransformation of RPV. Therefore, some effects of drugs modulating CYP3A4 enzyme activity on plasma concentrations of RPV 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 RPV, and with CYP3A4 inhibitors such as ketoconazole, which increased the exposure of RPV (m2.7.2, Section 3.3.3.2.2).

3.5.3.3. TAF

The potential for CYP enzymes to metabolize TAF was assessed (AD-120-2004). Metabolism of TAF was not detected by CYP1A2, CYP2C8, CYP2C9, CYP2C19 or CYP 2D6. Tenofovir alafenamide was slowly metabolized by CYP3A4 at a rate of 1.9 min^{-1} which was 26.6% of the positive control, testosterone. While TAF is a weak inhibitor of CYP3A in vitro, it is not a clinically meaningful inhibitor or inducer of CYP3A.

3.5.4. In Vivo Metabolism

3.5.4.1. FTC

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 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} (TEIN/93/0015, TEIN/93/0016, TOX063). 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.

3.5.4.2. RPV

Rilpivirine 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 RPV 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 3). In plasma of animals and human, unchanged RPV was more abundant than all metabolites combined.

3.5.4.3. TAF

Based on the studies from mouse, rat, dog and human (AD-120-2008, AD-120-2012, AD-120-2021, GS-US-120-0109), endogenous purine metabolites including hypoxanthine, xanthine, allantoin, and uric acid were observed in all species. Tenofovir accounted for a majority of drug related material in plasma, urine, and feces from all species except for human plasma, in which uric acid was the predominant metabolite accounting for 73.9% of the total AUC over 96 hours. M18 was the major metabolite in rat bile accounted for 63% of total radioactivity recovered in bile. M18 and its oxidized metabolite, M16 were the major metabolites in dog bile accounted for 29 and 38% of total radioactivity recovered in bile. Various oxidative metabolites were found in dog bile. No metabolites unique to human were observed.

Tenofovir alafenamide-related metabolites were also monitored in kidney, liver, and nasal turbinate from mice (AD-120-2012). Most of the radioactivity was associated with TFV in kidney and liver and xanthine (M7) was the major identified metabolite in nasal turbinates. In dog, TAF-related metabolites were monitored in bone and liver and most of the radioactivity in these tissues was associated with TFV (AD-120-2008).

M18 (isopropylalaninyl TFV) and M28 (alaninyl TFV) are considered to be intermediate metabolites during intracellular conversion of TAF to TFV. In the metabolite profiling study in dog, M28 was not detected in this study although it has been qualitatively detected previously in dog plasma at 15 minutes post dose {23907}. M18 was detected as a minor metabolite in plasma, urine, and liver. Relatively high level of M18 was observed in bile. Low levels of M28 were observed in rat and mouse plasma and relatively high levels were in rat bile.

3.5.5. FTC/RPV/TAF

While RPV is primarily metabolized by CYP3A, TAF and FTC do not interact with drug metabolizing enzymes as inhibitors or inducers at clinically relevant concentrations. Therefore, metabolic drug interactions between these agents are unlikely.

Emtricitabine and TAF are analogs of 2 different nucleosides, cytidine and adenosine, respectively, and do not share a common intracellular metabolism pathway for pharmacological activation through phosphorylation. In experiments where both drugs were incubated together at concentrations higher than achieved in the plasma, the intracellular activation of TFV to its active diphosphate was not negatively influenced by the presence of FTC, and the activation of

FTC to FTC-triphosphate, was not negatively affected by the presence of TFV (PC-164-2002). Also, because the 2 drugs form analogs of different nucleotides, there should be no competition for incorporation by HIV-1 RT and subsequent chain termination. This was confirmed in vitro in antiviral assays where strong synergy between the 2 compounds was observed (PC-183-2004). Similarly, because of the highly restricted substrate specificity of the enzymes catalyzing the phosphorylation of FTC and TFV, inhibition of pharmacological activation by RPV is unlikely and, again, there is no evidence for antagonism in antiviral assays in vitro.

3.6. Excretion

3.6.1. Route and Extent of Excretion

3.6.1.1. FTC

The primary route of elimination of [^{3}H]FTC and [^{14}C]FTC was via renal excretion of parent drug after oral and IV administration in mice, rats, and cynomolgus monkeys {4570}, (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.

3.6.1.2. RPV

The routes and extent of RPV 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]RPV was mainly excreted in feces as unchanged RPV 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 RPV in urine was negligible. Therefore, the renal clearance of RPV is negligible. Rilpivirine 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).

3.6.1.3. TAF and TFV

Following oral dosing of mice, rats, and dogs with [^{14}C]TAF, the majority of radiolabel is recovered in the feces or urine in all species (AD-120-2011, AD-120-2020, AD-120-2007). The excretion of [^{14}C]TAF was determined after administration of a single 5-mg/kg oral dose of [^{14}C]TAF to bile duct-intact and BDC male Sprague-Dawley rats (AD-120-2020). In BDC rats, means of 72.6%, 23.2%, and 2.11% of the administered radioactivity were excreted in feces, urine, and bile, respectively, by 168 hours postdose. Recoveries of radioactivity in bile and urine from BDC rats indicated that at least 25% of the dose was absorbed. The mean overall recovery of radioactivity after oral dosing to BDC rats was 99.9%. The excretion of [^{14}C]TAF was

determined after administration of a single 15-mg/kg oral dose of [^{14}C]TAF to bile duct-intact and BDC male dogs (AD-120-2007). In BDC dogs, means of 42.7%, 26.5%, and 14.0% of the administered radioactivity were excreted in feces, urine, and bile, respectively, through 168 hours postdose. The elimination of a large amount of radioactivity in bile of BDC dogs indicates that biliary excretion is a major route of elimination of [^{14}C]TAF-derived radioactivity. The overall recovery of radioactivity in BDC dogs was 86.2%.

Renal excretion is the primary systemic route of elimination of TFV in all preclinical species tested. Following intravenous administration of [^{14}C]TFV, the majority of radioactivity was recovered in the urine in rats and dogs with 85.2% by 24 hours and 70.03% by 48 hours, respectively (96-DDM-1278-001; 96-DDM-1278-002).

3.6.2. Excretion into Breast Milk

Excretion into milk has not been evaluated for FTC.

No studies have been conducted to directly assess the excretion of RPV into milk. However, in the QWBA study in pregnant Sprague Dawley rats, some radioactivity was seen in the mammary glands (tissue/blood $AUC_{0–8h}$ ratio = 3), which indicates the potential for excretion of RPV-related radioactivity via the milk (TMC278-NC109).

In a dose range-finding pre- and postnatal developmental study (TMC278-NC168), it was found that pups were exposed to RPV 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.

Tenofovir was excreted into the breast milk of lactating rats and rhesus monkeys (R990202-PK; P2000116). The TFV milk to plasma ratios ranged from 0.11 to 0.24 in rats and 0.19 to 0.22 in rhesus monkeys.

3.6.3. FTC/RPV/TAF

Emtricitabine and TFV are almost exclusively eliminated by renal excretion. While TFV is a substrate for OAT1, OAT3, and MRP4, none of these transporters was inhibited by FTC. Rilpivirine is predominantly excreted in feces. Therefore, interactions within the components of the FTC/RPV/TAF FDC during excretion are unlikely.

3.7. Pharmacokinetic Drug Interactions

To aid in the interpretation of the data presented below and allow a quantitative estimate of the potential drug interaction liability from the IC_{50} values in the section below, the key human pharmacokinetic data from multiple clinical studies with the F/TAF FDC (m2.7.2, Section 3.3.1.3) are summarized in Table 4.

Table 4. Steady State Pharmacokinetic Parameters for FDC Components

Parameter	FTC	RPV[d]	TAF	TFV[d]
Dose (mg)	200	25	10[e]	–
C_{max} ($[I]_1$) (µM)	7.7	0.37	0.34[e]	0.053[e]
$C_{max,u}$ (µM)[a]		0.0037	0.068[e]	
$[I]_2$ (µM)[b]	3236	270	210[f]	–
$C_{hep, inlet}$ (µM)[c]	23.9	1.76	1.41[f]	–

$C_{max,u}$ = unbound concentration of drug at C_{max}; $[I]_1$ = inhibitor concentration corresponding to steady state C_{max}; $[I]_2$ = inhibitor concentration corresponding to theoretical maximum concentration in the intestinal lumen

a Steady state C_{max} × in vitro plasma fraction unbound (f_u, 20% for TAF, ~1% for RPV, and ~ 100% for FTC and TFV)
b Dose / 250 mL
c Estimated total hepatic inlet (portal vein) concentration calculated according to Obach et al. {34390}
d Values based on pooled Phase 3 trials with RPV (m2.7.2, Section 3.3.1.3.2)
e Value for boosted TAF dose based on E/C/F/TAF FDC
f Values estimated based on TAF 25 mg dose

3.7.1. Metabolic Drug Interactions

3.7.1.1. FTC

Emtricitabine was not an inhibitor of activities catalyzed by CYP1A2, 2A6, 2B6, 2C9, 2C19, 2D6, 2E1, or 3A in human hepatic microsomal fractions. Emtricitabine also did not show inhibition of the glucuronidation of 7-hydroxycoumarin, a general UGT substrate {15247}.

3.7.1.2. RPV

In vitro, RPV 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 RPV 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 RPV was observed (m2.7.2). In vitro, RPV 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 RPV was not expected.

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

The in vitro interaction of RPV with the metabolism of sertraline (substrate of multiple CYPs, monoamine oxidase and UDP-GT), paroxetine (CYP2D6), clarithromycin (CYP3A4), sildenafil (CYP3A4), omeprazole (CYP2C19 and CYP3A4), chlorzoxazone (CYP2E1), 17α-ethinylestradiol (Phase II metabolism), S-mephenytoin (CYP2C19), and norethindrone (different isoenzymes) was investigated in a pooled batch of human liver microsomes and the same was done for abacavir (alcohol dehydrogenase) in a pooled batch of human liver cytosol (TMC278-NC194).

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

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

3.7.1.3. TAF and TFV

The potential for TAF and TFV to inhibit human CYP-mediated drug metabolism was examined in vitro using hepatic microsomal fractions and enzyme-selective activities (AD-120-2003, V990172-104). The inhibitory activity of TAF with human liver microsomal CYP isozymes, CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A were assessed at concentrations up to 25 μM. The inhibition constant (IC_{50}) values calculated for CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, and CYP2D6 were greater than 25 μM. Tenofovir alafenamide weakly inhibited CYP3A-mediated oxidation of midazolam or testosterone with IC_{50} of 7.6 or 7.4 μM, respectively. The weak inhibition of CYP3A, however, is unlikely to be clinically relevant as TAF did not affect the exposure to CYP3A substrates, midazolam or rilpivirine (GS-US-120-1538, GS-US-120-1554). Tenofovir at 100 μM did not inhibit CYP1A2, CYP2C9, CYP2D6, CYP2E1, and CYP3A.

The potential for TAF to be a mechanism-based inhibitor of the human CYP enzymes, CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19 and CYP2D6 was assessed at TAF concentration at 50 μM (AD-120-2040). There was no evidence for time- or cofactor-dependent inhibition of any enzyme by TAF, with the maximum change in activity of 17.4% with CYP2C8 relative to control.

3.7.1.4. FTC/RPV/TAF

Coadministration of strong inducers or inhibitors of CYP3A may affect exposure to RPV. Incubation of TAF with the HIV-1 PIs ATV or DRV, or the CYP inhibitors, RTV or COBI, did not markedly affect the stability of TAF in intestinal subcellular fractions. Similarly, because of the high specificity of the enzymes catalyzing the phosphorylation of the nucleoside analogs, FTC and TFV are unlikely to interact with this process, and no antagonistic effects on antiviral potency have been seen in vitro (m2.7.2, Section 4.1).

The clinical drug-drug interaction studies are described in detail in the Summary of Clinical Pharmacology Studies (m2.7.2, Section 3.3.3.2).

3.7.2. Induction Liability

3.7.2.1. FTC

Emtricitabine did not activate human AhR or PXR at concentrations up to 50 μM (AD-162-2005).

3.7.2.2. RPV

The potential of RPV to induce CYP450 activities was determined in primary cultures from cryopreserved human hepatocytes originating from 3 different donors and compared to the data obtained with the positive controls omeprazole, rifampicin, and ethanol (TMC278-NC186). In addition, induction of CYP activities was also determined by measurement of mRNA expression levels by TaqMan real-time reverse transcription-polymerase chain reaction. Based on the observed fold-changes of mRNA expression and fold-induction of CYP activities, it can be concluded that RPV might be a very weak inducer of CYP1A2 (6-fold less than omeprazole) and CYP2B6 (4.5-fold less than rifampicin) in human hepatocytes. In addition, the results indicate that RPV appears to be a moderate inducer of CYP2C19 (1.4-fold less than rifampicin) and CYP3A4 (2-fold less than rifampicin) in human hepatocytes. No conclusion could be drawn for CYP2E1.

Rilpivirine in aqueous HPMC (0.5%) was administered for 3 months to male and female CD-1 mice at doses of 20, 80, and 320 mg/kg/day (TMC278-NC192). Rilpivirine base in PEG400/CA (10%) was administered to male and female Sprague Dawley rats at doses of 40, 120, and 400 mg/kg/day and to male and female beagle dogs at doses of 5, 10, and 40 mg/kg/day for 6 months (TMC278-NC193 and TMC278-NC140, respectively). The effect of RPV on hepatic enzyme activities was assessed in hepatic microsomal fractions from the RPV-treated animals. In mice, RPV was an inducer of the CYP4A forms in both male and female animals (up to 25- and 20-fold, respectively). Some induction was also seen with the CYP3A forms (up to 1.7-fold in both males and females). Rilpivirine treatment induced UDP-GT activity in male and female mice (up to 2.1- and 2.3-fold, respectively) and decreased GST activity in male mice to 44% at 320 mg/kg/day. In rats, RPV was an inducer of CYP4A forms in male rats (4.7-fold), whereas in female rats, RPV was an inducer of CYP3A forms (6-fold) and possibly also of CYP2B and CYP4A forms. Rilpivirine treatment had some effect on UDP-GT activity in male rats (induction of 1.3 fold only at high dose level) and on GST activity in female rats (induction of 1.5-fold). In the 2-week study, the results were similar. In dogs, treatment with RPV did not result in any induction of CYP1A1, CYP2B, CYP2E, CYP4A, UDP-GT, or GST activity. Rilpivirine produced some decrease in microsomal CYP3A-dependent testosterone 6β-hydroxylase activity, but this effect was confined to the 2 highest dose levels and was not dose-dependent.

Rilpivirine, 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.

Therefore, ex vivo induction studies in rodents showed that RPV 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, RPV 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, administration of RPV 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.

3.7.2.3. TAF

The induction of CYP, P-gp and UGT1A1 mRNA and CYP activity by TAF was assessed in cultured human hepatocytes from 3 separate donors treated with 1, 10, and 100 µM TAF once daily for 3 consecutive days (AD-120-2032). Due to cytotoxicity, the cell viability was significantly affected at 100 µM TAF and mixed responses to TAF with increased mRNA levels and reduced CYP activities were observed. At noncytotoxic concentrations of TAF (1 and 10 µM), no significant increases in the mRNA levels and the CYP activities were observed. After treatment with 10 µM TAF, the mRNA levels of CYP1A2 and CYP3A4 increased by 3.0- and 8.3-fold which correspond to 3% and 6% of the induction levels observed with the respective positive controls. Therefore, TAF showed little or no potential for CYP induction at clinically relevant concentration (1 µM). No significant induction of P-gp and UGT1A1 mRNA was observed (less than 2-fold).

The potential for TAF to induce human drug metabolizing enzymes and drug transporters through the activation of human AhR or human PXR was further evaluated in cell-based systems (AD-120-2005). For PXR activation, at 50 µM TAF the extent of activation of PXR was only 23% of the maximal effect of rifampicin and 15 µM TAF demonstrated activation of < 5% of the maximal induction elicited by rifampicin. Tenofovir alafenamide did not activate AhR up to 50 µM, the highest concentration tested. Therefore, TAF is unlikely to activate either of these human xenobiotic receptors supporting the in vitro induction results in human hepatocytes. Furthermore, TAF is unlikely to be a clinically relevant inducer as it did not affect the exposure to midazolam or rilpivirine (GS-US-120-1538, GS-US-120-1554).

3.7.3. Transporter Drug Interactions

The potential for FTC, RPV, TAF, and TFV to be substrates or inhibitors for transporters was evaluated in vitro and summarized in Table 5 and Table 6, respectively.

Table 5. Transporter Substrate Assessment of FTC/RPV/TAF Components

Transporter	FTC	RPV	TAF	TFV	Referenced Reports
P-gp	n	n	y	n	AD-236-2004, TMC278-NC104, AD-120-2018
BCRP	n	ND	y	n	AD-236-2005, AD-120-2018
OATP1B1	ND	ND	y	ND	AD-120-2022
OATP1B3	ND	ND	y	ND	AD-120-2022
OAT1	n	ND	n	y	AD-236-2010, PC-104-2010, PC-120-2018
OAT3	y	ND	n	y	AD-236-2010, PC-103-2001 PC-120-2018
OCT1	ND	ND	n	n	PC-103-2001, AD-120-2036
OCT2	n	ND	ND	n	AD-236-2011, PC-103-2001
MRP1	ND	ND	ND	n	PC-104-2014
MRP2	n	ND	ND	n	AD-104-2001
MRP4	ND	ND	ND	y	AD-104-2001

BCRP = breast cancer resistance protein; MRP1 2, 3, or 4 = multidrug resistance associated protein 1, 2, or 4; n = no; ND = not determined; OAT1 or 3 = organic anion transporter 1 or 3; OATP1B1 or B3 = organic anion transporting polypeptide 1B1 or B3; OCT1 or 2 = Organic cation transporter 1; P-gp = permeability glycoprotein; y = yes

Table 6. Transporter Inhibition Assessment of FTC/RPV/TAF Components

Transporter	IC_{50} (µM)				Referenced Reports
	FTC	RPV	TAF	TFV	
P-gp	>100	9.2	>100	>1000	AD-236-2003, TMC278-NC104, AD-120-2019
BCRP	>100	ND	>100	>100	AD-236-2003, AD-120-2019
BSEP	>100	ND	>100	>100	AD-236-2008, AD-120-2036
OATP1B1	>100	ND	>100	>100	AD-236-2006, AD-120-2019
OATP1B3	>100	ND	>100	>100	AD-236-2006, AD-120-2019
MATE1	>100	7.51	>100	>300	AD-236-2001, TMC278-FK10420, AD-104-2012, AD-120-2036
OAT1	>100	ND	>100	33.8[a]	AD-236-2007, AD-120-2036
OAT3	>100	ND	>100	>1000	AD-236-2007, PC-103-2001, AD-120-2036
OCT1	>100	ND	>100	>100	AD-236-2008, PC-103-2001, AD-120-2036
OCT2	>100	5.46	>100	>300	AD-236-2001, TMC278-FK10042, AD-120-2036
MRP1	ND	ND	ND	>500	PC-104-2014
MRP2	>100	ND	ND	>100	AD-236-2012, AD-104-2001
MRP4	>100	ND	ND	>1000[b]	AD-236-2007

BCRP = breast cancer resistance protein; BSEP = bile salt excretory pump; MATE1 or 2-K = multidrug and toxin extrusion protein 1 or 2-K; MRP1 2, 3, or 4 = multidrug resistance associated protein 1, 2, or 4; ND = not determined; OAT1 or 3 = organic anion transporter 1 or 3; OATP1B1 or B3 = organic anion transporting polypeptide 1B1 or B3; OCT1 or 2 = Organic cation transporter 1; OCTN1 = organic cation transporter novel, type 1; P-gp = permeability glycoprotein

a Binding constant for uptake into CHO cells reported by Cihlar et al, 2009 {2520}.

b Imaoka et al 2007 {10260}

3.7.3.1. FTC

In vitro studies indicated that FTC is not a substrate or an inhibitor of any of the transporters tested except for being a substrate of OAT3. There is no clinical evidence for FTC to be involved in transporter-mediated drug interactions.

3.7.3.2. RPV

The potential of RPV to inhibit renal transporters, OCT2, MATE1 and MATE2-K was assessed in vitro (TMC278-FK10042 and TMC278-FK10420). Rilpivirine showed IC_{50} values for OCT2, MATE1, and MATE2-K of 5.46, 7.51, and < 0.05 μM, respectively. Since the estimated clinical unbound plasma C_{max} for RPV is 3.5 nM, the effect of RPV on OCT2 and MATE1 is unlikely to be clinically relevant but it cannot be excluded that RPV would inhibit MATE2-K at clinically relevant concentrations. Renal clearance is, however, negligible for RPV and it is not known what the intracellular RPV concentrations are at the local level of the MATEs' site of action. Metformin is a known substrate for OCT2 and MATEs and coadministration with RPV did not affect the plasma exposure or urine concentrations of metformin (TMC278IFD1004). OCT2, MATE1, and MATE2-K are known to be involved in renal secretion of creatinine {28345}. Small increases in serum creatinine have been observed in humans treated with RPV, likely due to inhibition of renal transporters for creatinine, but these are not considered to be clinically important {22839}.

3.7.3.3. TAF

In vitro studies demonstrated that TAF and TFV do not inhibit any of the transporters tested at clinically relevant concentrations. Therefore, TAF and TFV are unlikely to be perpetrators of transporter-mediated drug interactions.

Tenofovir alafenamide is a substrate for intestinal efflux transporters, P-gp and BCRP. An increase in TAF absorption was observed in the presence of efflux transport inhibitors, CsA or COBI in vitro (AD-120-2013). The effect of CsA on TAF oral bioavailability was also assessed in vivo in dogs (AD-120-2035). Following oral administration of TAF at 2 mg/kg to untreated or pretreated dogs with 75 mg CsA, the CsA pretreatment increased the plasma exposure to TAF and oral bioavailability by approximately 10-fold, while the PK profile of TFV was not significantly affected by CsA. Consistent with the increased TAF plasma exposure, the exposure to TFV-DP in PBMCs isolated from the CsA pretreated dogs was approximately 2-fold higher than that in cells from untreated animals. These results suggest that coadministration of efflux inhibitors increases TAF absorption and may potentiate the antiviral effect by increasing the TFV-DP levels in PBMCs.

Tenofovir alafenamide was found to be a substrate for hepatic uptake transporters, OATP1B1 and OATP1B3. Exposure to TAF may be affected by inhibitors of these transporters or by genetic polymorphisms that affect the transport activities. Unlike TFV, TAF is not a substrate for renal transporters, OAT1 and OAT3.

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 OAT1 and MRP4 acting in series as the major uptake and efflux transporters in proximal tubules, respectively (PC-103-2001, AD-104-2001, AD-104-2002), {2520}, {7299}, {8418}. Human organic anion transporter type 3 may play a secondary role in the tubular uptake of TFV. Neither P-gp nor MRP2 appear to be involved in the tubular efflux of TFV. As the primary transporter handling the tubular uptake of TFV, human OAT1 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 human OAT1-mediated transport of TFV, indicating a low potential for renal interactions with TFV due to inhibition of this pathway (PC-104-2010 and PC-104-2011, respectively) {2520}. Furthermore, the PIs ATV, lopinavir, and RTV 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.

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

3.7.3.4. FTC/RPV/TAF

Rilpivirine was shown to have P-gp inhibitor properties with an apparent IC_{50} value of 9.2 µM (3.4 µg/mL). Inhibition of transepithelial permeation of P-gp substrates cannot be excluded, but a possible effect is unlikely to be clinically relevant. P-gp inhibitors are not expected to play a role in modulating the intestinal absorption of RPV, given the limited role of efflux transporters in the epithelial permeability of RPV. RPV did not affect plasma exposure of P-gp substrates including digoxin and TAF in clinical drug interaction studies (TMC278IFD1001 and GS-US-120-1554, respectively).

Renal excretion of TFV is facilitated by basolateral uptake by OAT1 and OAT3 and apical efflux by the MRP4 efflux transporter. There is no evidence for inhibition of TFV renal excretion by FTC. The presence of 90 µM COBI in the Caco-2 bidirectional permeability assay, TAF forward permeability increased 4.6-fold and the efflux ratio significantly decreased suggesting P-gp-mediated drug interaction (AD-120-2013).

3.7.4. FTC/RPV/TAF Pharmacokinetic Drug Interactions

While RPV is primarily metabolized by CYP 3A, FTC, TAF and TFV are not clinically relevant substrates, inhibitors, or inducers. Tenofovir and FTC do not inhibit each other's pharmacological activation through phosphorylation. Thus, the drug interactions through drug metabolizing enzymes within the three compounds are unlikely. Coadministration of strong inducers or inhibitors of CYP3A may affect exposure to RPV.

Emtricitabine, TAF, and TFV do not inhibit any of the transporters tested at clinically relevant concentrations in vitro. Therefore, FTC, TAF, and TFV are unlikely to be a perpetrator of transporter-mediated drug interactions. There is no evidence for inhibition of TFV renal excretion by FTC as it shows undetectable inhibition of OAT1, OAT3, and MRP4 in vitro.

Renal excretion of TFV is facilitated by basolateral uptake by OAT1 and OAT3 and apical efflux by the MRP4 efflux transporter. There is no evidence for inhibition of TFV renal excretion by FTC as it shows undetectable inhibition of OAT1, OAT3, and MRP4 in vitro.

While RPV was found to inhibit P-gp with IC_{50} of 9.2 μM in vitro, it is not a clinically relevant inhibitor as exposure of digoxin and TAF was not affected by RPV (m2.7.2, Section 3.3.3.2.3).

Rilpivirine was found to be an inhibitor of OCT2, MATE1, and MATE2-K in vitro. However, inhibition of OCT2 and MATE1 by RPV is unlikely to be clinically relevant based on IC_{50} values greater than 10-fold higher than the C_{max} not bound to plasma protein. In addition, inhibition of MATE2-K by RPV may not result in marked drug-drug interactions with xenobiotics which are substrates for both MATE1 and MATE2-K. Drug interactions through inhibition of these transporters by RPV within the components of the FTC/RPV/TAF FDC are unlikely as FTC and TAF were bioequivalent when administered as the FTC/RPV/TAF or the E/C/F/TAF FDCs (GS-US-336-1159).

3.8. Summary of Pharmacokinetics

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

FTC

Emtricitabine shows high passive permeability and is unlikely to be affected when administered with TAF. While TAF is an efflux substrate in the intestine, absorption is unlikely to be affected by FTC as intestinal efflux transport is not inhibited by FTC. Drug interactions between FTC and TAF that affect distribution would not be expected from the data available. The plasma protein binding is moderate for TAF and very low for FTC and TFV. Therefore, interactions through binding displacement would not be anticipated.

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.

RPV

Human colon carcinoma-derived Caco-2 cells revealed that RPV can be classified as a compound with an intermediate permeability. Passive transcellular diffusion was proposed as a mechanism for RPV absorption. However, the solubility, and in the case of suspension and solid dosage forms, possibly also dissolution seem to limit the rate and extent of absorption. After oral

administration of TMC278 base, the absolute oral bioavailability of RPV was 32%, 54%, 31%, and 24% in rats, rabbits, dogs, and monkeys, respectively. With citric acid in the formulation, the absolute bioavailability in dogs was increased by a factor of 2.6 and was 80% at 5 mg/kg, whereas at 80 mg/kg, there was no effect of citric acid on exposure. In rats, the bioavailability remained the same at 40 mg/kg with or without citric acid and increased with citric acid at 400 mg/kg.

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

In rats, tissue distribution of [^{14}C]RPV 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 RPV and its metabolites to melanin. In pregnant rats, there was distribution of [^{14}C] RPV 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.

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

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

Rilpivirine 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 RPV and to a lesser extent glutathione conjugation were the predominant pathways. In rats the glutathione conjugation pathway is the predominant pathway, whereas in dog and man, oxidation of RPV is the predominant one. No unique human metabolites were observed. In plasma of animals and human, unchanged RPV was more abundant than any metabolite.

In rodents dosed with [^{14}C]RPV, total radioactivity was rapidly excreted, whereas in dogs, excretion was relatively slow. In all animal species and human, the predominant route of excretion was via feces (> 85%) and generally, the majority of the total radioactivity eliminated was unchanged RPV. 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 RPV in urine was negligible. In rats, biliary excretion was limited (18%–25% of the dose) and the amount of unchanged RPV in bile was negligible. In rats, there was indication that RPV was excreted in milk.

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

Rilpivirine was shown to have P-gp inhibitor properties with an apparent IC_{50} value of 9.2 µM (3.4 µg/mL). Inhibition of transepithelial permeation of P-gp substrates cannot be excluded, but a possible effect is unlikely to be clinically relevant. P-gp inhibitors are not expected to play a role in modulating the intestinal absorption of RPV, given the limited role of efflux transporters in the epithelial permeability of RPV. RPV did not affect plasma exposure to P-gp substrates including digoxin and TAF in clinical drug interaction studies (TMC278IFD1001 and GS-US-120-1554, respectively).

Ex vivo induction studies in rodents showed that RPV is an inducer of CYP3A-family (up to 1.7-fold in mice and up to 6-fold in rats) and CYP4A-family (up to 25-fold in mice and up to 4.7-fold in rats). Additionally, RPV induced UDP-GT activity in mice (up to 2.3-fold) and to a lesser extent in rats (up to 1.3-fold only at high dose in males). In dogs, treatment with RPV did not result in any enzyme induction.

The recommended dose of RPV in HIV-infected treatment-naive patients is 25 mg once daily. At this dose level, mean C_{max} (Week 4 to 8) was 0.13 µg/mL and mean AUC_{0-24h} (Week 48; population pharmacokinetics) was 2.4 µg.h/mL (TMC278-TiDP6-C209, TMC278-TiDP6-C215). These values were compared with those obtained at the highest doses tested in animal species.

At the end of administration, the C_{max} and AUC ratios (animal/human) ranged from 1.9 to 2.4 in monkeys; 25 to 42 in dogs; 97 to 115 in pregnant rabbits; 7.5 to 48 in male rats; 35 to 123 in female rats; 63 to 100 in pregnant rats; 21 to 70 in juvenile rats; and 210 to 446 in mice.

TAF/TFV

Renal excretion is the primary systemic route of elimination of TFV in all preclinical species tested. Tenofovir alafenamide is a prodrug of TFV which is intracellularly converted to its pharmacologically active TFV-DP by cellular enzymes including cathepsin A in PBMCs or carboxylesterase 1 in hepatocytes. Tenofovir alafenamide generates sufficient exposure in nonclinical species chosen for assessment of toxicology. Consistent with dose-dependent permeability observed in vitro, the oral bioavailability of TAF increased with increasing dose in dogs and the observed oral bioavailability was 14.3% at the 10 mg/kg dose {23907}. Hepatic extraction of TAF was estimated to be approximately 65% in dog.

Tenofovir alafenamide is not an inhibitor or an inducer of UGT1A1 or CYP enzymes known to metabolize xenobiotics except for weak inhibition observed for CYP3A in vitro. However, the weak inhibition of CYP3A is not clinically relevant as TAF did not affect the exposure to CYP3A substrates, midazolam or rilpivirine in clinical drug-drug interaction studies (GS-US-120-1538 and GS-US-120-1554, respectively). Tenofovir alafenamide is unlikely to be a perpetrator of transporter-mediated drug interactions. Since TAF is a substrate for intestinal efflux transporters P-gp and BCRP and hepatic uptake transporters OATP1B1 and OATP1B3, TAF exposure may be affected by inhibitors and by inducers of the intestinal efflux transporters and inhibitors or genetic polymorphisms of OATPs. Tenofovir alafenamide was not a substrate for renal transporters OAT1 and OAT3. While TFV is a substrate for OAT1, OAT3, and MRP4, none of these transporters was inhibited by FTC. Therefore, interactions between the compounds during excretion are unlikely.

FTC/RPV/TAF

Based on the data supporting the individual components and the extensive clinical data with the FTC/RPV/TDF FDC and FTC/TAF containing regimens, adverse pharmacokinetic interactions that would negatively affect pharmacological efficacy are not anticipated. While RPV is primarily metabolized by CYP 3A, FTC, TAF and TFV are not clinically relevant substrates, inhibitors, or inducers. Thus, the drug interactions through drug metabolizing enzymes within the three compounds are unlikely. Coadministration of strong inducers or inhibitors of CYP3A may affect exposure to RPV. Emtricitabine and TAF are analogs of 2 different nucleosides, cytidine and adenosine, respectively, and do not share a common intracellular metabolism pathway for pharmacological activation through phosphorylation. RPV did not affect plasma exposure to P-gp substrates including digoxin and TAF in clinical drug interaction studies (TMC278IFD1001 and GS-US-120-1554, respectively). While plasma protein binding is high for RPV and moderate for TAF, the binding was very low for FTC and TFV. Therefore, interactions through

binding displacement would not be anticipated. Emtricitabine and TFV are almost exclusively eliminated by renal excretion. While TFV is a substrate for OAT1, OAT3, and MRP4, none of these transporters was inhibited by FTC. Rilpivirine is predominantly excreted in feces. Therefore, interactions within the components of the FTC/RPV/TAF FDC during excretion are unlikely. The pharmacokinetic assessment of FTC, RPV, and TAF supports the safe use of these agents in combination.

4. TOXICOLOGY

Comprehensive nonclinical programs with FTC, RPV and TAF have been completed. These studies have characterized the single and repeat dose toxicity, mutagenicity, carcinogenicity (TDF studies in place of TAF), and reproductive toxicity of each the individual agents, and the toxicity of the FTC/TDF combination. The nonclinical toxicology studies discussed in this section provide an adequate basis to evaluate potential toxicities of the individual components and the 3-drug combination, and for comparing and interpreting results from clinical studies.

4.1. Single Dose Toxicity

Emtricitabine has demonstrated minimal acute toxicity in rodents (oral LD_{50} > 4000 mg/kg and IV LD_{50} > 200 mg/kg; TTEP/93/0020; TTEP/93/0023; TTEP/93/0021; TTEP/93/0024).

No formal single-dose studies were conducted with RPV 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 (TMC278-Exp5538). Rats dosed with an oral maximum feasible single dose of 800 mg/kg TMC278 base in PEG400 showed no treatment-related effects. 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.

The single dose NOAEL for a single oral dose TAF as GS-7340-02 in the rat was determined to be > 1000 mg/kg (R990185). The no observed effect level (NOEL) in dogs administered a single dose of TAF was 30 mg/kg (treatment-related clinical signs, renal lesions in the kidneys at 90 and 270 mg/kg (D990181).

No single-dose studies have been performed with the combination of FTC, RPV, and TAF. Coadministration is unlikely to provide significant information based on clinical data with the FDC.

4.2. Repeat Dose Toxicity

4.2.1. FTC

A series of GLP oral repeat-dose toxicity studies were conducted with FTC in mice (4 weeks [TOX599 and TOX599 addendum; TOX118] and 26 weeks [TOX022 and TOX628]), rats (13 weeks [TOX097]), and cynomolgus monkeys (4 weeks [TOX600 and TOX600 addendum], 13 weeks [TOX627], and 52 weeks [TOX032]).

Effects associated with the administration of FTC in the toxicology studies were confined to high-dose groups. Changes in red blood cell (RBC) parameters, interpreted as a mild, reversible anemia occurred at the highest dose in several studies (ie, 1- and 6-month mouse; 3-month rat;

and 12-month 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 (12 months). The exposures based on plasma AUC values at the NOEL doses in the animals were approximately 27-fold (mice), 27-fold (rats), and 7.5-fold (monkeys) higher than the AUC in patients treated with FTC at 200 mg once daily in the EVG/COBI/FTC/TDF FDC.

4.2.2. RPV

Systemic toxicity of TMC278 base or RPV 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. In this section only pivotal studies are summarized.

Mice

The doses of RPV in the 3-month oral gavage study with CD-1 mice were 20, 80, or 320 mg/kg/day (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 (TMC278-Exp5692). There were no mortalities associated with RPV, 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 RPV 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 (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 (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 (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 RPV. 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 (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 (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.

4.2.3. TAF

The repeat-dose oral toxicity of TAF has been studied in mice, (2 weeks [TX-120-2006] and 13 weeks [TX-120-2007]), rats (6-7 days [R2000044, R000139], 4 weeks [R990182] and 26 weeks [TOX-120-001]), dogs (4-weeks [D990175] and 39 weeks [TOX-120-002]) and monkeys (4 weeks [P2000114]). In chronic studies, kidneys (karyomegaly, tubular degeneration), and bone (atrophy of metaphyseal cancellous bone) were the primary target organs. TAF also appeared to increase biochemical markers of bone turnover and decrease serum 1, 25-dihydroxy- and 25-hydroxyvitamin D_3 at doses in rats and dogs.

4.2.3.1. Kidney

Renal tubular karyomegaly was observed in rats and dogs orally administered TAF. Focal areas of minimal renal cortical tubular basophilia and associated minimal nuclear karyomegaly were present in rats administered 400 mg/kg/day for 4 weeks and 100 mg/kg/day for 26 weeks. Renal tubular karyomegaly and/or basophilia were observed in dogs administered 3 and 10 mg/kg/day for 4 weeks and dogs administered 6 or 18/12 mg/kg/day for at least 13 weeks.

Renal cortical tubular degeneration/regeneration findings were limited to animals administered 6 or 18/12 mg/kg/day for at least 13 weeks in the 39-week dog toxicity study. Similar findings of renal cortical tubular degeneration/regeneration and karyomegaly were present in dogs administered either 6 or 18/12 mg/kg/day for 39 weeks. These changes were minimal to slight in affected males and females at 6 mg/kg/day. In high-dose males (18/12 mg/kg/day) the severity ranged from mild to moderate. Similar lesions (karyomegaly and tubular degeneration) but of only minimal severity were also present in 2 males administered 2 mg/kg/day of TAF for 39 weeks. After a 13-week recovery period, treatment-related histology changes were still observed in the kidney but were of reduced incidence and severity.

4.2.3.2. Bone

Atrophy of metaphyseal cancellous bone was observed in rats administered TAF at 100 mg/kg/day for 26 weeks. TAF also increased biochemical markers of bone turnover and decrease serum 1,25-dihydroxy- and 25-hydroxyvitamin D_3 in rats ($\geq$ 25 mg/kg/day) and dogs ($\geq$ 37.5 mg/kg/day for 6 days). In the 39-week dog study, bone mineral density changes at 18/12 mg/kg/day were most likely due to body weight loss, but these changes were accompanied by a slight but significant decrease in serum 1,25-dihydroxyvitamin D_3 in males only and a significant increase in 25-hydroxyvitamin D_3 in females only.

4.2.3.3. Other

TAF administered by oral gavage for up to 13 weeks to mice at ≥ 10 mg/kg/day resulted in adverse degenerative (olfactory) and acute inflammatory (infiltrate neutrophil) changes in the nasal mucosa. Because these changes were not observed in rats, dogs or monkeys for longer durations of administration, the relevance to humans is unknown and the risk of nasal inflammation in humans is very low.

TAF had no discernible electrocardiographic effect at the low dose of 2 mg/kg/day. Although TAF showed some potential to prolong the PR interval in the 39-week dog study at 18/12 mg/kg/day, the slight change was considered secondary to poor clinical condition and consistent with the significant decreases in T3 {29101}, {29104}. After the 13-week recovery period, serum T3 values returned to levels similar to the control group animals at the end of the study. No PR prolongation or any change in ECG results occurred in the safety pharmacology study that evaluated a TAF dose up to 100 mg/kg (D2000006) or in the thorough QT study (GS-US-120-0107).

At 18/12 mg/kg/day in dogs, the highest dose tested, a minimal infiltration of histiocytes was present in some organs (eye [choroid plexus, ciliary body], lung, and spleen) in some animals. In-life ophthalmologic examinations were normal. These infiltrates were seen primarily in perivascular regions in the ciliary body, and less frequently in the choroid layer subjacent to the border of the peripheral retina with the ciliary body at the level of the ora ciliaris retinae (equivalent to the ora serrata in humans). Based on their bilateral nature and typical locations in perivascular regions of the ciliary body and junction of the choroid layer with the margin of the peripheral retina, these infiltrates were considered to be consistent with an increased incidence and/or size of minor, random perivascular infiltrates of mononuclear cells commonly associated with maintenance of the blood-eye barrier. No mononuclear cell infiltrates were observed in dogs following the 13 week recovery period, suggesting these are minor, dose-dependent, reversible changes in dogs associated with maintenance of the blood-eye barrier during dosing with no functional consequence. There were no drug-related effects on ophthalmic exams or microscopic exams of ocular tissue observed in repeat-dose toxicity studies in mice (up to 13 weeks), rats (up to 26 weeks), and nonhuman primates (4 weeks) or in the 4-week dog toxicology study. Distribution of [^{14}C]TAF to eyes has been assessed in mice, rats, and dogs (AD-120-2011, AD-120-2020, and D990173-BP). Melanin binding has specifically been assessed by comparing distribution in pigmented and non-pigmented mice (C57 black and CD-1, respectively) and rats (Long Evans and Sprague-Dawley, respectively). [^{14}C]TAF-related radioactivity distributed poorly to the eyes of rats and dogs (C_{max} in eyes <8% that observed in plasma). Transient exposure to low levels of [^{14}C]TAF-related radioactivity was observed in the eyes of rats decreasing to undetectable levels at 8 hours postdose. No difference in distribution was observed between Sprague-Dawley and Long Evans rats, including in the skin and eyes, suggesting no binding to melanin. The distribution of [^{14}C]-TAF to eyes in mice was higher than other species studied (C_{max} in eyes 15%-20% that observed in plasma). More persistent exposures in eye lens, eye uveal tract, and eyes were observed in C57 black mice compared to CD-1 mice. However, no difference in distribution between pigmented and nonpigmented skin was observed illustrating that [^{14}C]TAF-related radioactivity was not selectively associated with melanin-containing tissues. The minimal infiltration of histiocytes observed in the eye of dogs administered the

highest dose of TAF occurred at 3.7- and 17-fold higher exposure to TAF and TFV, respectively, than that observed in human subjects administered a 25-mg dose of TAF and does not correlate with the tissue distribution where TAF was found to poorly penetrate across the blood brain and blood retinal barrier in dogs. Based on the evidence from tissue distribution and toxicology studies, Gilead concludes that the risk of posterior uveitis in humans is very low.

TAF is unlikely to cause mitochondrial toxicity. TAF did not affect the amount of mitochondrial DNA levels up to 1 μM (approximately 2-fold higher than C_{max} after a 25 mg TAF dose), the highest concentration tested, in HepG2 cells in a 10-day assay (PC-120-2006). The active metabolite of TAF, tenofovir diphosphate, is highly discriminated as a substrate by mitochondrial DNA polymerase γ relative to the natural substrate, adenosine triphosphate (ATP) (> 10,000 fold) {4923}. Therefore, TAF is unlikely to inhibit mitochondrial DNA polymerase γ under clinical relevant conditions.

The nonclinical toxicity studies demonstrate that there was no adverse effect of TAF for up to 26 weeks in the rat, up to 39 weeks in the dog, and 4-weeks in the monkey at doses producing TFV systemic exposure levels in animals 14-, 4- and > 22- fold greater, respectively, than those observed in patients treated with the recommended clinical dose of EVG/COBI/FTC/TAF.

4.2.4. FTC/TDF

Two 14-day oral gavage GLP studies were conducted to investigate the potential toxicity of FTC/TDF, and to qualify potential impurities in nondegraded and degraded FTC/TDF tablets following daily oral administration to rats for a minimum of 14 days (TX-164-2001 and TX-164-2005). There were no toxicologically significant differences between groups treated with nondegraded and degraded FTC/TDF, and no exacerbation of toxicity with the FTC/TDF combination compared to data with the individual agents.

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 (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 activated partial thromboplastin time and creatinine. Minimal tubular epithelial necrosis and slight to moderate tubular epithelial regeneration were seen in animals administered 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.

4.2.5. FTC/RPV/TAF

Administration of FTC/RPV/TAF in combination is unlikely to exacerbate known toxicities of the individual agents. The only significant effect of FTC identified in repeat-dose toxicity studies was a minor anemia at dose levels constituting large multiples of clinical exposure (110-fold in mice; 21-fold in monkeys). Bone marrow is not a target of RPV or TAF. 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. The principal target organs of toxicity following oral administration of TAF were the kidney (karyomegaly, tubular degeneration) and bone. Because neither FTC nor RPV have shown any potential for bone toxicity in chronic rat and dog toxicity studies, exacerbation of any TAF effects on bone is not expected. 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 TAF, renal toxicity is not anticipated to be an issue with the FTC/RPV/TAF combination product.

Administration of FTC and TAF in combination is unlikely to exacerbate known toxicities of the individual agents based on the FTC and TDF combination toxicity studies. No new or more marked toxicities occurred in two 14-day rat toxicology studies and a 4-week dog study with the combination (TX-164-2001, TX-164-2005, TX-164-2004).

The ample nonclinical safety database on these drugs, including combination toxicity studies with FTC and TDF, indicates further toxicological investigations are unlikely to yield new data relevant to humans. Studies with the combinations seem unwarranted given the lack of pharmacokinetic interactions and significant overlapping toxicities.

4.3. Genotoxicity

4.3.1. FTC

Emtricitabine was not genotoxic in the reverse mutation bacterial test (Ames test) (18637-0-409R; MUT203; K01-3154), mouse lymphoma (TOX012), or mouse micronucleus assays (TOX011).

4.3.2. RPV

Genotoxicity testing of TMC278 base or RPV comprised nonmammalian (bacterial) reverse gene mutation (Ames) tests (TMC278-Exp5540, TMC278-Exp5693, TMC278-NC279, TMC278-NC335), 2 mammalian (mouse lymphoma) forward mutation assays (TMC278-Exp5539, TMC278-NC336) and 1 in vivo mouse bone marrow micronucleus assay (TMC278-Exp5538).

Rilpivirine was tested up to the maximum concentration that allowed scoring due to precipitation. No increased mutation frequency or increased frequency of structural chromosomal aberrations was noted. In the Ames test with human S9 (TMC278-NC279), metabolites M30/M31, that are formed following Michael addition at the [redacted] moiety of RPV, were present at the same level as determined in mass balance study (C119) where ~3% of the administered dose was found in feces (m2.6.6). 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 $AUC_{0\text{-}6h}$ was 307 and 287 µg.h/mL for males and females, respectively; TMC278-Exp5538).

4.3.3. TAF

Tenofovir alafenamide was not genotoxic in a battery of in vitro and in vivo assays. The in vitro assays included gene mutation assays with bacterial strains (*Salmonella typhimurium*, *Escherichia coli;* V990212), and a L5178Y gene mutation assay in mouse lymphoma cells (V990213). The in vivo evaluation consisted of a mouse bone marrow micronucleus study at oral doses of 500, 1000, and 2000 mg/kg (M2000113).

4.3.4. FTC/TDF

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 (TX-164-2002; TX-164-2003).

4.3.5. FTC/RPV/TAF

FTC, RPV and TAF were negative in genotoxicity studies. The combination of the 3 components is therefore not expected to have an altered genotoxicity profile as compared with that of the individual agents.

4.4. Carcinogenicity

Per separate agreements with the FDA [redacted]) and with the CHMP ([redacted]). [redacted]. Conclusions of the carcinogenicity studies with TDF are provided below.

4.4.1. FTC

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 (23-fold the human systemic exposure at the therapeutic dose of 200 mg/day; TOX109) or in rats at doses up to 600 mg/day/day (28-fold the human systemic exposure at the therapeutic dose; TOX108).

4.4.2. RPV

In the mouse carcinogenicity study, RPV 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 (TMC278-NC120). 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 RPV from 20 mg/kg/day and above. The incidences of carcinomas and of adenomas for all groups treated with RPV 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 RPV 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, RPV 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 (TMC278-NC123). 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 RPV; whereas in the males the incidence of tumors was equal to the maximum historical background incidence recorded.

In the 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 RPV 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.

4.4.3. TAF/TDF

Long-term oral carcinogenicity studies of TDF in mice and rats were carried out at exposures up to approximately 10 times (mice) and 4 times (rats) those observed in humans at the 300 mg therapeutic dose in the EVG/COBI/FTC/TDF FDC (M990205; R990204). Female mice showed a low incidence of liver adenomas at the highest dose of 600 mg/kg/day. Rats did not show any carcinogenic potential in the long-term study.

4.4.4. FTC/RPV/TAF

Emtricitabine and TDF/TFV have demonstrated low carcinogenic potential in conventional 2-year bioassays at exposures that exceeded (TDF) or far exceeded (FTC) human exposures at the therapeutic doses. Carcinogenicity studies with RPV demonstrated an increased incidence of hepatocellular and thyroid tumors that are not considered relevant for humans. Combination dosing would be unlikely to change these profiles as no exposure difference would be expected and no exacerbation of toxicity/genotoxicity is expected.

4.5. Reproductive Toxicity

4.5.1. FTC

Emtricitabine did not affect fertility in male rats at approximately 140-fold or in male and female mice at approximately 60-fold higher exposures than in humans administered the recommended 200 mg daily dose (TTEP/95/0028, TOX036). There were no adverse effects in embryo-fetal development studies in mice at exposures approximately 60-fold higher and in rabbits at exposures approximately 120-fold higher than human exposures (TOX037, TOX038). In the pre/postnatal study in mice, F_1 dams at 1000 mg/kg/day had slightly longer estrous cycles than controls, but fertility was normal in the offspring exposed daily from before birth (in utero) through sexual maturity at daily exposures of approximately 60-fold higher than human exposures at the recommended 200 mg daily dose (TOX039).

4.5.2. RPV

Fertility

For a male fertility study, RPV 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 (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 RPV. 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 RPV. The NOAEL for male fertility was at least 1600 mg/kg/day.

Female Sprague Dawley rats were dosed with RPV once daily, by oral gavage, at 0 (vehicle), 40, 120, or 400 mg/kg/day in a dose volume of 10 mL/kg (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

Rilpivirine 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 (TMC278-NC105). The dams were necropsied on GD 21. There were no mortalities associated with RPV. 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.

Rilpivirine 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 (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

Rilpivirine 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 (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.

4.5.3. TAF

Reproductive tissues were examined in repeat-dose toxicology studies in the rat, dog, and monkey. There were no treatment-related histologic alterations or changes in organ weights in the rat and the dog following chronic daily dosing, or in the monkey following 28 days of daily oral administration.

The TAF fumarate (GS-7340-03) oral rat fertility study (TX-120-2012) data indicate dose related decreases in body weight gain in males and females, but no drug related changes occurred in male or female fertility endpoints including mating index, fertility index, sperm motility, sperm concentration, number of corpora lutea, implantation sites, early and late resorptions and live fetuses at doses up to 160 mg free base equivalents/kg/day. The TAF NOAEL for reproductive and early embryonic toxicity was 160 mg/kg/day.

There was no effect on fetal viability or fetal development in pregnant rats administered doses of GS-7340-02 up to 250 mg/kg/day (TX-120-2002), or in pregnant rabbits administered GS-7340-02 up to 100 mg/kg/day (TX-120-2005). The highest doses were maternally toxic. In the rat, decreased fetal body weight associated with some minor transitory delays in the rate of ossification was observed at 250 mg/kg/day, a maternally toxic dose. At the NOAEL for embryo-fetal development of approximately 100 mg/kg/day in rats, AUC_{tau} values for TAF and TFV on Day 17 were 0.2 and 17.4 µg·h/mL, respectively. At the NOAEL for embryo-fetal development of 100 mg/kg/day in rabbits, AUC_{tau} values for TAF and TFV on Day 20 were 11.0 and 27.3 µg·h/mL, respectively.

Tenofovir DF, but not TAF, has been tested in a perinatal/postnatal study. Per separate agreements with the FDA ([redacted]) and with the CHMP ([redacted]) [redacted] There was an alteration of the estrous cycle in female rats in the perinatal study in rats (R990202). The NOEL for behavioral, reproductive, and development toxicity was 150 mg/kg/day. Maternally toxic doses (≥ 450 mg/kg/day) had effects on pup survival, pup body weights, and sexual maturation.

4.5.4. FTC/RPV/TAF

4.5.4.1. Fertility and Early Embryonic Development

The reproductive and developmental NOELs/NOAELs for FTC, RPV and TFV were generally at exposure levels above human exposures. With no expected toxicologic interactions with the FTC/RPV/TAF FDC, studies with FTC/RPV/TAF FDC are not considered necessary.

4.5.4.2. Embryo-Fetal Development

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

4.5.4.3. Pre- and Postnatal Development

Slightly longer estrous cycles were observed in first (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. NOELs/NOAELs for FTC, RPV, and TAF were at exposures above human exposures. As with the other reproductive toxicity tests, a repeat of this test with the FTC/RPV/TAF FDC is unlikely to add any new information.

4.6. Juvenile Toxicity

4.6.1. FTC

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.

4.6.2. RPV

Rilpivirine 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 (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 RPV 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. Rilpivirine induced more prominent tertiary follicles in the ovaries of animals that did not ovulate and induced ovulation in 3 animals. The effects by RPV 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.

Cynomolgus Monkeys

In the 8-week immature female cynomolgus monkey study (TMC278-NC248), RPV 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.

4.6.3. TAF/TFV

Although no specific juvenile toxicity studies have been conducted with TAF or TDF, data are available from efficacy studies of TFV in SIV-infected and non-infected rhesus macaques {1787}, {7311}, {12968}. These studies included 12 gravid rhesus macaques, and more than 85 infant and juvenile rhesus macaques treated from ages ranging from 1 day to 7.5 years at initiation of dosing. This age range covers the human equivalent of prenatal, infant, juvenile and adolescent phases of growth. The duration of treatment ranged from 12 weeks to 13 years. Clinically relevant renal and bone pathology (including reduced bone mineral density, joint swellings, and bone fractures) occurred only in animals in which TFV was chronically administered at 30 mg/kg/day by daily subcutaneous injection. Exposure levels (TFV AUC 150 μg·h/mL) at this dose were more than 564-fold higher than those of adults after a 25 mg dose of TAF (30-fold higher than those of adults subjects after a 300 mg/day dose of TDF). Effects in rhesus monkeys were reversible by decreasing or stopping exposure. Administration of lower doses of TFV (10 mg/kg/day, ~15 μg·h/mL) did not cause renal dysfunction or abnormal bone density or growth.

Tenofovir administered to newborn or infant rhesus monkeys at doses of 4 to 30 mg/kg/day 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}. 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 were no clinical, radiographic, or dual-emission X-ray absorptiometry scan {7311} findings of an adverse effect on bone. The mean AUC associated with this dosage (18 μg·h/mL) 68-fold greater than the human AUC_{ss} following a 25 mg/day dose of TAF.

4.6.4. FTC/RPV/TAF

No specific studies were deemed necessary with the FTC/RPV/TAF FDC. Comprehensive reproductive and developmental toxicity studies were conducted with FTC, RPV and TAF/TDF. No overlapping toxicity was identified.

4.7. Local Tolerance

No local tolerance studies have been conducted with FTC. Rilpivirine tested negative for the potential to cause phototoxicity in vitro (TMC278-NC188) and to cause skin irritation in rabbits (TMC278-NC159). Rilpivirine was classified as a moderate eye irritant in an in vitro test (TMC278-NC202). Local toxicity studies concluded that TAF was predicted to be a noncorrosive/nonsevere eye irritant (TX-120-2013), and nonirriating/noncorrosive to rabbit skin under semi-occluded conditions (TX-120-2011).

The FTC/RPV/TAF tablet is intended for oral use. No local tolerance studies were conducted for the FTC/RPV/TAF combination. Evaluation of local tolerance to the GI tract has been conducted during the repeat-dose oral toxicity studies with each of the individual agents.

4.8. Other Toxicity Studies

4.8.1. Antigenicity

Rilpivirine tested negative for the potential to cause delayed-type hypersensitivity in the mouse local lymph node assay (TMC278-NC199). TAF showed no potential for sensitization (TX-120-2014).

4.8.2. Immunotoxicity

The immunotoxicity of FTC was evaluated in a 28-day study in CD rats at doses up to 1000 mg/kg/day (TOX146). There were no adverse effects of FTC during the dosing period and FTC did not affect the immunoglobulin M (IgM) antibody titers to sheep RBCs at any of the doses administered. The NOEL for immunotoxicity was 1000 mg/kg/day.

The lack of effects on the PFC-assay in the 1-month rat study (TMC278-TOX5692) indicated that RPV had no immunotoxic potential.

Data from repeat-dose toxicity studies with FTC, RPV or TAF (hematology, lymphoid organ weights, microscopy of lymphoid tissues, bone marrow cellularity) did not suggest immunotoxic potential. There were no notable effects of the combination of FTC/TDF on immune cells or NK cell assay values in a 4-week dog study (TX-164-2004). No further studies were deemed necessary with the FTC/RPV/TAF FDC.

4.8.3. Impurities/Degradation Products

4.8.3.1. FTC

The process impurities and degradation products of FTC have been qualified in animal studies. The major degradation product, 不純物 FA*, was qualified in 2 genotoxicity studies (TOX151; TOX152) using a batch of FTC that contained 1% (w/w) of the 不純物 FA* 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 FA*) of 50/1 mg/kg/day, 150/3 mg/kg/day, and 450/9 mg/kg/day (TOX153).

A 28-day mouse bridging study (TX-162-2001) was performed to qualify impurities in FTC (specifically 不純物FE*). There was no toxicity of FTC at doses of 50, 150, and 450 mg/kg/day.

Based on their impurity profiles, the multiple GLP batches of FTC tested in the toxicology program are considered, in composite, to be representative of the GMP material and support the specified limits of impurities and degradation products proposed for commercial production (m3.2.P.5.5, Characterization of Impurities [FTC/RPV/TAF Tablets] and m3.2.P.5.6, Justification of Specifications [FTC/RPV/TAF Tablets]).

4.8.3.2. RPV

The RPV 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A* and 不純物B*, have been evaluated upon spiking into the drug substance at a level of 4%. Qualification comprised a bacterial reverse mutation Ames test (TMC278-NC308, TMC278-NC309) a mouse lymphoma assay (TMC278-NC311, TMC278-NC312), and a 1-month oral rat study (TMC278-NC314). The presence of the impurities at 4% did not modify the effects of RPV in any of the tests. The third impurity, 不純物C* , is the [redacted] isomer of RPV. 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 RPV 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C* is not considered relevant. The impurities qualified at the level of 4% according to ICH Q3A occurred in representative RPV drug substance batches manufactured according to the proposed commercial synthesis method for less than 0.1% for 不純物B*, less than 0.25% for 不純物A*, and less than 0.25% for the non-qualified [redacted] isomer of RPV, 不純物C* .

Three further trace impurities, 不純物G*, 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G*, namely 不純物H*, and of 不純物E* and 不純物F* was tested in an Ames test (TMC278-NC179, TMC278-NC165, TMC278-1646_0016315). Only 不純物G*, tested as its HCl salt 不純物H*, showed a mutagenic potential. It is therefore concluded that 不純物G* is a genotoxic impurity, whereas the potential impurities 不純物E* (TMC278- 1646_0015298) and 不純物F* (TMC278-1646_0015299) with structural analogy to 不純物G* did not induce an increased mutation frequency in the Ames test. Probably, the [redacted] moiety of 不純物G* caused the genotoxic effect. The maximum allowable level of genotoxic impurity 不純物G* 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. [redacted]. [redacted]) with daily treatment for longer than 12 months. The levels of 不純物G* in representative RPV drug substance batches manufactured according to the proposed commercial synthesis method is less than 5 ppm (m3.2.P.5.5, Characterization of Impurities [FTC/RPV/TAF Tablets] and m3.2.P.5.6, Justification of Specifications [FTC/RPV/TAF Tablets]).

4.8.3.3. TAF

No metabolite toxicology studies were conducted as there are no unique human TAF metabolites. The hydrolytic pathway of TAF to TFV via TFV monoamidate is similar to that observed in vivo; monophenyl TFV, phenol, and TFV are known metabolites. Isopropanol, l-alanine, and phenol are formed at nontoxic levels.

Two 1-month impurity qualification studies were conducted in rats to evaluate potential drug substance impurities. Administration of 97.7% pure and 83.1% pure GS-7340-02 by oral gavage for 14 days to male rats was well tolerated at dose levels of 5 and 50 mg/kg/day (TX-120-2008). No test article-related findings were noted, and no differences were found between the 2 lots tested. The NOAEL for both lots is 50 mg kg/day (40 mg f.b.e./kg/day).

The second impurity qualification study evaluated daily administration of GS-7340-03 via oral gavage to male and female rats for at least 28 days (TX-120-2021). Three lots of GS-7340-03 were each administered at 25 and 50 mg/kg/day (free base equivalents). Test article 1 was 99.3% pure GS-7340-03. Test article 2 was 98% pure GS-7340-03 containing 11 spiked known and potential impurities. Test article 3 was 97.8% pure GS-7340-03 containing 4 spiked potential impurities. Control animals were administered the vehicle control article. Administration of GS-7340-03 drug substance with 3 different impurity profiles by oral gavage for 28 days to rats was well tolerated at dose levels of 25 and 50 mg/kg/day. There were no significant clinical or histopathological differences between the 3 lots tested. The NOAEL for all 3 lots is 50 mg f.b.e./kg/day.

Based on their impurity profiles, the multiple GLP batches of TAF tested in the toxicology program are considered, in composite, to be representative of the GMP material and support the specified limits of impurities and degradation products proposed for commercial production (m3.2.P.5.5, Characterization of Impurities [FTC/RPV/TAF Tablets] and m3.2.P.5.6, Justification of Specifications [FTC/RPV/TAF Tablets]).

4.8.3.4. FTC/TDF

Four degradation products not present in the individual drug substances have been observed in FTC/TDF tablets placed on accelerated stability at high temperature. The trivial name for these degradation products are 不純物TK* , 不純物TL* , 不純物FB* and 不純物FC* . The adducts of FTC/TDF form when [redacted], formed by the [redacted] of TDF (不純物TE*), reacts with one molecule of each TDF and FTC to form an adduct. FTC may undergo [redacted] and may additionally [redacted] group of the [redacted], thereby creating 不純物FD* . While 不純物FD* has the potential to exist as 4 diastereomers, only 2 of these diastereomers have been observed in FTC/TDF containing products. The 2 observed diastereomers of 不純物FD* are GS-9237 (不純物FB*) and GS-492127 (不純物FC*).

Two 14-day GLP oral toxicity studies have been conducted in rats to qualify impurities and degradants in the FTC/TDF tablets (TX-164-2001, TX-164-2005). The second qualification study (TX-164-2005) was conducted to verify the qualification of 不純物FB* and 不純物FC* as these degradants were identified later in development by virtue of a new analytical assay. In these studies, rats were administered 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 were 0/0, 20/30, 67/100, and 200/300 mg/kg/day FTC/TDF in both studies. Although there were slight differences in the findings from both studies, there were no new toxicities or exacerbation of previously defined toxicities, and there was no difference in toxicity between non-degraded and degraded material. The NOAEL in the initial study (TX-164-2001) was considered to be 67/100 mg/kg/day, and 200/300 mg/kg/day FTC/TDF in the second study (TX-164-2005).

The qualification of degradation products is summarized in m3.2.P.5.6, Justification of Specifications [FTC/RPV/TAF Tablets]. The impurities and degradation products in the 2 active ingredients, FTC and TDF, as well as the tableted drug product have been identified and qualified in toxicology studies. The safety margins support the specified limits proposed for these impurities and degradation products.

4.8.3.5. FTC/RPV/TAF

The FTC/RPV/TAF FDC is a bilayer tablet. There are no unique impurities or degradants in the FTC/RPV/TAF tablets.

The impurities and degradation products present in FTC, RPV, and TAF and in FTC/RPV/TAF tablets have been qualified through toxicology studies which employed drug substance from normal productions batches, laboratory scale batches with enhanced levels of impurities, or samples subjected to forced degradation conditions (high heat and humidity) (m3.2.P.5.5, Characterization of Impurities [FTC/RPV/TAF Tablets] and m3.2.P.5.6, Justification of Specifications [FTC/RPV/TAF Tablets]).

4.9. Summary of Toxicology and Target Organ Effects

4.9.1. Target Organ Effects

4.9.1.1. FTC

No specific concerns were identified in the safety pharmacology, genotoxicity, carcinogenicity and reproductive toxicity studies with FTC. The only significant effect of FTC identified at dose levels constituting large clinical multiples was a minor anemia.

4.9.1.2. RPV

Mortality

No RPV-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 RPV-related mortalities occurred at 320 mg/kg/day. The cause of these deaths could not be established.

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. 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.

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.

Liver

Liver effects in mice and rats 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, 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 RPV has a low potential for inducing hepatotoxicity (Draft Non-Clinical Guideline for Drug-Induced Hepatotoxicity. European Medicines Agency. CHMP. Doc. Ref. [redacted]).

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 RPV once daily (Table 8). Long-term safety data do not indicate an adverse effect of RPV on liver (m2.7.4).

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. 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 RPV 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 RPV 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.

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 do not indicate any effect of RPV on plasma T_4 concentrations.

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. As the effects on the pituitary gland and TSH are secondary to the effects of RPV on rodent-specific T_4 clearance, they bear no relevance for man. Long-term safety data do not indicate any effect of RPV on plasma T_4 concentrations.

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. In the carcinogenicity study with CD-1 mice, 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 RPV once daily in man (Table 8).

Dogs treated for 12 months with 40 mg/kg/day showed nephritis in males and mineralization in the corticomedullary region in females. 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 RPV once daily in man (Table 8).

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. Long-term safety data indicate a small, but consistent increase of the serum creatinine concentration. 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.

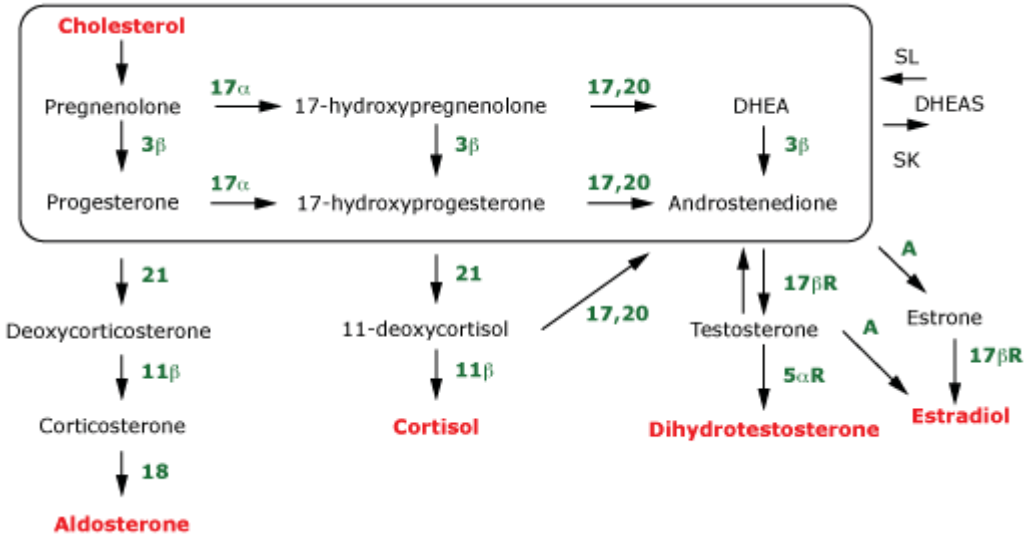
Adrenal Glands

In all species involved in nonclinical safety studies with RPV, except rabbits, an effect on adrenal gland has been demonstrated. The levels of adrenal hormones or their precursors and ACTH in serum of rats and dogs, and the results of the in vitro studies with guinea pig primary cell cultures and homogenate of adrenal gland cortex of dog indicate that RPV partially inhibits 21-hydroxylase, also known as CYP450 isozyme 21 (CYP21). 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 showed that RPV 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 4. 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 RPV on adrenal gland and its hormonal biosynthetic pathways are more prominent than the effects on gonads and their pathways. This may be explained by the affinity of RPV for adrenal gland in repeat-dose studies in dogs and of radiolabel in the rat single-dose QWBA studies with [^{14}C] RPV. 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 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 shows no clinically relevant effects of inhibition of CYP21 and CYP17 in adults.

Ovaries

The effects in ovaries of dogs after at least 1 month of treatment with RPV were characterized by increased numbers of tertiary follicles, (cystic) luteinized follicles, and in some dogs by corpora lutea. 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 RPV. 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 RPV 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 RPV. The compound had a clear effect on adrenal CYP21 and CYP17 in that species as shown by the increases in serum progesterone and 17α-hydroxy-progesterone 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 shows no clinically relevant effects on the estrous cycle.

<u>Testes</u>

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. 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 RPV 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 showed no clinically relevant effects.

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, RPV 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. 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.

Coagulation

In male rats, increased values for APTT and PT were noted in the 2-week pilot study with RPV at doses of 1500 mg/kg/day and higher and in the 6-month study at all doses of RPV 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 RPV once daily (Table 8). Moreover, long-term safety data from TMC278-C204 showed no clinically relevant effects on coagulation.

Genotoxicity and Carcinogenicity

Genotoxicity tests, in vitro and in vivo, have shown RPV to be free of a genotoxic potential. Nevertheless, the carcinogenicity studies with RPV in mice and rats induced hepatocellular adenomas and carcinomas and in rats follicular adenomas and carcinomas in the thyroid.

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 RPV was already evident in the repeat-dose general toxicity studies. Ex vivo hepatic enzyme activity evaluated in the 3-month mouse study and the 6-month rat study showed that RPV caused strong induction of the CYP4A family in male and female mice and male rats, and induction of the CYP3A family in female rats. 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 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 RPV 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 RPV 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 RPV 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. The NOAEL in the male fertility study was at least 1600 mg/kg/day, associated with an AUC_{0-24h} of approximately 85 µg.h/mL obtained from 2-week oral rat studies with RPV. The NOAEL in the female fertility study is at least 400 mg/kg/day, associated with an AUC_{0-24h} of approximately 100 µg.h/mL obtained from 2-week oral rat studies with RPV. The exposures at NOAEL in rats are at least 35 times higher than the exposure at the recommended dose of 25 mg RPV once daily in man.

The results of the embryo-fetal developmental studies in rats and rabbits with the highest feasible exposures to RPV demonstrated the absence of a potential for teratogenicity. The maternal and fetal NOAELs in rats were established at 40 mg/kg/day, associated with a maternal AUC_{0-24h} of 37 µg.h/mL. In rabbits, the fetal NOAEL was established at 10 mg/kg/day, associated with a maternal AUC_{0-24h} 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 RPV once daily in man. The safety of RPV has not been assessed in pregnant women in well controlled clinical trials. Therefore, women of child bearing potential should only use RPV 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 RPV during pregnancy and lactation. The NOAEL in the peri- and postnatal developmental study is at least 400 mg/kg/day, associated with a maternal AUC_{0-24h} of approximately 100 µg.h/mL obtained from 2-week oral rat studies with RPV. This exposure is at least 40 times higher than the exposure at the recommended dose of 25 mg RPV once daily in man. In animals, no studies have been conducted to assess directly the excretion of RPV into the milk. In humans it is not known if RPV 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 RPV.

Juvenile Toxicity

In the rat juvenile toxicity study with pups from RPV-treated mothers dosed by gavage from LDs 12 to 25, no effects were noted. The exposure of these pups was similar to that of adult rats dosed with the same dose of RPV. 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. Therefore, it is concluded that RPV will not induce different effects in children and adolescents from those it has in adults.

4.9.1.3. TAF

No specific concerns were identified in the safety pharmacology, genotoxicity, carcinogenicity and reproductive toxicity studies with TAF.

Kidney

Renal tubular karyomegaly was observed in rats orally administered TAF. Focal areas of minimal renal cortical tubular basophilia and associated minimal nuclear karyomegaly were present in rats administered 400 mg/kg/day for 4 weeks and 100 mg/kg/day for 26 weeks. Renal tubular karyomegaly and/or basophilia were observed in dogs administered 3 and 10 mg/kg/day for 4 weeks and dogs administered 6 or 18/12 mg/kg/day for at least 13 weeks.

Renal cortical tubular degeneration/regeneration findings were limited to animals administered 6 or 18/12 mg/kg/day for at least 13 weeks in the 39-week dog toxicity study. Similar findings of renal cortical tubular degeneration/regeneration and karyomegaly were present in dogs administered either 6 or 18/12 mg/kg/day for 39 weeks. These changes were minimal to slight in affected males and females at 6 mg/kg/day. In high-dose males (18/12 mg/kg/day) the severity ranged from mild to moderate. Similar lesions (karyomegaly and tubular degeneration) but of only minimal severity were also present in 2 males administered 2 mg/kg/day of TAF for 39 weeks. After a 13-week recovery period, treatment-related histology changes were still observed in the kidney but were of reduced incidence and severity.

Bone

Atrophy of metaphyseal cancellous bone was observed in rats administered TAF at 100 mg/kg/day for 26 weeks. TAF also increased biochemical markers of bone turnover and decrease serum 1, 25-dihydroxyvitamin D_3 and 25-hydroxyvitamin D_3 in rats (≥ 25 mg/kg/day) and dogs (≥ 37.5 mg/kg/day for 6 days). In the 39-week dog study, bone mineral density changes at 18/12 mg/kg/day may have been secondary to body weight loss but these changes were accompanied by a slight but significant decrease in serum 1, 25-dihydroxyvitamin D_3 in males only and a significant increase in 25-hydroxyvitamin D_3 in females only.

Other

TAF administered by oral gavage for up to 13 weeks to mice at ≥ 10 mg/kg/day resulted in adverse degenerative (olfactory) and acute inflammatory (infiltrate neutrophil) changes in the nasal mucosa. Because these changes were not observed in rats, dogs or monkeys for longer durations of administration, the relevance to humans is unknown and the risk of nasal inflammation in humans is very low.

TAF had no discernible electrocardiographic effect at the low dose of 2 mg/kg/day. There was some evidence at 6 and 18/12 mg/kg/day for an effect to slightly prolong PR intervals (~13-24%) which was considered secondary to poor clinical condition and associated with significant decreases in T_3 {29101}, {29104}. After the 13-week recovery period, serum T_3 values returned to levels similar to the control group animals at the end of the study. No PR prolongation or any change in ECG results occurred in the safety pharmacology study that evaluated a TAF dose up to 100 mg/kg or in the thorough QT study.

At 18/12 mg/kg/day in dogs, the highest dose tested, a minimal infiltration of histiocytes was present in some organs (eye (choroid plexus, ciliary body), lung, and spleen) in some animals. In-life ophthalmologic examinations were normal. These infiltrates were seen primarily in perivascular regions in the ciliary body, and less frequently in the choroid layer subjacent to the border of the peripheral retina with the ciliary body at the level of the ora ciliaris retinae (equivalent to the ora serrata in humans). Based on their bilateral nature and typical locations in perivascular regions of the ciliary body and junction of the choroid layer with the margin of the peripheral retina, these infiltrates were considered to be consistent with an increased incidence and/or size of minor, random perivascular infiltrates of mononuclear cells commonly associated with maintenance of the blood-eye barrier. No mononuclear cell infiltrates were observed in dogs following the 13 week recovery period, suggesting these are minor, dose-dependent, reversible changes in dogs associated with maintenance of the blood-eye barrier during dosing with no functional consequence. There were no drug-related effects on ophthalmic exams or microscopic exams of ocular tissue observed in repeat-dose toxicity studies in mice (up to 13 weeks), rats (up to 26 weeks), and nonhuman primates (4 weeks) or in the 4-week dog toxicology study. Distribution of [^{14}C]-TAF to eyes has been assessed in mice, rats, and dogs. Melanin binding has specifically been assessed by comparing distribution in pigmented and non-pigmented mice (C57 black and CD-1, respectively) and rats (Long Evans and Sprague-Dawley, respectively). [^{14}C]-TAF-related radioactivity distributed poorly to the eyes of rats and dogs (C_{max} in eyes <8% that observed in plasma). Transient exposure to low levels of [^{14}C]-TAF-related radioactivity was observed in the eyes of rats decreasing to undetectable levels at 8 hours postdose. No difference in distribution was observed between Sprague-Dawley and Long Evans rats, including in the skin and eyes, suggesting no binding to melanin. The distribution of [^{14}C]-TAF to eyes in mice was higher than other species studied (C_{max} in eyes 15%-20% that observed in plasma). More persistent exposures in eye lens, eye uveal tract, and eyes were observed in C57 black mice compared to CD-1 mice. However, no difference in distribution between pigmented and nonpigmented skin was observed illustrating that [^{14}C]-TAF-related radioactivity was not selectively associated with melanin-containing tissues. The minimal infiltration of histiocytes observed in the eye of dogs administered the highest dose of TAF occurred at 3.7- and 17-fold higher exposure to TAF and TFV, respectively, than that observed in human subjects administered a 25-mg dose of TAF and does not correlate with the tissue distribution where TAF was found to poorly penetrate across the blood brain and blood retinal barrier in dogs. Based on the evidence from tissue distribution and toxicology studies, Gilead concludes that the risk of posterior uveitis in humans is very low.

TAF is unlikely to cause mitochondrial toxicity. TAF did not affect the amount of mtDNA levels up to 1 μM (approximately 2-fold higher than C_{max} after a 25 mg TAF dose), the highest concentration tested, in HepG2 cells in a 10-day assay. The active metabolite of TAF, tenofovir diphosphate is highly discriminated as a substrate by mitochondrial DNA polymerase γ relative to the natural substrate, ATP (> 10,000 fold) {4923}. No toxicity indicative of mitochondrial toxicity was observed in nonclinical or clinical studies. Therefore, TAF is unlikely to inhibit mitochondrial DNA polymerase γ under clinical relevant conditions.

4.9.1.4. FTC/RPV/TAF

The only significant effect of FTC identified in repeat-dose toxicity studies was a minor anemia at dose levels constituting large multiples of clinical exposure (110-fold in mice; 21-fold in monkeys).

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.

The principal target organs of toxicity following oral administration of TAF were the kidney (karyomegaly, tubular degeneration) and bone. Neither FTC nor RPV have shown any potential for bone toxicity in chronic rat and dog toxicity studies, exacerbation of any TAF effects on bone is not expected. Given that kidney effects have been observed with RPV in mice and dogs only at high dose levels and exposures, that the routes of excretion differ for RPV and TAF, and the long term safety data with FTC/RPV/TDF, renal toxicity is not anticipated to be an issue with the FTC/RPV/TAF combination product.

4.9.2. Safety Margins

Safety margins for FTC are calculated based on population PK derived from E/C/F/TDF clinical studies. The FTC and TAF components of FTC/RPV/TAF were shown to be bioequivalent to E/C/F/TAF FDC (GS-US-366-1159). TFV was not measured. Because TFV is a more relevant analyte for nonclinical comparisons, the TAF and TFV margins presented below are based on E/C/F/TAF (150/150/200/10 mg) Phase 3 clinical data. The rilpivirine component (25 mg) of FTC/RPV/TAF was shown to be bioequivalent to Edurant (25mg) in the same study (GS-US-366-1159); safety margins are based on data from Edurant clinical studies.

4.9.2.1. FTC

Cross-species comparisons of FTC exposure (expressed based on AUC_{ss} levels) for the major target organs are shown in Table 7.

The NOELs obtained in the toxicity studies represent systemic exposures in animals well in excess of those expected in humans administered the daily recommended dose of 200 mg.

Table 7. Estimated Safety Margins of Emtricitabine Based on AUCss When Comparing Animal No-Effect-Level (NOEL)

Target Organ Effect	Species	Study Duration	NOEL (mg/kg/day)	AUC_{ss} (µg·h/mL) NOEL	Margin Relative to Human AUC_{ss}
			FTC		
Anemia	Mouse	6 months	500	350	27X
	Rat	3 months	600	346	27X
	Monkey	1 year	200	98	7.5 X

Human AUC_{tau} (13 µg·h/mL) following a 200 mg/day dose of FTC (m2.7.2, Table 49).

4.9.2.2. RPV

The RPV component (25 mg) of FTC/RPV/TAF was shown to be bioequivalent to Edurant (25mg) (GS-US-366-1159). The RPV safety margins 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 RPV once daily from the pooled data from Phase 3 studies C215, C209. At this dose, the C_{max} of RPV 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 8.

Table 8. Ratio Animal/Man Exposures at NOAEL or LOAEL

Species	Type study	NOAEL (mg/kg/day)		LOAEL (mg/kg/day)		$AUC_{0\text{-}24h}$ (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 m2.7.2, Table 50

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 RPV with

adrenal steroidogenesis could be found in long term safety data of TMC278-C204 and in the Phase 3 studies, C209 and C215 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. Rilpivirine 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.

4.9.2.3. TAF

Cross-species comparisons of exposure (expressed based on AUC_{ss} levels) for the major target organs are shown in Table 9.

Dog was the most sensitive species to renal and bone effects of TAF. The NOEL for renal effects in monkeys is greater than 30 mg/kg/day. The rat and dog showed some loss of bone mineral density at relatively high doses; however, clinically evident osteomalacic lesions occurred only in juvenile monkeys in which TFV was chronically administered at 30 mg/kg/day by daily subcutaneous injection. Tenofovir exposure levels (AUC 150 µg·h/mL) at this dose were more than 564-fold higher than those of adults after a 25 mg dose of TAF.

Table 9. Estimated Safety Margins of TAF Based on AUCss When Comparing Animal No-Adverse-Effect-Level (NOAEL)

Target Organ Effect	Species	Study/Dose Duration	TAF NOAEL (mg/kg/day)	AUC_{ss} (µg·h/mL) NOAEL	Margin Relative to Human AUC_{ss}
				TFV/ TAF	TFV[a]/TAF[b]
Nasal Turbinate Toxicity	Mouse	13 Weeks	<10	<0.213/NC	<0.7/NA
Renal Toxicity	Rat	26 weeks	25	3.8/NC	13/NA
	Dog	39 weeks	2	1.2/0.08	4/0.4
	Monkey	4-weeks	≥30	≥5.9/1.0	>20/5
Bone Mineral Loss	Rat	26 weeks	25	3.8/NC	13/NA
	Dog	39 weeks	2	1.2/0.08	4/0.4
	Monkey	4-weeks	≥30	≥5.9/1.0	>20/5
Fertility[c]	Rat	Up to 10 weeks	160	NA	NA
Embryo fetal development[c]	Rat	12 days	84	17.4/0.2	59/1
	Rabbit	14 days	100	27.3/11	93/53
Perinatal/postnatal[c]	Rat	27 days (Gestation day 7 to Lactation day 20)	150 (TDF)	7.84/NA	27/NA

NA = not applicable; NC = insufficient data to calculate

a Predicted safety margin for TFV human exposure is based on pooled PK data from E/C/F/TAF Phase 3 pivotal studies GS-US-292-0104 and GS-US-292-0111 where the mean TFV AUC_{ss} = 0.293 µg.h/mL; m2.7.2, Table 52

b Predicted safety margin for TAF human exposure is based on pooled PK data from E/C/F/TAF Phase 3 pivotal studies GS-US-292-0104 and GS-US-292-0111 where the mean TAF AUC_{ss} = 0.206 µg.h/mL; m2.7.2, Table 51

c NOAEL for reproductive endpoints provided; AUC data is for maternal exposure; the peri/postnatal study was conducted with TDF not TAF

5. INTEGRATED OVERVIEW AND CONCLUSIONS

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*.

5.2. Justification for Text in Labeling

The proposed prescribing information for the FTC/RPV/TAF 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 TAF include: (1) use in patients with severe hepatic impairment, (2) use during pregnancy and lactation, and (3) potential for carcinogenicity, (4) 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 for FTC/RPV/TAF:

1. The potential for hepatotoxicity appears to be low. There was no substantive hepatotoxicity identified in the nonclinical studies with FTC and TAF. The nature and degree of the observed hepatic effects support the conclusion that RPV has a low potential for inducing hepatotoxicity. Administration of 25 mg TAF to non-HIV infected subjects with mild or moderate hepatic impairment resulted in TAF and TFV plasma PK exposure parameters comparable to those in subjects with normal hepatic function. No RPV dose adjustment is required in patients with mild or moderate hepatic impairment.

2. Animal data indicate that FTC, RPV and TAF do not cause reproductive or embryo-fetal toxicity. Emtricitabine has 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 unknown if RPV and TAF are secreted in milk.

3. In long-term carcinogenicity studies of FTC, no drug-related increases in tumor incidence were found in mice or in rats. 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. Rilpivirine is not genotoxic. 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 (liver adenomas) at exposures 10 times those in humans. While the mechanism of this tumor formation is uncertain, the findings are unlikely to be of relevance to humans.

4. 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.

In addition to the items addressed above, which are product specific, other appropriate warnings have been included in the proposed Prescribing Information. 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.

5.3. Overall Conclusions

The overall nonclinical program including the data from the combination and individual agent studies is considered adequate to support the efficacy and safety of FTC/RPV/TAF FDC tablet. Emtricitabine, RPV, and TFV, have potent antiretroviral activity against wild-type and many drug-resistant strains of HIV-1 in vitro and in vivo. The pharmacologic basis to recommend the FTC/RPV/TAF FDC tablet for the treatment of HIV infection is scientifically sound based on the nonclinical in vitro and in vivo efficacy data for the individual components and the combination of the agents presented in this dossier.

Consistent with high hepatic extraction of TAF, high levels of radioactivity were observed in the liver; high radioactivity was also measured in the kidney. Distribution studies in dogs showed 5.7 to 15-fold higher ^{14}C-radioactivity in lymphoid tissues (iliac, axillary, inguinal and mesenteric lymph nodes, and spleen) 24 hours following administration of an equivalent dose of [^{14}C]-TAF relative to [^{14}C]-TDF {7415}. The concentration of TAF in dogs was relatively high also in lungs, thyroid, spleen, skeletal muscle, bone marrow, and some other tissues relative to TDF. Since the clinical TAF dose (25 mg) is approximately 8% of the TDF dose (300 mg), accumulation of TAF and/or its metabolites in these tissues should be similar (or less) to that with TDF. No clinically relevant toxicity has been observed related to TAF tissue distribution. There was no substantive TAF-related toxicity observed in the liver, lungs, spleen, skeletal muscle or bone marrow in the 9-month dog toxicity study. Although there were reversible decreases in serum T3 in the high dose dogs, no TAF-related histopathological alterations were observed in the thyroid of dogs in the 9-month toxicity study; the thyroid changes observed after RPV administration are rat specific and not clinically relevant. Information from nonclinical studies with TAF should be considered in the context of their clinical data and post marketing clinical experience within antiretroviral (ARV) combination therapy for the treatment of HIV-1 infection; no signs/symptoms of liver toxicity have been noted in clinical trials of TAF-containing regimens to date (m2.7.4).

Based on the data supporting the individual components and the extensive clinical data with the FTC/RPV/TDF FDC and FTC/TAF-containing regimens, adverse pharmacokinetic interactions that would negatively affect pharmacological efficacy are not anticipated. While RPV is primarily metabolized by CYP3A, FTC, TAF and TFV are not clinically relevant substrates, inhibitors, or inducers. Thus, drug interactions through drug metabolizing enzymes within these three compounds are unlikely. Coadministration of strong inducers or inhibitors of CYP3A may

affect exposure to RPV. Emtricitabine and TAF are analogs of 2 different nucleosides, cytidine and adenosine, respectively, and do not share a common intracellular metabolism pathway for pharmacological activation through phosphorylation. Rilpivirine did not affect plasma exposure to P-gp substrates including digoxin and TAF in a clinical drug interaction study. While plasma protein binding is high for RPV and moderate for TAF, the binding was very low for FTC and TFV. Therefore, interactions through binding displacement would not be anticipated. Emtricitabine and TFV are almost exclusively eliminated by renal excretion. While TFV is a substrate for OAT1, OAT3, and MRP4, none of these transporters was inhibited by FTC. Rilpivirine is predominantly excreted in feces. Therefore, interactions within the components of the FTC/RPV/TAF FDC during excretion are unlikely. The pharmacokinetic assessment of FTC, RPV, and TAF supports the safe use of these agents in combination.

Because significant pharmacokinetic interactions are unlikely and the target organ profiles of FTC, RPV and TAF have no clinically significant overlapping toxicity, administration of the combination product is unlikely to exacerbate known toxicities of the individual agents. The only significant effect of FTC identified in repeat-dose toxicity studies was a minor anemia at dose levels constituting large multiples of clinical exposure (110-fold in mice; 21-fold in monkeys). Bone marrow is not a target of TAF. 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. The principal target organs of toxicity following oral administration of TAF were the kidney (karyomegaly, tubular degeneration) and bone. Dog was the most sensitive species to renal and bone effects of TAF. The NOEL for renal effects in monkeys is greater than 30 mg/kg/day. The rat and dog showed some loss of bone mineral density at relatively high doses; however, clinically evident osteomalacic lesions occurred only in juvenile monkeys in which TFV was chronically administered at 30 mg/kg/day by daily subcutaneous injection. Tenofovir exposure levels (AUC 150 μg·h/mL) at this dose were more than 564-fold higher than those of adults after a 25 mg dose of TAF. Neither FTC nor RPV have shown any potential for bone toxicity in chronic rat and dog toxicity studies; exacerbation of any TAF effects on bone is not expected. 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 TAF, renal toxicity is not anticipated to be an issue with the FTC/RPV/TAF FDC.

Neither FTC, nor RPV or TAF had positive findings in genotoxicity studies; the FTC/RPV/TAF FDC is not anticipated to alter the genotoxicity profiles of the individual agents. Emtricitabine and TDF/TFV have demonstrated low carcinogenic potential in conventional 2-year bioassays. Carcinogenicity studies with RPV demonstrated an increased incidence of hepatocellular and thyroid tumors that are not considered relevant for humans. Combination dosing would not be expected to change these profiles, and no exacerbation of toxicity is expected. Emtricitabine,

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

Administration of FTC and TAF in combination is unlikely to exacerbate known toxicities of the individual agents based on the FTC and TDF combination toxicity studies. No new or more marked toxicities occurred in two 14-day rat toxicology studies and a 4-week dog study with the combination.

Identified impurities and degradants have been assessed as part of the routine toxicology or qualification studies with the individual agents. Although no specific toxicology studies with TAF metabolites were conducted, cytotoxicity and bioavailability of metabolites were conducted to support manufacturing activities. The cytotoxicity of two TAF metabolites, M18 (GS-645552) and M28 (GS-652829), had no cytotoxicity up to the highest tested concentration (57 μM).

In summary, comprehensive nonclinical pharmacology/virology, pharmacokinetic, and toxicology programs were conducted with FTC, RPV and TAF. Although no peri- postnatal study or carcinogenicity studies were conducted with TAF, these studies conducted with TDF resulted in higher TFV exposures than what could have been achieved with TAF administration. The ample nonclinical safety database on these drugs indicates further toxicological investigations are unlikely to yield new data relevant to humans. Studies with the combinations are unwarranted given the lack of pharmacokinetic interactions and significant overlapping toxicities. The absence of nonclinical safety studies with the FTC/RPV/TAF combination is in accordance with the FDA Guidance for Industry, Nonclinical Safety Evaluation of Drug or Biologic Combinations, March 2006 and 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/TAF FDC. Because the target organ profiles are different, and there is no evidence of genotoxicity, carcinogenicity, or reproductive toxicity, administration of the FTC/RPV/TAF combination product is unlikely to introduce new toxicities or to exacerbate known toxicities of the individual agents. Additionally, the clinical safety data available from the approved FTC/TDF regimens, including FTC/RPV/TDF and with the F/TAF-containing FDCs supports the safety of the FTC/RPV/TAF FDC for the treatment of HIV-1 infection.

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Justification for Absent Nonclinical Data in Module 4

Section		Comment
4.2.1.4	Pharmacodynamic Drug Interactions	No TAF or FTC studies are included, as drug interactions are discussed in the clinical m2.7.2 Section 2.5.
4.2.2.5	Excretion	No FTC studies are included in this Section, however Report TOX063 in Section 4.2.2.4, covers both the metabolism and excretion of FTC.
4.2.2.7	Other Pharmacokinetic (PK) Studies	"Other" PK studies were not conducted. All PK study information is included elsewhere in the dossier (see m2.6.5).
4.2.3.4.1	Carcinogenicity (including supportive toxicokinetics evaluations): Long term studies	As agreed with the EMA and CHMP [redacted]), carcinogenicity studies are not required for TAF due to the lack of TAF exposure in rats and TgRasH2 mice, and lower TFV exposure in rats and mice administered TAF compared to after administration of TDF.
4.2.3.4.2	Carcinogenicity (including supportive toxicokinetics evaluations): Short- or medium-term studies	Short- or medium-term carcinogenicity studies were not performed since long term carcinogenicity studies relevant to each component were conducted and are reported in m4.2.3.4.1 (see also m2.6.6). All supportive or dose-range finding studies are included elsewhere in the dossier.
4.2.3.4.3	Carcinogenicity (including supportive toxicokinetics evaluations): Other studies	"Other" carcinogenicity studies were not performed since long term carcinogenicity studies relevant to the components were conducted and are reported in m4.2.3.4.1 (see also m2.6.6). All supportive or dose-range finding studies are included elsewhere in the dossier.
4.2.3.5.3	Prenatal and postnatal development, including maternal function	As agreed with the EMA and CHMP [redacted] a perinatal/postnatal study in rats is not required for TAF registration due to the lack of TAF exposure in rats and lower TFV exposure compared to after TDF administration.
4.2.3.5.4	Studies in which the offspring (juvenile animals) are dosed and/or further evaluated	No specific juvenile toxicity studies are considered warranted with FTC in view of the results of the repeat-dose studies and that FTC is approved for use in humans from 4 months and above (see m2.6.6 and m2.4 Section 4.6.1). No specific studies on offspring were conducted with TAF (see m2.6.6 and m2.4 Section 4.6.3), however data are available from efficacy studies of TFV in nonhuman primates. Report TMC278-NC168 in Section 4.2.3.5.3 covers pre and post natal development toxicity and juvenile dose finding for RPV.
4.2.3.6	Local Tolerance	No FTC studies are included, as based on the extensive clinical data for FTC, local tolerance studies were considered unnecessary.

Section		Comment
4.2.3.7.1	Antigenicity	No studies were conducted with FTC. Based on the extensive clinical data for FTC, antigenicity studies were considered unnecessary. One Report that covers antigenicity is included for RPV (Report TMC278-NC199) in Section 4.2.3.6.
4.2.3.7.2	Immunotoxicity	No studies were conducted with TAF based upon an assessment of the data from the repeat-dose toxicity studies.
4.2.3.7.3	Mechanistic Studies (if not included elsewhere)	Based on the extensive pharmacology, toxicology and clinical data, mechanistic studies with FTC were considered unnecessary.
4.2.3.7.4	Dependence	Low levels of each component were detected in brain following {^{14}C} distribution studies. There was no evidence of dependency in nonclinical studies with FTC, RPV or TAF. Nucleos(t)ide analogues and NNRTIs as a class have no known properties that would suggest development of dependence. Therefore no specific studies on dependency were conducted with any of the components (see m2.6.6).
4.2.3.7.5	Metabolites	FTC does not undergo extensive first-pass or systemic metabolism, and is eliminated primarily by renal excretion of unchanged drug, therefore no studies were conducted. There are no unique human RPV metabolites, therefore no evaluation was necessary.