SECTION 2.6.—NONCLINICAL SUMMARY

SECTION 2.6.1—INTRODUCTION

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

Gilead Sciences

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TABLE OF CONTENTS

SECTION 2.6.1—INTRODUCTION 1
TABLE OF CONTENTS 2
GLOSSARY OF ABBREVIATIONS AND DEFINITION OF TERMS 3
NOTE TO REVIEWER 4
1. NONCLINICAL SUMMARY 6
1.1. Introduction 6
1.1.1. Emtricitabine 6
1.1.2. Rilpivirine 7
1.1.3. Tenofovir Alafenamide 7
1.1.4. Emtricitabine/Rilpivirine/Tenofovir Alafenamide 8
1.1.5. References 10

GLOSSARY OF ABBREVIATIONS AND DEFINITION OF TERMS

ARV	antiretroviral
CatA	cathepsin A
CHMP	Committee for Medicinal Products for Human Use
COBI	cobicistat, GS-9350 (Tybost®, Gilead)
dATP	deoxyadenosine triphosphate
DNA	deoxyribonucleic acid
E/C/F/TAF	elvitegravir, cobicistat, emtricitabine, and tenofovir alafenamide (coformulated)
FDC	fixed-dose combination
FTC	emtricitabine, (Emtriva®, Gilead)
FTC/TDF	emtricitabine/tenofovir disoproxil fumarate, TVD, (Truvada®, Gilead)
FTC-TP	emtricitabine triphosphate
HIV-1	human immunodeficiency virus type 1
NNRTI	nonnucleoside reverse transcriptase inhibitor
NRTI	nucleoside reverse transcriptase inhibitor
NtRTI	nucleotide reverse transcriptase inhibitor
OAT	organic anion transporter
PBMC	peripheral blood mononuclear cell
PMPA	(*R*)-9-(2-Phosphonoylmethoxypropyl)adenine; tenofovir
QT	interval between the start of the Q wave and the end of the T wave on ECG
QTcF	QT interval corrected for heart rate using Fredericia formula
RPV	rilpivirine (TMC278, Edurant®, Janssen)
RT	reverse transcriptase
STB	elvitegravir/cobicistat/emtricitabine/tenofovir disoproxil fumarate (coformulated), (Stribild®, Gilead)
TAF	tenofovir alafenamide (GS-7340)
TDF	tenofovir disoproxil fumarate (Viread®, Gilead)
TFV	tenofovir, PMPA
TFV-DP	tenofovir diphosphate

NOTE TO REVIEWER

This application is being submitted for a fixed dose combination (FDC) that contains nucleoside reverse transcriptase inhibitor (NRTI) emtricitabine (FTC, F, Emtriva®), the nonnucleoside reverse transcriptase inhibitor (NNRTI) rilpivirine (RPV, R, Edurant®) and and the nucleotide reverse transcriptase inhibitor (NtRTI) tenofovir alafenamide: the F/R/TAF FDC (200/25/25 mg) tablet. All nonclinical studies to support the F/R/TAF FDC application are included with no cross referencing to data previously submitted. This comprises all nonclinical tests utilizing FTC, RPV or TAF, including relevant combination studies, eg, FTC/tenofovir disoproxil fumarate (TDF); and other studies necessary to support the proposed product labeling for the treatment of human immunodeficiency virus type 1 (HIV-1) infection. Links to other modules and study reports are in blue text.

[redacted]
GS-7340-03,
GS-7340-03 (TAF fumarate)
GS-7340-03
GS-7340-03
[redacted] Although all doses are provided as both the respective form and the free base equivalents (f.b.e.), the following conversion table is provided as a reference.

GS-7340 Equivalents		
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
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[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]

The following conversions are also provided to aid the reviewer:

- FTC 1 µM = 0.247 µg/mL
- RPV 1 µM = 0.366 µg/mL
- TAF (GS-7340) 1 µM = 0.477 µg/mL
- TFV 1 µM = 0.287 µg/mL

1. NONCLINICAL SUMMARY

1.1. Introduction

This application is being submitted for the registration of a fixed dose combination (FDC) tablet that contains the nucleoside reverse transcriptase inhibitor (NRTI) emtricitabine (FTC, F, Emtriva®), the nonnucleoside reverse transcriptase inhibitor (NNRTI) rilpivirine (RPV, R, Edurant®) and the nucleotide reverse transcriptase inhibitor (NtRTI) tenofovir alafenamide (TAF, GS-7340) fumarate (GS-7340-03); the FTC/RPV/TAF (F/R/TAF, 200/25/25 mg) tablet.

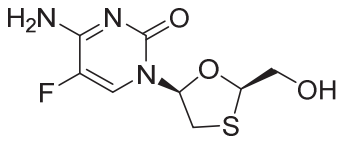
Tenofovir alafenamide is a prodrug of tenofovir (TFV). Tenofovir alafenamide is metabolized by hydrolases, including carboxyl esterase 1 and cathepsin A (CatA), and has minimal interaction with typical xenobiotic metabolizing enzymes. Because TAF is more stable in plasma than the TFV prodrug tenofovir disoproxil fumarate (TDF, Viread®), higher TFV levels are achieved in HIV target cells including lymphocytes and macrophages. The subsequent metabolism via CatA results in formation of 4-fold (3- to 7-fold at 90% confidence interval) higher intracellular levels of the active phosphorylated metabolite tenofovir diphosphate (TFV-DP) in peripheral blood mononuclear cells (PBMCs) and 90% lower circulating levels of TFV relative to TDF. The higher TFV-DP levels lead to more effective suppression of viral replication in clinical studies.

The FTC/RPV/TAF FDC tablet contains the same doses of FTC and RPV that are currently approved for use in adults. To facilitate the evaluation of the F/R/TAF FDC, nonclinical virology studies of FTC, RPV, TAF and TFV are described in detail in m2.7.2 (Summary of Clinical Pharmacology Studies), Section 4.1 (Nonclinical Virology), together with the clinical virology data contained in m2.7.2, Section 4.2 (Clinical Virology). Comprehensive programs of nonclinical studies have been conducted with FTC, RPV and TAF. Information from all nonclinical studies with FTC, RPV, and TAF should be considered in the context of the substantial clinical experience with FTC, RPV and TDF within antiretroviral (ARV) combination therapy for the treatment of HIV-1 infection. The nonclinical data discussed within this document support the proposed use of the FTC/RPV/TAF FDC for the treatment of HIV-1 infection.

1.1.1. Emtricitabine

Emtricitabine (FTC) is an NRTI. It is the active ingredient in Emtriva 200-mg capsules and 10 mg/mL oral solution that are approved in combination with other ARV products for the treatment of HIV-1 infection in adults and children aged 4 months and older. Emtricitabine is marketed as Emtriva and is a component of Truvada® (FTC/TDF), Complera®/Eviplera® (FTC/RPV/TDF), and Stribild® (elvitegravir/cobicistat/FTC/TDF, E/C/F/TAF).

The chemical structure of FTC is as follows:

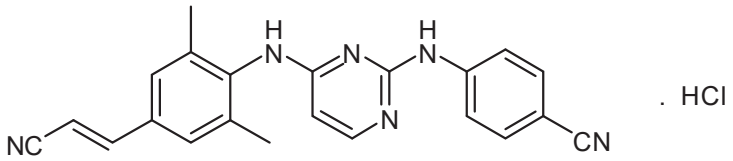


Following absorption, FTC is phosphorylated by cellular enzymes to emtricitabine triphosphate (FTC-TP), the active metabolite, an analog of 2'-deoxycytidine triphosphate. Emtricitabine triphosphate inhibits the activity of HIV-1 reverse transcriptase through high affinity binding, competing with the natural substrate 2'-deoxycytidine 5'-triphosphate. Emtricitabine triphosphate is efficiently incorporated into the nascent (viral) DNA chain by HIV-1 reverse transcriptase (RT) resulting in termination of DNA synthesis due to the lack of a hydroxyl group at the 3'- position of the sugar moiety of FTC, which in turn inhibits viral replication. In a clinical study, the intracellular half-life of FTC-TP in PBMCs was 39 hours. Intracellular triphosphate levels increased with dose, but reached a plateau at doses of 200 mg or greater. Emtricitabine has activity against retroviruses and hepadnaviruses.

1.1.2. Rilpivirine

Rilpivirine (Tibotec Medicinal Compound 278 [TMC278], RPV), a substituted diarylpyrimidine derivative, is a potent NNRTI with in vitro activity against wild type HIV-1 and HIV-1 NNRTI-resistant mutants, which has been developed for long term treatment of HIV-infected treatment-naive adults in combination with commercialized ARV agents. Rilpivirine is marketed as Edurant and is a component of Complera/Eviplera (FTC/RPV/TDF).

The chemical name for RPV is 4-[[4-[[4-[(E)-2-cyanovinyl]-2,6-dimethylphenyl]amino]-2-pyrimidinyl]amino]benzonitrile hydrochloride. The molecular formula is $C_{22}H_{18}N_6$.HCl and its molecular weight is 402.88 Dalton. Rilpivirine has the following structural formula:

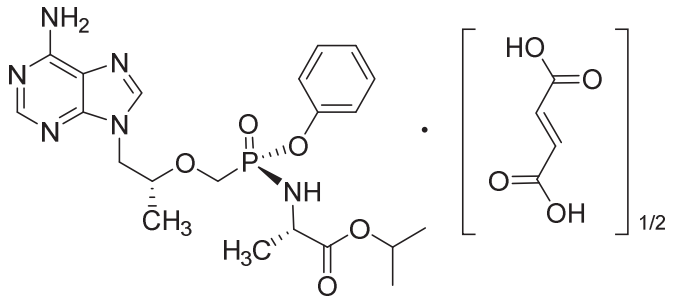


Rilpivirine has a median effective concentration (EC_{50}) in vitro for HIV-1/IIIb subtypes ranging from 0.13 to 0.73 nM (0.05 to 0.27 ng/mL) and for HIV-1 Group M ranging from 0.07 to 1.01 nM (0.03 to 0.37 ng/mL).

1.1.3. Tenofovir Alafenamide

Tenofovir alafenamide is a prodrug of TFV (PMPA), a NtRTI. The first generation tenofovir prodrug is TDF, the active ingredient in Viread, which is approved as 245-mg film-coated tablets for the treatment of HIV-1 infection in adults and adolescents in combination with other ARV products and as 123-, 163-, and 204-mg film-coated tablets and 33 mg/g granules for use in children aged 2 to less than 12 years.

The chemical structure of TAF fumarate is shown below.



Tenofovir is poorly absorbed from the intestine because of the presence of the negative charges associated with the phosphonate group. Prodrugs are, therefore, needed to mask the charge and improve oral bioavailability. Tenofovir alafenamide is more stable in plasma than TDF, resulting in higher levels of TFV-DP in HIV-1 target cells including lymphocytes and macrophages. Tenofovir alafenamide is predominantly hydrolyzed to TFV by CatA-mediated cleavage in HIV-target cells {13119}, {10427}. During clinical studies, administration of TAF resulted in subsequent formation of > 4-fold (3-7-fold at 90% confidence interval) higher intracellular levels of TFV-DP in PBMCs and 90% lower circulating levels of TFV relative to TDF {7415}, {13119}, {22029}. This active metabolite, TFV-DP {1574}, competes with natural 2'-deoxyadenosine triphosphate (dATP) for incorporation by the HIV-1 or hepatitis B virus RT and, once incorporated, results in chain-termination {21}, {1131}. Thus, the clinical dose of TAF is much lower than the clinical dose of TDF. Unlike TFV, TAF does not interact with and is not a substrate for the renal transporters organic anion transporter 1 or 3 (OAT1 or OAT3), and TAF exhibits no OAT-dependent cytotoxicity in human epithelial kidney cells transiently expressing these transporters. Therefore, TAF is unlikely to accumulate in renal proximal tubules in an OAT-dependent manner. Since TAF is unlikely to contribute to renal tubular cell loading of TFV, intracellular TFV concentrations in renal cells are likely to correlate with plasma TFV levels, which, at the 10-mg dose yields approximately 90% lower circulating levels of TFV relative to TDF. The lower systemic levels of TFV and the higher intracellular levels of TFV-DP translate into less risk of nephrotoxicity and less decrease in bone mineral density, which are known risks with TDF administration {21762}, {22031}, {30895}.

1.1.4. Emtricitabine/Rilpivirine/Tenofovir Alafenamide

Comprehensive nonclinical pharmacology/virology, pharmacokinetic, and toxicology programs were undertaken with FTC, RPV, and TAF. Emtricitabine, RPV and TAF are potent and selective inhibitors of HIV-1. All 3 drugs show potent ARV activity against diverse subtypes of HIV-1 in vitro. Emtricitabine and TAF are phosphorylated intracellularly through non overlapping pathways, and in combination show no antagonism for the formation of their active metabolites. Rilpivirine does not require modification for activity. Dual-drug combinations of FTC, RPV, and TAF consistently show additive to synergistic anti-HIV-1 activity in vitro and no evidence of cytotoxicity. The resistance profiles for the individual agents of FTC, RPV, and TAF have been well characterized. Cross-resistance between the NRTI and NNRTI classes is minimal.

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 study in healthy subjects. At the 25-mg dose of RPV, the observed change in QTcF was not considered clinically relevant, and the combination product is not anticipated to exacerbate the cardiovascular effect seen with RPV alone. No additional safety pharmacology studies are considered necessary with the FTC/RPV/TAF combination.

Adverse pharmacokinetic interactions that would negatively affect pharmacological efficacy are not anticipated. This is based on the discrete routes of absorption and elimination demonstrated for each compound and the differences in physicochemical properties between the compounds which influence drug distribution. Emtricitabine does not undergo extensive first-pass or systemic metabolism, and is eliminated primarily by renal excretion of unchanged drug. The total body clearance of FTC exceeds the glomerular filtration rate, suggesting the drug is actively secreted by renal tubules into the urine. In all animal species, the predominant route of [^{14}C] RPV 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. Renal excretion is the primary systemic route of elimination of TFV in all preclinical species tested.

The toxicity profiles of FTC, RPV and TAF differ substantially with no clinically significant overlapping toxicity. Neither FTC, nor RPV or TAF had positive findings in genotoxicity studies. 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. Emtricitabine, RPV, and TAF have not shown significant adverse effects in reproductive and developmental toxicity studies. Therefore, administration of the FTC/RPV/TAF combination product is unlikely to introduce new toxicities or to exacerbate known toxicities of the individual agents.

The absence of nonclinical safety studies with the F/R/TAF combination is in accordance with the United Stated Food and Drug Administration (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). 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. Additionally, the extensive clinical safety data available from the clinical trials with the FTC/RPV/TDF FDC, and from FTC/TAF-containing regimens support the safety of the FTC/RPV/TAF FDC for the treatment of HIV-1 infection.

1.1.5. References

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2.6 NONCLINICAL SUMMARY

SECTION 2.6.2—PHARMACOLOGY WRITTEN SUMMARY

EMTRICITABINE/RILPIVIRINE/TENOFOVIR ALAFENAMIDE FIXED-DOSE COMBINATION (FTC/RPV/TAF FDC)

Gilead Sciences

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TABLE OF CONTENTS

SECTION 2.6.2—PHARMACOLOGY WRITTEN SUMMARY 1
TABLE OF CONTENTS 2
LIST OF IN-TEXT TABLES 4
LIST OF IN-TEXT FIGURES 4
LIST OF ABBREVIATIONS 5
NOTE TO REVIEWER 7
1. BRIEF SUMMARY 8
2. PRIMARY PHARMACODYNAMICS 14
2.1. FTC 14
2.2. RPV 14
2.3. TAF 14
2.3.1. Intracellular Metabolism of TAF 15
2.3.2. Effects of Protease Inhibitors of HIV and HCV, and Host Cell Proteases on Cathepsin A-mediated Activation of TAF 16
3. SECONDARY PHARMACODYNAMICS 18
3.1. FTC 18
3.1.1. Effect on Cellular DNA Polymerases 18
3.1.2. In Vitro Receptor Binding Potencies 18
3.1.3. In Vitro Autonomic Pharmacology Effects on Peripheral Autonomic Receptors 18
3.1.4. In Vitro Cytotoxicity 19
3.1.5. Mitochondrial Toxicity 19
3.2. RPV 21
3.2.1. Effect on Cellular DNA Polymerases 21
3.2.2. In Vitro Receptor Binding Potencies and Neurotransmitter Uptake Inhibition 21
3.2.3. In Vivo Effect on Gastric Antisecretory Activity 21
3.2.4. In Vitro Cytotoxicity 22
3.3. TAF 24
3.3.1. Effect of TFV Diphosphate on Cellular DNA Polymerases 24
3.3.2. In Vitro Receptor Binding Potencies of TDF and TFV 25
3.3.3. In Vitro Cytotoxicity 25
3.3.4. Mitochondrial Toxicity 32
3.4. FTC/TFV 36
3.4.1. In Vitro Cytotoxicity 36
3.4.2. Mitochondrial Toxicity 38
3.5. TFV 38
3.6. FTC/RPV/TAF 38
4. SAFETY PHARMACOLOGY 39
4.1. FTC 39
4.1.1. Overt Pharmacodynamic Effects 39
4.1.2. Central Nervous System 39
4.1.3. Cardiovascular System 41
4.1.4. Respiratory System 42
4.1.5. Renal System 42
4.1.6. Gastrointestinal System 42
4.2. RPV 42

4.2.1. Central Nervous System 42
4.2.2. Cardiovascular System 43
4.2.3. Respiratory System 49
4.3. TAF 49
4.3.1. Central Nervous System 49
4.3.2. Cardiovascular System 49
4.3.3. Gastrointestinal System 50
4.3.4. Renal System 50
4.4. FTC/RPV/TAF 50
5. PHARMACODYNAMIC DRUG INTERACTIONS 51
6. DISCUSSION AND CONCLUSIONS 52
6.1. FTC 52
6.2. RPV 52
6.3. TAF (and TFV) 52
6.4. Conclusions 54
7. REFERENCES 55

LIST OF IN-TEXT TABLES

Table 1. Effects of COBI, HIV-1 Protease Inhibitors, or HCV Protease Inhibitors on CatA-mediated Hydrolysis of TAF 17
Table 2. Cytotoxicity of FTC in Comparison to 3TC and ZDV 19
Table 3. Ratio of Mitochondrial DNA to Cellular DNA in Molt-4 Cells 20
Table 4. In Vitro TMC278 and NNRTI CC_{50} Values 22
Table 5. In Vitro TMC278 and NNRTI TC_{50} Values 23
Table 6. In Vitro TMC278 and NNRTI CC_{50} Values on MT-4 23
Table 7. Kinetic Inhibition Constants of TFV-DP Against DNA Polymerases α, β, γ, δ, and ε Versus HIV-1 Reverse Transcriptase 24
Table 8. Relative Efficiencies of Incorporation into DNA of TFV-DP and NRTI-Triphosphates by Human DNA Polymerases α, β, and γ 25
Table 9. In Vitro Cytotoxicity of TAF, GS-7339, TDF, and TFV in Resting and Dividing PBMCs 26
Table 10. In Vitro Cytotoxicity of TAF and other HIV Inhibitors in Human T-Lymphoblastoid and Hepatic Cell Lines 27
Table 11. In Vitro Hematopoietic Toxicity of TAF in Comparison with 5-Fluorouracil 28
Table 12. In Vitro Hematopoietic Toxicity of TFV in Comparison with Other NRTIs 29
Table 13. In Vitro Cytotoxicity of TAF and TFV in the Presence and Absence of Organic Anion Transporters 1 and 3 in Human Epithelial Kidney Cells 30
Table 14. Profiles of TFV, Cidofovir, and Adefovir in In Vitro Models of Renal Proximal Tubular Toxicity 31
Table 15. In Vitro Cytotoxicity of TAF in Primary Osteoblasts 32
Table 16. Effect of TAF on Mitochondrial DNA Levels in HepG2 Cells 33
Table 17. Effect of TFV and Other NRTIs on Mitochondrial DNA Content in Differentiated Human Renal Proximal Tubular Epithelial Cells 35
Table 18. Effects of TFV and Other NRTIs on the In Vitro Production of Lactic Acid 36
Table 19. Effect of COBI, FTC, and EVG on the Cytotoxicity of TFV in Human RPTECs 37
Table 20. Effect of FTC (+COBI and EVG) on the Cytotoxicity of TFV in 293T Human Embryonic Kidney Cells Transiently Expressing Renal Transporters 38

LIST OF IN-TEXT FIGURES

Figure 1. Intracellular Activation of TAF in Lymphoid Cells and Tissues 15
Figure 2. Intracellular TAF Metabolites in CD4+ T cells and Monocyte-derived Macrophages from Different Donors 16
Figure 3. Chemical Structures of the Diastereomers TAF and GS-7339 26
Figure 4. Effect of TFV and Other NRTIs on Mitochondrial DNA Content in Human Liver and Skeletal Muscle Cells 34

LIST OF ABBREVIATIONS

3TC	lamivudine
ABC	abacavir
AIDS	acquired immune deficiency syndrome
ATV	atazanavir
AUC	area under the curve
CatA	cathepsin A
CC_{50}	drug concentration that results in a 50% reduction in cell viability
C_{max}	maximum concentration
CNS	central nervous system
COBI	cobicistat (GS-9350), Tybost®, Gilead Sciences
COXII	cytochrome c oxidase II
CPP	coronary perfusion pressure
CYP3A	cytochrome P450 3A
d4T	stavudine
dCTP	deoxycytidine triphosphate
ddC	zalcitabine
ddI	didanosine
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
dNTP	2'-deoxynucleoside triphosphates
DRV	darunavir
EC_{50}	half maximal effective concentration or concentration of compound inhibiting virus replication by 50%
EC_{95}	concentration of compound inhibiting virus replication by 95%
ECG	electrocardiogram
EFV	efavirenz
EtOH	ethanol
EU	European Union
EVG	elvitegravir (JTK-303), Vitekta®, Gilead Sciences
fbe	free base equivalent
FDC	fixed-dose combination
FIAU	fialuridine
FLT	alovudine, 3'-dideoxy-3'-fluorothymidine
FTC, 524W91	emtricitabine (Emtriva®, Gilead Sciences)
FTC-TP	emtricitabine 5'-triphosphate
GI	gastrointestinal
GLP	Good Laboratory Practice
GS-8374	Gilead's investigational HIV protease inhibitor
HBV	hepatitis B virus
HCV	hepatitis C virus
HEK	human embryonic kidney
hERG	human ether-à-go-go related gene

LIST OF ABBREVIATIONS (CONTINUED)

HIV-1	human immunodeficiency virus type 1
HS	human serum
IC_{50}	inhibitory concentration
K_I	affinity constant for enzyme inactivation
K_i	affinity constant for enzyme inhibition
IL-2	interleukin-2
INSTI	integrase strand transfer inhibitor
LDH	lactate dehydrogenase
LPV	lopinavir
MC	methyl cellulose
MDM	monocyte-derived macrophage
MRP4	multidrug resistance protein 4
MSD	multiplicative standard deviations
mtDNA	mitochondrial deoxyribonucleic acid
ND	not determined
NDP	nucleoside disphosphate
NFV	nelfinavir
NNRTI	nonnucleoside reverse transcriptase inhibitor
NRTI	nucleoside reverse transcriptase inhibitor
NtRTI	nucleotide reverse transcriptase inhibitor
OAT1/3	organic anion transporter 1/3
PBMC	peripheral blood mononuclear cell
PI	protease inhibitor
Pol	polymerase
PUR	puromycin
QT	interval between the start of the Q wave and the end of the T wave on ECG
QTc	QT interval corrected for heart rate
RAL	raltegravir
RPTECs	renal proximal epithelial tubule cells
RPV, TMC278	rilpivirine (Edurant®)
RT	reverse transcriptase
RTV, r	ritonavir
SD	standard deviation
SI	selectivity index
SkMC	skeletal muscle cell
TAF	tenofovir alafenamide, formerly GS-7340
TDF	tenofovir disoproxil fumarate
TFV	tenofovir
TFV-DP	tenofovir diphosphate
TFV-MP	tenofovir monophosphate
XTT	2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide, tetrazolium
ZDV	zidovudine

NOTE TO REVIEWER

This document contains a summary of nonclinical pharmacology studies conducted in support of a fixed-dose combination (FDC) that contains the active substances emtricitabine (FTC), rilpivirine (RPV), and tenofovir alafenamide (TAF). The FDC tablet is referred to as emtricitabine/rilpivirine/tenofovir alafenamide (FTC/RPV/TAF) throughout this document.

As TAF is a new chemical entity, this nonclinical summary contains all available data on this new component. Because FTC and RPV are approved antiretroviral agents, data included for these components are limited to key analyses relevant to and supporting the efficacy and safety claims in the FDC prescribing information. Nonclinical studies have utilized TAF in most cases; however, relevant studies with the parent drug tenofovir (TFV) are also provided as applicable. Links to all study reports included in the dossier are highlighted in blue text

In order to simplify the review, the order of presentation in each section follows the general format: FTC, RPV, TAF (and TFV), and combination studies. Given the lack of relevant effects in the in vitro studies with the individual agents (FTC, RPV, TFV, or TAF) or in two-drug combination studies, no additional pharmacology studies have been conducted for the FTC/RPV/TAF combination.

The following conversions are provided to aid the reviewer:

- FTC 1 μM = 0.247 μg/mL
- RPV 1 μM = 0.366 μg/mL
- TAF (GS-7340) 1 μM = 0.477 μg/mL
- TFV 1 μM = 0.287 μg/mL

1. BRIEF SUMMARY

This dossier is being submitted in support of an application for a fixed-dose combination (FDC) that contains emtricitabine (FTC, Emtriva®), rilpivirine (RPV, Edurant®), and tenofovir alafenamide (TAF, formerly GS-7340): the FTC/RPV/TAF FDC (200/25/25 mg).

Comprehensive programs of nonclinical pharmacology studies with FTC, RPV, and TAF (including studies with tenofovir [TFV]) have been conducted. All of the definitive safety pharmacology studies 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.

To facilitate the evaluation of the FTC/RPV/TAF FDC, nonclinical virology studies of FTC, RPV, TAF and TFV are described in detail in m2.7.2 (Summary of Clinical Pharmacology Studies), Section 4.1 (Nonclinical Virology) together with the clinical virology data contained in m2.7.2, Section 4.2 (Clinical Virology). The secondary pharmacodynamics (excluding all virology data) and safety pharmacology of FTC, RPV, TAF and TFV are described in detail in this module. The order of presentation in each section follows the general format: FTC, RPV, TAF, and combination studies.

The nonclinical data discussed within this document support the proposed use of the FTC/RPV/TAF FDC for the treatment of HIV-1 infection. All information from nonclinical pharmacology studies that is of relevance to the prescriber and patient has been included in the proposed prescribing information.

FTC

Emtricitabine is a nucleoside reverse transcriptase inhibitor (NRTI), developed by Gilead Sciences, that is marketed as a once-daily capsule (200 mg) and 10 mg/mL oral solution. Emtricitabine is a synthetic analogue of the naturally occurring pyrimidine nucleoside, 2′-deoxycytidine, which is structurally similar to lamivudine.

Intracellularly, FTC is sequentially phosphorylated to FTC 5’-monophosphate and 5’-diphosphate, and finally to FTC 5'-triphosphate (FTC-TP), the intracellular form active as an antiviral substance. Emtricitabine 5’-triphosphate inhibits viral polymerases by direct binding competition with the natural deoxyribonucleotide substrate (deoxycytidine triphosphate; dCTP) resulting in chain termination of the nascent viral deoxyribonucleic acid (DNA) during HIV-1 reverse transcription. Emtricitabine triphosphate is a very weak inhibitor of mammalian DNA polymerases α, β, ε, and mitochondrial DNA polymerase γ. The EC_{50} of FTC of laboratory adapted strains of HIV-1 and HIV-2 ranged from 0.0013 to 0.5 μM and 0.08 to 1.5 μM, respectively, depending on cell type and virus strain used in the assay.

The primary pharmacodynamic, secondary virology, and drug interaction pharmacodynamic studies of FTC are presented in detail in m2.7.2, Section 4.1.

For FTC, no cytotoxicity was observed in vitro in human PBMC, MT-2, HepG2, CEM, and Vero cells at concentrations up to 100 μM. Emtricitabine was also found to be nontoxic to human bone marrow progenitor cells and in lymphocyte and monocytic cells in vitro.

Emtricitabine showed a low potential for mitochondrial toxicity. Emtricitabine did not reduce mitochondrial DNA in Molt-4 cells or affect cell growth, lactic acid concentration, or mitochondrial DNA content in human HepG2 cells and MT-2 cells. Emtricitabine had no effect on mitochondrial ultrastructure.

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

RPV

Rilpivirine (RPV; TMC278), a diarylpyrimidine derivative, is a nonnucleoside reverse transcriptase inhibitor (NNRTI) active against wild type and NNRTI-resistant HIV-1 that is marketed as a single agent (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 “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 “TMC278.” TMC278 base has been applied in the early phases of development and TMC278 in the later phases, after selection of the final chemical form.

The primary pharmacodynamic, secondary virology, and drug interaction pharmacodynamic studies of RPV are presented in detail in m2.7.2, Section 4.1.

The in vitro cytotoxicity experiments performed in cell lines of various origins confirmed that the TMC278 CC_{50} was > 5 μM. A selectivity index of approximately 8,000 indicated that TMC278 was a potent and specific inhibitor of HIV-1.

The testing on a variety of receptors, human DNA polymerases, stomach ATPase, and gastric acidity did not reveal any significant effects secondary to the primary anti-HIV effect of TMC278.

TMC278 did not cause any inhibition in vitro of α- or β-adrenergic, dopaminergic, muscarinergic, serotonergic, opioid, interleukin, or chemokine receptors (at up to 10 μM). TMC278 showed no agonistic or antagonistic activity on histamine H2 receptors in the isolated guinea pig right atrium and no inhibition of adenosine triphosphatase (ATPase) in isolated pig stomach. In vivo, TMC278 did not cause any significant inhibition of pentagastrin-induced gastric acidity in rats. Therefore, TMC278 is essentially devoid of any clinically relevant secondary pharmacodynamic effect at unbound concentrations that exceed the median maximum systemic exposure to total TMC278 in man (0.55 μM or 0.2 μg/mL) following an oral dose of 25 mg once daily.

The standard battery of cardiovascular safety studies showed a concentration-dependent inhibition of TMC278 on the rapidly activating rectifying potassium current (I_{Kr}) from 33% at 0.3 µM (0.11 µg/mL) to 80% at 3 µM (1.1 µg/mL). However, no relevant effects by TMC278 were noted on other cardiovascular or electrocardiographic parameters in vitro in the right atrium of the guinea pig; in vivo in anesthetized guinea pigs and dogs given a single intravenous dose; or in conscious instrumented or telemetered dogs given a single oral dose.

Delayed-onset (after 11 days of treatment) prolongation of the QT-interval corrected for heart rate according to the Fridericia equation (QTcF) was reported in the clinical thorough QT (TQT) study, TMC278-TiDP6-C131 (C131). To investigate the mechanism of action of the QTc prolongation and of the delayed onset, additional nonclinical studies were done. Moreover, the potential of TMC278 to induce proarrhythmic effects, in particular torsade des pointes (TdP), was evaluated.

Additional studies showed inhibition of the slowly activating rectifying potassium current (IKs) from 17% at 1 µM (0.37 µg/mL) up to 73% at 10 µM (3.7 µg/mL), with an IC_{50} of 3.1 µM (1.15 µg/mL). In addition, the transient outward potassium current (I_{to}) was reduced by 14% at 0.3 µM (0.11 µg/mL) and up to 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 human ether à go-go (hERG) channel by TMC278 was observed from 1 µM (0.37 µg/mL), and above. 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 had maximum measured plasma concentrations of TMC278 ranging from 0.6 to 0.9 µg/mL. This concentration was similar to the steady state median maximum measured plasma concentration at 75 mg of TMC278 once daily, a dose that caused delayed-onset QTc-prolongation in TQT study TMC278-TiDP6-C131.

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

TMC278 had no effect on respiratory parameters in anesthetized dogs (single 1-hour intravenous infusion of 5 mg/kg) or conscious telemetered dogs (single oral dose of 20 mg/kg).

No compound-related neurological, behavioral changes, or delayed neurotoxicity were observed in a modified Irwin test in rats (single oral dose up to 400 mg/kg) or in conscious telemetered (single oral dose up to 160 mg/kg) or instrumented dogs (single oral dose of 20 mg/kg).

Overall, TMC278 had no effects on secondary pharmacodynamic parameters or on the core battery of safety pharmacology tests, apart from inhibitory effects on some cardiac potassium currents and channels and moderate QT-prolongation in the rabbit ventricular wedge. The observed inhibition of trafficking of the hERG channel may be involved in the delayed onset of the QTcF-prolongation observed in the clinical TQT study TMC278-TiDP6-C131. However, pharmacokinetic mechanisms may also be involved. Importantly, the effects in nonclinical

models allow the conclusion that TMC278 has only a marginal potential to induce proarrhythmic effects.

TAF

Tenofovir alafenamide is an investigational prodrug of TFV, a nucleotide reverse transcriptase inhibitor (NtRTI). In HIV-target cells including lymphocytes and macrophages, TAF is hydrolyzed to TFV by cathepsin A (CatA) cleavage {13119}, {10427}, resulting in higher intracellular levels of TFV-diphosphate (DP) and lower circulating levels of TFV relative to tenofovir disoproxil fumarate (TDF, Viread) clinically {17137}, {1574}. Tenofovir-DP is an inhibitor of HIV and hepatitis B virus (HBV) reverse transcriptases by direct binding competition with the natural deoxyribonucleotide substrate (deoxyadenosine triphosphate; dATP) resulting in chain termination of the elongating viral DNA {21}, {1131}. Tenofovir-DP is a very weak inhibitor of mammalian DNA polymerases α, β, δ, ε, and mitochondrial DNA polymerase γ. Tenofovir alafenamide 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}.

The primary pharmacodynamic, secondary virology, and drug interaction pharmacodynamic studies of TFV/TAF are presented in detail in m2.7.2, Section 4.1.

Tenofovir alafenamide is more stable in plasma than TDF, but rapidly converts to TFV inside cells. Assessment of the intracellular metabolism of TAF in CD4+ T lymphocytes and monocyte-derived macrophages (MDMs) showed efficient conversion of the prodrug to the active metabolite TFV-DP. The intracellular conversion of TAF to TFV-DP was consistent across immune cells derived from demographically diverse donors. Lysosomal carboxypeptidase CatA plays an essential role in the intracellular activation of TAF; therefore, a variety of protease inhibitors (PIs) were screened for possible effects on CatA-mediated intracellular activation and antiviral activity of TAF {20795}. Compounds assessed included HIV and hepatitis C virus (HCV) PIs, the pharmacokinetic enhancer cobicistat (COBI), as well as host serine PIs used as anti-diabetic and anti-coagulant agents. Of the agents tested, the covalent HCV PIs telaprevir and boceprevir, which are known to inhibit CatA, were the only compounds that changed the antiretroviral efficacy for TAF in primary CD4+ T lymphocytes (reduced 23-fold and 3-fold, respectively). These data support the co-administration of the tested PIs, with the exception of telaprevir or boceprevir, in combination with TAF, without negatively affecting its clinical pharmacology and intracellular conversion to TFV.

Tenofovir alafenamide showed low cytotoxicity in resting and dividing PBMCs, T-lymphoblastoid cells, and hepatocellular carcinoma (HepG2) cells, and provided > 1997-fold selectivity or greater 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.

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 therapeutic exposure, comparable intracellular 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.

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; Study GS-US-120-0104). Thus, TAF has a low potential for inhibiting mtDNA synthesis and inducing NRTI-related mitochondrial toxicities.

Safety pharmacology studies demonstrated that TAF has no effect on the rat CNS and renal system or dog cardiovascular system. There was reduced gastric emptying in rats dosed at 1000 mg/kg, but not at 100 mg/kg.

FTC/TFV

Combination studies of up to 5 μM FTC and 50 μM TFV had no effect on cell viability and there was no evidence of synergistic cellular toxicity for the combination of FTC and TFV in vitro. The combination of FTC and TFV had no time- or concentration-dependent effects on cytotoxicity or mitochondrial parameters in HepG2 liver cells.

FTC/RPV/TAF

No relevant cytotoxicity or mitochondrial toxicity was observed with FTC, RPV, TAF, or TFV. When tested in vitro at pharmacologically relevant concentrations, the cytotoxicity of TFV in renal proximal epithelial tubule cells (RPTECs) was not affected when combined with FTC. Rilpivirine has shown a low potential for in vitro cytotoxicity in a variety of human cell types. The potential for mitochondrial toxicity of RPV was low by in vitro assessment of the inhibitory activity on human polymerase γ. 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 cytotoxicity or mitochondrial toxicity with the FTC/RPV/TAF combination is low.

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. 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 potential to slightly prolong PR intervals (~13–24%) 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}. No PR prolongation or any change in ECG results occurred in the dog safety pharmacology study that evaluated a TAF dose up to 100 mg/kg (m2.6.3, Section 4.4, D2000006) or in the thorough QT clinical study (m2.7.2, Section 2.2.1.1 [GS-US-120-0107]).

There are no clinically significant overlapping toxicities with FTC, RPV, and TAF; therefore, no additional studies are considered necessary with the FTC/RPV/TAF combination. Overall, the pharmacological assessment of FTC, RPV, and TAF (and TFV) supports the effective use of FTC, RPV, and TAF together in combination therapy for HIV-1 disease.

2. PRIMARY PHARMACODYNAMICS

The primary pharmacodynamic studies evaluating the antiviral activity of FTC, RPV, and TAF are described in detail in the nonclinical virology summary contained in m2.7.2, Section 4.1.1.

2.1. FTC

Emtricitabine 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 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}. The primary pharmacodynamics of FTC are described in detail in the integrated virology summary contained in m2.7.2, Section 4.1.1.

2.2. RPV

Rilpivirine (TMC278) is a nonnucleoside reverse transcriptase (RT) inhibitor that does not require modification for antiviral activity. Crystal structures of the binding site between TMC278 and the HIV-1 RT complex revealed that TMC278, 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}. TMC278 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). The primary pharmacodynamics of RPV are described in detail in the integrated virology summary contained in m2.7.2, Section 4.1.1.

2.3. TAF

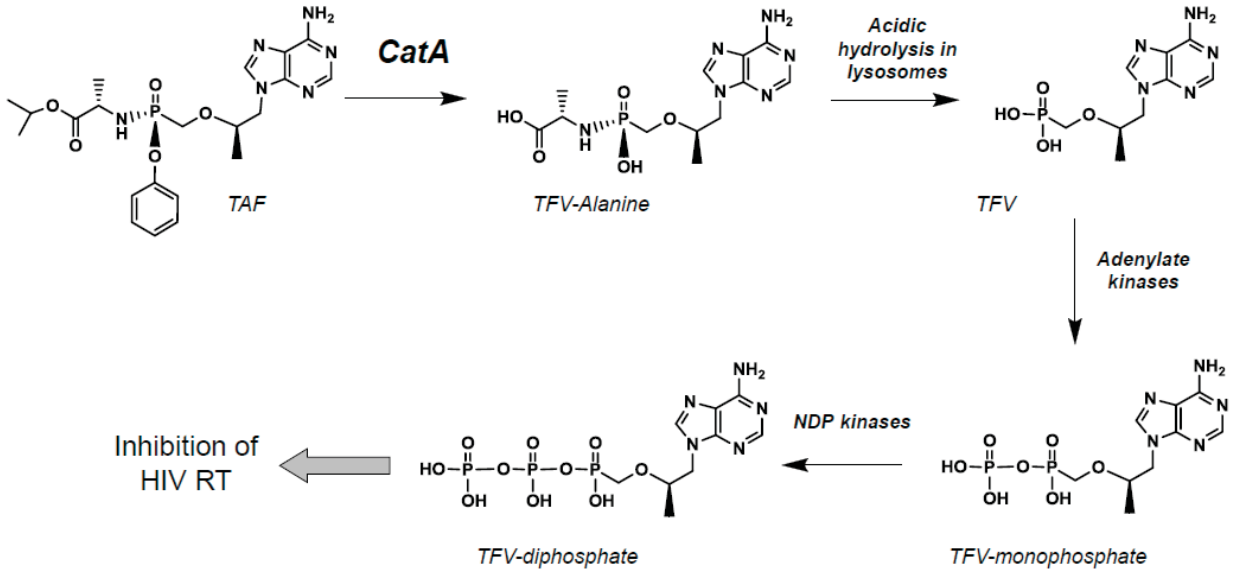
Tenofovir alafenamide is predominantly hydrolyzed to TFV by CatA cleavage in target lymphoid cells and macrophages {13119}, {10427}, resulting in higher intracellular levels of TFV-DP in vivo and lower circulating levels of TFV relative to TDF {17137}. Intracellularly, TFV is metabolized to the active metabolite, TFV-DP {1574}, a competitive inhibitor of HIV-1 RT that terminates the elongation of the viral DNA chain {21}, {1131}. Tenofovir alafenamide 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}.

The intracellular activation and activity of TAF are described below. The primary pharmacodynamics of TAF are described in more detail in the integrated virology summary contained in m2.7.2, Section 4.1.1.

2.3.1. Intracellular Metabolism of TAF

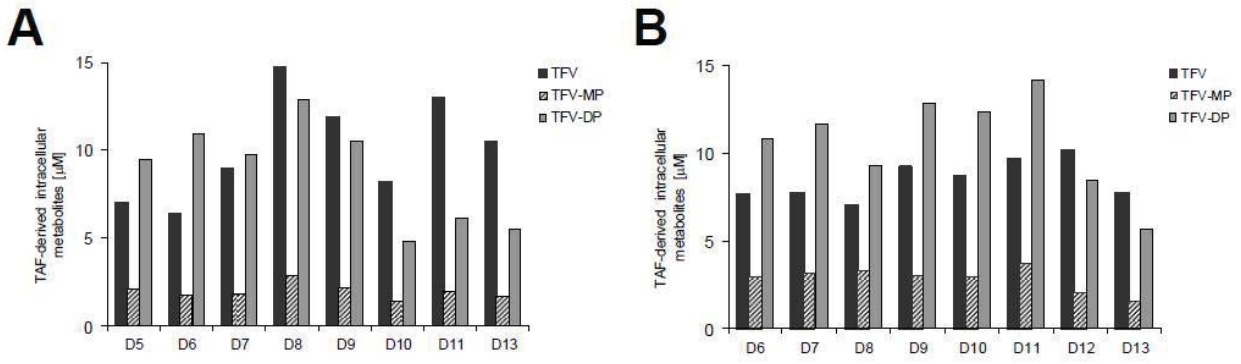
The lysosomal carboxypeptidase CatA plays an essential role in the intracellular activation of TAF in lymphoid cells and tissues (Figure 1). Cathepsin A levels and the intracellular activation of TAF-were evaluated in primary CD4+ T lymphocytes and MDMs isolated from PBMCs from 13 donors of variable gender, age, and ethnicity (m2.6.3, Section 1.3, PC-120-2017). Cathepsin A activity was determined by measuring the rate of conversion of TAF to TFV-alanine in extracts prepared from quiescent and PHA/interleukin-2 (IL-2) activated CD4+ T cells and MDMs from each donor. For both primary cell types, the level of active CatA was similar across the donors. The mean (± standard deviation [SD]) rate of TFV-alanine formation was similar between quiescent and activated CD4+ T cells extracts (2.7 ± 0.9 vs 3.0 ± 0.6 pmol/min•µg, respectively), with ≤ 3-fold differences between donors. Cathepsin A activity was approximately 2-fold greater in MDMs compared with CD4+ T cells. The mean (± SD) rate of TFV-alanine formation was 7.1 ± 3.3 pmol/min•µg in MDMs (range: 3.1 to 13.9 pmol/min•µg across the donors). In both primary cell types, the intracellular accumulation of TAF metabolites and conversion of TAF to TFV-DP were consistent across the 8 demographically diverse donors (Figure 2).

Figure 1. Intracellular Activation of TAF in Lymphoid Cells and Tissues



Cat A = cathepsin A; NDP = nucleoside diphosphate; RT = reverse transcriptase; TAF = tenofovir alafenamide; TFV = tenofovir
Source: Figure copied from {29240}; Report PC-120-2017

Figure 2. Intracellular TAF Metabolites in CD4+ T cells and Monocyte-derived Macrophages from Different Donors



D= Donor; TAF = tenofovir alafenamide; TFV = tenofovir; TFV-MP = tenofovir monophosphate; TFV-DP = tenofovir diphosphate
Following incubation for 4 hours with 1µM TAF, the formation and quantity of intracellular TAF metabolites in CD4+ T cells (A) and MDMs (B) were determined by HPLC combined with mass spectrometry.
Source: Figure copied from {29240}; Report PC-120-2017

2.3.2. Effects of Protease Inhibitors of HIV and HCV, and Host Cell Proteases on Cathepsin A-mediated Activation of TAF

Because certain viral PIs have been shown to be potent inhibitors of CatA, the potential for drug-drug interactions between TAF and antiviral PIs was evaluated in biochemical assays using purified CatA (m2.6.3, Section 1.3, PC-120-2001, {18608}). The HIV PIs DRV, ATV, lopinavir (LPV), and RTV did not inhibit CatA-mediated hydrolysis of TAF up to a concentration of 50 µM, well above the clinical C_{max} of each drug (Table 1). Similarly, HCV PIs TMC-435, BI-201355, MK-5172, GS-9256, and GS-9451 showed little-to-no inhibition of CatA, with IC_{50} values ranging from 25 to > 50 µM. On the other hand, both telaprevir and boceprevir, 2 irreversible inhibitors of the HCV protease, were identified as potent inhibitors of CatA-mediated hydrolysis of TAF, with IC_{50} values of 0.3 and 0.2 µM, respectively. When adjusted for plasma binding, these IC_{50} values are 6- to 8-fold below the clinical maximum concentration (C_{max}) levels observed in patients.

In conclusion, the tested HIV PIs, host serine PIs, and the majority of HCV PIs exhibit minimal potential to interfere with the intracellular activation of TAF. These data support the co-administration of the tested therapeutic PIs, with the exception of telaprevir and boceprevir, in combination with TAF, without negatively affecting its clinical pharmacology and intracellular conversion to TFV.

Table 1. Effects of COBI, HIV-1 Protease Inhibitors, or HCV Protease Inhibitors on CatA-mediated Hydrolysis of TAF

Compound	IC_{50} ± SD (µM)[a]	C_{max} (µM)[b] Total Drug	C_{max} (µM)[c] Free Fraction
COBI or HIV-1 PIs			
DRV	> 50	8.9	1.6
ATV	> 50	6.3	0.7
LPV	> 50	15.2	0.3
RTV	> 50	1.3	0.02
COBI	> 50	2.2	0.2
HCV PIs			
Telaprevir	0.3 ± 0.17	5.2	1.5
Boceprevir	0.2 ± 0.02	3.3	1.3
TMC-435	> 50	13.3	< 0.002
BI-201335	25 ± 7	20	0.08
MK-5172	50	2.5	0.06
GS-9256	> 50	10.5	0.004
GS-9451	50	1.7	0.04

ATV = atazanavir; COBI = cobicistat; DRV = darunavir; HCV = hepatitis C virus; IC_{50} = 50% inhibitory concentration; LPV = lopinavir; PI = protease inhibitor; RTV = ritonavir; SD = standard deviation

a Data represent mean ± SD values from at least 2 independent experiments

b {14702}, {17624}, {3084}, {15365}, {17733}, {15365}, {17653}, {18607}, {18606}, {18605}, {18609}

c Concentration of free drug at C_{max} based on serum protein binding as determined by Gilead Sciences.

Source: Report PC-120-2001

3. SECONDARY PHARMACODYNAMICS

The antiviral activity of FTC, RPV, and TAF against other viruses is described in detail in the nonclinical virology summary contained in m2.7.2, Section 4.1.2.

The remaining secondary pharmacodynamic effects of FTC, RPV, TAF (and TFV) and FTC/TFV are described below in Sections 3.1 to 3.5.

3.1. FTC

3.1.1. Effect on Cellular DNA Polymerases

The inhibition of human HeLa cell DNA polymerases α, β, γ, and ε by FTC-TP was examined under steady-state conditions (m2.7.2, Section 4.1 [TEZZ/93/0007]) {4541}. Activated calf thymus DNA was used as the template for analysis of each enzyme. Under these conditions, FTC-TP was a weak inhibitor of each of the human DNA polymerases when compared with HIV-1 RT. Apparent K_i values were 6.0 μM for polymerase α; 17 μM for polymerase β; 6.0 μM for polymerase γ; and 150 μM for polymerase ε.

Inhibition of human DNA polymerase γ (Pol γ) is one of the proposed mechanisms for nucleoside analogue-derived toxicity. Therefore the potential for FTC-TP and lamivudine (3TC) 5'-triphosphate to serve as substrates for Pol γ was investigated using pre steady-state kinetics (m2.7.2, Section 4.1 [TPI 9501]). For dCTP (the natural substrate), FTC-TP, and 3TC 5'-triphosphate, the order of incorporation efficiency is dCTP (k_{pol}/K_d = 40 $\mu M^{-1}s^{-1}$) > 3TC 5'-triphosphate (k_{pol}/K_d = 0.014 $\mu M^{-1}s^{-1}$) > FTC-TP (k_{pol}/K_d = 0.0006 $\mu M^{-1}s^{-1}$). The low rate of incorporation and poor binding affinity of FTC-TP makes it the least favorable substrate for Pol γ in this analysis.

3.1.2. In Vitro Receptor Binding Potencies

The effects of FTC (524W91) on the specific binding of various radioactively labeled ligands were studied in 19 different receptor binding assays (m2.6.3, Section 1.4, TPZZ/93/0002). Tissues from Sprague Dawley rats were obtained in all but 2 assays. A heart preparation was obtained from beagle dogs for use as a calcium release channel-binding assay, and platelets which were isolated from New Zealand White rabbits were used for a platelet activating factor assay. Emtricitabine had no pharmacologically significant binding affinity to the receptors tested.

3.1.3. In Vitro Autonomic Pharmacology Effects on Peripheral Autonomic Receptors

A variety of isolated muscle preparations were used in vitro to assess effects of FTC on autonomic function and peripheral receptors (m2.6.3, Section 1.4, TPZZ/92/0055). Emtricitabine (0.1 μM or 1.0 μM) had little or no direct effect on various isolated muscle preparations and had no major inhibitory effects on the contractile responses to acetylcholine, norepinephrine, serotonin, isoproterenol, arachidonic acid, histamine, bradykinin, and angiotensin II.

3.1.4. In Vitro Cytotoxicity

3.1.4.1. Cytotoxicity in Human Cells

The cytotoxicity of FTC has been evaluated extensively in vitro. In all cell lines examined, cell growth was not affected at concentrations of FTC up to 200 μM. Results are shown in Table 2.

Table 2. Cytotoxicity of FTC in Comparison to 3TC and ZDV

Cells	CC_{50} (μM)		
	FTC	3TC	ZDV
MT-4	> 100[a], > 200[b]	> 100[a], > 33[b]	20[a], > 100[b]
PBMC	> 100[a]	> 100[a]	> 100[a]
CEM	> 100[a], > 100[b]	> 100[a], > 100[b]	14.3[a], > 6[b]
Vero	> 100[a]	> 100[a]	28.0[a]
IM9	> 100[b]	> 100[b]	70[b]
Molt 4	> 100[b]	> 100[b]	10[b]
HepG2 2.2.15	> 200[b], > 200[c]	> 200[b]	> 200[b]

3TC = lamivudine; FTC = emtricitabine; ZDV = zidovudine
a Data from reference: {4533}
b Data from reference: {4531}
c Data from reference: {4535}

Because of the apparent correlation between toxicity to bone marrow progenitor cells in vitro and bone marrow suppression in vivo, human bone marrow progenitor colony-forming assays were performed. The concentration of FTC required to inhibit the formation of granulocyte-macrophage colonies by 50% (CC_{50}) was 300 ± 40 μM (n = 6). The CC_{50} for erythroid colonies was 220 ± 8 μM (n = 6).

3.1.5. Mitochondrial Toxicity

As a variety of clinical symptoms observed in patients with HIV treated with prolonged NRTI therapy may be linked to mitochondrial toxicity, the potential for mitochondrial toxicity of FTC was evaluated. HepG2 cells were incubated with FTC at concentrations ranging between 0.1 and 10 μM for 2 weeks (m2.6.3, Section 1.4, {4550}), and MT-2 cells were incubated with FTC at concentrations up to 100 μM for up to 8 weeks (m2.6.3, Section 1.4, TPI 11963). Under these conditions, FTC had no adverse effects on cell growth, mtDNA synthesis, or lactic acid production. In a separate study conducted in HepG2 cells exposed to concentrations of FTC ranging from 0.1 to 10 μM for 7 days, no effects on mitochondrial morphology were observed by transmission electron microscopy (m2.6.3, Section 1.4, 233 {6081}).

The inhibition of mitochondrial DNA synthesis was also assessed in an in vitro cell culture assay using Molt-4 cells (a T-lymphoblast cell line). The ratio of mitochondrial to cellular DNA was determined after exposure of cells to clinically relevant concentrations of FTC (0.1, 1, 10, and 100 μM) (m2.6.3, Section 1.4, TGZZ/93/0016 and TGZZ/93/0023). The ratio of mtDNA to genomic DNA for Molt-4 cells treated with several nucleoside analogues is shown in (Table 3).

Treatment of Molt-4 cells with various concentrations of zalcitabine (ddC) resulted in a reduction of the mtDNA content of the cells. After 5 days of treatment with 0.05 μM ddC, there was an 80% reduction in the ratio of mitochondrial to cellular DNA; however, there was no apparent increase in doubling time of the treated cells (data not shown). At 0.5 μM ddC, there was a 92% reduction in the ratio of mitochondrial to cellular DNA.

Emtricitabine did not reduce the ratio of mitochondrial to cellular DNA when tested at concentrations of up to 100 μM after 7 days of continuous cell exposure.

Table 3. Ratio of Mitochondrial DNA to Cellular DNA in Molt-4 Cells

Compound	N	Concentration (μM)	Days	Mitochondrial DNA/Cellular DNA (Percent of Control)
Zalcitabine (ddC)	2	0.05	5	20 ± 10
		0.1	5	11 ± 8
		0.5	5	8 ± 1
		5.0	5[a]	ND
Emtricitabine (FTC)	2	0.1	7	104 ± 10
		1.0	7	121 ± 11
		10.0	7	98 ± 15
		100.0	7	123 ± 19
Zidovudine (ZDV)	1	0.5	9	130
		5.0	9	174
Fialuridine (FIAU)	2	0.1	7	82 ± 27
		0.5	7	89 ± 37
		5.0	2[a]	173 ± 78
Alovudine (FLT)	1	0.05	7	41
		0.1	7	17
		0.5	7	14
		5.0	5[a]	7
Stavudine (d4T)	1	0.5	9	113
		5.0	9	60

ND = not determined
a Cell death
b Standard deviation, n > 1
Source: Reports TGZZ/93/0016 and TGZZ/93/0023

Thymidine analogues were also examined. After 7 days of treatment with 0.05 μM FLT (alovudine, 3'-dideoxy-3'-fluorothymidine), there was a 59% reduction in the ratio of mitochondrial to cellular DNA. After 9 days exposure to 5 μM d4T (stavudine, (2R,5S)-1-(2,5-dihydro)-5-(hydroxymethyl)-2-furyl thymidine), there was a 40% reduction in the ratio of

mitochondrial to cellular DNA. In contrast, fialuridine (FIAU) caused no reduction in the ratio of mitochondrial to cellular DNA after 7 days exposure to 0.1 or 0.5 μM of drug; however, significant cell death was noted after exposure to 5 μM FIAU for 2 days. Zidovudine (3'-azido-3'-deoxythymidine, ZDV) at 0.5 or 5.0 μM caused no significant decrease in the ratio of mitochondrial to cellular DNA after 9 days exposure. It should be noted that cell death occurred at 5 μM ddC, FLT, and FIAU. Cell death was not noted in ZDV- or FTC-treated cells at concentrations up to 100 μM.

The lack of impact of FTC on mitochondrial DNA content and function is consistent with its very low affinity for γ polymerase (Section 3.1.1).

3.2. RPV

3.2.1. Effect on Cellular DNA Polymerases

TMC278 had no effects on DNA synthesis by human polymerase α, β, or γ at concentrations up to 1000 μM as determined by PCR (m2.6.3, Section 1.5, TMC278-1646_0005343).

3.2.2. In Vitro Receptor Binding Potencies and Neurotransmitter Uptake Inhibition

The interaction of TMC278 base with 19 receptors in tissue or cellular preparations was studied at a concentration of 10 μM (3.7 μg/mL) in buffer containing 1% DMSO (m2.6.3, Section 1.5, TMC278-870219). TMC278 did not cause any significant inhibition of binding to α- or β-adrenergic, dopaminergic, muscarinergic, serotonergic, opioid, interleukin, or chemokine receptors.

No agonistic or antagonistic activity of TMC278 was noted on histamine H2 receptors in the isolated guinea pig right atrium at 30 μM (11 μg/mL) (m2.6.3, Section 1.5, TMC278-NC204[2]). No inhibition of adenosine triphosphatase (ATPase) by TMC278 was evident in isolated pig stomach at 10 μM (3.7 μg/mL) (m2.6.3, Section 1.5, TMC278-NC204[3]). The incubation in these 2 studies was done in buffer containing 0.1% DMSO.

3.2.3. In Vivo Effect on Gastric Antisecretory Activity

TMC278 was evaluated for possible gastric antisecretory activity in the pentagastrin-stimulated gastric acidity assay (m2.6.3, Section 1.5, TMC278-NC204[1]). Three groups of 5 male Wistar rats fasted overnight were treated intraperitoneally with TMC278 at 9.1 mg/kg at a volume of 5 mL/kg in vehicle (2% Tween 80 in 0.9% NaCl in water), or with the positive control cimetidine (10 mg/kg). Thirty minutes later, gastric acid production was stimulated by intraperitoneal injection of 5 μg/kg pentagastrin dissolved in distilled water. TMC278 caused a 20% increase in gastric acidity compared to vehicle. The positive control reduced acidity by 74%. The significance of the slightly increased gastric acidity effect by TMC278 is considered limited.

3.2.4. In Vitro Cytotoxicity

3.2.4.1. Cytotoxicity in Human Cells

The in vitro effects of TMC278 on the cell proliferation and the viability of HeLa (epithelial cervix, adenocarcinoma), HepG2 (epithelial liver, hepatoblastoma), HEp-2 (epithelial cervix adenocarcinoma), MRC-5 (normal fetal lung fibroblast), and A549 (epithelial lung carcinoma) cells were investigated (m2.6.3, Section 1.5, TMC278-IV2-AVMR). EFV, ETR, and NVP were tested in the same assays.

The CC_{50} value (as a measure of inhibition of cell proliferation) was determined as the concentration of a compound that resulted in a drop of 50% cell growth compared to the control. The median CC_{50} values of TMC278, EFV, ETR, and NVP on the different cells were measured at Day 3 and Day 5. The data presented in Table 4 show that the median CC_{50} values for TMC278 range between 17.34 to 34.51 µM (6.35 to 12.65 µg/mL) and between 16.90 to 35.59 µM (6.19 to 13.04 µg/mL) at Day 3 and Day 5, respectively. Median CC_{50} values for EFV, ETR, and NVP are all > 40 µM, except for NVP on MRC-5 cells.

Table 4. In Vitro TMC278 and NNRTI CC_{50} Values

Cells	Median CC_{50} (µM) (IQR)			
	TMC278	**EFV**	**ETR**	**NVP**
Day 3				
A549	25.48 (25.05 - 25.69)	> 40	> 40	> 40
HeLa	17.34 (17.22 - 17.37)	> 40	> 40	> 40
HEp-2	34.38 (31.88 - 35.43)	> 40	> 40	> 40
HepG2	33.18 (32.52 - 33.21)	> 40	> 40	> 40
MRC-5	34.51 (32.84 - 34.86)	> 40	> 40	33.03 (32.02 - 33.74)
Day 5				
A549	26.70 (26.60 - 27.52)	> 40	> 40	> 40
HeLa	16.90 (16.41 - 18.51)	> 40	> 40	> 40
HEp-2	26.27 (25.46 - 28.88)	> 40	> 40	> 40
HepG2	27.13 (26.99 - 30.83)	> 40	> 40	> 40
MRC-5	35.59 (35.34 - 37.79)	> 40	> 40	35.96 (35.09 - 36.03)

Cell lines (MRC-5, HepG2, HeLa, HEp-2, and A549) were seeded one day before compound addition (Day −1), in flat-bottom 96-well plates. Cells were incubated at 37°C in a 5% CO_2 atmosphere in the presence of 2-fold serial dilutions of the compounds. Rezasurin (50 µl) was added for 6 hours incubation at 37°C. The CC_{50} of TMC278 was estimated by the rezasurin assay on Day 0 (control), Day 3, and Day 5. The experiments were performed in triplicate. IQR = interquartile range

The TC_{50} value (as a measure of inhibition of cell viability) was defined as the concentration of a compound that resulted in a 50% reduction in cell growth on Day 3 or Day 5 compared to Day 0. Median TC_{50} values of TMC278, EFV, ETR, and NVP on the different cells were determined at Day 3 and Day 5. The data presented in Table 5 show that the median TC_{50} values for TMC278

range between 33.94 to > 40.00 µM (12.44 to > 14.66 µg/mL) and between 31.93 to > 40.00 µM (11.70 to > 14.66 µg/mL) at Day 3 and Day 5, respectively. Median TC50 values for EFV, ETR, and NVP at Day 3 and Day 5 are all > 40 µM.

Table 5. In Vitro TMC278 and NNRTI TC_{50} Values

Cells	Median TC_{50} (µM) (IQR)			
	TMC278	EFV	ETR	NVP
Day 3				
A549	> 40	> 40	> 40	> 40
HeLa	33.94 (33.89 - 34.17)	> 40	> 40	> 40
HEp-2	> 40	> 40	> 40	> 40
HepG2	> 40	> 40	> 40	> 40
MRC-5	> 40	> 40	> 40	> 40
Day 5				
A549	> 40	> 40	> 40	> 40
HeLa	31.93 (31.77 - 32.65)	> 40	> 40	> 40
HEp-2	> 40	> 40	> 40	> 40
HepG2	> 40	> 40	> 40	> 40
MRC-5	> 40	> 40	> 40	> 40

Cell lines (MRC-5, HepG2, HeLa, HEp-2, and A549) were seeded one day before compound addition (Day −1), in flat-bottom 96-well plates. Cells were incubated at 37°C in a 5% CO_2 atmosphere in the presence of 2-fold serial dilutions of the compounds. Rezasurin (50 µl) was added for 6 hours incubation at 37°C. The CC_{50} of TMC278 was estimated by the rezasurin assay on Day 0 (control), Day 3, and Day 5. The experiments were performed in triplicate.

Median CC_{50} values for TMC278, EFV, ETR, and NVP on MT-4-LTR-EGFP cells were measured at Day 3. The median CC_{50} for TMC278 was 5.91 µM, whereas it was > 32 µM for EFV, ETR, and NVP (Table 6).

Table 6. In Vitro TMC278 and NNRTI CC_{50} Values on MT-4

Cells	Median CC_{50} (µM) (IQR) (n)			
	TMC278	EFV	ETR	NVP
MT-4	5.91 (5.02–8.31) (381)	40.02 (36.46–43.06) (805)	> 64.00 (820)	> 32.00 (457)

A selectivity index (ratio of the CC_{50} to the EC_{50} value);(EC_{50} = 0.73 nM and CC_{50} = 5.91 µM) of 8096 was calculated in MT-4 cells, indicating that TMC278 is a potent and selective inhibitor of HIV-1 in vitro (m2.6.3, Section 1.5, TMC278-IV1-AVMR).

3.3. TAF

3.3.1. Effect of TFV Diphosphate on Cellular DNA Polymerases

As described in Section 2.3, TAF is predominantly hydrolyzed to TFV by CatA cleavage in target lymphoid cells {13119}, {10427}, resulting in high intracellular levels of TFV-DP in vivo {17137}. The in vitro specificity of TFV-DP for viral polymerases relative to its interaction with mammalian DNA polymerases was determined.

Table 7 summarizes the inhibitory effects of TFV-DP on DNA synthesis catalyzed by the mammalian DNA polymerases α, β, and γ, and by the rat DNA polymerases δ and ε {1131}, {2516}. The K_m for the natural substrate dATP is also shown. TFV-DP showed specificity for HIV-1 RT with K_i/K_m ratios 4- to 170-fold higher for mammalian DNA polymerases compared with HIV-1 RT. The K_i/K_m ratio was very high (85.3) for mtDNA polymerase γ, suggesting a low potential of TFV to interfere with the synthesis of mtDNA {1131}. Additional studies have shown that 1 mM TFV-DP exhibited little effect on the in vitro replication of SV40 DNA indicating a significant specificity of TFV-DP toward the viral RT in comparison to the host DNA replication complex {2517}. Similar conclusions of strong specificity of TFV-DP toward HIV-1 RT have been made using pre-steady state enzyme kinetic experiments {2518}.

Table 7. Kinetic Inhibition Constants of TFV-DP Against DNA Polymerases α, β, γ, δ, and ε Versus HIV-1 Reverse Transcriptase

Enzyme	K_i (μM)	K_m dATP (μM)	K_i/K_m
Human DNA pol α	5.2	2.7	1.92
Human DNA pol β	81.7	5.6	14.6
Human DNA pol γ	59.5	0.7	85.3
Rat DNA pol δ/PCNA	7.1	0.7	10.2
Rat DNA pol ε	95.2	6.1	15.6
HIV-1 RT	0.21	0.42	0.50

DNA = deoxyribonucleic acid; PCNA = proliferating cell nuclear antigen; pol = polymerase; RT = reverse transcriptase
Data from references: {1131}, {2516}

In order to evaluate TFV-DP as a potential substrate for host polymerases, its incorporation efficiency into a DNA primer/template by human DNA polymerases α, β, and γ relative to the natural dNTPs has been determined and compared with that of the triphosphates of other NRTIs {13797}. TFV-DP showed similar or lower incorporation by DNA polymerases α and β compared with ddATP (the active metabolite of didanosine [ddI]), ddCTP, 3TC-TP, and d4T-TP (Table 8). Importantly, DNA pol γ incorporates TFV-DP into a DNA primer/template with a very low efficiency (0.06%) relative to the natural substrate. This observation confirms the conclusions from the inhibition studies above that TFV-DP has a low potential for host polymerase inhibition.

Table 8. Relative Efficiencies of Incorporation into DNA of TFV-DP and NRTI-Triphosphates by Human DNA Polymerases α, β, and γ

dNTP Analog	Relative Efficiency of Incorporation (%)[a]		
	Pol α	Pol β	Pol γ
TFV-DP	1.4	1.3	0.06
ddATP	0.25	80	20
ddCTP	0.1	125	25
3TC-TP	0.05	9.0	0.13
d4T-TP	6.3	142	8.0

dNTP = 2'-deoxynucleoside triphosphates
a Relative efficiency of incorporation (%) = 100 × $[V_{max}(\text{dNTP analog})/K_m(\text{dNTP analog})]/[V_{max}(\text{dNTP})/K_m(\text{dNTP})]$.
Data from reference: {2005}

3.3.2. In Vitro Receptor Binding Potencies of TDF and TFV

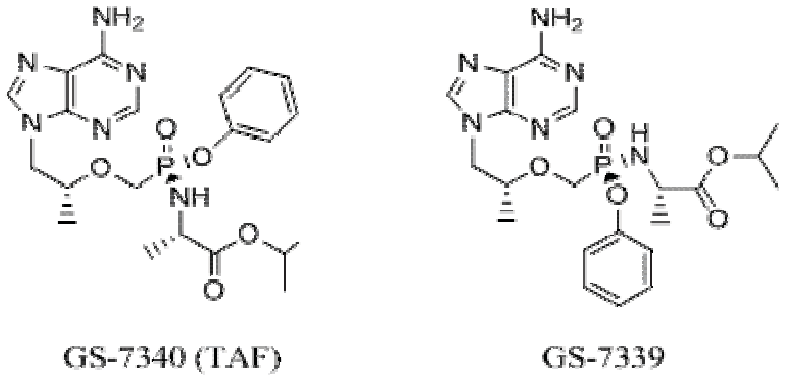
A primary screen was used to determine the effect of the major metabolite of TAF, TFV, and the other prodrug of TFV, TDF, on the inhibition or stimulation of binding in a series of 111 protein targets (neuroreceptors, ion channels, transporters, and nuclear receptors) (m2.6.3, Section 1.6, V2000020). The protein target was incubated in the presence of 10 µM TFV or TDF. The effect on the binding of the endogenous ligand was then determined. Responses of > 50% stimulation or inhibition were considered significant. There was no significant inhibition or stimulation of ligand binding to its protein target by either TFV or TDF. The results of this study demonstrate that neither TFV nor TDF significantly interacts with any of the 111 protein targets tested.

3.3.3. In Vitro Cytotoxicity

3.3.3.1. Cytotoxicity in Human PBMCs and Cell Lines

The cytotoxicity profiles (CC_{50} values) of TAF, its stereoisomer GS-7339 (Figure 3), TDF, and TFV were investigated in resting and dividing human PBMCs following 5 days of continuous drug incubation (m2.6.3, Section 1.6, PC-120-2009). The maximum concentrations of drugs used were 100, 100, 50, and 2000 µM, for TAF, GS-7339, TDF, and TFV, respectively. Notably, TAF is only present at significant levels in the systemic circulation for less than 2 hours {25765}; therefore TAF doses used in this in vitro study were supra-therapeutic in concentration and duration. CC_{50} values for TAF ranged from 6.8 µM in dividing PBMCs to 25.1 µM in resting PBMCs (Table 9), leading to a high SI of > 1,900 in dividing PBMCs when compared with the EC_{50} value of 3.6 nM (m2.7.2, Section 4.1.1., PC-120-2004). The higher CC_{50} value obtained for GS-7339 compared with TAF indicates an even lower potential for cytotoxicity for the diastereomer and is supported by the limited conversion of the stereoisomer to TFV-DP {7415}. Overall, TAF showed a favorable toxicity profile in resting and dividing PBMCs.

Figure 3. Chemical Structures of the Diastereomers TAF and GS-7339



Source: Report PC-120-2009

Table 9. In Vitro Cytotoxicity of TAF, GS-7339, TDF, and TFV in Resting and Dividing PBMCs

Class	Drug	Cytotoxicity CC_{50} (µM)[a]	
		Resting PBMCs	Dividing PBMCs
NtRTI	TAF	25.1 ± 11.5	6.8 ± 1.8
	GS-7339	> 124.6	> 186.2
	TDF	69.7 ± 22.1	19.6 ± 5.2
	TFV	> 2652	2150 ± 532

CC_{50} = drug concentration that results in a 50% reduction in cell viability; NtRTI = nucleotide reverse transcriptase inhibitor; PBMC = peripheral blood mononuclear cell; TAF = tenofovir alafenamide; TDF = tenofovir disoproxil fumarate; TFV = tenofovir
a Mean ± SD values from PBMCs isolated from up to 9 donors.
Source: Report PC-120-2009

The cytotoxicity profiles (CC_{50} values) of TAF, TDF, TFV, and a panel of clinically relevant antiretroviral inhibitors were also evaluated in 2 T-lymphoblastoid cell lines (MT-2 and MT-4) following 5 days of exposure (m2.6.3, Section 1.6, PC-120-2007). TAF had no observed cellular toxicity up to the highest tested compound concentration (53 µM) in the MT-2 cells. CC_{50} values for TAF ranged from 23.2 to > 53.0 µM in the 2 T-lymphoblastoid cell lines (Table 10). Based on observed CC_{50} and EC_{50} values, TAF exhibited SIs relative to antiviral activity of 1997 to > 3607 in T-lymphoblastoid cell lines, which was consistent with the results in PBMCs described above. Overall, TAF showed low cytotoxicity, and had a similar cytotoxicity profile in T-cell lines compared with other clinically relevant antiretroviral inhibitors.

The cytotoxicity profiles (CC_{50} values) of TAF, TDF, TFV, and a panel of clinically relevant antiretroviral inhibitors were also evaluated in a hepatic cell line (HepG2) following 5 days of exposure (m2.6.3, Section 1.6, PC-120-2007). The CC_{50} value for TAF was > 44.4 µM in the hepatic cell line (Table 10). TAF had no observed cellular toxicity up to the highest tested compound concentration (44.4 µM) in HepG2 cells. Overall, TAF had a similar cytotoxicity profile in hepatic cells compared with other clinically relevant antiretroviral inhibitors.

Table 10. In Vitro Cytotoxicity of TAF and other HIV Inhibitors in Human T-Lymphoblastoid and Hepatic Cell Lines

Class	Drug	Cytotoxicity CC_{50}, µM (MSD)[a]		
		Hepatic	T-Cell	
		HepG2	MT-2	MT-4
NtRTI	TAF	>44.4 (1)	>53.0 (1)	23.2 (1.13)
	TDF	>44.4 (1)	37.1 (1.02)	22.9 (1.04)
	TFV	>44.4 (1)	7605 (1.06)	6264 (1.13)
NRTI	FTC	>44.4 (1)	>53.0 (1)	>53.0 (1)
	3TC	>44.4 (1)	>53.0 (1)	>53.0 (1)
	ABC	>44.4 (1)	40.7 (1.02)	>53.0 (1)
	ZDV	>44.4 (1)	>53.0 (1)	>53.0 (1)
	ddI	>44.4 (1)	>53.0 (1)	>53.0 (1)
	ddC	>44.4 (1)	>53.0 (1)	>53.0 (1)
NNRTI	EFV	10.1 (1.05)	25.4 (1.03)	26.4 (1.04)
INSTI	RAL	>44.4 (1)	>53.0 (1)	>53.0 (1)
PI	ATV	>44.4 (1)	>53.0 (1)	>53.0 (1)
Control	PUR[b]	1.0 (1.12)	0.4 (1.05)	0.2 (1.06)

3TC = lamivudine; ABC = abacavir; ATV = atazanavir; CC_{50} = drug concentration that results in a 50% reduction in cell viability; ddC = zalcitabine; ddI = didanosine; DRV = darunavir; EFV = efavirenz; FTC = emtricitabine; INSTI = integrase strand transfer inhibitor; NNRTI = nonnucleoside reverse transcriptase inhibitor; NRTI = nucleoside reverse transcriptase inhibitor; NtRTI = nucleotide reverse transcriptase inhibitor; PI = protease inhibitor; RAL = raltegravir; TAF = tenofovir alafenamide; TDF = tenofovir disoproxil fumarate; TFV = tenofovir; ZDV = zidovudine

a All cells were treated for 5 days. Cytotoxicity CC_{50} values represent geometric of independent experiments (n=3) generated using 384-well assays. Multiplicative standard deviations (MSD) are shown in parenthesis.

b Puromycin (PUR) was used as a positive control in cytotoxicity assays.

Source: Report PC-120-2007

3.3.3.2. Hematopoietic Toxicity

3.3.3.2.1. Hematopoietic Toxicity of TAF

The effects of TAF were investigated on human myeloid and erythroid progenitor cells using bone marrow from 3 human donors (m2.6.3, Section 1.6, PC-120-2016). Two TAF exposure conditions were evaluated: a continuous incubation for 14 days and a 12-hour pulse incubation with washout for 14 days. The continuous incubation was evaluated as a comparator (the standard procedure for these assays). The 12-hour pulse with washout was evaluated to better mimic the limited in vivo TAF plasma exposure (T_{max} = 38 minutes, $t_{1/2}$ = 25 minutes) at concentrations significantly above the mean TAF plasma C_{max} of 0.484 µM observed at steady state in clinical studies {25765}. The effects of continuous and 12-hour pulse incubation (in liquid media) with TAF on human erythroid and myeloid progenitor proliferation cultured in MethoCult™ 84434 media using 3 different prequalified frozen bone marrow lots were examined.

In the continuous incubation, the extrapolated IC_{50} was 3.3 µM for erythroid progenitor cell proliferation; the IC_{50} was > 3 µM for myeloid progenitor cell proliferation. Overall, TAF showed IC_{50} values in the continuous incubation in a range similar to other primary cells, such as activated PBMCs (IC_{50} = 6.8 µM; m2.6.3, Section 1.6, PC-120-2009). In the 12-hour pulse incubation exposure, the TAF IC_{50} was > 3 µM (the highest concentration tested) for erythroid and myeloid progenitor cell proliferation. Therefore, the IC_{50} values for TAF under continuous and pulsed exposure conditions were at least 7-fold higher than the clinical C_{max}, supporting a favorable profile in myeloid and erythroid progenitor cells.

Table 11. In Vitro Hematopoietic Toxicity of TAF in Comparison with 5-Fluorouracil

Bone Marrow Lot	Continuous Incubation				12-Hour Pulse Incubation			
	Erythroid IC_{50} (µM)		Myeloid IC_{50} (µM)		Erythroid IC_{50} (µM)		Myeloid IC_{50} (µM)	
	TAF	5-FU	TAF	5-FU	TAF	5-FU	TAF	5-FU
BM07B21195	> 3	3.20	3.30[ex]	3.97	> 3	20.34	> 3	51.41
BM10A33225	> 3	3.89	3.92[ex]	2.49	> 3	118.80[ex]	> 3	91.15
BM10AOF3062	> 3	4.09	>3	1.06	> 3	69.43	> 3	67.05

5-FU = 5-fluorouracil; ex = extrapolated value
Source: Report PC-120-2016

3.3.3.2.2. Hematopoietic Toxicity of TFV

The hematopoietic toxicity of TFV and 4 other NRTIs (ZDV, d4T, ddC, and 3TC) was evaluated in human $CD34^+$ bone marrow progenitor stem cells exposed to specific cytokines, which programmed their differentiation and expansion into the erythroid and myeloid lineages {4077}. The expansion of the 2 lineages in the presence of the tested drugs was determined with progenitor cells from 2 independent donors by immunofluorescence detection of lineage-specific cell surface markers. Irrespective of the donor, TFV, at concentrations as high as 200 µM, showed no significant effect on the expansion of the erythroid lineage from the progenitor stem cells as determined by the level of expression of glycophorin A (Table 12). Likewise, TFV showed only limited effects on the expansion of the myeloid lineage based on the expression of CD11b, with the inhibition being more pronounced in progenitor cells from donor 2 (CC_{50} of 85 µM). Likewise, 3TC exhibited only a weak cytotoxicity against both the erythroid and myeloid lineages, with a moderate degree of inhibition observed at a concentration of 200 µM. In contrast, ZDV and d4T produced notable suppression of the erythroid and myeloid lineage expansion. Zalcitabine caused by far the most severe suppression of both the erythroid and myeloid lineages with CC_{50} values ranging from < 0.06 to 0.38 µM.

Table 12. In Vitro Hematopoietic Toxicity of TFV in Comparison with Other NRTIs

Drug	Hematopoietic Toxicity – CC_{50} [µM][a]			
	Myeloid Lineage		Erythroid Lineage	
	Donor 1	Donor 2	Donor 1	Donor 2
TFV	> 200	85	> 200	> 200
ZDV	49	3.6	0.85	0.62
d4T	200	10.5	5.0	3.3
ddC	0.38	0.24	0.14	< 0.06
3TC	> 200	140	> 200	170

3TC = lamivudine; CC_{50} = drug concentration that results in a 50% reduction in cell viability; d4T = stavudine; ddC = zalcitabine; TFV = tenofovir; ZDV = zidovudine

a Concentration of each drug inhibiting production of the myeloid or erythroid lineage from progenitor stem cells by 50%. The results are from a single experiment performed in triplicate.

Data from reference: {4077}

3.3.3.3. Renal Transporter-Dependent Cytotoxicity

The cytotoxicity of TAF and TFV was assessed in human HEK293T cells transiently expressing OAT1 and OAT3 (m2.6.3, Section 1.6, PC-120-2018). Cells were incubated with serial dilutions of TFV or TAF for 4 days. TFV was more cytotoxic in OAT1- and OAT3-expressing cells compared with control transporter null cells (> 21- and > 3.6-fold change in CC_{50} values, respectively) (Table 13). Due to greater cellular permeability, the cytotoxicity of TAF was greater than TFV in control cells. However, there was little to no change in cytotoxicity associated with TAF in OAT1- and OAT3-expressing cells compared with control transporter null cells (0.5- to 3.5-fold change in CC_{50} values, respectively). The minor increase in CC_{50} value for TAF in OAT3-expressing cells compared with control cells was not associated with an increase in TAF intracellular levels, and similar changes in cytotoxicity were observed for puromycin and gemcitabine (other drugs that are not transported by OAT3). 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). Taken together, these results indicate that TAF does not interact with the renal transporters OAT1 or OAT3, and exhibits no OAT-dependent cytotoxicity in human epithelial kidney cells transiently expressing these transporters.

Table 13. In Vitro Cytotoxicity of TAF and TFV in the Presence and Absence of Organic Anion Transporters 1 and 3 in Human Epithelial Kidney Cells

Compound	CC_{50} (μM)[a] (Fold Change)[b]			HIV-1 EC_{50} (μM)[c]	Selectivity Index (OAT1)[d]	Selectivity Index (OAT3)[d]
	Control Cells	OAT1-Expressing Cells	OAT3-Expressing Cells			
TFV	> 2000 (1.0)	94 ± 71 (> 21.3)	553 ± 174 (> 3.6)	6.7 ± 2.2	14	82
TAF	163 ± 42 (1.0)	319 ± 56 (0.5)	47 ± 17 (3.5)	0.011 ± 0.003	29,000	4270

CC_{50} = drug concentration that results in a 50% reduction in cell viability; OAT = organic anion transporter; TAF = tenofovir alafenamide; TFV = tenofovir

a Data represent mean ± SD from 5 independent experiments performed in triplicate.

b Control CC_{50}/OAT CC_{50}

c EC_{50} values were calculated 3 days after infection of activated primary human CD4+ T lymphocytes with pseudotyped HIV-1 containing a luciferase reporter and incubation with serial dilutions of TFV or TAF.

d CC_{50}/EC_{50}

Source: Table modified from {29239}; Report PC-120-2018

3.3.3.4. Renal Proximal Tubule Epithelial Cells

Effects of TFV have been studied in several in vitro models for renal proximal tubular toxicity and compared with those of cidofovir and adefovir in order to better understand the in vivo differences in nephrotoxicity observed between the 3 structurally related nucleotide analogs. In normal human RPTECs, TFV showed a negligible effect on cell growth with a CC_{50} of > 2,000 μM (Table 14). Moreover, TFV did not exhibit any marked effect on the long-term viability of quiescent RPTECs during a 25-day incubation (Table 14). In contrast, the half-life of quiescent RPTECs in the presence of cidofovir and adefovir was approximately 10 and 21 days, respectively (m2.6.3, Section 1.6, P4331-00037). In a separate study, TFV did not cause significant changes in cell viability in RPTECs after 22 days at concentrations up to 300 μM {9864}.

Integrity of the proximal tubule epithelium is essential for maintaining the selective barrier between blood and urine. As shown in Table 14, TFV at concentrations as high as 3 mM did not significantly affect the in vitro integrity of the differentiated proximal tubule epithelium when assessed by measuring the transepithelial resistance after a 10-day drug incubation {2520}. By comparison, cidofovir and adefovir reduced the tubular epithelium integrity by 50% at 105 μM and 1.2 mM, respectively.

Human renal OAT1, a protein localized in the basolateral membrane of the renal proximal tubule epithelium, has been implicated in the etiology of cidofovir- and adefovir-associated nephrotoxicity {2087}. OAT1 has also been shown to induce the cytotoxicity of TFV by enhancing its intracellular accumulation in kidney cells. Unlike TFV, TAF does interact with and is not a substrate for OAT1 {29239}.

Transport kinetics experiments revealed similar transport efficiency (calculated as V_{max}/K_m ratio) for cidofovir, adefovir, and TFV (Table 14) {2520} suggesting that a lack of interference with essential intracellular function(s) rather than a difference in renal transport is responsible for the improved nephrotoxicity profile of TFV.

Table 14. Profiles of TFV, Cidofovir, and Adefovir in In Vitro Models of Renal Proximal Tubular Toxicity

In vitro Assay	Tenofovir	Cidofovir	Adefovir
Inhibition of RPTECs growth; CC_{50} [µM][a]	> 2,000	260	495
Viability of RPTECs; $t_{1/2}$ [days][b]	> 25	9.7	21
Integrity of RPTEC epithelium; $CTER_{50}$[c] [µM]	> 3,000	110	1,100
Efficiency of human OAT1-mediated transport [V_{max}/K_m]	3.26	1.77	1.93

CC_{50} = drug concentration that results in a 50% reduction in cell viability; OAT1 = human organic anion transporter 1; RPTEC = renal proximal tubule epithelial cell
a CC_{50} was determined after 4 days incubation.
b In the presence of 500 µM drug.
c $CTER_{50}$, concentration reducing the transepithelial resistance of RPTEC monolayer cultured on microporous membrane by 50%. Epithelium integrity was evaluated after 10 days incubation.
Source: Report P4331-00037, {2520}

3.3.3.5. Primary Osteoblasts

Antiretrovirals, including TDF, have been associated with decreases in bone mineral density in clinical studies; therefore, the cytotoxic effect of clinically relevant TAF concentrations on PBMCs and primary osteoblasts was assessed in vitro (m2.6.3, Section 1.6, PC-120-2008). Drug loading studies with PBMCs determined that a 2-hour pulse and washout of 124 to 370 nM of TAF achieved intracellular TFV-DP levels comparable to those observed in vivo with a 25 mg dose of TAF. This dosing of TAF in vitro also aligned with the in vivo TAF plasma C_{max} of 484 nM. Comparable TFV-DP levels were achieved in primary osteoblasts with 3 days of daily 2-hour pulses at TAF concentrations similar to those used for PBMCs (100 to 400 nM).

No change in cell viability was observed in either primary osteoblasts or PBMCs. The mean TAF CC_{50} in primary osteoblasts with a 2-hour pulse and washout was > 500 µM, which is > 1033 times higher than the TAF plasma C_{max} (Table 15). In contrast, the cytotoxicity of 2 PIs, nelfinavir (NFV) and LPV, were 3.4 and 1.8 times higher than their respective C_{max} values (not adjusted for protein binding). In summary, primary osteoblasts were not preferentially loaded by TAF relative to PBMCs, and achieved comparable TFV-DP levels as PBMCs in vitro. Furthermore, there was no change in osteoblast or PBMC viability at clinically relevant TAF concentrations. In contrast, the CC_{50} values of the HIV-1 PIs NFV and LPV were only 3.4- and 1.8-fold higher than their respective C_{max} values (not taking into account plasma protein binding). Both of these PIs are documented to exhibit toxicity in cell lines and are associated with BMD decreases in vivo {17724}, {14194}.

Table 15. In Vitro Cytotoxicity of TAF in Primary Osteoblasts

Clinical Data		Osteoblast In Vitro Assay Data				Ratio
Drug	**C_{max} (µM)**	**Drug**	**Treatment**	**N**	**CC_{50} (µM)[a]**	**CC_{50}/C_{max}**
TAF 25 mg QD	0.484 (TAF)[b]	TAF	2-hour pulse	5	>500	>1033
	0.05 (TFV)[b]	TFV	Continuous	4	>1000	>20000
TDF 300 mg QD	1 (TFV)[c]	TFV	Continuous	4	>1000	>1000
NFV 1250 mg BID	7 (NFV)[d]	NFV	Continuous	4	23.5 ± 4.5	3.4
LPV 800 mg QD[e]	18.7 (LPV)[f]	NFV	Continuous	4	33.5 ± 3.8	1.8

CC_{50} = drug concentration that results in a 50% reduction in cell viability; LPV = lopinavir; NFV = nelfinavir; TAF = tenofovir alafenamide; TDF = tenofovir disoproxil fumarate
a Mean ± SD
b {20420}
c {26885}
d {27712}
e Boosted with RTV 200 mg
f {25768}
Source: Report PC-120-2008

3.3.3.6. TAF metabolites/degradants

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 (m2.6.3, Section 1.6, 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.

3.3.4. Mitochondrial Toxicity

HIV-infected patients treated with NRTIs have exhibited a range of clinical side effects including myopathy, sensory neuropathy, lactic acidosis, and hepatic steatosis {7180}, {5552}. It is believed that NRTI-induced mitochondrial toxicity plays a major role in these adverse symptoms. Many NRTIs, such as ddC, ddI, and d4T, can cause the depletion of mtDNA in cells due to the inhibition of mtDNA polymerase γ {3320}, {7538}. In contrast, previous studies have demonstrated a minimal effect of TFV on the mitochondrial DNA synthesis in vitro {3320}, {7538}.

3.3.4.1. Effect of TAF on Mitochondrial DNA Content

The potential for TAF to induce mtDNA depletion was evaluated in HepG2 cells (m2.6.3, Section 1.6, PC-120-2006). A quantitative real-time polymerase chain reaction assay was performed to measure the relative levels of mtDNA in HepG2 cells treated with the drug. In this assay, HepG2 cells treated with TAF (0.1, 0.3, or 1.0 µM) for 10 days exhibited no significant reduction in mtDNA compared with untreated cells (Table 16). In contrast, cells treated with ddC

(0.2, 2.0, or 20.0 μM) exhibited a dose-dependent decrease in mtDNA content. These data are consistent with the established lack of inhibition of the mitochondrial DNA polymerase γ by the active metabolite TFV-DP and suggest that TAF has a low potential for inhibiting mtDNA synthesis and inducing NRTI-related mitochondrial toxicities.

Table 16. Effect of TAF on Mitochondrial DNA Levels in HepG2 Cells

Drug	Concentration (μM)	Relative Amount of mtDNA (% mtDNA)[a]	p-value compared with DMSO (Control)[b]
DMSO (control)	-	100.0 ± 15.3	-
TAF	0.1	86.4 ± 30.5	0.190
	0.3	88.1 ± 35.5	0.294
	1.0	94.6 ± 17.3	0.318
ddC	0.2	86.7 ± 24.2	0.127
	2.0	11.5 ± 6.2	< 0.0001
	20.0	6.6 ± 1.5	< 0.0001

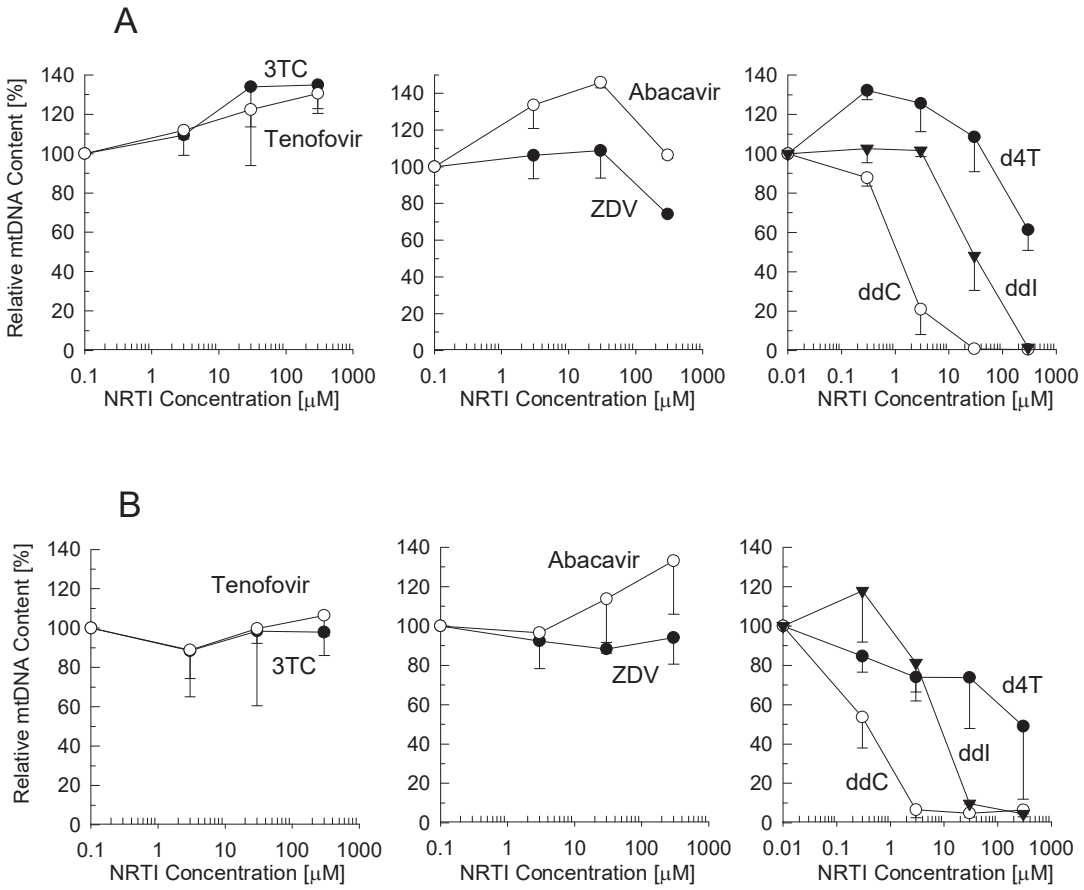
ddC = zalcitabine; DMSO = dimethylsulfoxide; mtDNA = mitochondrial DNA; TAF = tenofovir alafenamide
a Data represent the mean ± SD of 3 independent experiments performed in triplicate.
b Paired, 2-tailed Student's t-test
Source: Report PC-120-2006

3.3.4.2. Effect of TFV on the Synthesis of Mitochondrial DNA

Hybridization analyses to quantify mitochondrial DNA and chromosomal DNA levels were performed to assess any relative impairment in mtDNA synthesis with TFV. In HepG2 cells following a 9-day treatment with TFV at concentrations ranging from 3 to 300 mM, no effect of TFV on the synthesis of mtDNA was observed (Figure 4 A). In contrast, ddC and ddI showed marked depletion of mtDNA in HepG2 cells. Stavudine and ZDV showed less pronounced effects, with a reduction of relative mtDNA content of 30% to 40% at 300 μM. Similar to TFV, 3TC and ABC did not significantly change the relative levels of mtDNA (Table 17; m2.6.3, Section 1.6, P1278-00042).

Treatment of skeletal muscle cells (SkMCs) with TFV and other NRTIs yielded very similar conclusions. Proliferating SkMCs did not show any decrease in mtDNA levels following a 9-day incubation with up to 300 μM of TFV (Figure 4 B). Lamivudine, ABC, and ZDV also did not deplete mtDNA in these cells. Stavudine moderately reduced mtDNA, while ddC and ddI showed marked depletion of mtDNA in SkMCs. Similar effects of the tested drugs, including no depletion of mtDNA by TFV, were observed upon a 3-week treatment of quiescent nonproliferating SkMCs (Table 17; m2.6.3, Section 1.6, P1278-00042).

Figure 4. Effect of TFV and Other NRTIs on Mitochondrial DNA Content in Human Liver and Skeletal Muscle Cells



HepG2 human liver cells (A) and human skeletal muscle cells (B) were incubated with various drug concentrations for 9 days and DNA content was determined by hybridization analysis. Data are the means ± standard deviation from two independent experiments.
Source: Report P1278-00042

Effects of the drug on mtDNA in human RPTECs were also characterized. As shown in Table 17, no changes in relative mtDNA levels were observed following up to a 21-day treatment of differentiated RPTECs with TFV or ZDV. While d4T produced minor decrease in mtDNA content, ddC and ddI showed the most pronounced effects on mtDNA in RPTECs (Table 17; m2.6.3, Section 1.6, P1278-00042). In a separate study, levels of mtDNA or cytochrome c oxidase II (COXII) mRNA were not affected by TFV treatment of RPTECs for 22 days at concentrations up to 300 μM {9864}.

Table 17. Effect of TFV and Other NRTIs on Mitochondrial DNA Content in Differentiated Human Renal Proximal Tubular Epithelial Cells

Drug	Drug Concentration [μM]	Content of mtDNA [% of control][a]	
		12 days	21 days
TFV	300	118 ± 5.9	109 ± 9.2
ZDV	200	109 ± 3.0	104 ± 14.7
d4T	200	92.3 ± 13.9	77.0 ± 10.9
ddC	2	12.1 ± 1.1	5.1 ± 0.6
ddI	40	47.5 ± 5.3	25.7 ± 0.1

d4T = stavudine; ddC = zalcitabine; ddI = didanosine; mtDNA = mitochondrial DNA; TFV = tenofovir; ZDV = zidovudine
a Relative content of mtDNA in RPTECs after 12- and 21-day drug treatment given as mean ± standard deviation from a representative experiment performed in duplicates.
Source: Report P1278-00042

Overall, the relative effects of the drugs on mtDNA content were similar in all human cell types tested, and correlated well with the efficiencies of incorporation into DNA of the respective dNTP analogs by DNA polymerase γ determined in vitro (see Section 3.3.1).

Production of Lactic Acid

Lactic acid production is one of the widely used mitochondrial markers. Drug-related deficiencies in the mitochondrial oxidative phosphorylation system may induce a shift in the pyruvate/lactate ratio leading to increased production of lactic acid {2522}. As shown in Table 18, TFV does not increase the lactic acid production in HepG2 cells and SkMCs after 3- and 6-day incubations, respectively, relative to the untreated controls. Similarly, no effect was observed with 3TC. However, ZDV produced a concentration-dependent increase in the lactate production in both cell types tested (m2.6.3, Section 1.6, P1278-00042). Lactic acid production appears to be one of the few methods by which mitochondrial toxicity can be detected for ZDV in vitro. There were no measurable increases with TFV at concentrations up to 300 μM, and the likelihood of TFV causing clinical lactic acidosis is low.

Table 18. Effects of TFV and Other NRTIs on the In Vitro Production of Lactic Acid

Drug	Concentration (µM)	Lactic Acid Production (mg /10^6 cells)[a]	
		HepG2 cells[b]	SkMCs[b]
None	--	1.61 ± 0.25 (100)	7.53 ± 0.83 (100)
TFV	30	1.34 ± 0.18 (83)	7.23 ± 1.21 (96)
	300	1.62 ± 0.06 (101)	8.79 ± 1.97 (116)
ZDV	30	2.26 ± 0.04 (141)	10.39 ± 0.56 (138)
	300	3.32 ± 0.05 (207)	21.94 ± 4.04 (291)
3TC	30	1.92 ± 0.67 (119)	7.29 ± 1.47 (97)
	300	1.94 ± 0.14 (121)	8.13 ± 0.95 (108)

3TC = lamivudine; TFV = tenofovir; ZDV = zidovudine
a Extracellular lactate production given as a mean ± standard deviation from a representative experiment performed in duplicate. Data in parentheses represent percentage change from the no drug control.
b HepG2 cells and SkMCs were incubated with drugs for 3 and 6 days, respectively.
Source: Report P1278-00042

3.4. FTC/TFV

3.4.1. In Vitro Cytotoxicity

The combination of TFV and FTC has also been studied in vitro for potential synergistic cellular toxicity in the MT-2 cell line (m2.6.3, Section 1.7, PC-164-2002). In combination studies of up to 5 µM FTC and 50 µM TFV, no effect on cell viability was observed using a XTT-based enzymatic assay. In all drug combinations, cell viability values were > 90% of controls that did not contain any drugs. Thus, there was no evidence of synergistic cellular toxicity for the combination of TFV with FTC in vitro. These results are in agreement with the high CC_{50} values for each individual drug (> 100 µM) and the overall high selectivity ratios for the individual drugs with reference to their anti-HIV-1 activities (> 100-fold; see m2.7.2, Section 4.1).

3.4.1.1. In Vitro Cytotoxicity in Human Renal Proximal Tubule Epithelial Cells

The potential in vitro cytotoxicity of TFV alone or in combination with FTC (+COBI and EVG) was investigated in primary human RPTECs, either (m2.6.3, Section 1.7, PC-236-2012). Following a 5-day treatment, TFV was not cytotoxic to RPTECs from 2 independent donors at the highest concentrations tested (4000 µM) using either a cell viability or a lactate dehydrogenase release (LDH) readouts (Table 19). Tenofovir in combination with FTC (+COBI and EVG) did not affect the cytotoxicity of TFV in RPTECs when tested at concentrations corresponding to their respective peak plasma levels in HIV-infected patients treated with a clinical dose of each compound.

Table 19. Effect of COBI, FTC, and EVG on the Cytotoxicity of TFV in Human RPTECs

Compound	CC_{50} (µM)[a]	
	Cell Viability	LDH Release
TFV	> 4000	> 4000
COBI	26.2 ± 5.3	39.4 ± 0.8
EVG	13.7 ± 0.1	32.7 ± 0.1
FTC	> 100	> 100
TFV + COBI (2 µM)[b] + EVG (4.5 µM)[b] + FTC (8 µM)[b]	> 4000	> 4000
TFV + COBI (0.06 µM)[c] + EVG (1.2 µM)[c] + FTC (0.49 µM)[c]	> 4000	> 4000

LDH = lactate dehydrogenase

a The results represent mean ± SD from 4 independent experiments performed in RPTECs from 2 separate donors. CC_{50} values were determined in parallel from both cell viability (Cell Titer Glo) and from lactate dehydrogenase release readouts.

b The tested concentrations of COBI, EVG, and FTC correspond to peak plasma levels (C_{max}) in HIV-infected patients treated with a clinical dose of each compound.

c The tested concentrations of COBI, EVG, and FTC correspond to trough plasma levels (C_{min}) in HIV-infected patients treated with a clinical dose of each compound.

Source: Report PC-236-2012

3.4.1.2. In Vitro Cytotoxicity in Human Embryonic Kidney Cells Transiently Expressing Renal Transporters

The potential effect of FTC (+COBI and EVG) on the cytotoxicity of TFV was investigated in an in vitro model consisting of human embryonic kidney 293T cells co-expressing renal transporters OAT1 and MRP4, which are known to mediate TFV active renal secretion (m2.6.3, Section 1.7, PC-236-2013). Tenofovir cytotoxicity was measured in these cells either alone or in combination with FTC (+COBI and EVG). Following a 4-day treatment, TFV showed minimal cytotoxicity in control cells that did not express the renal transporters (CC_{50} > 2000 µM) (Table 20).
The cytotoxicity of TFV in cells expressing OAT1 was markedly increased due to its active intracellular accumulation (CC_{50} = 78.7 ± 1.3 µM). The OAT1-mediated cytotoxicity of TFV was reduced upon co-expression of MRP4 in the same cells due to increased efflux of the drug (CC_{50} = 299.5 ± 81.3 µM). Combination of TFV with either COBI alone or FTC (+COBI and EVG) at their pharmacologically relevant concentrations showed no significant change in the cytotoxicity of TFV in cells transiently expressing OAT1 alone, or OAT1 and MRP4 together.

Table 20. Effect of FTC (+COBI and EVG) on the Cytotoxicity of TFV in 293T Human Embryonic Kidney Cells Transiently Expressing Renal Transporters

Transient Gene Expression	Cytotoxic Effect, CC_{50} (µM)[a]			
	Puromycin	TFV	TFV + COBI (2.1 µM)[b]	TFV + COBI (2.1 µM)[b] + EVG (4.5 µM)[b] + FTC (8.2 µM)[b]
Negative Control	0.35 ± 0.02	> 2000	> 2000	> 2000
OAT1	0.21 ± 0.10	78.7 ± 1.3	68.3 ± 0.4	68.0 ± 4.1
OAT1 + MRP4	0.22 ± 0.06	299.5 ± 81.3	230.9 ± 82.6	228.8 ± 51.2

MRP4 = multidrug resistance protein 4; OAT1 = organic anion transporter 1
a The results represent mean ± SD from 3 independent experiments performed in transiently transfected 293T cells. CC_{50} values were determined from a cell viability (Cell Titer Glo) readout.
b The tested concentration of FTC, COBI, and EVG correspond to their respective peak plasma levels (C_{max}) in HIV-infected patients treated with a clinical dose of each compound.
Source: Report PC-236-2013

3.4.2. Mitochondrial Toxicity

In vitro combination studies have been conducted in HepG2 cells to evaluate the potential mitochondrial toxicity of FTC and TFV (as well as other nucleosides) (m2.6.3, Section 1.7, TX-104-2001). HepG2 cells were exposed to FTC and TFV (as well as other nucleosides), either alone or in combination. HepG2 cells were treated for up to 25 days with concentrations of NRTIs equal to 1 and 10 times the maximal therapeutic plasma levels. Assay endpoints included cell growth, extracellular production of lactic acid, relative cellular content of mtDNA and mtDNA-encoded COX II, and intracellular lipid accumulation.

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

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

3.5. TFV

Given the lack of relevant effects in the aforementioned in vitro studies which included the individual agents (FTC, TFV, or TAF), no additional secondary pharmacodynamics studies have been conducted for TFV alone.

3.6. FTC/RPV/TAF

Given the lack of relevant effects in the in vitro studies with the individual agents (FTC, RPV, TFV, or TAF) or with the combination of FTC/TFV, no additional secondary pharmacodynamic studies have been conducted or are necessary for the FTC/RPV/TAF combination.

4. SAFETY PHARMACOLOGY

In vitro and in vivo safety pharmacology data for FTC, RPV, and TAF are presented in Sections 4.1 to 4.3. As discussed in Section 4.4, additional safety pharmacology studies on the FTC/RPV/TAF combination are considered unwarranted.

4.1. FTC

4.1.1. Overt Pharmacodynamic Effects

In vivo studies on the central nervous system (mice and rats), cardiovascular system (rats and dogs), respiratory system (mice, rats and dogs), renal system (rats), and gastrointestinal system (mice) were conducted. The studies were conducted prior to the implementation of ICH S7A (published in November 2000), but followed good scientific practices and established methods and protocols.

4.1.1.1. Mice

In a single-dose modified Irwin screen with toxicity observations, male ICR mice (10/dose) were given FTC orally at 0 (distilled water), 10, 30, or 100 mg/kg in a dose volume of 20 mL/kg, then observed for 7 days to collect data on behavioral effects (m2.6.3, Section 4.2, 477). Emtricitabine did not affect behavior at any dose. In a subsequent single-dose general pharmacology study, male CD-1 mice (4/dose) were given FTC orally at 0 (0.5% MC), 100, 250, 500, 750, or 1000 mg/kg, then observed for 7 days to collect data on body weight, rectal temperature, and behavior (m2.6.3, Section 4.2, TPZZ/93/0001). Emtricitabine did not affect body weight, rectal temperature, or behavior at any dose.

4.1.1.2. Rats

In a single-dose general pharmacology study, male CD (SD) rats (4/dose) were given FTC orally at 0 (0.9% saline), 250, 500, or 1000 mg/kg, then observed for 7 days to collect data on body weight, rectal temperature, and behavior (m2.6.3, Section 4.2, TPZZ/93/0001). Emtricitabine did not affect body weight, rectal temperature, or behavior at any dose. In a separate single-dose study, male Wistar rats (5/dose) were given FTC orally at 0 (distilled water), 10, 30, or 100 mg/kg in a dose volume of 10 mL/kg (m2.6.3, Section 4.2, 477). Rectal temperature was measured before dosing at intervals up to 2 hours postdose. Again, FTC did not affect rectal temperature at any dose.

4.1.2. Central Nervous System

4.1.2.1. Mice

In a single-dose modified Irwin screen with toxicity observations, male ICR mice (10/dose) were given FTC orally at 0 (distilled water), 10, 30, or 100 mg/kg, then observed for 7 days to collect data on neurological and autonomic effects (m2.6.3, Section 4.2, 477). Emtricitabine did not produce neurological or autonomic effects at any dose. In a subsequent single-dose general

pharmacology study, male CD-1 mice (4/dose) were given FTC orally at 0 (0.5% MC), 100, 250, 500, 750, or 1000 mg/kg, then observed for 7 days to collect data on reflexes (m2.6.3, Section 4.2, TPZZ/93/0001). Emtricitabine did not affect reflexes at any dose.

In a single-dose spontaneous locomotor activity study, male ICR mice (8/dose) were given FTC orally at 0 (distilled water), 10, 30, or 100 mg/kg (m2.6.3, Section 4.2, 477). Spontaneous activity was recorded at 15-minute intervals for 2 hours postdose. Emtricitabine did not affect spontaneous locomotion at any dose.

In a single-dose motor coordination (rotorod) study, male ICR mice (10/dose) were given FTC orally at 0 (distilled water), 10, 30, or 100 mg/kg (m2.6.3, Section 4.2, 477). Ability to remain on a rotating rod was evaluated at 0.5, 1, and 2 hours postdose. Emtricitabine did not affect motor coordination at any dose.

In a single-dose hexobarbital potentiation (sleeping time) study, male ICR mice (10/dose) were given FTC orally at 0 (distilled water), 10, 30, or 100 mg/kg, and then anesthetized with hexobarbital 1 hour later (m2.6.3, Section 4.2, 477). Time to recovery from anesthesia (return of righting reflex) was measured. Emtricitabine did not affect duration of anesthesia at any dose.

In a single-dose anticonvulsant activity study, male ICR mice (10/dose) were given FTC orally at 0 (distilled water), 10, 30, or 100 mg/kg, then given a maximal electrical shock at 1 hour postdose (m2.6.3, Section 4.2, 477). The occurrence of death or tonic/clonic convulsions was recorded. Emtricitabine did not affect mortality and had no anticonvulsant activity at any dose. In a second single-dose anticonvulsant activity study, male ICR mice (10/dose) were given FTC orally at 0, 10, 30, or 100 mg/kg, then given metrazole at 100 mg/kg at 1 hour postdose (m2.6.3, Section 4.2, 477). The occurrence of death or tonic/clonic convulsions was recorded. Again, FTC did not affect mortality and had no anticonvulsant activity at any dose.

In a single-dose proconvulsant activity study, male ICR mice (10/dose) were given FTC orally at 0 (distilled water), 10, 30, or 100 mg/kg, then given a subthreshold electrical shock at 1 hour postdose (m2.6.3, Section 4.2, 477). The occurrence of death or tonic/clonic convulsions was recorded. Emtricitabine did not affect mortality and had no proconvulsant activity at any dose. In a second single-dose proconvulsant activity study, male ICR mice (10/dose) were given FTC orally at 0 (distilled water), 10, 30, or 100 mg/kg, then given metrazole at 70 mg/kg at 1 hour postdose (m2.6.3, Section 4.2, 477). The occurrence of death or tonic/clonic convulsions was recorded. Again, FTC did not affect mortality and had no proconvulsant activity at any dose.

In a single-dose analgesic activity study, male ICR mice (10/dose) were given FTC orally at 0 (distilled water), 10, 30, or 100 mg/kg (m2.6.3, Section 4.2, 477). The time to tail-flick response to an uncomfortable heat stimulus was recorded before and 1 hour after dosing. Emtricitabine had no analgesic activity at any dose. In a second single-dose analgesic activity study, male ICR mice (10/dose) were given FTC orally at 0 (distilled water), 10, 30, or 100 mg/kg, then given phenylquinone at 2 mg/kg at 1 hour postdose (m2.6.3, Section 4.2, 477). The number of writhes occurring during the next 10 minutes was recorded. Again, FTC had no analgesic activity at any dose. In a subsequent single-dose general pharmacology study, male CD-1 mice (4/dose) given FTC orally at 0 (0.5% MC), 100, 250, 500, 750, or 1000 mg/kg were evaluated for analgesia by the tail-flick test (m2.6.3, Section 4.2, TPZZ/93/0001). Once again, FTC had no analgesic activity at any dose.

4.1.2.2. Rats

In a single-dose general pharmacology study, male CD (SD) rats (4/dose) were given FTC orally at 0 (0.9% saline), 250, 500, or 1000 mg/kg, and then observed for 7 days to collect data on reflexes and analgesia (m2.6.3, Section 4.2, TPZZ/93/0001). Emtricitabine did not affect reflexes and had no analgesic activity at any dose.

In a single-dose conditioned avoidance response study, trained ovariectomized female Long-Evans rats (6/dose) were given FTC by intraperitoneal injection at 0 (0.5% MC), 30, or 100 mg/kg, then tested for their ability to respond to an audio-visual cue to avoid foot-shock (m2.6.3, Section 4.2, TPZZ/93/0119). Emtricitabine did not affect conditioned avoidance response at any dose.

4.1.3. Cardiovascular System

4.1.3.1. Effects on Isolated Cardiac Muscle of Rat, Guinea Pig, and Cat

Emtricitabine was tested on different cardiac preparations to determine its effect on cardiac function in vitro (m2.6.3, Section 4.1, TPZZ/92/0056). Parameters measured following treatment with 1 μM FTC included: cardiac chronotropy (rat and guinea pig), cardiac inotrophy (cat and guinea pig), and the incidence of ventricular arrhythmias (rat). Results from these in vitro studies suggested that FTC was free of negative cardiac effects at 1 μM.

4.1.3.2. Rats

In a single-dose cardiovascular effects study, conscious male Wistar rats (5/dose) were given FTC orally at 0 (distilled water), 10, 30 or 100 mg/kg (m2.6.3, Section 4.2, 477). Heart rate and arterial blood pressure were measured before dosing and at 5, 30, and 60 minutes postdose. Emtricitabine did not affect heart rate or blood pressure at any dose. In a second single-dose cardiovascular effects study, conscious male CD (SD) rats (8/dose) were given FTC orally at 0 (0.5% MC) or 250 mg/kg (m2.6.3, Section 4.2, TPZZ/92/0057). Heart rate and blood pressure were measured at intervals for 4 hours postdose. Again, FTC did not affect heart rate or blood pressure.

4.1.3.3. Dogs

In a single-dose cardiovascular and respiratory effects study, 4 anesthetized male beagle dogs were given FTC intravenously (formulated in 5% dextrose in water) as consecutive bolus injections of 1.0, 2.5, 5, 10, and 20 mg/kg (cumulative dose = 38.5 mg/kg) over an hour, then monitored for 30 more minutes (m2.6.3, Section 4.2, TPZZ/92/0076). The average plasma concentration of FTC at 30 minutes postinfusion was 34.6 mg/mL. Heart rate, arterial blood pressure, and lead II ECG were measured at intervals, and blood pressure responses to norepinephrine, acetylcholine, carotid artery occlusion, and vagal nerve stimulation were evaluated 30 minutes after the last dose. Emtricitabine did not affect the ECG, cardiovascular function parameters, or blood pressure response to stimuli at any dose or time point.

4.1.4. Respiratory System

4.1.4.1. Mice

In a single-dose general pharmacology study, male CD-1 mice (4/dose) were given FTC orally at 0 (0.5% MC), 100, 250, 500, 750, or 1000 mg/kg, then observed for 7 days to collect data on respiratory rate (m2.6.3, Section 4.2, TPZZ/93/0001). Emtricitabine did not affect respiratory rate at any dose.

4.1.4.2. Rats

In a single-dose general pharmacology study, male CD (SD) rats (4/dose) were given FTC orally at 0 (0.9% saline), 250, 500, or 1000 mg/kg, and then observed for 7 days to collect data on respiratory rate. Emtricitabine did not affect respiratory rate at any dose (m2.6.3, Section 4.2, TPZZ/93/0001).

4.1.4.3. Dogs

In a single-dose cardiovascular and respiratory effects study, 4 anesthetized male beagle dogs were given FTC intravenously as consecutive bolus injections of 1, 2.5, 5, 10, and 20 mg/kg (cumulative dose = 38.5 mg/kg) over an hour, then monitored for 30 more minutes (m2.6.3, Section 4.2, TPZZ/92/0076). The average plasma concentration of FTC at 30 minutes postinfusion was 34.6 mg/mL. Respiratory rate and respiratory minute volume were measured at intervals. Emtricitabine did not affect respiratory function parameters at any dose or time point.

4.1.5. Renal System

In a single-dose renal function study, male Long Evans-derived rats (6/dose) were given FTC orally at 0 (distilled water), 10, 30, or 100 mg/kg and urine was collected for 6 hours postdose to measure pH, volume, and electrolyte concentrations (m2.6.3, Section 4.2, 477). Emtricitabine did not affect urine output, pH, or electrolyte excretion at any dose.

4.1.6. Gastrointestinal System

In a single-dose GI motility study, male ICR mice (10/dose) were given FTC orally at 0 (distilled water), 10, 30, or 100 mg/kg (m2.6.3, Section 4.2, 477), then given a charcoal suspension orally at 1 hour postdose and killed 15 minutes later to record intestinal transit of charcoal. Emtricitabine did not affect GI motility at any dose.

4.2. RPV

4.2.1. Central Nervous System

4.2.1.1. Rats

TMC278 was tested in a modified test according to Irwin {9454} in Sprague Dawley rats (5 males/group) in order to evaluate the neurofunctional integrity (m2.6.3, Section 4.4, TMC278-Exp5560). TMC278 base, dissolved in PEG400 with citric acid, was administered

orally by gavage in a volume of 10 mL/kg at 40, 120, and 400 mg/kg. Mortality, observations of motor-affective and sensori-motor behavior and neurologic and autonomic functioning upon manipulation and made at cage side, general clinical signs, and body weight were evaluated during a 7-day observation period following the single oral administration.

No mortality was seen in any group and no adverse clinical signs were noted up to 400 mg/kg. 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. 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.

Data from a separate toxicokinetic study in male rats with the same formulation and oral doses (m2.6.7, FK 4243 [Addendum to TMC278-Exp5535]) indicated that T_{max} values were reached within 3 hours (40 mg/kg) or within 8 hours (120 and 400 mg/kg) after dosing. Mean C_{max} values were 1.6, 2.8, and 9.9 µg/ml at 40, 120, and 400 mg/kg, respectively (m2.6.5, Section 4.2.6).

4.2.1.2. Dogs

4.2.1.2.1. Instrumented, Awake Dogs

Behavioral effects of a single oral dose of 20 mg/kg of TMC278 base dissolved in PEG400 were assessed upon cage-side observations in instrumented awake dogs (m2.6.3, Section 4.4, TMC278-CPF654).

TMC278 had no overt effects on behavior. The median maximum plasma concentration of TMC278 was 1.5 µg/mL.

4.2.1.2.2. Telemetered Conscious Dogs

Four telemetered male beagle dogs were dosed orally via gavage at escalating doses of 0 (vehicle), 20, 80, and 160 mg/kg of TMC278 base dissolved in PEG400 plus citric acid (m2.6.3, Section 4.4, TMC278-Exp5555), with 1 week dosing intervals.

TMC278 did not influence locomotor activity at cage side observations over 12 hours following dosing. For exposure data, see Section 4.2.2.3.3.

4.2.2. Cardiovascular System

Additional cardiovascular safety studies were conducted to investigate the mechanism of action of the delayed onset of the prolongation of the QT-interval corrected for heart rate according to Fridericia {15639} (QTcF) noted in the thorough clinical QTc-prolongation (TQT) study, TMC278-TiDP6-C131 (C131). In this study, TMC278 was given to healthy subjects for 11 days. Another objective of the additional nonclinical studies was to evaluate the proarrhythmic potential of TMC278 at doses that gave QT-prolongation.

As a result of duration-related reduced responses to TMC278 in some of the in vitro studies, recovery of freshly prepared solutions of TMC278 base in the buffers used in these studies was evaluated using [^{14}C]TMC278 base. The radioactive concentration of a TMC278 base stock solution of 10 mM (10 MBq/mL) in DMSO was 99.1% of the theoretical radioactive concentration. However, the radioactive concentration of a solution of 10 µM and 3 µM of TMC278 base in Tyrode buffer containing 0.1% and 0.03% DMSO was 84.7% and 58.0% of the theoretical radioactive concentration, respectively (TMC278-NC338). Further studies (TMC278-TiDP6-NC381) showed that recovery could be significantly improved by adding 5% bovine serum albumin to the solutions, indicating that the low recovery in the serum-free conditions was likely due to adsorption of TMC278 base to the equipment. This conclusion is in line with the high (more than 99%) degree of plasma protein binding of TMC278 in all species including man (see m2.6.4, Section 4.1.2). As the in vitro models were validated with protein-free incubation fluids, it was decided not to add serum albumin. The results of the studies are presented with the nominal bath concentrations.

4.2.2.1. In Vitro

Chinese hamster ovary (CHO) cells were stably transfected with the hERG encoding for the I_{Kr} channel (m2.6.3, Section 4.3, TMC278-CPF730). These cells were incubated with TMC278 base at nominal bath concentrations of 0.1, 0.3, and 3 µM (0.037, 0.111, and 1.11 µg/mL) in buffer containing 0.1 % DMSO. Astemizole and terfenadine were used as positive controls.

TMC278 caused a concentration-dependent inhibition of I_{Kr} ranging from 10% at 0.1 µM (0.037 µg/mL) to 80% at 3 µM (1.11 µg/mL).

4.2.2.2. Guinea Pigs

4.2.2.2.1. Cardiac Contractility in Isolated Guinea Pig Right Atrium

Rate and force of (spontaneous) contraction (RC and FC, respectively) and effective refractory period (ERP), defined as the frequency of external stimulations that does not elicit continuously a contraction, were evaluated in 2 series of experiments with isolated spontaneously beating right atrium of guinea pigs. In the first series, incubations occurred with TMC278 base at increasing concentrations of 0.01, 0.03, and 0.1 µM (0.004, 0.011, and 0.037 µg/mL) in buffer containing 0.01%, 0.03%, 0.1% DMSO, respectively. In the second series, increasing concentrations of 1, 3, and 10 µM (0.369, 1.11, and 3.69 µg/mL) TMC278 base in buffer containing 1%, 3%, and 10% DMSO were tested (m2.6.3, Section 4.3, TMC278-N168576). The ERP was determined after the highest concentration in each of the series was applied.

TMC278 caused a concentration-dependent decrease of the rate of spontaneous contractions to 86%, 72%, and 44% of baseline at 1, 3, and 10 µM (0.369, 1.11, and 3.69 µg/mL), respectively. The compound at 0.1 and 10 µM (0.037 and 3.69 µg/mL) had no effects on ERP compared to vehicle. TMC278 did not cause any effects on the force of the spontaneous contraction compared to vehicle over the full range of concentrations.

4.2.2.2.2. Anesthetized Guinea Pigs

In this assay, anesthetized guinea pigs were used to evaluate the effects of TMC278 base relative to vehicle on heart rate and mean arterial blood pressure and electrophysiological parameters (m2.6.3, Section 4.4, TMC278-CPF643). These parameters comprised the duration of the QRS-complex and of the PQ, RR, and QT interval. The duration of QT interval corrected for heart rate using Bazett's formula {14029} ($QT = QT/\sqrt{RR}$) was calculated. In addition, the incidence of cardiac conduction disturbances, such as type II and III atrioventricular blocks and intraventricular bundle branch block, was recorded. Moreover, the occurrence of ventricular arrhythmias was evaluated on the basis of occurring ventricular premature beats, VT and VF. A group of 7 animals received a series of 6 intravenous injections of increasing concentrations of TMC278 dissolved in PEG400 at a volume of 0.5 mL/kg with a 15 minute interval. Individual animals received doses ranging from 0.16 up to 5 mg/kg (cumulative dose of 9.87 mg/kg over 75 minutes). Plasma, heart, and lung tissue of the first 3 animals were sampled immediately after the last injection for determination of TMC278.

TMC278 had no effect on heart rate and mean arterial blood pressure, and had no statistically significant effect on the duration of the PQ and the QRS intervals of the ECG. The QT interval tended to be increased (+18% versus +10% with solvent) after administration of the highest dose of 5 mg/kg. After correction for heart rate using Bazett's formula, no drug- induced effect was observed on QTc. No differences in the incidence of cardiac conduction disturbances were observed. Plasma levels of TMC278 reached a median value of 9.2 µg/mL, 5 minutes after the last injection. The concentration of TMC278 in lung tissue was 4 times higher than that in heart tissue (47.7 µg/g and 11.7 µg/g, respectively).

4.2.2.2.3. Telemetered Guinea Pigs

The potential of orally administered TMC278 to inhibit in vivo trafficking of the hERG channel was evaluated through the occurrence of delayed-onset QT-prolongation in a repeat-dose telemetered guinea pig model ({15702}, m2.6.3, Section 4.4, TMC278-NC327). The dose of TMC278 was selected in a preliminary pharmacokinetics study as that giving a C_{max} value of approximately 2 times the human C_{max} at 75 mg TMC278 once daily (0.466 µg/mL) in a clinical Phase 2b study (C204). This dose gave approximately 10 milliseconds QT prolongation in the TQT study C131, at a steady state median maximum plasma concentration of 0.636 µg/mL. TMC278 was suspended in an aqueous mixture of hydroxypropylmethylcellulose (HPMC; 0.5%) and Tween 80, and dosed by gavage at 10 mg/kg for 16 consecutive days. Recordings of heart rate, body temperature, and ECG made for 4 hours after dosing were evaluated. From ECGs, the durations of the QRS complex, and the PQ and QT interval (with or without correction for heart rate according to Bazett {14029} or Fridericia {15639}) were reported every 30 minutes.

TMC278 at a dose of 10 mg/kg/day for 16 consecutive days, which gave mean C_{max} values of 0.689 to 0.911 µg/mL throughout the dosing period, had no notable effects on the measured ECG parameters, heart rate, or body temperature.

4.2.2.3. Dogs

4.2.2.3.1. Anesthetized Dogs

Groups of 4 anesthetized beagle dogs (m2.6.3, Section 4.4, TMC278-CPF648) received an intravenous infusion for 1 hour at a flow of 2 mL/kg of a formulation of 2.5 mg TMC278 base/mL in PEG400 (dose: 5 mg TMC278 base/kg) or the vehicle, PEG400. The effects of TMC278 base were compared with those of the vehicle, and evaluated during infusion and for 3 hours following administration on general and regional cardio- hemodynamic, ECG, cardio-electrophysiological, pulmonary, and arterial blood parameters. The general hemodynamic parameters comprised heart rate, systolic and diastolic aortic and pulmonary artery pressure, systolic pressure rate product, cardiac output, and stroke volume. Moreover, left ventricular (LV) pressure parameters were determined, including end diastolic pressure (LVEDP), and at maximum positive and negative isovolumic rate of change (LV dp/dt max and LV dp/dt min, respectively); contractility index (LV dp/dt max/pd); and the time constant of relaxation. The regional hemodynamic parameters were common carotid artery blood flow, systemic and pulmonary vascular resistance, common carotid arterial vascular resistance, systolic and diastolic intracranial pressure, and cerebral perfusion pressure. Electrocardiogram morphology was evaluated and the duration of the QRS complex and of the PQ and QT interval was determined; the latter also corrected for heart rate according to Bazett, Fridericia, and Van de Water {15700}. Cardio-electrophysiological effects were evaluated on the morphology and the duration of the right endocardial monophasic action potential (MAP, at 90% of repolarization [APD90]). The pulmonary parameters and arterial blood parameters are presented in Section 4.2.3.1.1.

TMC278 had no effects on the ECG or cardio-electrophysiological parameters. From cardio-hemodynamic parameters, systemic vascular resistance (−25% versus baseline; vehicle: +29% versus baseline), pulmonary vascular resistance (−20% versus baseline; vehicle: +31% versus baseline), and cardiac output (+31% versus baseline; vehicle: −12% versus baseline) were affected at the end of the infusion. The vehicle, PEG400, had its own cardio-hemodynamic effects. The mean plasma concentration of TMC278 at the end of the infusion was 2.62 µg/mL and decreased to 0.484 µg/mL, 3 hours after cessation of the infusion. The results on the pulmonary and arterial blood parameters are presented in Section 4.2.3.1.1.

4.2.2.3.2. Instrumented, Awake Dogs

Instrumented dogs were allocated to 2 groups of 7 dogs each. One group was administered a single oral dose of 20 mg/kg of TMC278 base dissolved in PEG400 and the second group was dosed with vehicle (PEG400) (m2.6.3, Section 4.4, TMC278-CPF654).

TMC278 had no effects on heart rate, blood pressure, systolic pressure rate product, LV dp/dt max, LV dp/dt min, LV dp/dt max/pd, cardiac output, stroke volume, systemic vascular resistance, and ECG intervals. No differences in the morphology of the ECG were observed between the 2 groups. The median maximum plasma concentration of TMC278 was 1.5 µg/mL.

4.2.2.3.3. Telemetered Conscious Dogs

Four telemetered male beagle dogs were administered orally via gavage escalating doses of 0 (vehicle), 20, 80, and 160 mg/kg of TMC278 base dissolved in PEG400 plus citric acid (m2.6.3, Section 4.4, TMC278-Exp5555). The interval between administrations was 1 week. Cardio-hemodynamic (heart rate, systolic and diastolic blood pressure, and pressure rate product) and ECG parameters (RR, PQ, and QT intervals) recorded for 12 hours following dosing were evaluated.

TMC278 did not show any effects on cardio-hemodynamic or ECG parameters. Plasma samples taken during the days of dosing were rejected following erroneous processing. Exposure to TMC278 was evaluated on the basis of oral single-dose toxicokinetic data from a separate dog study (m2.6.7, FK4244 [Addendum to TMC278-Exp5534]), with the same formulation at oral doses of 20, 40, and 80 mg TMC278/kg. The mean maximal plasma concentrations of TMC278 were fairly comparable between the different dose groups: 1.5, 1.7, and 1.5 µg/mL, respectively. The T_{max} values were variable, occurring up to 24 hours after dosing. The AUC_{0-24h} values also did not differ between the 3 dose levels (18.1, 26.8, and 18.8 µg.h/mL, respectively).

4.2.2.4. Additional Mechanistic Studies

4.2.2.4.1. Membrane Currents Involved in Cardiac Action Potential Depolarization and Repolarization

The effects of TMC278 base were studied on ion channels involved in the depolarization and repolarization phase of the cardiac action potential. The parameters evaluated included the slowly activating rectifying potassium current (I_{Ks}), the transient outward potassium current (I_{to}), the inward rectifying potassium current (I_{K1}), the fast sodium current (I_{Na}), and the high threshold L-calcium current ($I_{Ca,L}$). The effects on the currents were studied at single cell level using the single-electrode-whole-cell configuration of the patch clamp technique. Positive controls were used in each study.

The effect of TMC278 base at nominal bath concentrations of 0.3 to 10 µM (0.11 to 3.7 µg/mL) in a buffer containing 0.3% DMSO was studied in a CHO cell line transfected with human KvLQT1 and minK, 2 subunits encoding for the I_{Ks} channel (m2.6.3, Section 4.3, TMC278-NC342). The positive control was HMR 1556. TMC278 caused a concentration-dependent block of I_{Ks} from 1 µM (0.37 µg/mL) and above, with an IC_{50} of 3.1 µM (1.15 µg/mL).

In addition, TMC278 base was tested in the human embryonic kidney (HEK) 293 cells (m2.6.3, Section 4.3, TMC278-NC331) transfected with human cDNA encoding for channels for I_{Na}, I_{Ks}, I_{to}, $I_{Ca,L}$, and I_{K1}. The positive controls used were lidocaine for I_{Na}, chromanol 293B for I_{Ks}, flecinide for I_{to}, nifedipine for $I_{Ca,L}$, and $BaCl_2$ for I_{K1}. TMC278 base at nominal concentrations of 0.1, 0.3, and 1 µM (0.037, 0.11, and 0.37 µg/mL) caused 19.1% inhibition of I_{Ks} at 1 µM (0.37 µg/mL), and 13.6% and 35.5% inhibition of I_{to} at 0.3 and 1µM (0.1 and 0.37 µg/mL), respectively. TMC278 had no biologically relevant effects on I_{K1}, I_{Na}, or $I_{Ca,L}$ at 1 µM.

4.2.2.4.2. Trafficking of hERG Channel

One of the mechanisms possibly responsible for the delayed-onset QTcF-prolongation observed in clinical TQT study C131 is inhibition by TMC278 of the synthesis, assembly in the endoplasmic reticulum, and/or transport of the hERG channel to the cell membrane. This effect - also known as trafficking - on the most prominent ion channel involved in the repolarization of the cardiac action potential was assessed in the HERG-Lite® test {15701}, m2.6.3, Section 4.3, TMC278-NC330). In this test, the effects of TMC278 on the expression of the wild type hERG channel (hERG-WT) or a single mutant channel (hERG-SM) at the surface of HEK293 cells transfected with these channels were determined, following overnight incubation. The mildly misfolded mutant channels show reduced trafficking. However, hERG channel blockers have shown to act as pharmacological chaperones for the mutant channels in stabilizing their correct conformation and rescuing their expression by allowing their export from the endoplasmic reticulum and movement to the cell surface. So, in this test the potential of TMC278 to inhibit trafficking of the hERG- WT channel was evaluated in parallel with its potential to behave as a hERG blocker by rescuing the surface expression of the hERG-SM channel. TMC278 base was incubated at nominal bath concentrations of 1, 10, and 30 µM (0.37, 3.7, and 11.1 µg/mL) in 0.1% DMSO in aqueous buffer. Geldanamycin (1 µM) served as positive control for trafficking of hERG-WT. Astemizole (1 µM) was used as positive control hERG blocker.

On the basis of the reduced expression of the hERG-WT on the cell membrane (29% and 36% of control expression at 10 and 30 µM (3.7 and 11.0 µg/mL), respectively), it is concluded that TMC278 has the potential to reduce trafficking of the hERG channel. The overexpression of hERG-SM on the cell membrane of 146%, 155%, and 213% of control values at TMC278 base concentrations of 1, 10, and 30 µM (0.37, 3.7, and 11.0 µg/mL), respectively, is an indication of the potential of TMC278 to inhibit the hERG channel. This result is in line with that of the patch clamp study with CHO cells expressing the hERG channel (Section 4.2.2.1, TMC278-CPF730).

4.2.2.4.3. Electrophysiological Parameters of the Isolated Arterially Perfused Rabbit Left Ventricular Wedge

Electrophysiological and other cardiophysiological parameters were studied in isolated, arterially-perfused rabbit ventricular wedge preparations (m2.6.3, Section 4.3, TMC278-NC341). The effects of TMC278 base at concentrations increasing from 10 nM to 10 µM (3.7 ng/mL to 3.7 µg/mL) were evaluated relative to vehicle control (buffer containing 0.1% DMSO). Parameters determined were the duration of the QT and QRS intervals; the time between the peak and the end of the T wave (Tp-Te) as a measure of transmural dispersion of repolarization (TDR); and the Tp-Te/QT ratio (rTp-Te) as an indicator of the potential to induce phase 2 early afterdepolarizations (EADs). Moreover, the force of contraction (FC), in-excitability (inability of the preparation to follow the stimulation), and the occurrence of ventricular tachycardia (VT) and ventricular fibrillation (VF) were recorded. The potential to induce torsade de pointes (TdP) was assessed semiquantitatively on the basis of QT duration, TDR measured as (Tp-Te)/QT, and the occurrence of phase 2 EADs.

TMC278 caused 6% and 9% prolongation of the QT-interval from baseline at nominal bath concentrations of 1 and 10 µM (0.37 and 3.7 µg/mL), respectively. At 10 µM (3.7 µg/mL) only, the assessment of the potential to induce TdP resulted in a marginal score of 0.5. No other significant or physiologically relevant changes were noted in any of the other parameters.

4.2.3. Respiratory System

4.2.3.1. Dogs

4.2.3.1.1. Anesthetized Dogs

Respiration pressure and flow, tidal volume, expiratory airway resistance, dynamic lung compliance, and arterial blood parameters (pH, oxygen tension and saturation, CO_2 tension, and concentration of electrolytes, hemoglobin, glucose, lactate, chloride, and bicarbonate) were studied in groups of 4 anesthetized beagle dogs (m2.6.3, Section 4.4, TMC278-CPF648). The animals received an intravenous infusion for 1 hour at a flow of 2 mL/kg of 2.5 mg TMC278 base/mL in PEG400, resulting in a dose of 5 mg/kg, or the vehicle PEG400. Parameters were recorded during the infusion and for 3 hours after the end of the administration.

None of the respiratory or arterial blood parameters were affected during or for 3 hours after the 1-hour intravenous infusion. The mean plasma concentration of TMC278 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.

4.2.3.1.2. Telemetered Conscious Dogs

Respiratory rate and tidal volume were not affected in 4 telemetered conscious male beagle dogs at 1, 2, and 4 hours after single oral (gavage) escalating doses of 0 (vehicle), 20, 80, and 160 mg TMC278 base/kg dissolved in PEG400 plus citric acid (m2.6.3, Section 4.4, TMC278-Exp5555), with a dosing interval of 1 week. For exposure data, see Section 4.2.2.3.3.

4.3. TAF

The safety pharmacology studies of TAF were conducted in accordance with GLP regulations. The in vitro hERG assay was also conducted in accordance with guidelines issues by the ICH.

In vivo safety pharmacology experiments were conducted using TAF as the monofumarate form (GS-7340-02) in 50 mM citric acid. 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.

4.3.1. Central Nervous System

The effect of TAF (as GS-7340-02) on the CNS was evaluated in male Sprague Dawley rats following administration of single oral doses of 0, 100, or 1000 mg/kg (80 or 800 mg free base equivalents [fbe]/kg) (m2.6.3, Section 4.6, R990188; [redacted] 56518). The no-effect dose for a pharmacological effect on the CNS of the male Sprague Dawley rat was 1000 mg/kg.

4.3.2. Cardiovascular System

4.3.2.1. In Vitro

TAF (as GS-7340-03) was evaluated at concentrations of 1 and 10 µM (fbe), and hERG inhibition was not statistically significant ($p < 0.05$) when compared with vehicle control values. The IC_{50} for the inhibitory effect of TAF on hERG potassium current was estimated to be greater than 10 µM (m2.6.3, Section 4.5, PC-120-2005; [redacted] 111213.[redacted]).

4.3.2.2. In Vivo

Oral administration of TAF (as GS-7340-02) to conscious instrumented male beagle dogs at dose levels of 30 or 100 mg/kg (24 and 80 mg fbe/kg) did not induce pharmacologic effects on heart rate, systemic blood pressure, or ECGs (m2.6.3, Section 4.6, D2000006; [redacted] 93205).

4.3.3. Gastrointestinal System

Administration of TAF (as GS-7340-02) to Sprague Dawley rats by oral gavage indicated that at 1000 mg/kg (800 mg fbe/kg), the rate of gastric emptying was reduced (m2.6.3, Section 4.6, R990187; [redacted] 56519). At 100 mg/kg (80 mg fbe/kg), there was no clear effect on gastric emptying. The reduction in charcoal transit through the intestine at the 2-hour time point at 1000 mg/kg may have been due to reduced gastric emptying. A dose of 100 mg/kg was considered to have had no effect on gastric emptying or intestinal motility.

4.3.4. Renal System

The effect of TAF (as GS-7340-02) on the renal system was evaluated in male Sprague Dawley rats following administration of single oral doses of 0, 100, or 1000 mg/kg (80 or 800 mg fbe/kg) (m2.6.3, Section 4.6, R990186; [redacted] 56520). Although urinary output of calcium was increased at 1000 mg/kg, this correlated with an increase in serum calcium concentration and indicated that the kidneys were functioning in order to reduce the serum calcium load. The no-effect dose for a pharmacological effect on the renal system of the male Sprague Dawley rat was 1000 mg/kg.

4.4. FTC/RPV/TAF

Emtricitabine and TAF had little effect on vital organ systems in safety pharmacology studies. 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 associated with 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 (m2.6.3, Section 4.6, D2000006; [redacted] 93205) or in the thorough QT study (m2.7.2, Section 2.2.1.1 [Study GS-US-120-0107]). Rilpivirine has shown the potential for QT prolongation, an effect confirmed in a thorough QT 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 small cardiovascular effect seen with the 25-mg RPV dose alone.

Overall, the pharmacological assessment of FTC, RPV, and TAF supports the effective use of these 3 agents at the proposed doses and together in combination therapy for HIV-1 disease. Additional safety pharmacology studies on the FTC/RPV/TAF combination are considered unwarranted.

5. PHARMACODYNAMIC DRUG INTERACTIONS

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

6. DISCUSSION AND CONCLUSIONS

6.1. FTC

A discussion of the nonclinical virology data of FTC, an NRTI, is provided in m2.7.2, Section 4.1.

Emtricitabine has shown no in vitro cytotoxicity in a variety of human cell types. It also shows no toxicity against hematopoietic progenitor cells. Emtricitabine has a high selectivity for HIV RT versus cellular DNA polymerases α, β, γ, and ε. Emtricitabine has also shown no effect on mitochondrial function as measured by mitochondrial DNA synthesis, cellular content of COX II, intracellular lipid accumulation, lactic acid production, and mitochondrial ultrastructure.

The results of the safety pharmacology program suggest that FTC has no adverse effects on vital organ systems at systemic (AUC) exposures approximately 10- to > 50-fold higher than human exposure at the recommended clinical dose.

6.2. RPV

A discussion of the nonclinical virology data of RPV, an NNRTI, is provided in m2.7.2, Section 4.1.

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.

The testing on a variety of receptors, human DNA polymerases, stomach ATPase, and gastric acidity did not reveal any significant effects secondary to the primary anti-HIV effect of TMC278.

Overall, TMC278 had no effects on secondary pharmacodynamic parameters or on the core battery of safety pharmacology tests, apart from inhibitory effects on some cardiac potassium currents and channels and moderate QT-prolongation in the rabbit ventricular wedge. The observed inhibition of trafficking of the hERG channel may be involved in the delayed onset of the QTcF-prolongation observed in the clinical TQT study C131. However, pharmacokinetic mechanisms may also be involved. Importantly, the effects in nonclinical models allow the conclusion that TMC278 has only a marginal potential to induce proarrhythmic effects.

6.3. TAF (and TFV)

A discussion of the nonclinical virology data of TAF, an NtRTI, is presented in m2.7.2, Section 4.1.

Tenofovir alafenamide is an investigational prodrug of TFV. Tenofovir alafenamide is predominantly hydrolyzed to TFV by CatA cleavage in HIV-target cells including lymphocytes {13119}, {10427}, resulting in higher intracellular levels of TFV-DP and lower circulating levels

of TFV relative to TDF clinically {17137}, {1574}. TFV-DP is an inhibitor of HIV-1 RT and HBV DNA polymerase that terminates the elongation of the viral DNA chain {21}, {1131}. TFV-DP is a very weak inhibitor of mammalian DNA polymerases α, β, δ, ε, and mitochondrial DNA polymerase γ.

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 2-fold (C_{max} = 0.48 μM; Study GS-US-120-0104). Thus, TAF has a low potential for inhibiting mtDNA synthesis and inducing NRTI-related mitochondrial toxicities.

The primary pharmacodynamic, secondary virology, and drug interaction pharmacodynamic studies of TAF (and TFV) are presented in detail in m2.7.2, Section 4.1.

Tenofovir alafenamide is more stable in plasma than TDF, but rapidly converts to TFV inside cells. Assessment of the intracellular metabolism of TAF in CD4+ T-cells and MDMs showed efficient conversion of TAF to the active metabolite TFV-DP. The intracellular conversion of TAF to TFV-DP was consistent across immune cells derived from demographically diverse donors. Lysosomal CatA plays an essential role in the intracellular activation of TAF; therefore, a variety of PIs were screened for possible effects on CatA-mediated intracellular activation of TAF {20795}. Compounds assessed included HIV and HCV PIs, the pharmacokinetic enhancer COBI, as well as host serine PIs used as antidiabetic and anticoagulant agents. Of the agents tested, the covalent HCV PIs telaprevir and boceprevir were the only ones that inhibited CatA-mediated hydrolysis of TAF, with IC_{50} values of 0.3 and 0.2 μM, respectively. These data support the co-administration of the tested therapeutic PIs, with the exception of telaprevir and boceprevir, in combination with TAF, without negatively affecting its clinical pharmacology and intracellular conversion to TFV.

Tenofovir alafenamide showed low cytotoxicity in resting and dividing PBMCs, T-lymphoblastoid cells, and hepatocellular carcinoma (HepG2) cells, and provided > 1997-fold selectivity or greater 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.

Unlike TFV, TAF did not interact with and is not a substrate for the renal transporters 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 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.

Tenofovir has also shown no effect on mitochondrial function as measured by mtDNA synthesis, cellular content of COX II, intracellular lipid accumulation, and lactic acid production.

Safety pharmacology studies demonstrated that TAF has no effect on the rat CNS and renal system or dog cardiovascular system. There was reduced gastric emptying in rats dosed at 1000 mg/kg, but not at 100 mg/kg.

Overall, the pharmacodynamic and pharmacological assessment of TAF supports the effective and safe use of TAF in combination therapy for the treatment of HIV-1 infection.

6.4. Conclusions

The cytotoxicity of TFV in RPTECs was not affected by combination with FTC when tested in vitro at pharmacologically relevant concentrations. Combination of TFV with FTC (+COBI and EVG) at their pharmacologically relevant concentrations showed no significant change in the cytotoxicity of TFV in cells transiently expressing OAT1 alone, or OAT1 and MRP4 together, suggesting that FTC is not likely to directly affect the toxicity potential of TFV in renal cells and tissues expressing the relevant renal transporters.

NRTIs currently carry a class labeling for mitochondrial toxicity; however, both FTC and TAF have shown a low potential for mitochondrial toxicity in repeat-dose mouse, rat, and dog toxicity studies. The potential for mitochondrial toxicity of RPV was low by in vitro assessment of the inhibitory activity on human polymerase γ. 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.

Rilpivirine has shown a low potential for in vitro cytotoxicity in a variety of human cell types. The selectivity index of approximately 8000 indicates that RPV is a potent and selective inhibitor of HIV-1.

Emtricitabine and TAF had little effect on vital organ systems in safety pharmacology studies. 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 associated with 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 (m2.6.3, Section 4.6, D2000006) or in the thorough QT study (m2.7.2, Section 2.2.1.1 [Study GS-US-120-0107]). Rilpivirine has shown the potential for QT prolongation, an effect confirmed in a thorough QT study in healthy subjects. At the 25-mg dose of RPV, the observed change in QTcF was not considered clinically relevant, and the combination product is not anticipated to exacerbate the small cardiovascular effect seen with the 25-mg RPV dose alone.

Given the favorable safety pharmacology profiles of FTC, RPV, and TAF, additional safety pharmacology studies on the FTC/RPV/TAF combination are considered unwarranted.

Overall, the pharmacodynamic and pharmacological assessment of FTC, RPV, and TAF (and TFV) supports the effective and safe use of the FTC/RPV/TAF FDC at the proposed doses for the treatment of HIV-1 disease.

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2.6 NONCLINICAL SUMMARY

SECTION 2.6.3— PHARMACOLOGY TABULATED SUMMARY

EMTRICITABINE/RILPIVIRINE/TENOFOVIR ALAFENAMIDE FIXED-DOSE COMBINATION (FTC/RPV/TAF FDC)

Gilead Sciences

20 May 2015

TABLE OF CONTENTS

SECTION 2.6.3— PHARMACOLOGY TABULATED SUMMARY 1
TABLE OF CONTENTS 2
NOTE TO REVIEWER 3
1. PHARMACOLOGY OVERVIEW 4
1.1. Primary Pharmacodynamics of FTC 4
1.2. Primary Pharmacodynamics of RPV 4
1.3. Primary Pharmacodynamics of TAF 5
1.4. Secondary Pharmacodynamics of FTC 6
1.5. Secondary Pharmacodynamics of RPV 8
1.6. Secondary Pharmacodynamics of TAF 9
1.7. Secondary Pharmacodynamics of FTC/TFV 11
1.8. Safety Pharmacology of FTC 12
1.9. Safety Pharmacology of RPV 15
1.10. Safety Pharmacology of TAF 17
1.11. Pharmacodynamic Drug Interactions 18
2. PRIMARY PHARMACODYNAMICS 19
3. SECONDARY PHARMCODYNAMICS 20
4. SAFETY PHARMACOLOGY 21
4.1. In Vitro Studies with FTC 21
4.2. In Vivo Studies with FTC 23
4.3. In Vitro Studies with RPV 28
4.4. In Vivo Studies with RPV 31
4.5. In Vitro Studies with TAF 33
4.6. In Vivo Studies with TAF 34
5. PHARMACODYNAMIC DRUG INTERACTIONS 35
6. REFERENCES 36

NOTE TO REVIEWER

This document contains a summary of nonclinical pharmacology studies conducted in support of a fixed-dose combination (FDC) that contains the active substances emtricitabine (FTC), rilpivirine (RPV), and tenofovir alafenamide (TAF). The FDC tablet is referred to as emtricitabine/rilpivirine/tenofovir alafenamide (FTC/RPV/TAF) throughout this document.

As TAF is a new chemical entity, this nonclinical summary contains all available data on this new component while providing key data on FTC and RPV that support the efficacy and safety claims in the FDC prescribing information. Nonclinical studies have utilized TAF in most cases; however, relevant studies with the parent drug tenofovir (TFV) are also provided as applicable. Links to all study reports included in the dossier are highlighted in blue text.

In order to simplify the review, the order of presentation in each section follows the general format: FTC, RPV, TAF (and TFV), and combination studies. Given the lack of remarkable effects in the in vitro studies with the individual agents (FTC, RPV, TFV, or TAF) or in two-drug combination studies, no additional pharmacology studies have been conducted for the FTC/RPV/TAF combination.

The following conversions are provided to aid the reviewer:

- FTC 1 μM = 0.247 μg/mL
- RPV 1 μM = 0.366 μg/mL
- TAF (GS-7340) 1 μM = 0.477 μg/mL
- TFV 1 μM = 0.287 μg/mL

1. PHARMACOLOGY OVERVIEW

1.1. Primary Pharmacodynamics of FTC

The primary pharmacodynamics of FTC are described in detail in the nonclinical virology summary contained in m2.7.2, Section 4.1.

1.2. Primary Pharmacodynamics of RPV

The primary pharmacodynamics of RPV are described in detail in the nonclinical virology summary contained in m2.7.2, Section 4.1.

1.3. Primary Pharmacodynamics of TAF

Studies of the intracellular activation and activity of TAF are listed below. For additional information on the primary pharmacodynamics of TAF, refer to the nonclinical virology summary contained in m2.7.2, Section 4.1.

Test Article: TAF

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Study No./Reference
Effect of TAF on CatA Hydrolase Activity and TAF Antiretroviral Activity	No	Primary CD4+ T lymphocytes and macrophages	In vitro	Gilead Sciences, Inc., USA	PC-120-2017 Antiviral Therapy, 2014;10.3851/IMP2767. {29240}
Effect of TAF on CatA-mediated Activation and Antiretroviral Activity	No	Purified CatA and primary CD4+ T-lymphocytes	In vitro	Gilead Sciences, Inc., USA	PC-120-2001

CatA = cathepsin A; GLP = Good Laboratory Practice; TAF = tenofovir alafenamide
a An entry of "Yes" indicates that the study includes a GLP compliance statement.

1.4. Secondary Pharmacodynamics of FTC

Test Article: FTC

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Study No./Reference
In Vitro Receptor Binding Potencies of FTC	No	In vitro binding assay	In vitro	[redacted] USA	TPZZ/93/0002
In Vitro Autonomic Pharmacology Cholinergic (Muscarinic) Activity Alpha-adrenoceptor Activity Beta-adrenoceptor Activity Serotonin Receptor Activity	No	 Guinea pig ileum Rabbit aortic strips Guinea pig trachea and atria Rat fundus strips	In vitro	[redacted] USA	TPZZ/92/0055
Antiviral Activity vs. Human Hepatitis B Virus (HBV), Cytotoxicity	No	Human hepatoma cell line HepG2.2.15	In vitro	Emory University, Atlanta, GA, USA	Antimicrob Agents Chemother. 1994; 38:2172-2174 {4533}
Antiviral Activity vs. Human HBV, Cytotoxicity	No	Human hepatoma cell line HepG2.2.15	In vitro	Burroughs Wellcome Co., Research Triangle Park, NC 27709 USA	Antimicrob Agents Chemother. 1992; 36:2686-2692 {4535}
Cytotoxicity Assay	No	MT-4, CEM, IM9, Molt-4, and HepG2.2.15 cells	In vitro	Burroughs Wellcome Co., Research Triangle Park, NC 27709 USA	Antimicrob Agents Chemother. 1994; 38:868-871 {4531}
Mitochondrial Toxicity	No	Human hepatoma cell line HepG2	In vitro	University of Alabama, Birmingham, USA	Biochem. Pharmacol. 1996; 52:1577-1584 {4550}

Test Article: FTC

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Study No./Reference
An In Vitro Evaluation of the Effects on Cell Growth and Mitochondrial Functions in the MT2 Cell Line after Long Term Exposure to Antiviral Xenobiotics	No	Human T cell line, MT-2 cells	In vitro	Gilead Sciences, Inc., Foster City, CA USA	TPI 11963
Data from Clonogenic Assays CFU-GM and BFU-E and Mitochondrial Assays for TP0001 and TP0004 as Compared to AZT	No	Human bone marrow progenitor cells	In vitro	Gilead Sciences, Inc. USA	233 {6081}
Effect of FTC on Mitochondrial DNA	No	Molt-4 cell culture assay	In vitro	[redacted] USA	TGZZ/93/0016
Effect of FTC on Mitochondrial DNA	No	Molt-4 cell culture assay	In vitro	[redacted] USA	TGZZ/93/0023

FTC = emtricitabine, GLP = Good Laboratory Practice; HBV = hepatitis B virus
a An entry of "Yes" indicates that the study includes a GLP compliance statement.

1.5. Secondary Pharmacodynamics of RPV

Test Article: RPV

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Study No.
Polymerase Inhibition	No	Human Polymerase α, β, γ	In vitro	[redacted], USA	TMC278-1646_0005343
Receptor Binding	No	Cells & enzymes	In vitro	[redacted], France	TMC278-870219
Receptor Binding	No	Guinea pig right atrium	In vitro	[redacted], Taiwan	TMC278-NC204(2)
Receptor Binding	No	Pig stomach	In vitro	[redacted], Taiwan	TMC278-NC204(3)
Receptor Binding	No	Rat/Wistar	Intraperitoneal injection	[redacted], Taiwan	TMC278-NC204(1)
Cytotoxicity	No	HeLa, HepG2, HEp-2, MRC-5, and A549 cells	In vitro	Tibotec, Belgium	TMC278-IV2-AVMR
Cytotoxicity and Susceptibility Index	No	MT-4 cells	In vitro	Tibotec, Belgium	TMC278-IV1-AVMR

a An entry of "Yes" indicates that the study includes a GLP compliance statement.

1.6. Secondary Pharmacodynamics of TAF/TFV

Test Article: TAF, TFV

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Study No./Reference
Binding Screen to Neuroreceptors, Ion Channels, Transporters, Nuclear Receptors with TFV	No	Protein targets	In vitro	[redacted], Taiwan	V2000020
Cytotoxic Effect of TAF on PBMCs	No	PBMCs	In vitro	Gilead Sciences, Inc., Foster City, CA USA	PC-120-2009
Cytotoxicity Assay with TAF	No	MT-2, MT-4, and HepG2 cells	In vitro	Gilead Sciences, Inc., Foster City, CA USA	PC-120-2007
Cytotoxicity Assay with TFV	No	HepG2, human skeletal muscle cells (SKMC), human renal proximal tubule epithelial cells (RPTECs)	In vitro	Gilead Sciences, Inc., Foster City, CA USA	P4331-00037
Cytotoxicity Assay with TFV	No	RPTECs	In vitro	Hospital Universitari de Tarragona, Tarragona, Spain	Antimicrob. Agents Chemother., 2006;50 (11):3824-32. {9864}
Effects of TAF on Hematopoietic Progenitors	No	Erythroid and myeloid progenitors	In vitro	[redacted], Canada	PC-120-2016 GLD06A
Cytotoxicity Assay with TAF	No	HEK293T cells expressing renal OAT1 and OAT3 transporters and primary human CD4+ T lymphocytes	In vitro	Gilead Sciences, Inc., Foster City, CA USA	PC-120-2018 Antiviral Therapy, 2014;10.3851/IMP2770. {29239}
Cytotoxic Effect of TAF on Primary Osteoblasts	No	PBMCs and human proliferating osteoblast cells	In vitro	Gilead Sciences, Inc., Foster City, CA USA	PC-120-2008

Test Article: TAF, TFV

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Study No./Reference
Antiviral Activity and Cytotoxicity of TAF Metabolites	No	MT-2 and MT-4 cells	In vitro	Gilead Sciences, Inc., Foster City, CA USA	PC-120-2021
Mitochondrial Toxicity with TFV	No	HepG2, SKMC, RPTECs	In vitro	Gilead Sciences, Inc., Foster City, CA USA	P1278-00042
Effect of TAF on Mitochondrial DNA	No	HepG2 cells	In vitro	Gilead Sciences, Inc., Foster City, CA USA	PC-120-2006

CatA = cathepsin A; DNA = deoxyribonucleic acid; GLP = Good Laboratory Practice; OAT = organic anion transporter; PBMC = peripheral blood mononuclear cell; RPTECs = renal proximal tubule epithelial cells; SKMC = skeletal muscle cells; TAF = tenofovir alafenamide

a An entry of "Yes" indicates that the study includes a GLP compliance statement.

1.7. Secondary Pharmacodynamics of FTC/TFV

Test Article: FTC/TFV

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Study No.
Anti-HIV Synergy Studies of TFV and FTC	No	MT-2 cells	In vitro	Gilead Sciences, Inc., Foster City, CA USA	PC-164-2002
Mitochondrial Toxicity of TFV and FTC	No	HepG2 cells	In vitro	[redacted], Germany	TX-104-2001
Cytotoxicity Assay with TFV alone and in combination with COBI or EVG+COBI+FTC	No	RPTECs	In vitro	Gilead Sciences, Inc., Foster City, CA USA	PC-236-2012
Cytotoxicity Assay with TFV alone and in combination with COBI or EVG+COBI+FTC	No	Human embryonic kidney 293T cells transiently expressing OAT1 and MRP4 transporters	In vitro	Gilead Sciences, Inc., Foster City, CA USA	PC-236-2013

COBI = cobicistat; EVG = elvitegravir; FTC = emtricitabine; MRP4 = multidrug resistance protein 4; OAT = organic anion transporter; RPTECs = renal proximal tubule epithelial cells; TFV = tenofovir

a An entry of "Yes" indicates that the study includes a GLP compliance statement.

1.8. Safety Pharmacology of FTC

Test Article: FTC

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Study No.
In Vitro					
Isolated Guinea Pig Ileum	No	Guinea Pig, Duncan Hartley	In Vitro	[redacted]	477
Isolated Cardiac Muscle Isolated Perfused Rat Heart Electrically Driven Papillary and Atrial Muscle of the Cat Spontaneously-beating Guinea Pig Paired Atria	No	 Rat heart Cat papillary and atrial muscle strips Guinea pig paired atria	In Vitro	[redacted] USA	TPZZ/92/0056
In Vivo					
General High Dose Pharmacology Testing Results for FTC					
Modified Irwin Screen	No	Mouse, ICR	Oral	[redacted] Taiwan	477
CNS – Spontaneous Locomotor Activity	No	Mouse, ICR	Oral		
CNS – Motor Incoordination (Roto-rod Test)	No	Mouse, ICR	Oral		
CNS – Hexobarbital Potentiation (Sleeping Time)	No	Mouse, ICR	Oral		
CNS – Anticonvulsant Activity (Maximal Electroshock)	No	Mouse, ICR	Oral		

Test Article: FTC

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Study No.
CNS – Anticonvulsant Activity (Metrazole)	No	Mouse, ICR	Oral	[redacted] Taiwan	477
CNS – Preconvulsant Activity (Electroshock)	No	Mouse, ICR	Oral		
CNS – Preconvulsant Activity (Metrazole)	No	Mouse, ICR	Oral		
CNS –Analgesic Activity (Tail Flick)	No	Mouse, ICR	Oral		
CNS – Analgesic Activity (Phenylquinone Writhing)	No	Mouse, ICR	Oral		
CNS – Body Temperature	No	Rat, Wistar	Oral		
Cardiovascular Function	No	Rat Wistar	Oral		
Renal Function	No	Rat, Long Evans	Oral		
Gastrointestinal Motility	No	Mouse, ICR	Oral		
Overt Pharmacological Effects	No	Mouse, CD-1 Rat, CD(Sprague Dawley)	Oral gavage	[redacted] USA	TPZZ/93/0001
Conditioned Avoidance Response	No	Rat, Long Evans	Intraperitoneal	[redacted] USA	TPZZ/93/0119
Systolic Blood Pressure and Heart Rate	No	Conscious Normotensive Rat, CD (Sprague Dawley)	Oral	[redacted] USA	TPZZ/92/0057

Test Article: FTC

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Study No.
Cardiovascular, Respiratory and Autonomic Function					
Cardiovascular and Respiratory Effects in Spontaneously Breathing Dogs Anesthetized with Allobarbital-Urethane	No	Anesthetized Dogs, Beagle	Intravenous	[redacted] USA	TPZZ/92/0076
Effects on the Changes in Mean Arterial Blood Pressure Induced by Norepinephrine, Carotid Occlusion, Acetylcholine and Vagal Nerve Stimulation in the Anesthetized Dog	No	Anesthetized Dogs, Beagle	Intravenous		

a An entry of "Yes" indicates that the study includes a GLP compliance statement.

1.9. Safety Pharmacology of RPV

Test Article: RPV

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Study No.
In Vitro					
Cardiovascular (cardiac membrane potassium current)	No	I_{Kr} in transfected CHO cells expressing hERG	In vitro	[redacted], Germany	TMC278-CPF730
Cardiovascular (cardiac action potential)	No	Guinea pig isolated atrium	In vitro	Janssen Research Foundation, Belgium	TMC278-N168576
Cardiovascular (cardiac membrane potassium current)	No	I_{Ks} in transfected CHO cells expressing KvLQT1/minK	In vitro	J&J PRD, Belgium	TMC278-NC342
Cardiovascular (cardiac membrane ion currents)	No	I_{Ks} in transfected HEK293 cells expressing KvLQT1/minK	In vitro	[redacted]	TMC278-NC331
		I_{to} in transfected HEK293 cells expressing $hK_v4.3$			
		I_{K1} in transfected HEK293 cells expressing $hK_{ir}2.1$			
		I_{Na} in transfected HEK293 cells expressing $hNa_v1.5$			
		$I_{Ca,L}$ in transfected HEK293 cells expressing $hCa_v1.2$			

Test Article: RPV

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Study No.
Cardiovascular (cardiac membrane potassium current)	No	Surface expression of hERG channels in HEK293 cells	In vitro	[redacted]	TMC278-NC330
Cardiovascular (cardiac action potential)	No	Arterially perfused rabbit ventricular wedge	In vitro	J&J PRD, Belgium	TMC278-NC341
In Vivo					
CNS	Yes	Male Sprague Dawley rat	Oral gavage	J&J PRD, Belgium	TMC278-Exp5560
CNS and cardiovascular	No	Conscious instrumented dogs	Oral gavage	J&J PRD, Belgium	TMC278-CPF654
CNS, cardiovascular, and respiratory	Yes	Conscious telemetered male beagle dogs	Oral gavage	[redacted], Switzerland	TMC278-Exp5555
Cardiovascular (ECG and cardio-hemodynamic parameters)	No	Anesthetized Guinea pig, Dunkin Hartley	Intravenous	J&J PRD, Belgium	TMC278-CPF643
Cardiovascular (ECG, cardio-hemodynamic and respiratory parameters)	No	Telemetered Guinea pig, Dunkin Hartley	Oral gavage	J&J PRD, Belgium	TMC278-NC327
Cardiovascular (ECG, cardio-hemodynamic and respiratory parameters)	No	Anesthetized Dogs, Beagle	Intravenous	J&J PRD, Belgium	TMC278-CPF648

CNS = central nervous system; CRO = contract research organization; ECG = electrocardiogram; GLP = Good Laboratory Practice; HEK = human embryonic kidney; hERG = human ether-a-go-go related gene

a An entry of "Yes" indicates that the study includes a GLP compliance statement.

1.10. Safety Pharmacology of TAF

Test Article: TAF

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Gilead (CRO) Study No.
In Vitro					
Cardiovascular (hERG Inhibition)	Yes	Human embryonic kidney cells (HEK293)	In vitro	[redacted]	PC-120-2005 ([redacted] 111213.DPW)
In Vivo					
Cardiovascular	Yes	Conscious male beagle dog	Oral gavage	([redacted]), [redacted], Canada	D2000006 ([redacted] 93205)
CNS	Yes	Male Sprague Dawley rat	Oral gavage	([redacted]), [redacted], Canada	R990188 ([redacted] 56518)
Gastrointestinal	Yes	Male Sprague Dawley rat	Oral gavage	([redacted]), [redacted], Canada	R990187 ([redacted] 56519)
Renal	Yes	Male Sprague Dawley rat	Oral gavage	([redacted]), [redacted], Canada	R990186 ([redacted] 56520)

CNS = central nervous system; CRO = contract research organization; GLP = Good Laboratory Practice; hERG = human ether-a-go-go related gene
a An entry of "Yes" indicates that the study includes a GLP compliance statement.

1.11. Pharmacodynamic Drug Interactions

The pharmacodynamic drug interactions of FTC, RPV, TAF, and the combinations of FTC/RPV/TAF are described in detail in the nonclinical virology summary contained in m2.7.2, Section 4.1.

2. PRIMARY PHARMACODYNAMICS

Studies of the primary pharmacodynamics of FTC, RPV, TAF, and the combinations of FTC/RPV/TAF are presented in the nonclinical virology summary contained in m2.7.2, Section 4.1.

3. SECONDARY PHARMCODYNAMICS

Studies of the secondary pharmacodynamics of FTC, RPV, TAF, and the combination of FTC/TFV are listed in Sections 1.4 to 1.7.

4. SAFETY PHARMACOLOGY

4.1. In Vitro Studies with FTC

Test Article: FTC

Organ Systems Evaluated	Species / Strain	Method of Administration	Dose (µM or M)	Gender and n per Group	Noteworthy Findings	GLP[a]	Study No.
Contractile Responses	Isolated Guinea Pig Ileum	In Vitro	10, 30, 100 µM	6 ileum strips per group	FTC did not elicit any significant agonist effect upon guinea pig ilea or alter contractile responses induced by acetylcholine, histamine or $BaCl_2$	No	477
Isolated Perfused Rat Heart	Rat heart	In Vitro	10^{-4} M	—	Perfusion of isolated rat hearts at 10^4 M produced decreases in heart rate of 5 ± 4% that were similar to that of control hearts (6 ± 5% decrease). The incidence of ventricular premature extrasystoles in FTC-treated hearts was no greater than that observed in control hearts. No ventricular tachycardia or fibrillation occurred in hearts treated with FTC or vehicle control.	No	TPZZ/92/0056

Test Article: FTC

Organ Systems Evaluated	Species / Strain	Method of Administration	Dose (µM or M)	Gender and n per Group	Noteworthy Findings	GLP[a]	Study No.
Electrically Driven Papillary and Atrial Muscle of the Cat	Cat papillary and atrial muscle strips	In Vitro	10^{-4} M	7 muscle strips	Minor positive inotropism (12 ± 6% increase in developed tension) was observed on papillary muscle preparations (n = 7) after incubation for 30 minutes with FTC. Negligible positive inotropism (2 ± 3% increase in developed tension) occurred on cat atrial preparations (n = 5) following a 30 minute incubation. The maximum following rate at which atrial muscle would follow electrical stimulation was not affected by FTC.	No	TPZZ/92/0056
Spontaneously-beating Guinea Pig Paired Atria	Guinea pig paired atria	In Vitro	10^{-4} M	—	Fifteen-minute incubation with FTC produced no direct chronotropic or inotropic effects on spontaneously-beating guinea-pig atria.	No	TPZZ/92/0056

FTC = emtricitabine
a An entry of "Yes" indicates that the study includes a GLP compliance statement.

4.2. In Vivo Studies with FTC

Test Article: FTC

Organ Systems Evaluated	Species / Strain	Method of Administration	Dose (mg/kg)	Gender and n per Group	Noteworthy Findings	GLP[a]	Study No.
Modified Irwin Screen	Mouse, ICR	Oral	10, 30, 100	10 M	FTC did not cause any noteworthy findings on behavioral, autonomic, or neurological signs in animals at all doses.	No	477
CNS – Spontaneous Locomotor Activity	Mouse, ICR	Oral	10, 30, 100	8 M	FTC had no significant effect on spontaneous locomotor activity.	No	477
CNS – Motor Incoordination (Roto-rod Test)	Mouse, ICR	Oral	10, 30, 100	10 M	FTC had no significant effect on motor incoordination.	No	477
CNS – Hexobarbital Potentiation (Sleeping Time)	Mouse, ICR	Oral	10, 30, 100	10 M	FTC had no significant prolongation of hexobarbital sleeping time.	No	477
CNS – Anticonvulsant Activity (Maximal Electroshock)	Mouse, ICR	Oral	10, 30, 100	10 M	FTC had no significant effect on the number of animals experiencing clonic/tonic convulsions and mortality.	No	477
CNS – Anticonvulsant Activity (Metrazole)	Mouse, ICR	Oral	10, 30, 100	10 M	FTC had no significant effect on the number of animals experiencing clonic/tonic convulsions and mortality.	No	477
CNS – Preconvulsant Activity (Electroshock)	Mouse, ICR	Oral	10, 30, 100	10 M	FTC had no significant effect on the incidence of clonic/tonic convulsions and mortality.	No	477

Test Article: FTC

Organ Systems Evaluated	Species / Strain	Method of Administration	Dose (mg/kg)	Gender and n per Group	Noteworthy Findings	GLP[a]	Study No.
CNS – Preconvulsant Activity (Metrazole)	Mouse, ICR	Oral	10, 30, 100	10 M	FTC had no significant effect on the incidence of clonic/tonic convulsions and mortality.	No	477
CNS –Analgesic Activity (Tail Flick)	Mouse, ICR	Oral	10, 30, 100	10 M	FTC caused no significant increase in latency of the tail flick response.	No	477
CNS – Analgesic Activity (Phenylquinone Writhing)	Mouse, ICR	Oral	10, 30, 100	10 M	No significant attenuation of PQ-inducing writhing was observed after all tested doses.	No	477
CNS – Body Temperature	Rat, Wistar	Oral	10, 30, 100	10 M	FTC did not cause any significant change in body temperature.	No	477
General Pharmacology	Mouse, CD-1	Oral	100, 250, 500, 750, 1000	4 M	Mice exhibited no visible effects. Minimal respiratory rate effects were only noted at 750 mg/kg (21% decrease) and in the control animals (29% increase). Behavioral reflexes were unaffected at doses up to 1000 mg/kg.	No	TPZZ/93/0001

Test Article: FTC

Organ Systems Evaluated	Species / Strain	Method of Administration	Dose (mg/kg)	Gender and n per Group	Noteworthy Findings	GLP[a]	Study No.
General Pharmacology	Rat, CD (Sprague Dawley)	Oral	250, 500, 1000	4 M	Rats exhibited no visible effects. Minimal respiratory rate effects were only noted at 750 mg/kg (21% decrease) and in the control animals (29% increase). Behavioral reflexes were unaffected at doses up to 1000 mg/kg.	No	TPZZ/93/0001
Conditioned Avoidance Response	Rat, Long Evans	Intraperitoneal	30, 100	6 F	Control days – avoided shock on 95 ± 2 trials (mean ± SEM). Vehicle – avoided shock on 96 ± 1 trials. Emtricitabine 30 or 100 mg/kg – avoided shock 97 ± 1 and 94 ± 2 trials, respectively. In neither control, vehicle, nor the drug conditions did the rats fail to escape shock on the trials in which they failed to avoid. After 100 mg/kg, inter-trial crosses declined significantly (to 42 ± 12) but inter-trial crosses also declined significantly after vehicle injection (to 34 ± 8), an injection effect that has been seen before.	No	TPZZ/93/0119
Cardiovascular Function	Rat, Wistar	Oral	5, 10, 50	5 M	No significant effect on mean arterial blood pressure and heart rate was found.	No	477

Test Article: FTC

Organ Systems Evaluated	Species / Strain	Method of Administration	Dose (mg/kg)	Gender and n per Group	Noteworthy Findings	GLP[a]	Study No.
Systolic Blood Pressure and Heart Rate	Conscious Normotensive Rat, CD (Sprague Dawley)	Oral	250	8 M	No statistically significant differences in SBP or HR were found.	No	TPZZ/92/0057
Cardiovascular, Respiratory and Autonomic Function	Anesthetized Dogs, Beagle	Intravenous	Total cumulative dose 38.5	4 M	No statistically significant changes in mean arterial blood pressure or heart rate. No drug-related arrhythmias were observed in the Lead II electrocardiogram. There were no decreases in respiratory parameters. Increases in respiratory rate of up to 3.8 breaths/minute and minute volume of up to 0.5 l/minute were seen 10 minutes after dosing FTC; however, they were not statistically significant. The average plasma level of 2 FTC-treated dogs 30 minutes following the last dose was 140 mM. This represents 93-fold the anticipated anti-HIV/HBV IC_{50} values *in vitro* at a minimum.	No	TPZZ/92/0076

Test Article: FTC

Organ Systems Evaluated	Species / Strain	Method of Administration	Dose (mg/kg)	Gender and n per Group	Noteworthy Findings	GLP[a]	Study No.
Renal Function	Rat, Long Evans	Oral	10, 30, 100	6 M	FTC failed to cause any significant change in urine volume output, electrolyte excretion or pH values.	No	477
Gastrointestinal Motility	Mouse, ICR	Oral	10, 30, 100	10 M	No significant change in GI motility was observed.	No	477

CNS = central nervous system; F = female; FTC = emtricitabine; GI = gastrointestinal; HBV = hepatitis B virus; HIV = human immunodeficiency virus; IC_{50} = 50% inhibitory concentration; M = male

a An entry of "Yes" indicates that the study includes a GLP compliance statement.

4.3. In Vitro Studies with RPV

Test Article: RPV

Organ Systems Evaluated	Species / Strain	Method of Administration	Dose (µM)	Gender and n per Group	Noteworthy Findings	GLP[a]	Study Number
Cardiovascular (cardiac membrane potassium current)	I_{Kr} in transfected CHO cells expressing hERG	In vitro	0.1, 0.3, and 3 µM	—	0.1 µM: 10% inhibition 0.3 µM: 33% inhibition 3 µM: 80% inhibition	No	TMC278-CPF730
Cardiovascular (cardiac action potential)	Guinea pig isolated atrium	In vitro	0.01, 0.03, 0.1, 1, 3, and 10 µM	—	1 µM: RC 86% of baseline 3 µM: RC 72% of baseline 10 µM: RC 44% of baseline No effects on force of contraction compared to vehicle over the whole conc. range. No effects on ERP compared to vehicle at 0.1 and 10 µM	No	TMC278-N168576
Cardiovascular (cardiac membrane potassium current)	I_{Ks} in transfected CHO cells expressing KvLQT1/minK	In vitro	0.3, 1, 3, and 10 µM	—	1 µM: 17% inhibition 3 µM: 47% inhibition 10 µM: 73% inhibition IC50: 3.1 µM (1.15 µg/mL)	No	TMC278-NC342
Cardiovascular (cardiac membrane ion currents)	I_{Ks} in transfected HEK293 cells expressing KvLQT1/minK	In vitro	0.1, 0.3, and 1 µM	—	1 µM: 19.1% inhibition	No	TMC278-NC331

Test Article: RPV

Organ Systems Evaluated	Species / Strain	Method of Administration	Dose (µM)	Gender and n per Group	Noteworthy Findings	GLP[a]	Study Number
Cardiovascular (cardiac membrane ion currents) (continued)	I_{to} in transfected HEK293 cells expressing $hK_v4.3$	In vitro	0.1, 0.3, and 1 µM	—	0.3 µM: 13.6% inhibition 1 µM: 35.5% inhibition	No	TMC278-NC331 (continued)
	I_{K1} in transfected HEK293 cells expressing $hK_{ir}2.1$	In vitro	0.1, 0.3, and 1 µM	—	No effects	No	
	I_{Na} in transfected HEK293 cells expressing $hNa_v1.5$	In vitro	0.1, 0.3, and 1 µM	—	No effects	No	
	$I_{Ca,L}$ in transfected HEK293 cells expressing $hCa_v1.2$	In vitro	0.1, 0.3, and 1 µM	—	No effects	No	

Test Article: RPV

Organ Systems Evaluated	Species / Strain	Method of Administration	Dose (µM)	Gender and n per Group	Noteworthy Findings	GLP[a]	Study Number
Cardiovascular (cardiac membrane potassium current)	Surface expression of hERG channels in HEK293 cells	In vitro	1, 10, and 30 µM	—	1 µM: 146% expression hERG-SM 10 µM: 155% expression hERG-SM, 29% block hERG-WT 30 µM: 213% expression hERG-SM, 36% block hERG-WT TMC278 has the potential to reduce trafficking of the hERG channel and the potential to inhibit the hERG channel.	No	TMC278-NC330
Cardiovascular (cardiac action potential)	Arterially perfused rabbit ventricular wedge	In vitro	0.01, 0.1, 1, 10 µM	—	1 µM: QT-interval 106% of baseline 10 µM: QT-interval 109% of baseline. TdP score 0.5. No EADs, TdP, VT, VF, or inexcitability over the whole conc. range	No	TMC278-NC341

CHO = Chinese hamster ovary; CRO = contract research organization; ERP = effective refractory period (maximal frequency of stimulations not followed by contraction); HEK = human embryonic kidney; hERG = human ether-a-go-go related gene; hERG-WT = wild-type hERG channel; hERG-SM = chaperone resistant single mutant hERG channel; IC_{50} = 50% inhibitory concentration; RC = rate of spontaneous contraction; QT-interval = time between peak Q wave and end T wave; TdP = torsade des pointes; VF = ventricular fibrillation; VT = ventricular tachycardia

a An entry of "Yes" indicates that the study includes a GLP compliance statement.

4.4. In Vivo Studies with RPV

Test Article: RPV

Organ Systems Evaluated	Species / Strain	Method of Administration	Dose (mg/kg)	Gender and n per Group	Noteworthy Findings	GLP[a]	Study Number
CNS	Rat/Sprague Dawley	Oral gavage	10, 120, 400	5M	400 mg/kg: Slightly abnormal single behavioral parameter, 8 hrs after dosing. Slightly reduced pupil size, 2 hrs after dosing.	Yes	TMC278-Exp5560
CNS and cardiovascular	Conscious instrumented dog/Beagle	Oral gavage	20	3M and 11F	No effects Median C_{max} = 1.5 µg/mL (at 240 min)	No	TMC278-CPF654
CNS, cardiovascular, and respiratory	Conscious telemetered dog/Beagle	Oral gavage	20, 80, 160	4M	No effects on cardiac hemodynamic, electrophysiological, respiratory, or locomotor activity parameters.	Yes	TMC278-Exp5555
Cardiovascular (ECG and cardio-hemodynamic parameters)	Anesthetized Guinea pig/ Dunkin Hartley	Intravenous	0.16, 0.32, 0.64, 1.25, 2.5, 5	7F	No effects on HR, MABP, or ECG Plasma conc.:9.2 µg/mL Heart conc.: 47.7 µg/g Lung conc.: 11.7 µg/g All conc. 5 min after dosing at 5 mg/kg	No	TMC278-CPF643
Cardiovascular (ECG, cardio-hemodynamic and respiratory parameters)	Telemetered Guinea pig/ Dunkin Hartley	Oral gavage	10	6F	No effects on HR; ECG, BT Plasma C_{max} = 0.689 – 0.911 µg/mL throughout the dosing period	No	TMC278-NC327

Test Article: RPV

Organ Systems Evaluated	Species / Strain	Method of Administration	Dose (mg/kg)	Gender and n per Group	Noteworthy Findings	GLP[a]	Study Number
Cardiovascular (ECG, cardio-hemodynamic and respiratory parameters)	Anesthetized Dog/Beagle	Intravenous	5	4 (gender not reported)	SVR: -25% to BL; vehicle: +29% to BL PVR: -20% to BL; vehicle: +31% to BL Cardiac output: +31% to BL; vehicle: -12% to BL No effect on other parameters Plasma $C_{end\ of\ inf}$ = 2.62 µg/mL Plasma $C_{3h\ after\ inf}$ = 0.721 µg/mL	No	TMC278-CPF648

BL = baseline; BT = body temperature; C_{max} = maximal concentration; CNS = central nervous system; CRO = contract research organization; ECG = electrocardiogram; F = female; GLP = Good Laboratory Practice; HR = heart rate; M = male; MABP = mean arterial blood pressure; SVR = systemic vascular resistance; PVR = peripheral vascular resistance

a An entry of "Yes" indicates that the study includes a GLP compliance statement.

4.5. In Vitro Studies with TAF

Test Article: TAF (GS-7340-03)

Organ Systems Evaluated	Species / Strain	Method of Administration	Dose (µM)	Number per concentration	Noteworthy Findings	GLP[a]	Gilead (CRO) Study Number
Cardiovascular (hERG Inhibition)	Human embryonic kidney cells (HEK293)	In vitro	0, 1, and 10µM	3 replicates	No significant inhibition IC_{50} > 10 µM	Yes	PC-120-2005 ([redacted] 111213.DPW)

CRO = contract research organization; hERG = human ether-a-go-go related gene
a An entry of "Yes" indicates that the study includes a GLP compliance statement.

4.6. In Vivo Studies with TAF

Test Article: TAF (GS-7340-02)

Organ Systems Evaluated	Species / Strain	Method of Administration	Dose (mg/kg)	Gender and n per Group	Noteworthy Findings	GLP[a]	Gilead (CRO) Study Number
Cardiovascular	Dog/Beagle	Oral gavage	30, 100 (NOEL)	M 3/group	None	Yes	D2000006 ([redacted] 93205)
CNS	Rat/ Sprague Dawley CD (Crl: CD®(SD)BR)	Oral gavage	0, 100, 1000 (NOEL)	M 10/group	None	Yes	R990188 ([redacted] 56518)
Gastrointestinal	Rat/ Sprague Dawley CD (Crl: CD®(SD)BR)	Oral gavage	0, 100 (NOEL), 1000	M 9/group	At 1000 mg/kg, the rate of gastric emptying was reduced.	Yes	R990187 ([redacted] 56519)
Renal	Rat/ Sprague Dawley CD (Crl: CD®(SD)BR)	Oral gavage	0, 100, 1000 (NOEL)	M 10/group	None	Yes	R990186 ([redacted] 56520)

CNS = central nervous system; CRO = contract research organization; GLP = Good Laboratory Practice; GS-7340-02 = tenofovir alafenamide as the monofumarate form (1:1 ratio of GS-7340 to fumarate); M = male; NOEL = no-observed-effect level

a An entry of "Yes" indicates that the study includes a GLP compliance statement.

5. PHARMACODYNAMIC DRUG INTERACTIONS

Studies of the pharmacodynamic drug interactions of FTC, RPV, TAF, and the combinations of FTC/RPV/TAF are presented in the nonclinical virology summary contained in m2.7.2, Section 4.1.

6. REFERENCES

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