2.6 NONCLINICAL SUMMARY

SECTION 2.6.1—INTRODUCTION

ELVITEGRAVIR/COBICISTAT/EMTRICITABINE/
TENOFOVIR ALAFENAMIDE
FIXED-DOSE COMBINATION
(EVG/COBI/FTC/TAF [E/C/F/TAF] FDC)

Gilead Sciences

[redacted] 20[redacted]

TABLE OF CONTENTS

GLOSSARY OF ABBREVIATIONS AND DEFINITION OF TERMS

AAG	α1-acid glycoprotein
ARV	antiretroviral
ATV	atazanavir
CatA	cathepsin A
COBI	cobicistat (GS-9350), Tybost®
CYP3A	cytochrome P450 3A
dATP	deoxyadenosine triphosphate
DNA	deoxyribonucleic acid
DRV	darunavir
EC_{50}	concentration of compound inhibiting virus replication by 50%
EC_{95}	concentration of compound inhibiting virus replication by 95%
EU	European Union
EVG	elvitegravir (GS-9137; JTK-303), Vitekta®
E/C/F/TAF	elvitegravir, cobicistat, emtricitabine, and tenofovir alafenamide (coformulated)
FDA	Food and Drug Administration
FDC	fixed-dose combination
FTC	emtricitabine, Emtriva®
FTC/TDF	emtricitabine/tenofovir disoproxil fumarate, TVD, Truvada®
FTC-TP	emtricitabine triphosphate
HBV	hepatitis B virus
HIV-1	human immunodeficiency virus type 1
HS	human serum
HSA	human serum albumin
INSTI	integrase strand transfer inhibitor
MRP4	multidrug resistance associated protein 4
NDA	new drug application
NRTI	nucleoside reverse transcriptase inhibitor
NtRTI	nucleotide reverse transcriptase inhibitor
OAT	organic anion transporter
OATP	organic anion transporter polypeptide
PBMC	peripheral blood mononuclear cell
RT	reverse transcriptase
RTV	ritonavir
STB	elvitegravir/cobicistat/emtricitabine/tenofovir disoproxil fumarate (coformulated), Stribild®
TAF	tenofovir alafenamide (GS-7340)
TDF	tenofovir disoproxil fumarate, Viread®
TFV	tenofovir, PMPA
TFV-DP	tenofovir diphosphate
US	United States

1. NONCLINICAL SUMMARY

1.1. Introduction

This application is being submitted in support of a new drug application (NDA) for a fixed dose combination (FDC) that contains the integrase strand transfer inhibitor (INSTI) elvitegravir (EVG, E, Vitekta®), the pharmacokinetic enhancer cobicistat (COBI, C, Tybost®), the nucleoside reverse transcriptase inhibitor (NRTI) emtricitabine (FTC, F, Emtriva®), and the nucleotide reverse transcriptase inhibitor (NtRTI) tenofovir alafenamide (TAF, GS-7340) fumarate (GS-7340-03): the E/C/F/TAF FDC (150/150/200/10 mg) tablet. E/C/F/TAF is indicated for the treatment of human immunodeficiency virus, type 1 (HIV-1) infection in adult and pediatric patients 12 years of age and older without any known mutations associated with resistance to the individual components of E/C/F/TAF.

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 levels are achieved in HIV target cells. In HIV target cells, including lymphoid cells, TAF is metabolized by CatA, providing enhanced delivery of TFV, resulting in subsequent formation of 4-fold (3- to 7-fold at 90% confidence interval) higher intracellular levels of the active phosphorylated metabolite 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 lower circulating level of TFV is expected to result in reduced off-target effects of TFV and an improved safety profile as compared to TDF.

The E/C/F/TAF FDC tablet contains the same dosages of EVG, COBI, and FTC that are currently approved within Vitekta, Tybost, Emtriva, Truvada® (FTC/TDF), and Stribild® (E/C/F/TDF, STB) for use in adults (150 mg of EVG, 150 mg COBI, 200 mg of FTC).

Per the agreement reached between Gilead and the Food and Drug Administration (FDA; [redacted]), this NDA is supported by [redacted]. To assist the reviewer, [redacted]

To facilitate the evaluation of the E/C/F/TAF FDC, nonclinical virology studies of EVG, COBI, FTC, TAF, and TFV are described in detail in the integrated virology summary contained in m2.7.2, Section 4.1, together with the clinical virology data.

Comprehensive programs of nonclinical studies have been conducted with EVG, COBI, FTC, and TAF. Information from all nonclinical studies with EVG, COBI, FTC, and TAF should be considered in the context of the substantial clinical experience with FTC and TDF within

antiretroviral (ARV) combination therapy for the treatment of HIV-1 infection, experience with STB, and experience in the Phase 2 and 3 studies with the E/C/F/TAF FDC.

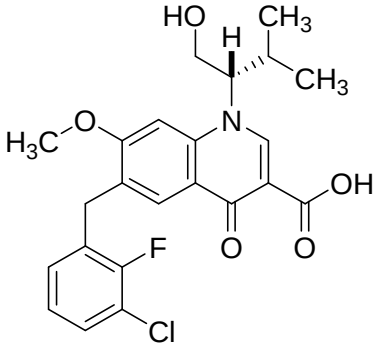
The nonclinical data discussed within this document support the proposed use of the E/C/F/TAF FDC as a complete single tablet regimen for the treatment of HIV-1 infection in adult and pediatric patients 12 years of age and older without any known mutations associated with resistance to the individual components of E/C/F/TAF. All information from nonclinical studies that is of relevance to the prescriber and patient has been included in the proposed Prescribing Information and Patient Package Insert.

1.1.1. Elvitegravir

Elvitegravir (Vitekta) is an INSTI, which has received marketing authorization in the United States (US) and European Union (EU) as 85- and 150-mg tablets to be coadministered with a ritonavir (RTV)-boosted protease inhibitor and with other ARV agents for the treatment of HIV-1 infection in adults. Elvitegravir is also a component of Stribild (STB, E/C/F/TDF 150/150/200/300 mg), which is marketed in the US and EU as a complete regimen for the treatment of HIV-1 infection in adults. Elvitegravir prevents integration of the HIV-1 genetic material into the host-cell genome.

Elvitegravir inhibited viral replication in laboratory strains and various clinical isolates of HIV-1 with mean EC_{50} (concentration of compound inhibiting virus replication by 50%) values of 0.38 nM against wild type HIV-1 in T-cell lines, 0.35 nM against HIV-1 macrophage-tropic virus in monocyte/macrophage cells, and 0.62 nM against clinical HIV-1 isolates in human PBMCs in vitro. The calculated EC_{95} value for EVG was 1.25 nM (0.61 ng/mL) in the absence of human serum (HS) components and 100 nM (44.8 ng/mL) in the presence of the HS components, human serum albumin (HSA) and α1-acid glycoprotein (AAG), in HIV-1 infected human PBMC cultures.

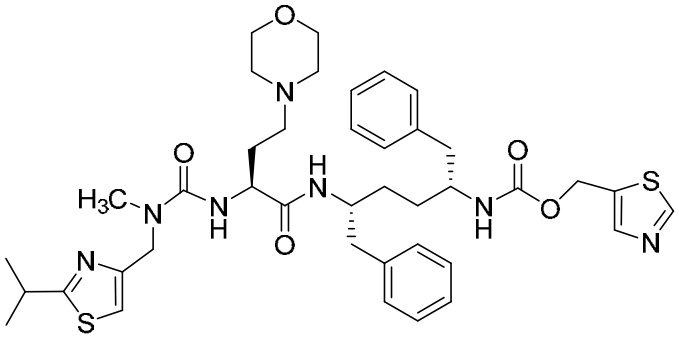
Elvitegravir has the following chemical structure:



1.1.2. Cobicistat

Cobicistat (Tybost) is a strong mechanism-based cytochrome P450 3A (CYP3A) inhibitor (a pharmacokinetic enhancer) that increases the systemic levels of coadministered agents metabolized by CYP3A enzymes, including EVG and the HIV protease inhibitors atazanavir (ATV) and darunavir (DRV). Tybost 150-mg tablets received marketing authorization in the US and EU as a pharmacokinetic enhancer of ATV 300 mg once daily or DRV 800 mg once daily as part of ARV combination therapy in adults with HIV-1 infection. Cobicistat is also a component of Stribild.

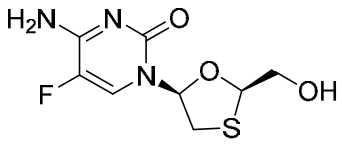
Cobicistat, a structural analogue of RTV, has the following chemical structure:



1.1.3. Emtricitabine

Emtricitabine (FTC) is a NRTI. It is the active ingredient in Emtriva 200-mg capsules and 10 mg/mL oral solution that have been approved in the US, the EU, and other countries worldwide in combination with other ARV agents for the treatment of HIV-1 infection. The international birthdate for FTC is 02 July 2003.

The chemical structure of FTC is as follows:



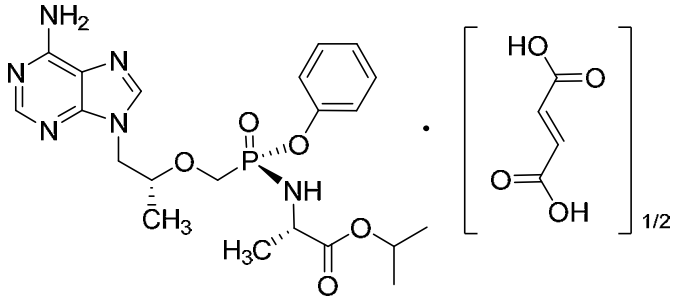
Following absorption, FTC is phosphorylated by cellular enzymes to emtricitabine triphosphate (FTC-TP), the active metabolite, an analog of 2'-deoxycytidine triphosphate. Emtricitabine 5'-triphosphate inhibits the activity of HIV-1 reverse transcriptase through high affinity binding, competing with the natural substrate 2'-deoxycytidine 5'-triphosphate. Emtricitabine 5'-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 in 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.

1.1.4. Tenofovir Alafenamide

Tenofovir alafenamide (TAF), a prodrug of TFV (PMPA), is a NtRTI. The first generation tenofovir prodrug is TDF, which is the active ingredient in Viread that has been approved in the US, the EU, and other countries worldwide as a once a day tablet (300 mg, equivalent to 245 mg tenofovir disoproxil), in combination with other ARV agents, for the treatment of HIV-1 infection.

The chemical structure of TAF fumarate is shown below.



Tenofovir is not well 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-diphosphate (DP) in HIV-1 target cells including lymphocytes and macrophages. TAF is predominantly hydrolyzed to TFV by CatA cleavage in HIV-target cells {13119}, {10427}, resulting in higher intracellular levels of TFV-DP relative to TDF {17137}. The active metabolite, TFV-DP {1574}, competes with natural 2'-deoxyadenosine triphosphate (dATP) for incorporation by the HIV-1 or hepatitis B virus (HBV) 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 are lower following the administration of TAF than of TDF. The lower systemic levels of TFV and the higher intracellular levels of TFV-DP have the potential to translate into less risk of nephrotoxicity and less decrease in bone mineral density, which are known risks with TDF administration {21762}, {22031}.

1.1.5. Elvitegravir/Cobicistat/Emtricitabine/Tenofovir Alafenamide

The proposed FDC is based on the complimentary pharmacological mechanisms of action of EVG, FTC, and TAF and the body of clinical experience with nucleoside/nucleotide reverse transcriptase inhibitors (N[t]RTIs) and INSTIs in HIV-infected patients. Combinations of these agents are not antagonistic and are synergistic in cell-based in vitro studies.

The intended positive pharmacokinetic interaction within the 4-drug combination is an increase in the bioavailability and a decrease in the rate of elimination of EVG due to inhibition of CYP3A activity by COBI, and a consequent profound reduction in the formation of M1 (GS-9202), the major oxidative metabolite of EVG. This interaction has been well characterized in vitro. Animal models are inappropriate to investigate this interaction due to the lack of mechanism-based inhibition by COBI in nonhuman species. The other potential drug interactions among the 4 components include inhibition of intestinal efflux of TAF by COBI and inhibition of organic anion transporter polypeptide (OATP)-mediated hepatic uptake of TAF by COBI and EVG. The increase in TAF exposure due to inhibition of intestinal efflux by COBI has been taken into account during the TAF clinical dose selection for the E/C/F/TAF FDC. The effect of OATP inhibition by COBI and EVG on TAF exposure is unlikely to be clinically significant as only a modest increase in exposure (not considered clinically relevant) of the OATP substrate, rosuvastatin, was observed when it was codosed with both EVG and COBI. In a Phase 1 clinical study, no statistically significant difference in FTC and TAF exposures were observed following multiple-dose administration of E/C/F/TAF and FTC/TAF 10 mg (GS-US-292-0103). Cobicistat does not inhibit OAT1 or multidrug resistance associated protein 4 (MRP4), the transporters responsible for the renal excretion of TFV and so will not interfere with the elimination of TFV.

The toxicity profiles of the 4 agents differ substantially with no clinically significant overlapping toxicity. Because the target organ profiles are different, and there is no evidence of genotoxicity, carcinogenicity, or reproductive toxicity, administration of the E/C/F/TAF combination product is unlikely to introduce new toxicities or to exacerbate known toxicities of the individual agents. The ample nonclinical safety databases on these drugs, including combination toxicity studies with EVG and COBI, and with FTC and TDF, strongly indicate further toxicological investigations are unlikely to yield new data relevant to humans. Additionally, the extensive clinical safety data available from the clinical trials with STB and with the E/C/F/TAF FDC support the safety of the new combination product for HIV-1 infection. The absence of nonclinical safety studies with the combination is in accordance with the FDA Guidance for Industry, Nonclinical Safety Evaluation of Drug or Biologic Combinations, March 2006 and the CHMP Guideline on the Non-Clinical Development of Fixed Combinations of Medicinal Products (EMEA/CHMP/SWP/258498/2005, January 2008). Extensive clinical safety data are available for the approved drugs FTC (Emtriva), TDF (Viread), the FTC/TDF FDC product (Truvada), and the E/C/F/TDF FDC product (STB, Stribild) and support the overall risk/benefit of this new E/C/F/TAF FDC product for HIV-1 infection.

1.1.6. References

21 Yokota T, Konno K, Shigeta S, Holý A, Balzarini J, De Clercq E. Inhibitory effects of acyclic nucleoside phosphonate analogues of hepatitis B virus DNA synthesis in HB611 cells. Antivir Chem Chemother 1994;5 (2):57-63.

1131 Cherrington JM, Allen SJW, Bischofberger N, Chen MS. Kinetic interaction of the diphosphates of 9-(2-phosphonylmethoxyethyl)adenine and other anti-HIV active purine congeners with HIV reverse transcriptase and human DNA polymerases α, β, and γ. Antivir Chem Chemother 1995;6 (4):217-21.

1574 Robbins BL, Srinivas RV, Kim C, Bischofberger N, Fridland A. Anti-human immunodeficiency virus activity and cellular metabolism of a potential prodrug of the acyclic nucleoside phosphonate 9-*R*-(2-phosphonomethoxypropyl)adenine (PMPA), Bis(isopropyloxymethylcarbonyl) PMPA. Antimicrob Agents Chemother 1998;42 (3):612-7.

10427 Birkus G, Wang R, Liu XH, Kutty N, MacArthur H, Cihlar T, et al. Cathepsin A is the major hydrolase catalyzing the intracellular hydrolysis of the antiretroviral nucleotide phosphonoamidate prodrugs GS-7340 and GS-9131. Antimicrob Agents Chemother 2007;51 (2):543-50.

13119 Birkus G, Kutty N, He GX, Mulato A, Lee W, McDermott M, et al. Activation of 9-[(R)-2-[[(S)-[[(S)-1-(Isopropoxycarbonyl)ethyl]amino] phenoxyphosphinyl]-methoxy]propyl]adenine (GS-7340) and other tenofovir phosphonoamidate prodrugs by human proteases. Mol Pharmacol 2008;74 (1):92-100.

17137 Markowitz M, Zolopa A, Ruane P, Squires K, Zhong L, Kearney BP, et al. GS-7340 Demonstrates Greater Declines in HIV-1 RNA than Tenofovir Disoproxil Fumarate During 14 Days of Monotherapy in HIV-1 Infected Subjects [Oral Presentaton / Paper 152LB]. 18th Conference on Retroviruses and Opportunistic Infections (CROI); 2011 February 27 - March 2; Boston, MA.

21762 VIREAD® (tenofovir disoproxil fumarate) Tablets and Powder for oral use. US Prescribing Information. Gilead Sciences, Inc. Foster City, CA. Revised August 2012.

22031 Department of Health and Human Services (DHHS). HHS Panel on Antiretroviral Guidelines for Adults and Adolescents Recommends a Fixed-Dose Combination Product of Elvitegravir/Cobicistat/Tenofovir/Emtricitabine as an Alternative Regimen in Antiretroviral Treatment-Naive Individuals with HIV-1 Infection. 2012:1-2.

2.6 NONCLINICAL SUMMARY

SECTION 2.6.2—PHARMACOLOGY WRITTEN SUMMARY

ELVITEGRAVIR/COBICISTAT/EMTRICITABINE/ TENOFOVIR ALAFENAMIDE FIXED-DOSE COMBINATION (EVG/COBI/FTC/TAF [E/C/F/TAF] FDC)

Gilead Sciences

[redacted] **20**[redacted]

TABLE OF CONTENTS

LIST OF IN-TEXT TABLES

LIST OF IN-TEXT FIGURES

LIST OF ABBREVIATIONS

3TC	lamivudine
ABC	abacavir
AIDS	acquired immune deficiency syndrome
ATV	atazanavir
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
CYP3A	cytochrome P450 3A
d4T	stavudine
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%
E/C/F/TAF	elvitegravir, cobicistat, emtricitabine, and tenofovir alafenamide (coformulated)
ECG	electrocardiogram
EFV	efavirenz
EVG	elvitegravir (JTK-303), Vitekta®, Gilead Sciences
fbe	free base equivalent
FDC	fixed-dose combination
FTC, 524W91	emtricitabine (Emtriva®, Gilead Sciences)
GLP	Good Laboratory Practice
HBV	hepatitis B virus
HCV	hepatitis C virus
HEK	human embryonic kidney
hERG	human ether-à-go-go related gene
HIV-1	human immunodeficiency virus type 1
IC_{50}	inhibitory concentration
K_i	affinity constant for enzyme inhibition
IL-2	interleukin-2
INSTI	integrase strand transfer inhibitor
LDH	lactate dehydrogenase

LIST OF ABBREVIATIONS (CONTINUED)

LPV	lopinavir
LV	left ventricular
MRP4	multidrug resistance protein 4
MSD	multiplicative standard deviations
mtDNA	mitochondrial deoxyribonucleic acid
NDP	nucleoside disphosphate
NFV	nelfinavir
NNRTI	nonnucleoside reverse transcriptase inhibitor
NRTI	nucleoside reverse transcriptase inhibitor
NtRTI	nucleotide reverse transcriptase inhibitor
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
RT	reverse transcriptase
RTV, r	ritonavir
SD	standard deviation
SI	selectivity index
SkMC	skeletal muscle cell
STB	elvitegravir/cobicistat/emtricitabine/tenofovir disoproxil fumarate (coformulated) (Stribild®, Gilead Sciences)
TAF	tenofovir alafenamide, formerly GS-7340
TDF	tenofovir disoproxil fumarate
TFV	tenofovir
TFV-DP	tenofovir diphosphate
TFV-MP	tenofovir monophosphate
ZDV	zidovudine

NOTE TO REVIEWER

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

As TAF is a new chemical entity, this nonclinical summary contains all available data on this new component. Per the agreement reached between Gilead Sciences, Inc. (Gilead) and the Food and Drug Administration (FDA;), this NDA is supported by .

. To assist the reviewer,

Given the lack of relevant effects in the in vitro studies with the individual agents (EVG, COBI, FTC, TFV, or TAF) or with the combination of EVG+COBI+FTC+TFV, no additional pharmacology studies have been conducted for the E/C/F/TAF combination.

The following conversions are provided to aid the reviewer:

- EVG (GS-9137, JTK-303) 1 µM = 0.448 µg/mL
- COBI (GS-9350) 1 µM = 0.776 µg/mL
- FTC 1 µM = 0.247 µg/mL
- TAF (GS-7340) 1 µM = 0.477 µg/mL
- TFV 1 µM = 0.287 µg/mL

1. BRIEF SUMMARY

This application is being submitted in support of a new drug application for a fixed-dose combination (FDC) that contains elvitegravir (EVG, E, Vitekta®), cobicistat (COBI, C, Tybost®), emtricitabine (FTC, F, Emtriva®), and tenofovir alafenamide (TAF, formerly GS-7340): the E/C/F/TAF FDC (150/150/200/10 mg). E/C/F/TAF is indicated for the treatment of HIV-1 infection in adults and pediatric patients 12 years of age and older without any known mutations associated with resistance to the individual components of E/C/F/TAF.

Comprehensive programs of nonclinical pharmacology studies with EVG, COBI, FTC, 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 E/C/F/TAF FDC, nonclinical virology studies of TFV/TAF are described in detail in the integrated virology summary contained in m2.7.2, Section 4.1, together with the clinical virology data. The secondary pharmacodynamics (excluding all virology data) and safety pharmacology of TFV/TAF and of the E/C/F/TFV combination are described in detail in this section.

The nonclinical data discussed within this document support the proposed use of the E/C/F/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.

EVG

Elvitegravir (Vitekta) is an integrase strand transfer inhibitor (INSTI) developed by Gilead Sciences that is approved in the United States (US) as 85- and 150-mg tablets to be coadministered with a ritonavir (RTV)-boosted protease inhibitor (PI) and with other antiretroviral agents for the treatment of HIV-1 infection. Elvitegravir is also a component of Stribild (EVG/COBI/FTC/ tenofovir disoproxil fumarate [TDF] 150/150/200/300 mg), which is approved in the US as a complete regimen for the treatment of HIV-1 infection. Elvitegravir prevents integration of the HIV-1 genetic material into the host-cell genome.

A comprehensive nonclinical pharmacology program was undertaken in support of the registration of EVG. The results of this evaluation were presented in detail in the original NDA and subsequent submissions for Vitekta and Stribild.

COBI

Cobicistat (Tybost) is a mechanism-based cytochrome P450 3A (CYP3A) inhibitor (a pharmacokinetic enhancer) that increases the systemic levels of co-administered agents metabolized by CYP3A enzymes, including EVG and the HIV protease inhibitors (PIs) atazanavir (ATV) and darunavir (DRV). Tybost 150 mg tablets, developed by Gilead Sciences, is

approved in the US as a pharmacokinetic enhancer of ATV 300 mg once daily or DRV 800 mg once daily as part of antiretroviral combination therapy in adults with HIV-1 infection. Cobicistat is also a component of Stribild.

A comprehensive nonclinical pharmacology program was undertaken in support of the registration of COBI. The results of this evaluation were presented in detail in the original NDA and subsequent submissions for Tybost and Stribild.

FTC

Emtricitabine (Emtriva) 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 phosphorylated by cellular enzymes to form the active metabolite, emtricitabine triphosphate.

A comprehensive nonclinical pharmacology program was undertaken in support of the registration of FTC. The results of this evaluation were presented in detail in the original NDA and subsequent submissions for Emtriva.

TAF (and TFV)

Tenofovir alafenamide is an investigational prodrug of TFV, a nucleotide reverse transcriptase inhibitor (NtRTI). In HIV-target cells including lymphocytes, TAF is hydrolyzed to TFV by cathepsin A (CatA) cleavage {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 reverse transcriptase (RT) and hepatitis B virus (HBV) DNA polymerase that terminates the elongation of the viral deoxyribonucleic acid (DNA) chain {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 peripheral blood mononuclear cells (PBMCs), and macrophages with 50% effective concentration (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 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 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 therapeutic 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 selectivity index (SI) (considering CC_{50} in renal human embryonic kidney [HEK] 293 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 mitochondrial DNA (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 central nervous system (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.

Summary

No relevant cytotoxicity or mitochondrial toxicity was observed with TAF alone or TFV in combination with EVG, COBI, and FTC. 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, EVG, and COBI. The combination of TFV with either COBI alone or EVG+COBI+FTC at their pharmacologically relevant concentrations showed no significant change in the cytotoxicity of TFV in cells transiently expressing OAT1 alone, or OAT1 and multidrug resistance protein 4 (MRP4) together, suggesting that EVG, COBI, and FTC are not likely to directly affect the toxicity potential of TFV in renal cells and tissues

expressing the relevant renal transporters. The potential for exacerbating cytotoxicity or mitochondrial toxicity with the E/C/F/TAF combination is low.

Elvitegravir, FTC, and TAF had little effect on vital organ systems in safety pharmacology studies. Minor changes were observed in the cardiovascular safety pharmacology study with COBI, but in a thorough QT/QTc clinical study (m1.4.4, GS-US-216-0107), a modest, dosing-related increase in PR interval was observed, but was not considered to be clinically significant. Thus, additional safety pharmacology studies on the E/C/F/TAF combination are considered unwarranted.

Overall, the pharmacological assessment of EVG, COBI, FTC, and TAF (and TFV) supports the effective use of EVG, COBI, FTC, and TAF together in combination therapy for HIV-1 disease.

2. PRIMARY PHARMACODYNAMICS

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

2.1. TAF

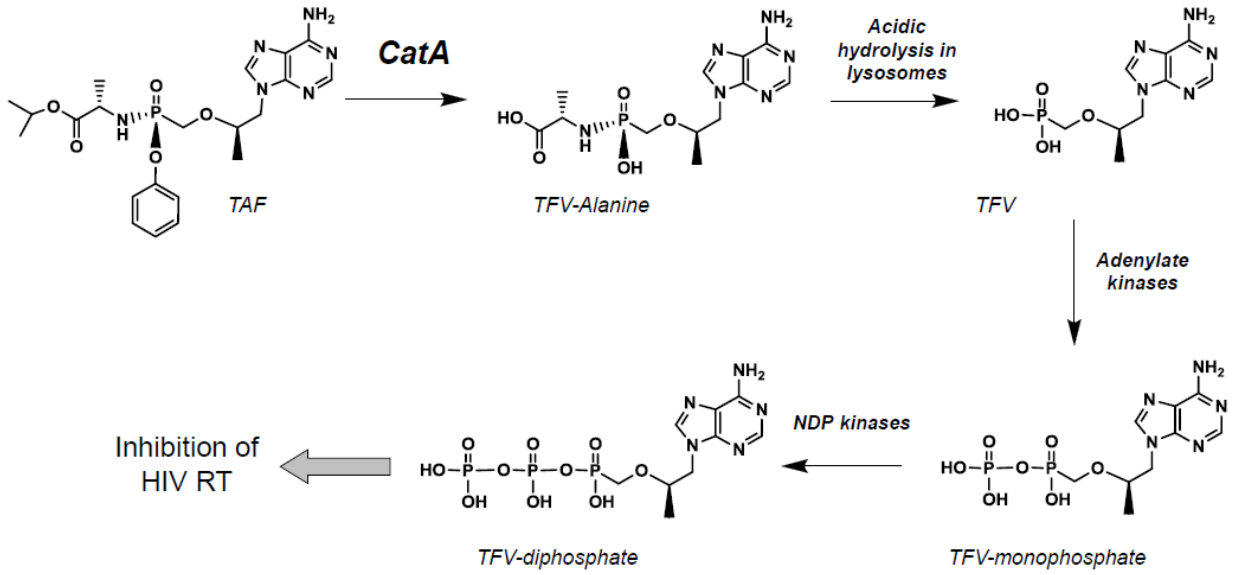
Tenofovir alafenamide is predominantly hydrolyzed to TFV by CatA cleavage in target lymphoid cells {13119}, {10427}, resulting in higher intracellular levels of TFV-DP in vivo and lower circulating levels of TFV relative to TDF {17137}. Intracellularly, TFV is metabolized to the active metabolite, TFV-DP {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.

2.1.1. Intracellular Metabolism of TAF

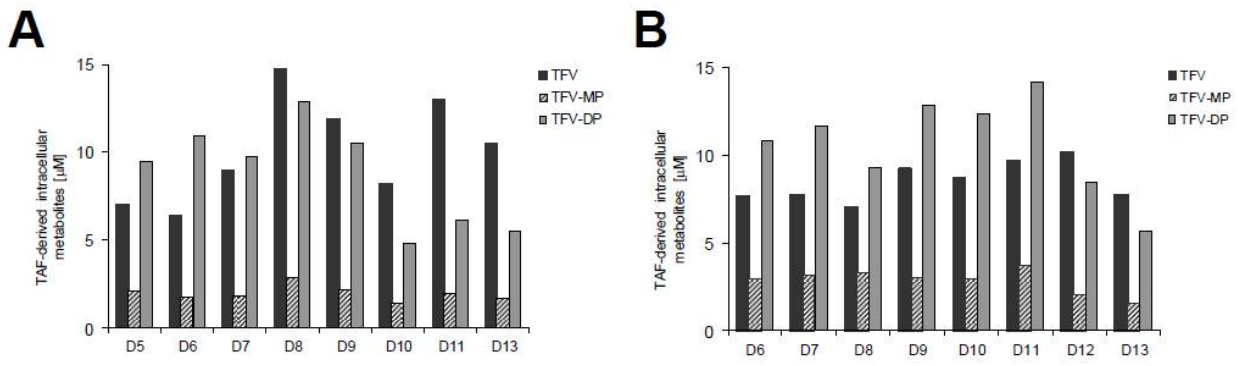
The lysosomal carboxypeptidase CatA plays an essential role in the intracellular activation of TAF in lymphoid cells and tissues (Figure 1). Cathepsin A levels and the intracellular activation of TAF were evaluated in primary CD4+ T lymphocytes and MDMs isolated from PBMCs from 13 donors of variable gender, age, and ethnicity (m2.6.3, Section 1.1, 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



TAF = tenofovir alafenamide; TFV = tenofovir; TFV-MP = tenofovir monophosphate; TFV-DP = tenofovir disphosphate
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

2.1.2. Effects of Inhibitors of HIV, 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 1.1, PC-120-2001). The HIV PIs DRV, ATV, lopinavir (LPV), and RTV, as well as the pharmacoenhancing agent COBI, 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 50% inhibitory concentration (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 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 TAF (and TFV) and E/C/F/TFV are described in Sections 3.1 and 3.2.

3.1. TAF

3.1.1. Effect of TFV Diphosphate on Cellular DNA Polymerases

As described in Section 2.1, 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 2 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 2. 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}, {8925}

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

	Relative Efficiency of Incorporation (%)[a]		
dNTP Analog	**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 (%) = $100 \times [V_{max}(\text{dNTP analog})/K_m(\text{dNTP analog})]/[V_{max}(\text{dNTP})/K_m(\text{dNTP})]$.
Data from reference: {2005}

3.1.2. In Vitro Receptor Binding Potencies of TDF and TFV

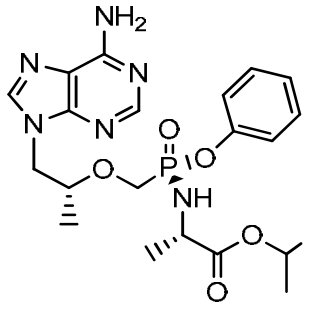
A primary screen was used to determine the effect of the major metabolite of TAF, TFV, and the other prodrug of TFV, TDF, on the inhibition or stimulation of binding in a series of 111 protein targets (neuroreceptors, ion channels, transporters, and nuclear receptors) (m2.6.3, Section 1.2, V2000020). 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.

3.1.3. In Vitro Cytotoxicity

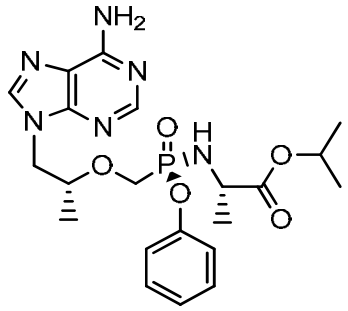
3.1.3.1. Cytotoxicity in Human PBMCs and Cell Lines

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 1.2, 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 4), 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 4.1.1.1.2.1, 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



GS-7340 (TAF)



GS-7339

Source: Report PC-120-2009

Table 4. 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 1.2, 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 5). 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 1.2, PC-120-2007). The CC_{50} value for TAF was > 44.4 µM in the hepatic cell line (Table 5). 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 5. In Vitro Cytotoxicity of TAF and other HIV Inhibitors in Human T-Lymphoblastoid and Hepatic Cell Lines

		Cytotoxicity CC_{50}, µM (MSD)[a]		
		Hepatic	T-Cell	
Class	Drug	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

3.1.3.2. Hematopoietic Toxicity

3.1.3.2.1. 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 1.2, 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 1.2, 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 6. 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

3.1.3.2.2. 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 7). 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 7. In Vitro Hematopoietic Toxicity of TFV in Comparison with Other NRTIs

	Hematopoietic Toxicity – CC_{50} [μM][a]			
	Myeloid Lineage		Erythroid Lineage	
Drug	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}

3.1.3.3. 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 1.2, 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 8). 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 8. 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

3.1.3.4. 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 9). Moreover, TFV did not exhibit any marked effect on the long-term viability of quiescent RPTECs during a 25-day incubation (Table 9). 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 1.2, 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 9, 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 9) {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 9. 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}

3.1.3.5. 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 1.2, 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 10). 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 10. 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

3.1.4. 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}.

3.1.4.1. Effect of TAF on Mitochondrial DNA Content

The potential for TAF to induce mtDNA depletion was evaluated in HepG2 cells (m2.6.3, Section 1.2, 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 11). 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 11. 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

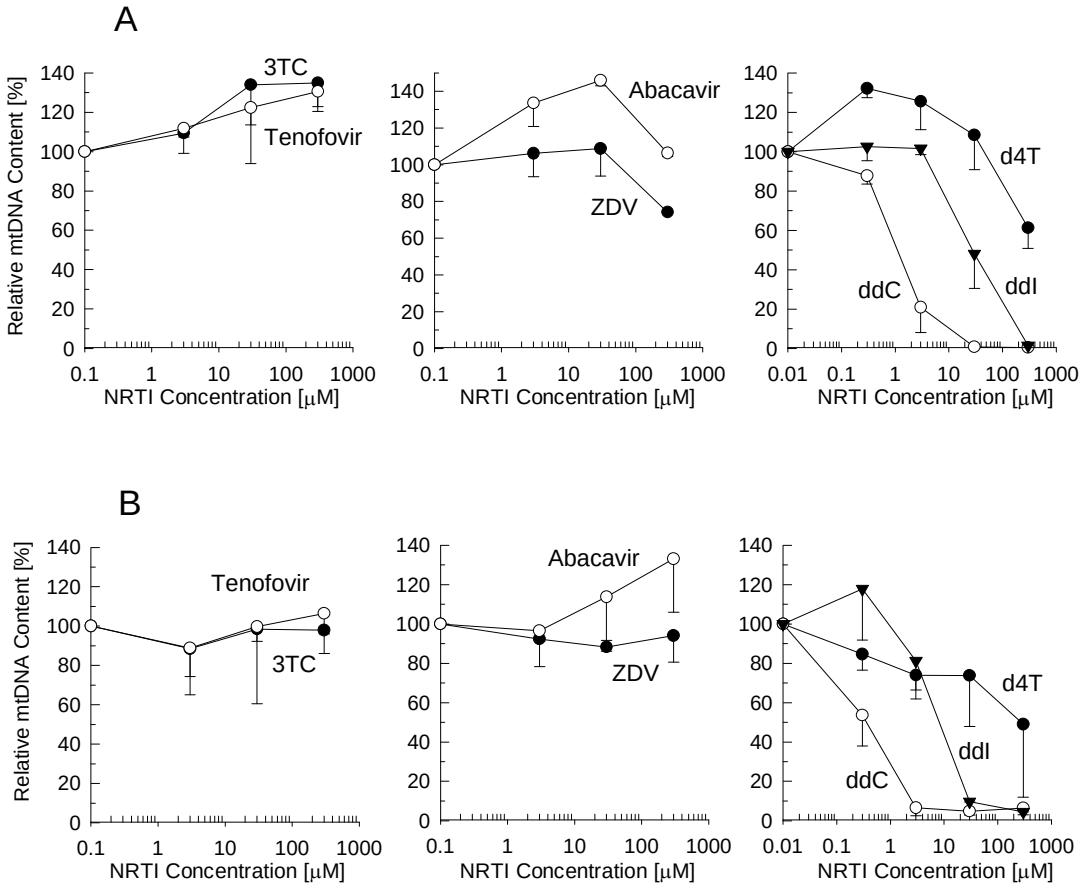
ddC = zalcitabine; DMSO = dimethylsulfoxide; mtDNA = mitochondrial DNA; TAF = tenofovir alafenamide
a Data represent the mean ± SD of 3 independent experiments performed in triplicate.
b Paired, 2-tailed Student's t-test
Source: Report PC-120-2006

3.1.4.2. 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 12; m2.6.3, Section 1.2, 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 12; m2.6.3, Section 1.2, 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 12, 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 12; m2.6.3, Section 1.2, 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 12. 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 3.1.1).

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 13, 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 1.2, 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 13. 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

3.2. TFV

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

The potential in vitro cytotoxicity of TFV was investigated in primary human RPTECs, either alone or in combination with COBI or EVG+COBI+FTC (m1.4.4, 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 14). Tenofovir + COBI alone or in combination with FTC 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 14. 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]	> 4000	> 4000
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

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

The potential effect of EVG, COBI, and FTC 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 (m1.4.4, PC-236-2013). Tenofovir cytotoxicity was measured in these cells either alone or in combination with EVG+COBI+FTC. Following a 4-day treatment, TFV showed minimal cytotoxicity in control cells that did not express the renal transporters (CC_{50} > 2000 µM) (Table 15). 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 EVG+COBI+FTC 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 15. Effect of EVG, COBI, and FTC 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 EVG, COBI, and FTC 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

3.3. E/C/F/TAF

Given the lack of relevant effects in the in vitro studies with the individual agents (EVG, COBI, FTC, TFV, or TAF) or with the combination of EVG+COBI+FTC+TFV, no additional secondary pharmacodynamic studies have been conducted for the E/C/F/TAF combination.

4. SAFETY PHARMACOLOGY

In vitro and in vivo safety pharmacology data for TAF are presented in Section 4.1. As discussed in Section 4.2, additional safety pharmacology studies on the E/C/F/TAF combination are considered unwarranted.

4.1. TAF

The safety pharmacology studies of TAF were conducted in accordance with GLP regulations. The in vitro human ether-à-go-go related gene (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.

4.1.1. 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 4.2, R990188; [redacted] 56518). The no-effect dose for a pharmacological effect on the CNS of the male Sprague Dawley rat was 1000 mg/kg.

4.1.2. Cardiovascular System

4.1.2.1. 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 4.1, PC-120-2005; [redacted] 111213.[redacted]).

4.1.2.2. 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 electrocardiograms (ECGs) (m2.6.3, Section 4.2, D2000006; [redacted] 93205).

4.1.3. 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 4.2, 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.

4.1.4. 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 free base equivalents [fbe]/kg) (m2.6.3, Section 4.2, 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.

4.2. E/C/F/TAF

Elvitegravir, FTC, and TAF had little effect on vital organ systems in safety pharmacology studies. Cobicistat showed the potential to decrease left ventricular (LV) function and prolong the PR interval in the isolated rabbit heart at ≥ 1 μM, which is approximately 11-fold above the anticipated clinical exposure at the COBI 150-mg dose (maximal plasma concentrations of approximately 1.4 μM and fraction unbound of 6.3% based on in vitro equilibrium dialysis). Further, as the fraction of unbound COBI is lower in plasma samples obtained in clinical studies (2.49% to 3.23%) compared with the in vitro studies, including clinical studies in subjects with moderate hepatic impairment or severe renal impairment (m1.4.4, GS-US-183-0133 and GS-US-216-0124, respectively]), the potential of COBI to decrease LV function and prolong PR is expected to be low in patients. In a thorough QT/QTc clinical study (m1.4.4, GS-US-216-0107), a modest, dosing-related increase in PR interval was observed, but was not considered to be clinically significant. 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 4.2, D2000006; [redacted] 93205) or in the thorough QT study (m2.7.2, Section 2.2.2.1 [Study GS-US-120-0107]).

Overall, the pharmacological assessment of EVG, COBI, FTC, and TAF supports the effective use of these 4 agents at the proposed doses and together in combination therapy for HIV-1 disease. Additional safety pharmacology studies on the E/C/F/TAF combination are considered unwarranted.

5. PHARMACODYNAMIC DRUG INTERACTIONS

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

6. DISCUSSION AND CONCLUSIONS

6.1. TAF (and TFV)

A discussion of the nonclinical virology data of TAF 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 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 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 this agent in combination therapy for the treatment of HIV-1 infection.

6.2. Discussion and Conclusions

The cytotoxicity of TFV in RPTECs was not affected by combination with FTC, EVG, and COBI when tested in vitro at pharmacologically relevant concentrations. Combination of TFV with either COBI alone or EVG+COBI+FTC 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 EVG, COBI, and FTC are 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 EVG was considered low based on assessment of the mtDNA levels in HepG2 liver cells. The potential for mitochondrial toxicity by COBI is also considered low. As EVG and COBI are not anticipated to significantly increase the exposure of FTC and TAF, the potential for exacerbating mitochondrial toxicity with the E/C/F/TAF combination is low.

Elvitegravir, FTC, and TAF had little effect on vital organ systems in safety pharmacology studies. Cobicistat showed the potential to decrease LV function and prolong the PR interval in the isolated rabbit heart at ≥ 1 μM, which is approximately 11-fold above the anticipated clinical exposure at the COBI 150-mg dose (maximal plasma concentrations of approximately 1.4 μM and fraction unbound of 6.3% based on in vitro equilibrium dialysis). Further, as the fraction of unbound COBI is lower in plasma samples obtained in clinical studies (2.49% to 3.23%) compared with the in vitro studies, including clinical studies in subjects with moderate hepatic impairment or severe renal impairment (m1.4.4, GS-US-183-0133 and GS-US-216-0124, respectively]), the potential of COBI to decrease LV function and prolong PR is expected to be low in patients. In a thorough QT/QTc clinical study (m1.4.4, GS-US-216-0107), a modest,

dosing-related increase in PR interval was observed, but was not considered to be clinically significant. 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 4.2, D2000006) or in the thorough QT study (m2.7.2, Section 2.2.2.1 [Study GS-US-120-0107]).

Given the favorable safety pharmacology profiles of EVG, FTC, and TAF, combination of these 3 agents with COBI is not expected to exacerbate the minor findings of COBI. Thus, additional safety pharmacology studies on the E/C/F/TAF combination are considered unwarranted.

Overall, the pharmacodynamic and pharmacological assessment of EVG, COBI, FTC, and TAF (and TFV) supports the effective and safe use of EVG, COBI, FTC, and TAF at the proposed doses together in combination for treatment of HIV-1 disease.

7. REFERENCES

References are available upon request.

21 Yokota T, Konno K, Shigeta S, Holý A, Balzarini J, De Clercq E. Inhibitory effects of acyclic nucleoside phosphonate analogues of hepatitis B virus DNA synthesis in HB611 cells. Antivir Chem Chemother 1994;5 (2):57-63.

1131 Cherrington JM, Allen SJW, Bischofberger N, Chen MS. Kinetic interaction of the diphosphates of 9-(2-phosphonylmethoxyethyl)adenine and other anti-HIV active purine congeners with HIV reverse transcriptase and human DNA polymerases a, b, and g. Antivir Chem Chemother 1995;6 (4):217-21.

1574 Robbins BL, Srinivas RV, Kim C, Bischofberger N, Fridland A. Anti-human immunodeficiency virus activity and cellular metabolism of a potential prodrug of the acyclic nucleoside phosphonate 9-R-(2-phosphonomethoxypropyl)adenine (PMPA), Bis(isopropyloxymethylcarbonyl) PMPA. Antimicrob Agents Chemother 1998;42 (3):612-7.

2005 Cihlar T, Chen MS. Incorporation of selected nucleoside phosphonates and anti-human immunodeficiency virus nucleotide analogues into DNA by human DNA polymerases a, b and g. Antivir Chem Chemother 1997;8 (3):187-95.

2087 Ho ES, Lin DC, Mendel DB, Cihlar T. Cytotoxicity of antiviral nucleotides adefovir and cidofovir is induced by the expression of human renal organic anion transporter 1. J Am Soc Nephrol 2000;11 (3):383-93.

2516 Kramata P, Birkus G, Otmar M, Votruba I, Holy A. Structural features of acyclic nucleotide analogs conferring inhibitory effects on cellular replicative DNA polymerases. Collection Symposium Series (Holy A and Tocik Z, eds), Institute of Organic Chemistry and Biochemistry, Academy of Sciences of Czech Republic, Prague, Czech Republic 1996;1:188-91.

2517 Pisarev VM, Lee S-H, Connelly MC, Fridland A. Intracellular metabolism and action of acyclic nucleoside phoshonates on DNA replication. Mol Pharmacol 1997;52 (1):63-8.

2518 Suo Z, Johnson KA. Selective inhibition of HIV-1 reverse transcriptase by an antiviral inhibitor, (R)-9-(2-Phosphonylmethoxypropyl)adenine. J Biol Chem 1998;273 (42):27250-8.

2520 Cihlar T, Ho ES, Lin DC, Mulato AS. Human renal organic anion transporter 1 (hOAT1) and its role in the nephrotoxicity of antiviral nucleotide analogs. Nucleosides Nucleotides Nucleic Acids 2001;20 (4-7):641-8.

2522 Brinkman K, Ter Hofstede HJ, Burger DM, Smeitink JAM, Koopmans PP. Adverse effects of reverse transcriptase inhibitors: mitochondrial toxicity as common pathway. AIDS 1998;12 (14):1735-44.

3084 Murphy RL, Brun S, Hicks C, Eron JJ, Gulick R, King M, et al. ABT-378/ritonavir plus stavudine and lamivudine for the treatment of antiretroviral-naive adults with HIV. AIDS 2001;15 (1):F1-F9.

3320 Birkus G, Hitchcock MJM, Cihlar T. Assessment of mitochondrial toxicity in human cells treated with tenofovir: comparison with other nucleoside reverse transcriptase inhibitors. Antimicrob Agents Chemother 2002;46 (3):716-23.

4077 Cihlar T, Birkus G, Greenwalt DE, Hitchcock MJM. Tenofovir exhibits low cytotoxicity in various human cell types: comparison with other nucleoside reverse transcriptase inhibitors. Antiviral Res 2002;54 (1):37-45.

5552 Carr A. Toxicity of antiretroviral therapy and implications for drug development. Nat Rev Drug Discov 2003;2 (8):624-34.

7180 Moyle G. Clinical manifestations and management of antiretroviral nucleoside analog-related mitochondrial toxicity. Clin Ther 2000;22 (8):911-36.

7415 Lee WA, He G-X, Eisenberg E, Cihlar T, Swaminathan S, Mulato A, et al. Selective intracellular activation of a novel prodrug of the human immunodeficiency virus reverse transcriptase inhibitor tenofovir leads to preferential distribution and accumulation in lymphatic tissue. Antimicrob Agents Chemother 2005;49 (5):1898-906.

7538 Pan-Zhou X-R, Cui L, Zhou X-J, Sommadossi J-P, Darley-Usmar VM. Differential effects of antiretroviral nucleoside analogs on mitochondrial function in HepG2 cells. Antimicrob Agents Chemother 2000;44 (3):496-503.

8925 White KL, Chen JM, Feng JY, Margot NA, Ly JK, Ray AS, et al. The K65R reverse transcriptase mutation in HIV-1 reverses the excision phenotype of zidovudine resistance mutations. Antivir Ther 2006;11:155-63.

9864 Vidal F, Domingo JC, Guallar J, Saumoy M, Cordobilla B, Sanchez de la Rosa R, et al. In vitro cytotoxicity and mitochondrial toxicity of tenofovir alone and in combination with other antiretrovirals in human renal proximal tubule cells. Antimicrob Agents Chemother 2006;50 (11):3824-32.

10427 Birkus G, Wang R, Liu XH, Kutty N, MacArthur H, Cihlar T, et al. Cathepsin A is the major hydrolase catalyzing the intracellular hydrolysis of the antiretroviral nucleotide phosphonoamidate prodrugs GS-7340 and GS-9131. Antimicrob Agents Chemother 2007;51 (2):543-50.

13119 Birkus G, Kutty N, He GX, Mulato A, Lee W, McDermott M, et al. Activation of 9-[(R)-2-[[(S)-[[(S)-1-(Isopropoxycarbonyl)ethyl]amino] phenoxyphosphinyl]-methoxy]propyl]adenine (GS-7340) and other tenofovir phosphonoamidate prodrugs by human proteases. Mol Pharmacol 2008;74 (1):92-100.

13797 Patterson TF, Boucher HW, Herbrecht R, Denning DW, Lortholary O, Ribaud P, et al. Strategy of following voriconazole versus amphotericin B therapy with other licensed antifungal therapy for primary treatment of invasive aspergillosis: impact of other therapies on outcome. Clin Infect Dis 2005;41 (10):1448-52.

14194 Duvivier C, Kolta S, Assoumou L, Ghosn J, Rozenberg S, Murphy RL, et al. Greater decrease in bone mineral density with protease inhibitor regimens compared with nonnucleoside reverse transcriptase inhibitor regimens in HIV-1 infected naive patients. AIDS 2009;27 (7):817-24.

14702 Boffito M, Miralles D, Hill A. Pharmacokinetics, efficacy, and safety of darunavir/ritonavir 800/100 mg once-daily in treatment-naive and -experienced patients. HIV Clin Trials 2008;9 (6):418-27.

15365 Mathias AA, German P, Murray BP, Wei L, Jain A, West S, et al. Pharmacokinetics and pharmacodynamics of GS-9350: a novel pharmacokinetic enhancer without anti-HIV activity. Clin Pharmacol Ther 2010;87 (3):322-9.

17137 Markowitz M, Zolopa A, Ruane P, Squires K, Zhong L, Kearney BP, et al. GS-7340 Demonstrates Greater Declines in HIV-1 RNA than Tenofovir Disoproxil Fumarate During 14 Days of Monotherapy in HIV-1 Infected Subjects [Oral Presentaton / Paper 152LB]. 18th Conference on Retroviruses and Opportunistic Infections (CROI); 2011 February 27 - March 2; Boston, MA.

17624 Reyataz® (atazanavir sulfate) Capsules. US Prescribing Information. Bristol-Myers Squibb Company, Princeton, NJ, USA. Revised February 2011.

17653 Victrelis® (boceprevir) Capsules. US Prescribing Information. Schering Corporation, Whitehouse Station, NJ, USA. Revised May 2011.

17724 Callebaut C, Stray K, Tsai L, Williams M, Yang ZY, Cannizzaro C, et al. In vitro characterization of GS-8374, a novel phosphonate-containing inhibitor of HIV-1 protease with a favorable resistance profile. Antimicrob Agents Chemother 2011;55 (4):1366-76.

17733 INCIVEKTM (telaprevir) Film Coated Tablets. US Prescribing Information. Vertex Pharmaceuticals Incorporated. Cambridge, MA. May 2011.

18605 Goldwater R, DeMicco M, Zong J, Chittick G, Yuen G, West S, et al. Safety, pharmacokinetics and antiviral activity of single oral doses of the HCV NS3 protease inhibitor GS-9256 [Poster Abstract 824]. 61st Annual Meeting of the American Association for the Study of Liver Diseases (AASLD); 2010 October 29th - November 2nd; Boston, MA.

18606 Lawitz E, Hill JB, Marbury T, Rodriguez-Torres M, DeMicco M, Quesada J, et al. Three-day, dose-ranging study of the HCV NS3 protease inhibitor GS-9451 [Poster Abstract 820]. 61st Annual Meeting of the American Association for the Study of Liver Diseases (AASLD); 2010 October 29th - November 2nd; Boston, MA.

18607 Manns MP, Bourliere M, Benhamou Y, Pol S, Bonacini M, Trepo C, et al. Potency, safety, and pharmacokinetics of the NS3/4A protease inhibitor BI201335 in patients with chronic HCV genotype-1 infection. J Hepatol 2011;54 (6):1114-22.

18609 Petry A, Brainard DM, Van Dyck K, Nachbar R, De Lepeleire I, Caro L, et al. Safety and antiviral activity of MK-5172, a novel HCV NS3/4a protease inhibitor with potent activity against known resistance mutants, in genotype 1 and 3 hcv-infected patients [Poster Abstract]. Presented at: 61st Annual Meeting of the American Association for the Study of Liver Diseases (AASLD); 2010; Boston, MA.

20420 Ruane P, DeJesus E, Berger D, Markowitz M, Bredeek UF, Callebaut C, et al. GS-7340 25 mg and 40 mg Demonstrate Greater Antiviral Activity Compared with TDF 300 mg in a 10-Day Monotherapy Study of HIV-1 Infected Patients [Presentation]. 19th Conference on Retroviruses and Opportunistic Infections (CROI); 2012 March 7th; Seattle, WA.

20795 Birkus G, Bam RA, Frey CR, Tsai L, Stray KM, Yant SR, et al. Intracellular Activation Pathways for GS-7340 and the Effect of Antiviral Protease Inhibitors [Poster J-115]. Presented at: 19th Conference on Retroviruses and Opportunistic Infections (CROI); 2012 March 5-8; Seattle, WA.

25765 Ruane PJ, Dejesus E, Berger D, Markowitz M, Bredeek UF, Callebaut C, et al. Antiviral Activity, Safety, and Pharmacokinetics/Pharmacodynamics of Tenofovir Alafenamide as 10-Day Monotherapy in HIV-1-Positive Adults. J Acquir Immune Defic Syndr 2013;63 (4):449-55.

25768 KALETRA (lopinavir/ritonavir) Tablet, Film Coated for Oral use KALETRA (lopinavir/ritonavir) Solution for Oral use. US Prescribing Information. AbbVie LTD. North Chicago, IL. Revised January 2013.

26885 VIREAD® (tenofovir disoproxil fumarate) tablets, for oral use VIREAD® (tenofovir disoproxil fumarate) powder, for oral use. U.S. Prescribing Information. Gilead Sciences, Inc. Foster City, CA. Revised October 2013.

27712 VIRACEPT® (nelfinavir mesylate) Tablets and Oral Powder. US Prescribing Information. Agouron Pharmaceuticals, Inc. La Jolla, CA. Revised May 2013.

29101 Kienle RD, Bruyette D, Pion PD. Effects of thyroid hormone and thyroid dysfunction on the cardiovascular system. Vet Clin North Am Small Anim Pract 1994;24 (3):495-507.

29104 Tribulova N, Knezl V, Shainberg A, Seki S, Soukup T. Thyroid hormones and cardiac arrhythmias. Vascular pharmacology 2010;52 (3-4):102-12.

29239 Bam RA, Yant SR, Cihlar T. Tenofovir alafenamide is not a substrate for renal organic anion transporters (OATs) and does not exhibit OAT-dependent cytotoxicity. Antivir Ther 2014:1-12.

29240 Bam RA, Birkus G, Babusis D, Cihlar T, Yant SR. Metabolism and antiretroviral activity of tenofovir alafenamide in CD4 T-cells and macrophages from demographically diverse donors. Antivir Ther 2014:1-18.

2.6 NONCLINICAL SUMMARY

SECTION 2.6.3— PHARMACOLOGY TABULATED SUMMARY

ELVITEGRAVIR/COBICISTAT/EMTRICITABINE/ TENOFOVIR ALAFENAMIDE FIXED-DOSE COMBINATION (EVG/COBI/FTC/TAF [E/C/F/TAF] FDC)

Gilead Sciences

[redacted] **20**

TABLE OF CONTENTS

NOTE TO REVIEWER

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

As TAF is a new chemical entity, this nonclinical summary contains all available data on this new component. Per the agreement reached between Gilead Sciences, Inc. (Gilead) and the Food and Drug Administration (FDA;), this NDA is supported by .

. To assist the reviewer, Links to all study reports included in the application are highlighted in blue text.

Given the lack of relevant effects in the in vitro studies with the individual agents (EVG, COBI, FTC, TFV, or TAF) or with the combination of EVG+COBI+FTC+TFV, no additional pharmacology studies have been conducted for the E/C/F/TAF combination.

The following conversions are provided to aid the reviewer:

- EVG (GS-9137, JTK-303) 1 µM = 0.448 µg/mL
- COBI (GS-9350) 1 µM = 0.776 µg/mL
- FTC 1 µM = 0.247 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 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: TFV, TAF

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Study No.
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.

1.2. Secondary Pharmacodynamics of TAF

Test Article: TFV, TAF

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Study No.
Binding Screen to Neuroreceptors, Ion Channels, Transporters, Nuclear Receptors	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	[redacted], 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: TFV, TAF

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Study No.
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.

1.3. Secondary Pharmacodynamics of TFV

Test Article: EVG, COBI, FTC, TFV

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Study No.
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.

1.4. 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.[redacted])
In Vivo					
Cardiovascular	Yes	Conscious male beagle dog	Oral gavage	[redacted], Canada	D2000006 ([redacted] 93205)
CNS	Yes	Male Sprague Dawley rat	Oral gavage	[redacted], Canada	R990188 ([redacted] 56518)
Gastrointestinal	Yes	Male Sprague Dawley rat	Oral gavage	[redacted], Canada	R990187 ([redacted] 56519)
Renal	Yes	Male Sprague Dawley rat	Oral gavage	[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.

1.5. Pharmacodynamic Drug Interactions

The pharmacodynamic drug interactions of 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 TAF and the combination of E/C/F/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 TAF and the combination of E/C/F/TFV are listed in Section 1.2.

4. SAFETY PHARMACOLOGY

4.1. 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.[redacted])

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.

4.2. In Vivo Studies with TAF

Test Article: 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 TAF are presented in the nonclinical virology summary contained in m2.7.2, Section 4.1.

6. REFERENCES

References are available upon request.

9864 Vidal F, Domingo JC, Guallar J, Saumoy M, Cordobilla B, Sanchez de la Rosa R, et al. In vitro cytotoxicity and mitochondrial toxicity of tenofovir alone and in combination with other antiretrovirals in human renal proximal tubule cells. Antimicrob Agents Chemother 2006;50 (11):3824-32.

29239 Bam RA, Yant SR, Cihlar T. Tenofovir alafenamide is not a substrate for renal organic anion transporters (OATs) and does not exhibit OAT-dependent cytotoxicity. Antivir Ther 2014:1-12.

29240 Bam RA, Birkus G, Babusis D, Cihlar T, Yant SR. Metabolism and antiretroviral activity of tenofovir alafenamide in CD4 T-cells and macrophages from demographically diverse donors. Antivir Ther 2014:1-18.