

## 2.4 NONCLINICAL OVERVIEW

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

Gilead Sciences

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**CONFIDENTIAL AND PROPRIETARY INFORMATION**

## TABLE OF CONTENTS

TABLE OF CONTENTS .....	2
LIST OF IN-TEXT TABLES .....	4
LIST OF IN-TEXT FIGURES .....	4
LIST OF ABBREVIATIONS .....	5
1. NONCLINICAL OVERVIEW .....	8
1.1. Overview of the Nonclinical Testing Strategy .....	9
1.1.1. FTC .....	9
1.1.2. TAF .....	10
1.1.3. F/TAF .....	11
2. PHARMACOLOGY/VIROLOGY .....	12
2.1. Primary Pharmacodynamics .....	12
2.2. Secondary Pharmacodynamics .....	13
2.2.1. In Vitro Cytotoxicity .....	13
2.2.2. Mitochondrial Toxicity .....	14
2.2.3. Off Target Activity .....	15
2.3. Safety Pharmacology .....	15
2.3.1. FTC .....	15
2.3.2. TAF .....	16
2.3.3. F/TAF .....	16
2.4. Pharmacodynamic Drug Interactions .....	16
2.5. Summary of Pharmacology .....	16
3. PHARMACOKINETICS .....	18
3.1. Analytical Methods .....	18
3.2. In Vitro Absorption and Single Dose Pharmacokinetics .....	18
3.2.1. FTC .....	18
3.2.2. TAF .....	18
3.2.3. F/TAF .....	19
3.3. Repeat Dose Pharmacokinetics .....	19
3.3.1. FTC .....	19
3.3.2. TAF .....	19
3.3.3. F/TAF .....	20
3.4. Distribution .....	20
3.4.1. Protein Binding .....	20
3.4.2. Tissue Distribution .....	20
3.4.3. Distribution in Pregnant Animals .....	21
3.4.4. F/TAF Distribution .....	21
3.5. Metabolism .....	21
3.5.1. Intracellular Metabolism .....	21
3.5.2. Routes of Metabolism .....	22
3.5.3. In Vitro Metabolism .....	24
3.5.4. In Vivo Metabolism .....	24
3.6. Excretion .....	25
3.6.1. Recovery in Excreta .....	25
3.6.2. Excretion into Breast Milk .....	26
3.7. Pharmacokinetic Drug Interactions .....	26
3.7.1. Metabolic Drug Interactions .....	27
3.7.2. Induction Liability .....	28
3.7.3. Transporter Drug Interactions .....	29

3.7.4.	F/TAF Pharmacokinetic Drug Interactions .....	32
3.8.	Summary of Pharmacokinetics.....	33
4.	TOXICOLOGY .....	35
4.1.	Single Dose Toxicity.....	35
4.2.	Repeat Dose Toxicity.....	35
4.2.1.	FTC .....	35
4.2.2.	TAF .....	35
4.2.3.	FTC/TDF.....	38
4.2.4.	F/TAF.....	38
4.3.	Genotoxicity.....	39
4.3.1.	FTC .....	39
4.3.2.	TAF .....	39
4.3.3.	FTC/TDF.....	39
4.3.4.	F/TAF.....	39
4.4.	Carcinogenicity .....	39
4.4.1.	FTC .....	40
4.4.2.	TAF .....	40
4.4.3.	F/TAF.....	40
4.5.	Reproductive Toxicity.....	40
4.5.1.	FTC .....	40
4.5.2.	TAF .....	40
4.5.3.	F/TAF.....	41
4.6.	Juvenile Toxicity.....	42
4.6.1.	FTC .....	42
4.6.2.	TAF/TFV.....	42
4.6.3.	F/TAF.....	42
4.7.	Local Tolerance.....	43
4.8.	Other Toxicity Studies .....	43
4.8.1.	Antigenicity.....	43
4.8.2.	Immunotoxicity .....	43
4.8.3.	Impurities/Degradation Products.....	43
4.9.	Summary of Toxicology and Target Organ Effects .....	46
4.9.1.	Target Organ Effects .....	46
4.9.2.	Safety Margins .....	48
5.	INTEGRATED OVERVIEW AND CONCLUSIONS.....	51
5.1.	Correlation of Nonclinical and Clinical Findings .....	51
5.2.	Justification for Text in Labeling .....	51
5.3.	Overall Conclusions .....	52
6.	REFERENCES .....	54

### LIST OF IN-TEXT TABLES

Table 1.	Steady State Pharmacokinetic Parameters for FDC Components .....	27
Table 2.	Transporter Substrate Assessment of F/TAF Components.....	29
Table 3.	Transporter Inhibition Assessment of F/TAF Components.....	30
Table 4.	Estimated Safety Margins of Emtricitabine Based on AUCss When Comparing Animal No-Effect-Level (NOEL) .....	49
Table 5.	Estimated Safety Margins of TAF Based on AUCss When Comparing Animal No- Adverse-Effect-Level (NOAEL) .....	50

### LIST OF IN-TEXT FIGURES

Figure 1.	Oxidative Metabolism of FTC.....	22
Figure 2.	Metabolites of TAF .....	23

## LIST OF ABBREVIATIONS

AhR	aryl hydrocarbon receptor (AHR gene product)
ARV	antiretroviral
ATP	adenosine triphosphate
ATV	atazanavir (Reyataz <sup>®</sup> , Bristol-Myers Squibb)
AUC	area under the curve
AUC <sub>ss</sub>	area under the plasma concentration curve at steady state
AUC <sub>tau</sub>	the area under the plasma concentration-time curve from time zero to time tau over a dosing interval at steady state (AUC <sub>0-tau</sub> ), where tau is the length of the dosing interval.
BCRP	breast cancer resistance protein (ABCG2)
BDC	bile-duct cannulated
BSEP	bile salt excretory pump
Caco-2	human colon carcinoma cell line
CatA	cathepsin A
CC <sub>50</sub>	drug concentration that results in a 50% reduction in cell viability
CD4	cluster determinant 4
cDNA	complimentary deoxyribonucleic acid
CHMP	Committee for Medicinal Products for Human Use
C <sub>max</sub>	maximum observed concentration of drug in serum, plasma, or peripheral blood mononuclear cells
C <sub>max,u</sub>	unbound concentration of drug at C <sub>max</sub>
CNS	central nervous system
COBI	cobicistat (GS-9350, [Tybost <sup>®</sup> , Gilead])
COX II	cytochrome c oxidase II
CsA	cyclosporine A
CYP	cytochrome P450
dATP	deoxyadenosine triphosphate
dCTP	deoxycytidine triphosphate
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DRV	darunavir (Prezista <sup>®</sup> , Janssen)
EC <sub>50</sub>	concentration of a compound inhibiting virus replication by 50%
EC <sub>95</sub>	concentration of a compound inhibiting virus replication by 95%
E/C/F/TAF	elvitegravir/cobicistat/emtricitabine/tenofovir alafenamide (coformulated)
ECG	electrocardiograph, electrocardiogram
EFV	efavirenz (Sustiva <sup>®</sup> , Bristol-Myers Squibb)
EMA	European Medicines Agency
EVG	elvitegravir (Vitekta <sup>®</sup> , Gilead)
EVG/COBI/FTC/TDF	elvitegravir/cobicistat/emtricitabine/tenofovir DF (coformulated); STB
EU	European Union

## LIST OF ABBREVIATIONS (CONTINUED)

F <sub>1</sub>	first generation
FDA	Food and Drug Administration
FDC	fixed-dose combination
FMO	flavin monooxygenase
FTC	emtricitabine (Emtriva <sup>®</sup> , Gilead)
FTC/TDF	emtricitabine/tenofovir DF, TVD (Truvada <sup>®</sup> , Gilead)
FTC-TP	emtricitabine triphosphate
GD	gestation day
GI	gastrointestinal
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
GS-7340	tenofovir alafenamide (TAF) free base
GS-7340-02	tenofovir alafenamide (TAF) monofumarate
GS-7340-03	tenofovir alafenamide (TAF) hemifumarate
GSI	Gilead Sciences, Inc. (Gilead)
HBV	hepatitis B virus
HEK	human embryonic kidney (cell line)
hERG	human ether-à-go-go related gene
HIV-1	human immunodeficiency virus type 1
HPMC	hydroxypropylmethylcellulose
[I] <sub>1</sub>	inhibitor concentration corresponding to steady state C <sub>max</sub>
[I] <sub>2</sub>	inhibitor concentration corresponding to theoretical maximum concentration in the intestinal lumen
IC <sub>50</sub>	concentration resulting in 50% of maximum inhibition
ICH	International Conference on Harmonization (of Technical Requirements for Registration of Pharmaceuticals for Human Use)
IgM	immunoglobulin M
IV	intravenous
K <sub>i</sub>	kinetic inhibition constant
K <sub>1</sub>	affinity constant for enzyme inactivation
k <sub>inact</sub>	theoretical maximum enzyme inactivation rate
LD	lactation day
LD <sub>50</sub>	the estimated dose that results in lethality in 50 percent of a group
MATE1	multidrug and toxin extrusion protein 1 (SLC47A1)
MATE2-K	multidrug and toxin extrusion protein 2-K (SLC47A2)
MRP1, 2, or 4	multidrug resistance related protein 1, 2, or 4
MTD	maximal tolerated dose
mtDNA	mitochondrial DNA
NDA	new drug application

## LIST OF ABBREVIATIONS (CONTINUED)

NNRTI	nonnucleoside reverse transcriptase inhibitor
NOAEL	no-observed-adverse-effect level
NOEL	no-observed-effect level
NRTI	nucleoside reverse transcriptase inhibitor
N(t)RTI	nucleoside or nucleotide reverse transcriptase inhibitor
OAT1	organic anion transporter 1 (SLC22A6)
OAT3	organic anion transporter 3 (SLC22A8)
OATP1B1	organic anion transporting polypeptide 1B1 (SLCO1B1)
OATP1B3	organic anion transporting polypeptide 1B3 (SLCO1B3)
OCT1	organic cation transporter 1
OCT2	organic cation transporter 2 (SLC22A2)
OCTN1	organic cation transporter novel, type 1 (SLC22A4)
PBMC	peripheral blood mononuclear cell
P-gp	permeability glycoprotein
PI	protease inhibitor
PND	postnatal day
PXR	pregnane X receptor
QT	interval between the start of the Q wave and the end of the T wave on ECG
QTc	QT interval duration corrected for heart rate
RBC	red blood cell
RNA	ribonucleic acid
RPTECs	renal proximal tubule epithelial cells
RT	reverse transcriptase
RTV	ritonavir (Norvir <sup>®</sup> , Abbvie)
S9	tissue post-mitochondrial (9,000 x g) supernatant
SI	selectivity index
SIV	simian immunodeficiency virus
STB	E/C/F/TAF (Stribild <sup>®</sup> , Gilead)
TAF	tenofovir alafenamide (GS-7340)
TDF	tenofovir disoproxil fumarate, tenofovir DF (Viread <sup>®</sup> , Gilead)
TFV	tenofovir, PMPA
TFV DP	tenofovir diphosphate
TVD	emtricitabine/tenofovir DF, FTC/TDF (Truvada <sup>®</sup> , Gilead)
UGT	uridine diphosphate glucuronosyltransferase
US	United States
ZDV	zidovudine, AZT (Retrovir <sup>®</sup> , GlaxoSmithKline)

## 1. NONCLINICAL OVERVIEW

This nonclinical overview is being submitted in support of a new drug application (NDA) for a fixed-dose combination (FDC) that contains the nucleoside reverse transcriptase inhibitor (NRTI) emtricitabine (FTC, Emtriva<sup>®</sup>), and the nucleotide reverse transcriptase inhibitor (NtRTI) tenofovir alafenamide (TAF, formerly GS-7340); the FTC/TAF (F/TAF, 200/10 mg, 200/25mg) tablet. The proposed indication for the F/TAF FDC is for use in combination with other antiretroviral (ARV) products for the treatment of human immunodeficiency virus, type 1 (HIV-1) infection in adult and adolescent patients 12 years of age and older.

Emtricitabine (Emtriva) is a nucleoside reverse transcriptase inhibitor (NRTI). Tenofovir disoproxil fumarate (TDF, Viread<sup>®</sup>) is the first generation prodrug of tenofovir (TFV). Tenofovir alafenamide is a prodrug of TFV that, compared to TDF, has a longer plasma half-life and achieves higher levels of TFV into target cells at lower exposure of circulating TFV. TAF has a unique metabolism that provides enhanced delivery of TFV to HIV-target cells including lymphocytes and macrophages, resulting in higher intracellular levels of the active phosphorylated metabolite tenofovir-diphosphate (TFV-DP), and, at the 10-mg dose, yields approximately 90% lower circulating levels of TFV relative to TDF {7415}, {13119}, {22029}. These features translate into less risk of nephrotoxicity and less decrease (or improvements) in bone mineral density, both of which are known risks with TDF administration {21762}, {22031}, {30895}.

The F/TAF FDC contains the same dose of FTC that is currently approved for use in adults within Emtriva, Truvada, Atripla, Complera/Eviplera, and Stribild (200 mg of FTC). A comprehensive nonclinical pharmacology/virology, pharmacokinetic, and toxicology program was conducted to support of the registration of FTC and TAF. Information from nonclinical studies with FTC, TAF, TDF or TFV, and FTC/TDF should be considered in the context of their clinical data and post marketing clinical experience within ARV combination therapy for the treatment of HIV-1 infection.

Drug interaction studies have shown that inhibition of intestinal efflux transporters (e.g. P-glycoprotein [P-gp] and Breast Cancer Resistance Protein [BCRP]) increases TAF exposure. The recommended F/TAF dose (200/10 mg or 200/25 mg) with each third agent is generally based on whether or not the co-administered ARV product has any clinically relevant boosting effect on TAF exposure; F/TAF 200/25 mg is recommended to be used with unboosted ARV products, and F/TAF 200/10 mg with boosted ARV products.

To facilitate the evaluation of the F/TAF application, nonclinical virology studies of FTC and TFV, TAF, and TDF are described in detail in the virology summary contained in m2.7.2, Section 4.1, together with the clinical virology data in Section 4.2, and summarized in m2.5, Section 4.



The nonclinical data discussed within this document support the favorable benefit/risk profile for the proposed use of F/TAF for the treatment of HIV-1 infection in patients 12 years of age and older in combination with other ARV products. All information from nonclinical studies that is relevant to the prescriber and patient has been included in the proposed Prescribing Information and Patient Labeling.

### **1.1. Overview of the Nonclinical Testing Strategy**

This document provides an overview of the nonclinical information that is relevant to the assessment of the F/TAF FDC. This document is structured as an overview of the studies in the various disciplines, including primary pharmacodynamics, secondary pharmacodynamics, safety pharmacology, pharmacokinetics, and toxicology. A critical assessment of the completeness and relevance of the nonclinical testing program and the key findings are included. An integrated safety assessment of F/TAF for the treatment of HIV-1 infection in patients  $\geq 12$  years old in combination with other ARVs is included in Section 5, “Integrated Overview and Conclusions,” of this document. Specific cross-disciplinary topics and proposals for the inclusion of nonclinical items in the product labeling are discussed throughout the text, as appropriate, and summarized at the end of the document.

All of the definitive safety pharmacology, toxicology, and toxicokinetic studies reported in this summary for FTC, TAF, and the FDC of FTC/TDF were conducted in accordance with guidelines issued by the International Conference on Harmonization (ICH) and with Good Laboratory Practice (GLP) or other applicable regulations promulgated by international health authorities. Pilot, exploratory, and mechanistic studies were either conducted in full compliance with GLP procedures or were conducted using appropriate protocols and documentation to assure data integrity.

#### **1.1.1. FTC**

Emtricitabine is a nucleoside reverse transcriptase inhibitor (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 for use in combination with other ARV products for the treatment of HIV-1 infection.

Emtricitabine is the (-) enantiomer of a thio analogue of cytidine, which differs from other cytidine analogues in that it has a fluorine in the 5-position. Intracellularly, FTC is phosphorylated by enzymes to form emtricitabine triphosphate (FTC-TP), the active metabolite. Emtricitabine is an NRTI that has activity against HIV and hepatitis B virus (HBV).

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

### 1.1.2. TAF

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

Tenofovir alafenamide is metabolized by cellular enzymes including carboxyl esterase 1 and cathepsin A (CatA) and has minimal interaction with typical xenobiotic metabolizing enzymes. Unlike tenofovir disoproxil fumarate (TDF, Viread), TAF is relatively stable in human plasma ( $t_{1/2}$  ~90 minutes in vitro {7415} and 25-40 minutes in clinical studies [m2.7.2]). Because TAF is more stable in plasma than TDF, higher levels are achieved in HIV-target cells including lymphocytes and macrophages, providing enhanced delivery of TFV. During clinical studies with the FDCs, administration of TAF resulted in subsequent formation of > 4-fold (3-7-fold at 90% confidence interval) higher intracellular levels of TFV-DP in peripheral blood mononucleated cells (PBMCs) and 90% lower circulating levels of TFV relative to TDF {7415}, {13119}, {22029}. These features translate into less risk of nephrotoxicity and less decrease (or improvements) in bone mineral density, both of which are known risks with TDF administration {21762}, {22031}, {30895}.

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

Per separate agreements with the Food and Drug Administration (FDA) and European Medicines Agency (EMA), [REDACTED]

The nonclinical toxicity studies demonstrate that there was no adverse effect of TAF for up to 26 weeks in the rat, up to 39 weeks in the dog, and 1 month in the monkey at doses producing

TFV systemic exposure levels in animals 13-, 4- and > 20-fold greater, respectively, than those in patients treated with the recommended clinical dose of E/C/F/TAF.

### **1.1.3. F/TAF**

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

The proposed FDC is based on the complimentary pharmacology of FTC and TAF and the body of clinical experience with nucleoside/nucleotide reverse transcriptase inhibitors (N[t]RTIs) in HIV-infected patients. Combinations of these agents in cell-based in vitro assays show favorable anti-HIV activity and no evidence for antagonism. The toxicity profiles of the 2 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 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 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 the FTC/TDF containing regimens and with the EVG/COBI/FTC/TAF FDC support the safety of the new combination product for the treatment of HIV-1 infection.

The absence of nonclinical safety studies with the F/TAF combination is in accordance with the FDA Guidance for Industry, Nonclinical Safety Evaluation of Drug or Biologic Combinations, March 2006 and the Committee for Medicinal Products for Human Use (CHMP) Guideline on the Non-Clinical Development of Fixed Combinations of Medicinal Products (EMA/CHMP/SWP/258498/2005, January 2008).

## 2. PHARMACOLOGY/VIROLOGY

### 2.1. Primary Pharmacodynamics

Nonclinical virology studies of FTC and TAF, and the FTC/TDF drug combination are described in detail in m2.7.2, Section 4.1, together with the clinical virology data in m2.7.2, Section 4.2 and summarized in m2.5, Section 4.

#### Mechanism of Action

Emtricitabine, an NRTI, is a synthetic analogue of the naturally occurring pyrimidine nucleoside, 2'-deoxycytidine. Intracellularly, FTC is converted through 3 phosphorylation reactions to its active tri-phosphorylated anabolite FTC-TP {4527}, {4535}. Emtricitabine triphosphate inhibits viral polymerases, including HIV-1 reverse transcriptase (RT) by direct binding competition with the natural deoxyribonucleotide substrate (deoxycytidine triphosphate; dCTP), and after incorporation into DNA, by DNA chain termination {4249}. The EC<sub>50</sub> of FTC against laboratory adapted strains of HIV-1 ranged from 0.001 to 0.62 μM depending on cell type and virus strain used in the assay {4534}, {4541}, {4526}. With clinical isolates of HIV-1, EC<sub>50</sub> values ranged from 0.002 to 0.028 μM {4534}.

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

Unlike tenofovir disoproxil fumarate (TDF, Viread), TAF is relatively stable in human plasma (t<sub>1/2</sub> ~90 minutes in vitro {7415} and 25-40 minutes in clinical studies [m2.7.2]), but rapidly converts to TFV inside cells {7415}. Assessment of the intracellular metabolism of TAF in various types of immune cells including cluster determinant 4 (CD4)<sup>+</sup> T-cells, lymphocytes, and monocytes showed efficient conversion of the prodrug to the active metabolite TFV-DP {20795}.

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

Additive to synergistic effects were observed in in vitro interaction studies of TFV, the active metabolite of TAF, with NRTIs (abacavir, FTC, lamivudine, stavudine, zalcitabine, zidovudine

[ZDV]), nonnucleoside reverse transcriptase inhibitors (NNRTIs) (delavirdine, efavirenz [EFV], nevirapine), PIs (amprenavir, indinavir, nelfinavir, ritonavir [RTV], saquinavir), and the IN inhibitor EVG {1649}. No antagonistic interactions were observed for any of these 2-drug combinations in a T lymphoblastoid cell line. Data show similar results for the in vitro interactions of TAF with several commonly coadministered ARVs (m1.4.4, PC-120-2002).

## **In Vivo Efficacy in Animal Models**

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

No additional studies for the F/TAF FDC are warranted in animal models in view of the extensive clinical experience with the use of FTC, and TDF, FTC/TDF containing regimens, and the E/C/F/TAF FDC for the treatment of HIV-1 infection.

In summary, FTC and TAF/TFV are potent and selective inhibitors of HIV-1. Both drugs show potent antiretroviral activity against diverse subtypes of HIV-1 in vitro. Emtricitabine and TAF/TFV are phosphorylated intracellularly through nonoverlapping pathways, and in combination show no antagonism for the formation of their active metabolites. Additive to synergistic effects were observed in in vitro interaction studies of TFV, the active metabolite of TAF, with NRTIs, NNRTIs, PIs and the IN inhibitor EVG {1649}.

## **2.2. Secondary Pharmacodynamics**

### **2.2.1. In Vitro Cytotoxicity**

For FTC, no cytotoxicity was observed in vitro in human PBMC, MT-2, HepG2, CEM, MOLT-4, and Vero cells at concentrations up to 100  $\mu$ M {4531}, {4534}. Emtricitabine was also found to be nontoxic to human bone marrow progenitor cells in vitro. For TFV in quiescent human PBMCs, no cytotoxic effect was detected at concentrations as high as 100  $\mu$ M {1574}. Low in vitro cytotoxicity of TFV was also demonstrated in human liver cells (HepG2), proliferating human skeletal muscle cells, or quiescent renal tubular epithelial cells (m1.4.4, P4331-00037). In addition, TFV showed no toxicity to myeloid and erythroid hematopoietic progenitor cells in vitro {4077}. Thus, FTC and TFV have a low order of cytotoxicity and a large therapeutic ratio in vitro.

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

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

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

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

The combination of TFV and FTC was studied for cytotoxicity in MT-2 cells. No cytotoxicity was observed at the highest concentrations tested, up to 50  $\mu$ M TFV and 5  $\mu$ M FTC (m1.4.4, PC-164-2002). Cytotoxicity studies were also conducted on the combination of TFV and FTC in HepG2 cells as detailed below; no cytotoxicity was observed (m1.4.4, TX-104-2001).

The cytotoxicity and antiviral activity of two TAF metabolites, M18 (GS-645552) and M28 (GS-652829), was evaluated in two T-lymphoblastoid cell lines (MT-2 and MT-4) following 5 days of compound exposure (m1.4.4, PC-120-2021). These metabolites are also degradants and the testing supported manufacturing activities. Both TAF metabolites had no cytotoxicity up to the highest tested concentration (57  $\mu$ M). Both metabolites/degradants showed weak inhibition of HIV-1 replication with 1723 to 2630-fold lower inhibitory potency relative to TAF ( $EC_{50}$  values of 7.41 to 21.04  $\mu$ M) for metabolite M28 and 121 to 130-fold lower inhibitor potency relative to TAF ( $EC_{50}$  values of 0.56 to 0.97  $\mu$ M) for metabolite M18.

Collectively, these data demonstrate that FTC, TFV, and TAF have low cytotoxicity and a large SI in vitro.

### **2.2.2. Mitochondrial Toxicity**

A variety of clinical symptoms observed in HIV patients treated with prolonged NRTI therapy appear to be linked to mitochondrial toxicity {2522}. Several representatives of this class of HIV drugs inhibit mitochondrial DNA polymerase  $\gamma$ , by direct binding and competition with the natural deoxyribonucleotide substrate, incorporation into DNA, leading to DNA chain termination. A variety of in vitro studies have been conducted to evaluate the potential of FTC, TFV and TAF to exert mitochondrial toxicity. Results from these studies suggest that FTC and TFV have limited capability to inhibit human DNA polymerases or to mediate cytotoxicity or mitochondrial damage ({4541}, {6053}, {1131}, {2516}). In vitro combination studies have also been conducted in HepG2 cells to further evaluate the potential mitochondrial toxicity of FTC and TFV (as well as other nucleosides, m1.4.4, TX-104-2001).

HepG2 cells were exposed to FTC and TFV (as well as other nucleosides), either alone or in combination. Assay endpoints included cell growth; extracellular production of lactic acid; relative cellular content of mtDNA and mtDNA-encoded cytochrome c oxidase II (COX II); and intracellular lipid accumulation. Tenofovir and FTC alone or in combination with each other or

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

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

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

These studies confirmed that the potential of FTC and TFV to interfere with mitochondrial functions is low, whether administered alone or in combination with other NRTIs. Further, because administration of TAF results in lower exposure to TFV compared to TDF, the potential for mitochondrial toxicity is also low with the F/TAF FDC. No additional nonclinical studies are therefore warranted with the combination of FTC and TAF.

### **2.2.3. Off Target Activity**

Emtricitabine had no pharmacologically significant binding affinity at 19 different receptors (m1.4.4, TPZZ/93/0002), showed little or no direct effect on various isolated muscle preparations (cholinergic, adrenergic, histaminergic, and serotonergic), and had no major inhibitory effects on the contractile responses to acetylcholine, norepinephrine, serotonin, isoproterenol, arachidonic acid, histamine, bradykinin, and angiotensin II (m1.4.4, TPZZ/92/0055).

Tenofovir and TDF showed no significant inhibition of or increased binding to a series of 111 protein targets (neuroreceptors, ion channels, transporters, and nuclear receptors) (m1.4.4, V2000020).

## **2.3. Safety Pharmacology**

### **2.3.1. FTC**

A comprehensive range of safety pharmacology studies revealed no treatment-related adverse effects on any organ system at systemic exposure levels much higher than those anticipated in patients at the recommended clinical dose (10- to more than 50-fold) (m1.4.4, 477, TPZZ/92/0056; TPZZ/93/0001; TPZZ/93/0119; TPZZ/92/0057). No effects on the cardiovascular system were reported in anesthetized dogs administered a cumulative dose of 38.5 mg/kg of FTC intravenously over a 1-hour period (m1.4.4, TPZZ/92/0076). In addition, there were no abnormalities reported on the ECG data obtained from the repeated-dose toxicity studies in monkeys, where AUC exposures were up to 26-fold higher than in humans administered the 200 mg dose (m1.4.4, TOX600; TOX627; TOX032).

### 2.3.2. TAF

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

The IC<sub>50</sub> for the inhibitory effect of TAF on hERG potassium current was estimated to be greater than 10 μM, far above human exposure (m1.4.4, PC-120-2005). There were no adverse effects detected in the CNS in rats dosed at 1000 mg/kg (m1.4.4, R990188), in the renal system in rats administered 1000 mg/kg (m1.4.4, R990186) or in the cardiovascular system of dogs dosed at 100 mg/kg (80 mg free base equivalents/kg) (m1.4.4, D2000006). There was reduced gastric emptying in rats dosed at 1000 mg/kg, but not at 100 mg/kg (m1.4.4, R990187).

### 2.3.3. F/TAF

A comprehensive safety pharmacology program has been conducted for both individual agents. While the study designs for these studies varied between the agents, the major organ systems were comprehensively evaluated. Emtricitabine and TAF had little effect on vital organ systems in safety pharmacology studies. Although TAF showed some potential to prolong the PR interval in the 39-week dog study at 18/12 mg/kg/day, the slight change was associated with decreased weight gain, bone and renal toxicity, and significant decreases in triiodothyronine (T<sub>3</sub>) {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 (m1.4.4, D-TX-D2000006) or in the thorough QT study (m1.4.4, GS-US-120-0107). Overall, the pharmacological assessment of FTC and TAF supports the effective use of these agents together in combination therapy for the treatment of HIV-1 infection. Additional safety pharmacology studies on the F/TAF FDC are considered unwarranted.

## 2.4. Pharmacodynamic Drug Interactions

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

## 2.5. Summary of Pharmacology

The nucleoside or nucleotide reverse transcriptase inhibitors (N[t]RTIs), FTC and TFV, have potent antiretroviral activity against wild-type and many drug-resistant strains of HIV-1 in vitro and in vivo. The nonclinical virology studies of FTC, TAF, and FTC/TDF are summarized and described in detail in the virology summary contained in m2.7.2, Section 4.1, together with the clinical virology data in m.2.7.2, Section 4.2, and summarized in m2.5, Section 4.

Emtricitabine and TDF have a high selectivity for HIV RT and are very weak inhibitors of mammalian DNA polymerases α, β, δ, ε, and mitochondrial DNA polymerase γ. Elvitegravir, FTC, and TDF have no pharmacologically significant off-target binding affinity to the receptors tested. Emtricitabine and TAF have low in vitro cytotoxicity in a variety of human cell types.



Nucleoside reverse transcriptase inhibitors currently carry a class labeling for mitochondrial toxicity; however, both FTC and TAF have shown a low potential for mitochondrial toxicity in long-term toxicity studies.

Emtricitabine and TAF had little effect on vital organ systems in safety pharmacology studies. Although TAF showed some potential to prolong the PR interval in the 39-week dog study at 18/12 mg/kg/day, the slight change was associated with decreased weight gain, bone and renal toxicity, and significant decreases in T3 {29101}, {29104}. No PR prolongation or any change in ECG results occurred in the safety pharmacology study that evaluated a TAF dose up to 100 mg/kg (m1.4.4, D-TX-D2000006) or in the thorough QT study (m1.4.4, GS-US-120-0107). Overall, the pharmacological assessment of FTC and TAF supports the effective use of both agents in combination therapy for the treatment of HIV-1 infection. Additional safety pharmacology studies on the F/TAF FDC are not warranted.

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

### **3. PHARMACOKINETICS**

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

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

#### **3.1. Analytical Methods**

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

#### **3.2. In Vitro Absorption and Single Dose Pharmacokinetics**

##### **3.2.1. FTC**

Single-dose pharmacokinetics of FTC have been studied in mice (m1.4.4, TEIN/93/0003, TEIN/93/0004; IUW00101), rats {4570}, and cynomolgus monkeys (m1.4.4, IUW00301, TEZZ/93/0019). In these species, FTC was rapidly and well absorbed with oral bioavailability ranging from 58% to 97% over the dose range of 10 to 600 mg/kg.

##### **3.2.2. TAF**

In Caco-2 cell monolayers, TAF showed a dose-dependent increase in forward permeability and a decrease in efflux ratio indicating saturable efflux transport. Addition of the efflux transport inhibitor, cyclosporin A (CsA) diminished the efflux ratio and increased the forward permeability (m1.4.4, AD-120-2037).

Single-dose plasma pharmacokinetics of TFV and/or TAF were evaluated following administration of TAF by dosing either GS-7340-02 or GS-7340-03 to male CD-1 mice or GS-7340-03 to both male and female 001178-W mice via oral gavage (m1.4.4, AD-120-2014, AD-120-2016), to rats via oral gavage (m1.4.4, AD-120-2015, R990130 and R2000065, to dogs via intravenous (IV) bolus of GS-7340-02 or oral administration of TAF as free base, its diastereomer GS-7339, the mixture GS-7171, or GS-7340-02 under fasted and under fed conditions (m1.4.4, 99-DDM-1278-001-PK, AD-120-2034). Tenofovir alafenamide was not detected in any of the rat studies. Additionally, the plasma PK profiles for TAF and TFV and TFV concentrations in PBMCs were determined in rhesus monkeys following a single oral dose of GS-7340-02 (m1.4.4, P2000087). Tenofovir alafenamide is generated at sufficient exposures in nonclinical species chosen for assessment of toxicology. Consistent with dose-dependent

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

### **3.2.3. F/TAF**

With respect to potential drug interactions within the combination that could affect absorption, FTC shows high passive permeability and so is unlikely to be affected when administered with TAF. The pharmacokinetic enhancers (“boosters”) COBI and RTV are weak inhibitors of intestinal efflux transporters, but high concentrations of COBI and RTV in the intestinal lumen, achievable briefly during absorption, inhibit P-gp (m1.4.4, AD-216-2072 and AD-216-2104). Tenofovir alafenamide is an efflux substrate in the intestine; therefore, absorption is increased in the presence of COBI and RTV due to inhibition of intestinal efflux transport. In the presence of 90 μM COBI in the Caco-2 bidirectional permeability assay, TAF forward permeability increased 4.6-fold and the efflux ratio significantly decreased suggesting P-gp mediated drug interaction (m1.4.4, AD-120-2013). Although formal nonclinical studies of the single dose pharmacokinetics of the F/TAF FDC have not been conducted, comprehensive pharmacokinetic clinical studies with the F/TAF FDC have been performed (m2.7.2).

## **3.3. Repeat Dose Pharmacokinetics**

### **3.3.1. FTC**

The multiple-dose pharmacokinetic parameters for FTC were derived as part of the repeat-dose toxicity studies in mice (80 to 3000 mg/kg/day; m1.4.4, TOX109; IUW00701; TOX599; TOX628), rats (60 to 3000 mg/kg/day; m1.4.4, TOX108; TOX097), and monkeys (40 to 2000 mg/kg/day; m1.4.4, TOX600; TOX627; TOX032) dosed for periods of 3 days to 104 weeks. In general, there were no significant differences in pharmacokinetics following single and multiple dosing. Systemic exposure to FTC ( $C_{max}$  and AUC) increased approximately proportionally with dose and was similar between males and females.

### **3.3.2. TAF**

The multiple-dose pharmacokinetics of TFV were characterized in a pharmacokinetic study in dogs orally administered TAF (m1.4.4, AD-120-2033) and in toxicokinetic studies following oral administration of TAF in mice (m1.4.4, TX-120-2007), rats (m1.4.4, R990182, TOX-120-001), dogs, (m1.4.4, D990175-PK, TOX-120-002) and monkeys (m1.4.4, P2000114-PK). After repeat dosing in mice or monkeys for up to 13 weeks or 4 weeks, respectively, no accumulation of TFV occurred; slight accumulation (up to ~3-fold) of TFV occurred in rats and dogs dosed for up to 26 and 39 weeks, respectively.

### **3.3.3. F/TAF**

With respect to potential drug interactions within the combination that could affect absorption, FTC shows high passive permeability and is unlikely to be affected when administered with TAF. While TAF is an efflux substrate in the intestine, absorption is unlikely to be affected by FTC as intestinal efflux transport is not inhibited by FTC. Although formal nonclinical studies of the repeat dose pharmacokinetics of the F/TAF FDC have not been conducted, comprehensive pharmacokinetic clinical studies on the combination have been performed (m2.7.2).

### **3.4. Distribution**

#### **3.4.1. Protein Binding**

##### **3.4.1.1. FTC**

The protein binding of FTC was very low (< 5%) in mouse, rabbit, monkey, and human plasma (m1.4.4, TBZZ/93/0025).

##### **3.4.1.2. TAF and TFV**

Since TAF is highly unstable in rodent plasma due to hydrolytic cleavage by plasma esterases, the extent of TAF binding to plasma was determined in dog and human plasma in vitro (m1.4.4, AD-120-2026). In vitro protein binding of TAF was moderate in dog and human plasma with the percent unbound values of 48.0% and 46.8%, respectively. These in vitro values were higher than those observed in multiple human ex vivo studies with the mean percent unbound TAF ranging from 14% to 23% in all subjects (m1.4.4, GS-US-120-0108 and GS-US-120-0114). Since the ex vivo results should be more clinically relevant than the in vitro values, percent unbound TAF of 20% was used for the assessments for potential drug interactions.

The protein binding of TFV was very low (< 10%) in the plasma and serum of humans and all other species examined (m1.4.4, P0504-00039).

#### **3.4.2. Tissue Distribution**

##### **3.4.2.1. FTC**

The tissue distribution of [<sup>14</sup>C]FTC was characterized in rats and cynomolgus monkeys after a single oral dose of 200 mg/kg (m1.4.4, TOX092 and TOX063, respectively). Emtricitabine was widely distributed in the body, with measurable concentrations found in all tissues within 1 hour following oral administration. Tissue concentrations generally declined in parallel with plasma concentrations, with no indication of accumulation in any tissue examined. Virtually no radioactivity remained in the body at 72 hours after dosing. The highest concentrations of FTC were found in the kidneys and liver. Concentrations in CNS tissues were 2% to 10% of the concentration in plasma.

### 3.4.2.2. TAF and TFV

Following oral administration of [ $^{14}\text{C}$ ]TAF to mouse (m1.4.4, AD-120-2011), rat (m1.4.4, AD-120-2020), and dog (m1.4.4, AD-120-2009, D990173-BP), [ $^{14}\text{C}$ ]TAF-derived radioactivity was widely distributed to most of the tissues in all species. Consistent with high hepatic extraction, high levels of radioactivity were observed in the liver; high radioactivity was also measured in the kidney. Low levels of radioactivity were observed in brain and testis in mouse. No melanin binding was observed in rats. Distribution trends in the pigmented uveal tract of the eye and pigmented skin suggested that [ $^{14}\text{C}$ ]TAF-related radioactivity was not selectively associated with melanin-containing tissues in the pigmented mouse. Distribution studies in dogs showed 5.7 to 15-fold higher  $^{14}\text{C}$ -radioactivity in lymphoid tissues (iliac, axillary, inguinal and mesenteric lymph nodes, and spleen) 24 hours following administration of an equivalent dose of [ $^{14}\text{C}$ ]-TAF relative to [ $^{14}\text{C}$ ]-TDF {7415}.

Following single intravenous administration of [ $^{14}\text{C}$ ]TFV in male rats, the highest concentrations of radioactivity were found in the kidney, liver, urine, and large intestine and trace amounts were observed in bone or bone marrow (m1.4.4, 95-DDM-1278-002).

### 3.4.3. Distribution in Pregnant Animals

Pharmacokinetic parameters for FTC in pregnant animals were generally similar to those reported for nonpregnant animals. While accumulation of TFV was observed after multiple dosing of TAF as GS-7340-02 up to 200 mg/kg/day in pregnant rats in the range-finding study (m1.4.4, TX-120-2001), no accumulation of TAF and TFV was observed up to 250 mg/kg/day in the embryo-fetal development study (m1.4.4, TX-120-2002). No accumulation occurred in pregnant rabbits in the range-finding study (m1.4.4, TX-120-2004) or the embryo-fetal development study (m1.4.4, TX-120-2005).

Placental transfer studies were conducted for TFV (rhesus monkeys) and FTC (mice and rabbits). Both drugs are transferred across the placenta, but did not concentrate in fetal tissues. Fetal/maternal exposure ratios, determined on appropriate gestation days (GDs) by the concentrations of TFV in serum and FTC in plasma and umbilical cord blood, were  $\leq 0.5$  (m1.4.4, 96-DDM-1278-005, TOX103, TOX038).

### 3.4.4. F/TAF Distribution

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

## 3.5. Metabolism

### 3.5.1. Intracellular Metabolism

Tenofovir alafenamide is subject to intracellular metabolism to TFV, which is further phosphorylated to the anabolites, TFV-MP and TFV-DP with TFV-DP being the pharmacologically active form. Intracellular metabolic activation of TAF in PBMCs or

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

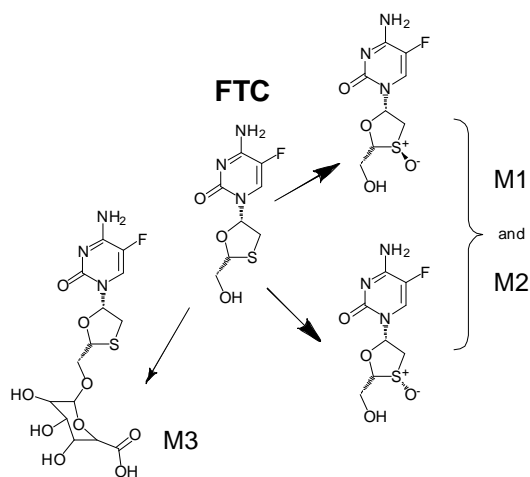
Emtricitabine and TFV are analogues of 2 different nucleosides, cytosine and adenosine, respectively, and do not share a common intracellular metabolism pathway. In experiments where both FTC and TFV were incubated together at concentrations higher than achieved in the plasma (10 µM), the intracellular phosphorylation of FTC and TFV to their active intracellular anabolites was not affected (m1.4.4, PC-164-2001).

### 3.5.2. Routes of Metabolism

#### 3.5.2.1. FTC

Emtricitabine is not subject to significant metabolism by CYP enzymes. Generation of a minor (~1%) sulfoxide metabolite (M1 and/or M2) was catalyzed by CYP3A4, and inhibitor studies suggested that at least one other enzyme, possibly flavin-containing monooxygenase, may play a role (m1.4.4, 15396 v1). A minor direct glucuronide metabolite, M3, was also detected (Figure 1) {4570}.

**Figure 1. Oxidative Metabolism of FTC**

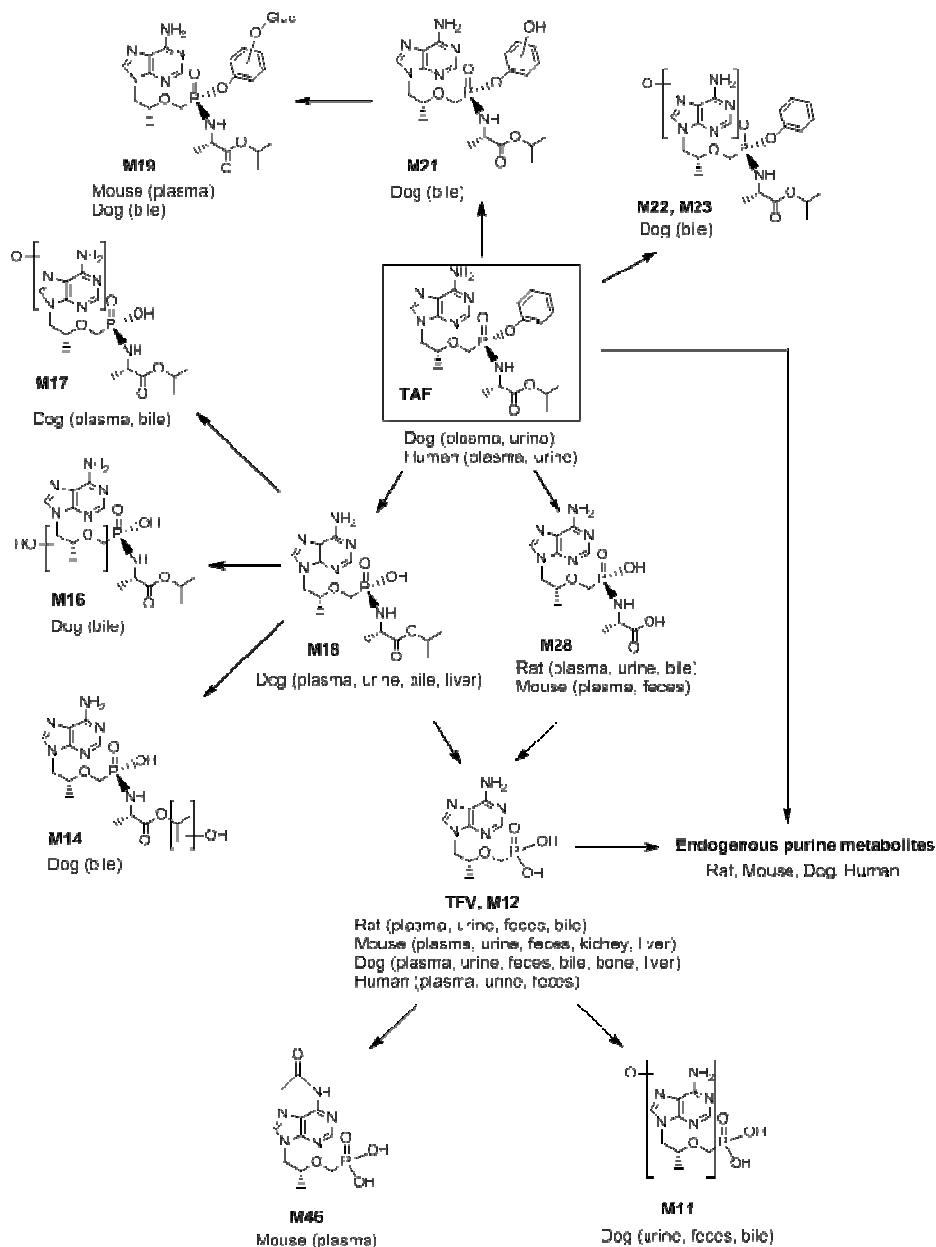


#### 3.5.2.2. TAF

The metabolic profiles of TAF were determined in plasma, urine, feces, kidney, liver, and nasal turbinate from mice (m1.4.4, AD-120-2012), in plasma, urine, bile, and feces from rats (m1.4.4,

AD-120-2021), and in plasma, urine, bile, feces, bone, and liver from dogs (m1.4.4, AD-120-2008). The metabolite profiles were also determined in human plasma, urine, and feces following administration of a single oral dose of [<sup>14</sup>C]TAF (GS-US-120-0109). Based on the results from mouse, rat, dog, and human, a proposed biotransformation pathway is summarized (Figure 2). Tenofovir alafenamide is also subject to intracellular metabolism to TFV, which is further phosphorylated to the anabolites, TFV-MP and TFV-DP, with TFV-DP being the pharmacologically active form.

**Figure 2. Metabolites of TAF**



### 3.5.2.3. F/TAF

Drug metabolizing enzymes do not contribute significantly to the elimination of FTC or TFV, so no interactions are anticipated.

## 3.5.3. In Vitro Metabolism

### 3.5.3.1. FTC

An in vitro metabolism study was performed to identify the potential human CYP enzyme(s) responsible for the metabolism of FTC using human liver microsomes and Bactosomes containing cDNA-expressed human CYP enzymes (m1.4.4, 15396v1). The results showed that FTC was relatively stable in the incubation medium. One minor metabolite (~1%) was detected only in incubations with cDNA-expressed CYP3A4 incubations. It was not formed by CYP1A2, 2A6, 2B6, 2D6, 2E1, 2C8, 2C9, or 2C19. Human hepatic microsomal incubations in the presence and absence of selective inhibitors of various CYPs confirmed the low rate of FTC metabolism, and due to incomplete inhibition by the CYP3A-selective inhibitor, ketoconazole, also suggested the possible involvement of flavin monooxygenases (FMOs) in the metabolism of FTC. In vitro glucuronidation of FTC was not detected.

### 3.5.3.2. TAF

The potential for CYP enzymes to metabolize TAF was assessed (m1.4.4, AD-120-2004). Metabolism of TAF was not detected by CYP1A2, CYP2C8, CYP2C9, CYP2C19 or CYP 2D6. Tenofovir alafenamide was slowly metabolized by CYP3A4 at a rate of  $1.9 \text{ min}^{-1}$  which was 26.6% of the positive control, testosterone. While TAF is a weak inhibitor of CYP3A in vitro, it is not a clinically meaningful inhibitor or inducer of CYP3A.

## 3.5.4. In Vivo Metabolism

### 3.5.4.1. FTC

Emtricitabine was not extensively metabolized and is eliminated primarily as unchanged drug by renal excretion in mice, rats, and cynomolgus monkeys. Over 90% of the radioactivity in mouse and rat urine and 64% of the radioactivity in monkey urine was unchanged drug. Only trace levels of metabolites were found in feces {4570}, {4251} (m1.4.4, TEIN/93/0015, TEIN/93/0016, TOX063). In all 3 species, metabolism accounted for only a minor percentage of FTC elimination. Emtricitabine is subject to Phase I metabolism (oxidation to a diastereomeric sulfoxide) and to some direct conjugation (glucuronidation of hydroxymethyl group) as minor metabolic routes.

### 3.5.4.2. TAF

Based on the studies from mouse, rat, dog and human (m1.4.4, AD-120-2008, AD-120-2012, AD-120-2021, GS-US-120-0109), endogenous purine metabolites including hypoxanthine, xanthine, allantoin, and uric acid were observed in all species. Tenofovir accounted for a majority of drug related material in plasma, urine, and feces from all species except for human



plasma, in which uric acid was the predominant metabolite accounting for 73.9% of the total AUC over 96 hours. M18 was the major metabolite in rat bile accounted for 63% of total radioactivity recovered in bile. M18 and its oxidized metabolite, M16 were the major metabolites in dog bile accounted for 29 and 38% of total radioactivity recovered in bile. Various oxidative metabolites were found in dog bile. No metabolites unique to human were observed.

Tenofovir alafenamide-related metabolites were also monitored in kidney, liver, and nasal turbinate from mice (m1.4.4, AD-120-2012). Most of the radioactivity was associated with TFV in kidney and liver and xanthine (M7) was the major identified metabolite in nasal turbinates. In dog, TAF-related metabolites were monitored in bone and liver and most of the radioactivity in these tissues was associated with TFV (m1.4.4, AD-120-2008).

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

#### 3.5.4.3. F/TAF

Emtricitabine and TAF are analogs of 2 different nucleosides, cytidine and adenosine, respectively, and do not share a common intracellular metabolism pathway for pharmacological activation through phosphorylation. The available data indicate no significant potential for metabolic interaction among the individual components based on metabolism. Consequently, no additional studies with the combination product were conducted.

### 3.6. Excretion

#### 3.6.1. Recovery in Excreta

##### 3.6.1.1. FTC

The primary route of elimination of [<sup>3</sup>H]FTC and [<sup>14</sup>C]FTC was via renal excretion of parent drug after oral and IV administration in mice, rats, and cynomolgus monkeys {4570}, (m1.4.4, TEIN/93/0015, TOX063, TEIN/93/0016, and TOX092, respectively). The majority of the FTC recovered in the feces after oral administration most likely represents unabsorbed drug, rather than biliary excretion. Although FTC is metabolized to only a minor extent, its metabolites are also excreted via the kidneys.

##### 3.6.1.2. TAF and TFV

Following oral dosing of mice, rats, and dogs with [<sup>14</sup>C]TAF, the majority of radiolabel is recovered in the feces or urine in all species (m1.4.4, AD-120-2011, AD-120-2020, AD-120-2007). The excretion of [<sup>14</sup>C]TAF was determined after administration of a single 5-mg/kg oral dose of [<sup>14</sup>C]TAF to bile duct-intact and BDC male Sprague-Dawley rats (m1.4.4, AD-120-2020). In BDC rats, means of 72.6%, 23.2%, and 2.11% of the administered

radioactivity were excreted in feces, urine, and bile, respectively, by 168 hours postdose. Recoveries of radioactivity in bile and urine from BDC rats indicated that at least 25% of the dose was absorbed. The mean overall recovery of radioactivity after oral dosing to BDC rats was 99.9%. The excretion of [<sup>14</sup>C]TAF was determined after administration of a single 15-mg/kg oral dose of [<sup>14</sup>C]TAF to bile duct-intact and BDC male dogs (m1.4.4, AD-120-2007). In BDC dogs, means of 42.7%, 26.5%, and 14.0% of the administered radioactivity were excreted in feces, urine, and bile, respectively, through 168 hours postdose. The elimination of a large amount of radioactivity in bile of BDC dogs indicates that biliary excretion is a major route of elimination of [<sup>14</sup>C]TAF-derived radioactivity. The overall recovery of radioactivity in BDC dogs was 86.2%.

Renal excretion is the primary systemic route of elimination of TFV in all preclinical species tested. Following intravenous administration of [<sup>14</sup>C]TFV, the majority of radioactivity was recovered in the urine in rats and dogs with 85.2% by 24 hours and 70.03% by 48 hours, respectively (m1.4.4, 96-DDM-1278-001; 96-DDM-1278-002).

#### 3.6.1.3. F/TAF

Emtricitabine and TFV are almost exclusively eliminated by renal excretion. While TFV is a substrate for OAT1, OAT3, and MRP4, none of these transporters was inhibited by FTC. Therefore, interactions between the compounds during excretion are unlikely.

### 3.6.2. Excretion into Breast Milk

Excretion into milk has not been evaluated for FTC.

Tenofovir was excreted into the breast milk of lactating rats and rhesus monkeys (m1.4.4, R990202-PK; P2000116). The TFV milk to plasma ratios ranged from 0.11 to 0.24 in rats and 0.19 to 0.22 in rhesus monkeys.

### 3.7. Pharmacokinetic Drug Interactions

To aid in the interpretation of the data presented below and allow a quantitative estimate of the potential drug interaction liability from the IC<sub>50</sub> values in the section below, the key human pharmacokinetic data from multiple clinical studies with the F/TAF FDC (m2.7.2, Section 3.2.1.4) are summarized in Table 1.

**Table 1. Steady State Pharmacokinetic Parameters for FDC Components**

Parameter	FTC	TAF <sup>d</sup>	TFV <sup>d</sup>
Dose (mg)	200	10	–
C <sub>max</sub> ([I] <sub>1</sub> ) (μM)	7.7	0.34	0.053
C <sub>max,u</sub> (μM) <sup>a</sup>		0.068	
[I] <sub>2</sub> (μM) <sup>b</sup>	3236	84	–
C <sub>hep, inlet</sub> (μM) <sup>c</sup>	23.9	0.76	–

C<sub>max,u</sub> = unbound concentration of drug at C<sub>max</sub>; [I]<sub>1</sub> = inhibitor concentration corresponding to steady state C<sub>max</sub>; [I]<sub>2</sub> = inhibitor concentration corresponding to theoretical maximum concentration in the intestinal lumen

a Steady state C<sub>max</sub> × in vitro plasma fraction unbound (f<sub>u</sub>, 20% for TAF and ~ 100% for FTC and TFV)

b Dose / 250 mL

c Estimated total hepatic inlet (portal vein) concentration calculated according to Obach et al. {34390}

d Value for boosted TAF dose based on E/C/F/TAF FDC

### 3.7.1. Metabolic Drug Interactions

#### 3.7.1.1. FTC

Emtricitabine was not an inhibitor of activities catalyzed by CYP1A2, 2A6, 2B6, 2C9, 2C19, 2D6, 2E1, or 3A in human hepatic microsomal fractions. Emtricitabine also did not show inhibition of the glucuronidation of 7-hydroxycoumarin, a general UGT substrate (m1.4.4, 15247).

#### 3.7.1.2. TAF and TFV

The potential for TAF and TFV to inhibit human CYP-mediated drug metabolism was examined in vitro using hepatic microsomal fractions and enzyme-selective activities (m1.4.4, AD-120-2003 and m1.4.4, V990172-104). The inhibitory activity of TAF with human liver microsomal CYP isozymes, CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A were assessed at concentrations up to 25 μM. The inhibition constant (IC<sub>50</sub>) values calculated for CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, and CYP2D6 were greater than 25 μM. Tenofovir alafenamide weakly inhibited CYP3A-mediated oxidation of midazolam or testosterone with IC<sub>50</sub> of 7.6 or 7.4 μM, respectively. The weak inhibition of CYP3A, however, is unlikely to be clinically relevant as TAF did not affect the exposure to CYP3A substrates, midazolam or rilpivirine (m5.3.3.4, GS-US-120-1538 and GS-US-120-1554). Tenofovir at 100 μM did not inhibit CYP1A2, CYP2C9, CYP2D6, CYP2E1, and CYP3A.

The potential for TAF to be a mechanism-based inhibitor of the human CYP enzymes, CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19 and CYP2D6 was assessed at TAF concentration at 50 μM (m1.4.4, AD-120-2040). There was no evidence for time- or cofactor-dependent inhibition of any enzyme by TAF, with the maximum change in activity of 17.4% with CYP2C8 relative to control.

### 3.7.1.3. F/TAF

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

The clinical drug-drug interaction studies are described in detail in the Summary of Clinical Pharmacology Studies (m2.7.2, Section 3.2.3.2).

## 3.7.2. Induction Liability

### 3.7.2.1. FTC

Emtricitabine did not activate human AhR or PXR at concentrations up to 50  $\mu$ M (m1.4.4, AD-162-2005).

### 3.7.2.2. TAF

The induction of CYP, P-gp and UGT1A1 mRNA and CYP activity by TAF was assessed in cultured human hepatocytes from 3 separate donors treated with 1, 10, and 100  $\mu$ M TAF once daily for 3 consecutive days (m1.4.4, AD-120-2032). Due to cytotoxicity, the cell viability was significantly affected at 100  $\mu$ M TAF and mixed responses to TAF with increased mRNA levels and reduced CYP activities were observed. At noncytotoxic concentrations of TAF (1 and 10  $\mu$ M), no significant increases in the mRNA levels and the CYP activities were observed. After treatment with 10  $\mu$ M TAF, the mRNA levels of CYP1A2 and CYP3A4 increased by 3.0- and 8.3-fold which correspond to 3% and 6% of the induction levels observed with the respective positive controls. Therefore, TAF showed little or no potential for CYP induction at clinically relevant concentration (1  $\mu$ M). No significant induction of P-gp and UGT1A1 mRNA was observed (less than 2-fold).

The potential for TAF to induce human drug metabolizing enzymes and drug transporters through the activation of human AhR or human PXR was further evaluated in cell-based systems (m1.4.4, AD-120-2005). For PXR activation, at 50  $\mu$ M TAF the extent of activation of PXR was only 23% of the maximal effect of rifampicin and 15  $\mu$ M TAF demonstrated activation of < 5% of the maximal induction elicited by rifampicin. Tenofovir alafenamide did not activate AhR up to 50  $\mu$ M, the highest concentration tested. Therefore, TAF is unlikely to activate either of these human xenobiotic receptors supporting the in vitro induction results in human hepatocytes. Furthermore, TAF is unlikely to be a clinically relevant inducer as it did not affect the exposure to midazolam or rilpivirine (m5.3.3.4, GS-US-120-1538 and GS-US-120-1554).

### 3.7.3. Transporter Drug Interactions

The potential for FTC, TAF, and TFV to be substrates or inhibitors for transporters was evaluated in vitro and summarized in Table 2 and Table 3, respectively.

**Table 2. Transporter Substrate Assessment of F/TAF Components**

Transporter	FTC	TAF	TFV	Cross Referenced Reports in m1.4.4
P-gp	n	y	n	AD-236-2004, AD-120-2018
BCRP	n	y	n	AD-236-2005, AD-120-2018
OATP1B1	ND	y	ND	AD-120-2022
OATP1B3	ND	y	ND	AD-120-2022
OAT1	n	n	y	AD-236-2010, PC-104-2010, PC-120-2018
OAT3	y	n	y	AD-236-2010, PC-103-2001 PC-120-2018
OCT1	ND	n	n	PC-103-2001, AD-120-2036
OCT2	n	ND	n	AD-236-2011, PC-103-2001
MRP1	ND	ND	n	PC-104-2014
MRP2	n	ND	n	AD-104-2001
MRP4	ND	ND	y	AD-104-2001

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

**Table 3. Transporter Inhibition Assessment of F/TAF Components**

Transporter	IC <sub>50</sub> (μM)			Cross Referenced Reports in m1.4.4
	FTC	TAF	TFV	
P-gp	>100	>100	>1000	AD-236-2003, AD-120-2019
BCRP	>100	>100	>100	AD-236-2003, AD-120-2019
BSEP	>100	>100	>100	AD-236-2008 AD-120-2036
OATP1B1	>100	>100	>100	AD-236-2006 AD-120-2019
OATP1B3	>100	>100	>100	AD-236-2006 AD-120-2019
MATE1	>100	>100	>300	AD-236-2001 AD-104-2012 AD-120-2036
OAT1	>100	>100	33.8 <sup>a</sup>	AD-236-2007, AD-120-2036
OAT3	>100	>100	>1000	AD-236-2007, PC-103-2001, AD-120-2036
OCT1	>100	>100	>100	AD-236-2008, PC-103-2001 AD-120-2036
OCT2	>100	>100	>300	AD-236-2001, AD-120-2036
MRP1	ND	ND	>500	PC-104-2014
MRP2	>100	ND	>100	AD-236-2012, AD-104-2001
MRP4	>100	ND	>1000 <sup>b</sup>	AD-236-2007,

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

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

b Imaoka et al 2007 {10260}

### 3.7.3.1. FTC

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

### 3.7.3.2. TAF

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

Tenofovir alafenamide is a substrate for intestinal efflux transporters, P-gp and BCRP. An increase in TAF absorption was observed in the presence of efflux transport inhibitors, CsA or COBI in vitro (m1.4.4, AD-120-2013). The effect of CsA on TAF oral bioavailability was also assessed in vivo in dogs (m1.4.4, AD-120-2035). Following oral administration of TAF at 2 mg/kg to untreated or pretreated dogs with 75 mg CsA, the CsA pretreatment increased the plasma exposure to TAF and oral bioavailability by approximately 10-fold, while the PK profile of TFV was not significantly affected by CsA. Consistent with the increased TAF plasma exposure, the exposure to TFV-DP in PBMCs isolated from the CsA pretreated dogs was approximately 2-fold higher than that in cells from untreated animals. These results suggest that coadministration of efflux inhibitors increases TAF absorption and may potentiate the antiviral effect by increasing the TFV-DP levels in PBMCs.

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

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

Results of in vitro transport studies indicate that the active tubular secretion of TFV is mediated by the human OAT1 and MRP4 acting in series as the major uptake and efflux transporters in proximal tubules, respectively (m1.4.4, PC-103-2001, AD-104-2001, AD-104-2002), {2520}, {7299}, {8418}. Human organic anion transporter type 3 may play a secondary role in the tubular uptake of TFV. Neither P-gp nor MRP2 appear to be involved in the tubular efflux of TFV. As the primary transporter handling the tubular uptake of TFV, human OAT1 has been assessed for its potential role in drug interactions between TFV and other renally secreted therapeutics including antibiotics, anti-inflammatory agents, and other antivirals (including PIs). Under physiologically relevant conditions, none of the tested drugs affected human OAT1-mediated transport of TFV, indicating a low potential for renal interactions with TFV due to inhibition of this pathway (m1.4.4, PC-104-2010 and PC-104-2011, respectively) {2520}. Furthermore, the PIs ATV, lopinavir, and RTV did not exhibit any effect on the active cellular elimination of TFV mediated by the MRP4 efflux pump {8418}. The results of in vitro drug interaction studies indicate that PIs are unlikely to exert any substantial effect on the accumulation of TFV in renal proximal tubules.

The results from in vitro studies investigating the contribution from MRP1 in tubular reabsorption of TFV (m1.4.4, PC-104-2014) indicated that MRP1 is not involved in the reabsorption of TFV at the basolateral membrane of proximal tubule cells.

### 3.7.3.3. F/TAF

Renal excretion of TFV is facilitated by basolateral uptake by OAT1 and OAT3 and apical efflux by the MRP4 efflux transporter. There is no evidence for inhibition of TFV renal excretion by FTC. As described in Section 3.2.2, in the presence of 90  $\mu$ M COBI in the Caco-2 bidirectional permeability assay, TAF forward permeability increased 4.6-fold and the efflux ratio significantly decreased suggesting P-gp-mediated drug interaction (m1.4.4, AD-120-2013).

### 3.7.4. F/TAF Pharmacokinetic Drug Interactions

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

For the F/TAF FDC, the clinical pharmacokinetic interaction studies with the components should be given the greatest consideration. Neither FTC nor TFV interact with drug metabolizing enzymes as substrates, inhibitors, or inducers (oxidative metabolism of FTC plays only a minor role in the elimination of the compound). TFV and FTC do not inhibit each other's pharmacological activation through phosphorylation. Thus, the drug interactions between the two compounds are unlikely.

As F/TAF FDC may be used in combination with boosted ARVs, the potential for drug-drug interactions with a pharmacokinetic enhancer, COBI or RTV was evaluated. Neither FTC nor TFV interact with drug metabolizing enzymes as substrates, inhibitors, or inducers (oxidative metabolism of FTC plays only a minor role in the elimination of the compound) and so will not take part in metabolic drug interactions with COBI or RTV. Cobicistat and RTV are shown to be weak inhibitors of efflux transporters, P-gp and BCRP (m1.4.4, AD-216-2072 and AD-216-2104). Since TAF, but not FTC, is a substrate for both P-gp and BCRP, high concentrations of COBI or RTV achieved briefly in the intestinal lumen can inhibit the intestinal efflux of TAF, thereby increasing its absorption (m1.4.4, AD-120-2013). Therefore, a lower TAF dose for the FDC (F/TAF, 200/10 mg) is recommended when used with boosted ARVs. Cobicistat and RTV showed either weak or undetectable inhibition of OAT1, OAT3, and MRP4 in vitro (m1.4.4, AD-216-2105). Consistently, transport of TFV by OAT1, OAT, and MRP4 was not meaningfully inhibited by COBI or RTV under physiological conditions and clinically relevant concentrations (m1.4.4, PC-236-2008 and PC-236-2009). In addition, COBI and RTV had no effect on the accumulation of TFV in human renal tissue slices at clinically relevant concentrations (m1.4.4, PC-236-2007). Since both COBI and RTV are inhibitors and TAF is a substrate of OATP transporters in vitro, the exposure to TAF may be affected by COBI or RTV via inhibition of hepatic uptake (m1.4.4, AD-216-2100 and AD-120-2019). The effects of differences in OATP1B1 and OATP1B3 activity are, however, not expected to be clinically relevant given the high passive permeability of TAF (m1.4.4, AD-120-2019). Because of the highly restricted substrate specificity of the enzymes catalyzing the phosphorylation of FTC and TFV, inhibition of pharmacological activation by COBI or RTV is unlikely.



### 3.8. Summary of Pharmacokinetics

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

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

Emtricitabine does not undergo extensive first-pass or systemic metabolism, and is eliminated primarily by renal excretion of unchanged drug. The total body clearance of FTC exceeds the glomerular filtration rate, suggesting the drug is actively secreted by renal tubules into the urine.

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

Tenofovir alafenamide is not an inhibitor or an inducer of UGT1A1 or CYP enzymes known to metabolize xenobiotics except for weak inhibition observed for CYP3A in vitro. However, the weak inhibition of CYP3A is not clinically relevant as TAF did not affect the exposure to CYP3A substrates, midazolam or rilpivirine in clinical drug-drug interaction studies (m2.7.2, Sections 2.5.2.3 and 2.5.2.4, [GS-US-120-1538 and GS-US-120-1554]). Tenofovir alafenamide is unlikely to be a perpetrator of transporter-mediated drug interactions. Since TAF is a substrate for intestinal efflux transporters P-gp and BCRP and hepatic uptake transporters OATP1B1 and OATP1B3, TAF exposure may be affected by inhibitors and by inducers of the intestinal efflux transporters and inhibitors or genetic polymorphisms of OATPs. Tenofovir alafenamide was not a substrate for renal transporters OAT1 and OAT3. While TFV is a substrate for OAT1, OAT3, and MRP4, none of these transporters was inhibited by FTC. Therefore, interactions between the compounds during excretion are unlikely.

Therefore, pharmacokinetic interactions between FTC and TAF are not anticipated as FTC, TAF, and TFV are not inhibitors or inducers of drug metabolizing enzymes and not inhibitors of all the transporters tested. In addition, FTC and TAF are analogs of 2 different nucleosides, cytidine and adenosine, respectively, and do not share a common intracellular metabolism pathway for pharmacological activation through phosphorylation. When F/TAF is used with other ARVs in combination with COBI or RTV, increase in TAF exposure is expected due to inhibition of intestinal efflux transporters and, therefore, a lower TAF dose for the FDC (F/TAF, 200/10 mg)

is recommended. No other clinically relevant drug interactions between COBI or RTV and F/TAF components are anticipated. No additional nonclinical pharmacokinetic studies are considered warranted with the F/TAF FDC in view of the results of extensive nonclinical and clinical pharmacokinetic studies of the individual components and the clinical studies with the F/TDF containing regimens and with the E/C/F/TAF FDC.

## 4. TOXICOLOGY

Comprehensive nonclinical programs with FTC and TAF have been completed. These studies have characterized the single and repeat dose toxicity, mutagenicity, carcinogenicity (TDF studies in place of TAF), and reproductive toxicity of each the individual agents, and the toxicity of the FTC/TDF combination. The nonclinical toxicology studies discussed in this section provide an adequate basis to evaluate potential toxicities of the individual components and the 2-drug combination, and for comparing and interpreting results from clinical studies.

### 4.1. Single Dose Toxicity

Emtricitabine has demonstrated minimal acute toxicity in rodents (oral LD<sub>50</sub> > 4000 mg/kg and IV LD<sub>50</sub> > 200 mg/kg; m1.4.4, TTEP/93/0020; TTEP/93/0023; TTEP/93/0021; TTEP/93/0024).

The single dose NOAEL for a single oral dose TAF as GS-7340-02 in the rat was determined to be > 1000 mg/kg (m1.4.4, R990200). The no observed effect level (NOEL) in dogs administered a single dose of TAF was 30 mg/kg (treatment-related clinical signs, renal lesions in the kidneys at 90 and 270 mg/kg (m1.4.4, D990181).

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

### 4.2. Repeat Dose Toxicity

#### 4.2.1. FTC

A series of GLP oral repeat-dose toxicity studies were conducted with FTC in mice (4 weeks [m1.4.4, TOX599 and TOX599 addendum; TOX118] and 26 weeks [m1.4.4, TOX022 and TOX628]), rats (13 weeks [m1.4.4, TOX097]), and cynomolgus monkeys (4 weeks [m1.4.4, TOX600 and TOX600 addendum], 13 weeks [m1.4.4, TOX627], and 52 weeks [m1.4.4, TOX032]).

Effects associated with the administration of FTC in the toxicology studies were confined to high-dose groups. Changes in red blood cell (RBC) parameters, interpreted as a mild, reversible anemia occurred at the highest dose in several studies (ie, 1- and 6-month mouse; 3-month rat; and 12-month monkey). The NOELs for the longest treatment period in each species were 500 mg/kg/day in mice (6 months), 600 mg/kg/day in rats (3 months), and 200 mg/kg/day in monkeys (12 months). The exposures based on plasma AUC values at the NOEL doses in the animals were approximately 27-fold (mice), 27-fold (rats), and 7.5-fold (monkeys) higher than the AUC in patients treated with FTC at 200 mg once daily in the EVG/COBI/FTC/TDF FDC.

#### 4.2.2. TAF

The repeat-dose oral toxicity of TAF has been studied in mice, (2 weeks [TX-120-2006] and 13 weeks [TX-120-2007]), rats (6-7 days [R2000044, R000139], 4 weeks [R990182] and

26 weeks [TOX-120-001]), dogs (4-weeks [D990175] and 39 weeks [TOX-120-002]) and monkeys (4 weeks [P2000114]). In chronic studies, kidneys (karyomegaly, tubular degeneration), and bone (atrophy of metaphyseal cancellous bone) were the primary target organs. TAF also appeared to increase biochemical markers of bone turnover and decrease serum 1, 25-dihydroxy- and 25-hydroxyvitamin D<sub>3</sub> at doses in rats and dogs.

#### 4.2.2.1. Kidney

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

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

#### 4.2.2.2. Bone

Atrophy of metaphyseal cancellous bone was observed in rats administered TAF at 100 mg/kg/day for 26 weeks. TAF also increased biochemical markers of bone turnover and decrease serum 1,25-dihydroxy- and 25-hydroxyvitamin D<sub>3</sub> in rats ( $\geq 25$  mg/kg/day) and dogs ( $\geq 37.5$  mg/kg/day for 6 days). In the 39-week dog study, bone mineral density changes at 18/12 mg/kg/day were most likely due to body weight loss, but these changes were accompanied by a slight but significant decrease in serum 1,25-dihydroxyvitamin D<sub>3</sub> in males only and a significant increase in 25-hydroxyvitamin D<sub>3</sub> in females only.

#### 4.2.2.3. Other

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

TAF had no discernible electrocardiographic effect at the low dose of 2 mg/kg/day. There was some evidence at 6 and 18/12 mg/kg/day for an effect to slightly prolong PR intervals (~13-24%) which was associated with significant decreases in T3 {29101}, {29104}. After the 13-week recovery period, serum T3 values returned to levels similar to the control group animals at the end of the study. No PR prolongation or any change in ECG results occurred in the safety

pharmacology study that evaluated a TAF dose up to 100 mg/kg (m1.4.4, D2000006) or in the thorough QT study (m1.4.4, GS-US-120-0107).

At 18/12 mg/kg/day in dogs, the highest dose tested, a minimal infiltration of histiocytes was present in some organs (eye [choroid plexus, ciliary body], lung, and spleen) in some animals. In-life ophthalmologic examinations were normal. The histiocytic infiltration observed in multiple organs was most likely an indirect drug effect due to general debilitation and was not observed in other repeat dose toxicity studies. There were no drug-related effects on ophthalmic exams or microscopic exams of ocular tissue observed in repeat-dose toxicity studies in mice (up to 13 weeks), rats (up to 26 weeks), and nonhuman primates (4 weeks) or in the 4-week dog toxicology study. The minimal to slight infiltration of mononuclear cells in the ocular posterior uvea occurred only in dogs administered the highest dose of TAF where compound-related toxicities occurred on clinical condition, weight gain, serum chemistry, bone mineral density, and other organ histopathology. Distribution of [<sup>14</sup>C]TAF to eyes has been assessed in mice, rats, and dogs (m1.4.4, AD-120-2011, AD-120-2020, and D990173-BP). Melanin binding has specifically been assessed by comparing distribution in pigmented and non-pigmented mice (C57 black and CD-1, respectively) and rats (Long Evans and Sprague-Dawley, respectively). [<sup>14</sup>C]TAF-related radioactivity distributed poorly to the eyes of rats and dogs ( $C_{\max}$  in eyes < 8% that observed in plasma). Transient exposure to low levels of [<sup>14</sup>C]TAF-related radioactivity was observed in the eyes of rats decreasing to undetectable levels at 8 hours postdose. No difference in distribution was observed between Sprague-Dawley and Long Evans rats, including in the skin and eyes, suggesting no binding to melanin. The distribution of [<sup>14</sup>C]TAF to eyes in mice was higher than other species studied ( $C_{\max}$  in eyes 15%-20% that observed in plasma). More persistent exposures in eye lens, eye uveal tract, and eyes were observed in C57 black mice compared to CD-1 mice. However, no difference in distribution between pigmented and nonpigmented skin was observed illustrating that [<sup>14</sup>C]TAF-related radioactivity was not selectively associated with melanin-containing tissues. The posterior uveitis in dogs administered the highest dose of TAF occurred at 3.7- and 17-fold higher exposure to TAF and TFV, respectively, than that observed in human subjects administered a 25-mg dose of TAF and does not correlate with the tissue distribution where TAF was found to poorly penetrate across the blood brain and blood retinal barrier in dogs. Because TAF has poor penetration across the blood brain and blood retinal barrier in dogs, it is unlikely that TAF directly caused the observed histiocytic infiltration in the posterior uvea. Based on the evidence from tissue distribution and toxicology studies, Gilead concludes that the risk of posterior uveitis in humans is very low.

TAF is unlikely to cause mitochondrial toxicity. TAF did not affect the amount of mitochondrial DNA levels up to 1  $\mu$ M (approximately 2-fold higher than  $C_{\max}$  after a 25 mg TAF dose), the highest concentration tested, in HepG2 cells in a 10-day assay (m1.4.4, PC-120-2006). The active metabolite of TAF, tenofovir diphosphate, is highly discriminated as a substrate by mitochondrial DNA polymerase  $\gamma$  relative to the natural substrate, adenosine triphosphate (ATP) (> 10,000 fold) {4923}. Therefore, TAF is unlikely to inhibit mitochondrial DNA polymerase  $\gamma$  under clinical relevant conditions.

The nonclinical toxicity studies demonstrate that there was no adverse effect of TAF for up to 26 weeks in the rat, up to 39 weeks in the dog, and 4-weeks in the monkey at doses producing

TFV systemic exposure levels in animals 14-, 4- and > 22- fold greater, respectively, than those observed in patients treated with the recommended clinical dose of E/C/F/TAF.

#### **4.2.3. FTC/TDF**

Two 14-day oral gavage GLP studies were conducted to investigate the potential toxicity of FTC/TDF, and to qualify potential impurities in nondegraded and degraded FTC/TDF tablets following daily oral administration to rats for a minimum of 14 days (m1.4.4, TX-164-2001 and TX-164-2005). There were no toxicologically significant differences between groups treated with nondegraded and degraded FTC/TDF, and no exacerbation of toxicity with the FTC/TDF combination compared to data with the individual agents.

A 4-week toxicity study was conducted with FTC and TDF in dogs to examine the possible exacerbation of renal toxicity with combination treatment and to assess possible effects on the immune system (m1.4.4, TX-164-2004). Male dogs were treated with vehicle, FTC alone (20 mg/kg/day), TDF alone (30 mg/kg/day), or a low dose (2/3 mg/kg/day) or high dose (20/30 mg/kg/day) of the combination. No adverse effects were observed in the FTC alone group or the low dose combination group. No remarkable changes were observed for immunophenotyping or natural killer cell assay values for any treatment group. Tenofovir DF at 30 mg/kg alone or in combination with 20 mg/kg FTC caused minimally increased activated partial thromboplastin time and creatinine. Minimal tubular epithelial necrosis and slight to moderate tubular epithelial regeneration were seen in animals administered TDF at 30 mg/kg alone or in combination with 20 mg/kg FTC. There were no overall differences in the incidences and mean severities of the renal findings between the 2 groups. Renal findings were reversible after a 4-week recovery period (examined for combination only). Systemic exposure (AUC) was not altered with combination dosing when compared to the agents dosed individually. The NOAEL for the combination of FTC/TDF is 2/3 mg/kg/day in dogs.

#### **4.2.4. F/TAF**

FTC and TAF exhibit different patterns of target organ toxicity. The only significant effect of FTC identified at dose levels constituting large clinical multiples was a minor anemia. The principal target organs of toxicity following oral administration of TAF were the kidney (karyomegaly, tubular degeneration) and bone.

The potential drug interactions of F/TAF with other ARVs include inhibition of intestinal efflux of TAF by COBI and other boosting agents and inhibition of OATP-mediated hepatic uptake of TAF, including by COBI and EVG. The recommended F/TAF tablet (200/10mg) will be based on the increase in TAF exposure due to inhibition of intestinal efflux by COBI. Cobicistat does not inhibit OAT1 or MRP4, the transporters responsible for the renal excretion of TFV and so will not interfere with the elimination of TFV.

Administration of FTC and TAF in combination is unlikely to exacerbate known toxicities of the individual agents based on the FTC and TDF combination toxicity studies. No new or more marked toxicities occurred in two 14-day rat toxicology studies and a 4-week dog study with the combination (m1.4.4, TX-164-2001; TX-164-2005; TX-164-2004).

The only toxicity observed in chronic animal studies with FTC was mild, reversible anemia in mice and minor decreases in erythrocyte counts/increases in mean corpuscular hemoglobin in monkeys at large multiples of clinical exposure (110-fold in mice; 21-fold in monkeys). These hematological findings are not considered relevant to clinical use FTC has not shown any potential for bone toxicity in chronic rat and dog toxicity studies; thus, exacerbation of any TAF effects on bone is not expected. The ample nonclinical safety database on these drugs, including combination toxicity studies with FTC and TDF, indicates further toxicological investigations are unlikely to yield new data relevant to humans.

### **4.3. Genotoxicity**

#### **4.3.1. FTC**

Emtricitabine was not genotoxic in the reverse mutation bacterial test (Ames test) (m1.4.4, 18637-0-409R; MUT203; K01-3154), mouse lymphoma (m1.4.4, TOX012), or mouse micronucleus assays (m1.4.4, TOX011).

#### **4.3.2. TAF**

Tenofovir alafenamide was not genotoxic in a battery of in vitro and in vivo assays. The in vitro assays included gene mutation assays with bacterial strains (*Salmonella typhimurium*, *Escherichia coli*; m1.4.4, V990212), and a L5178Y gene mutation assay in mouse lymphoma cells (m1.4.4, V990213). The in vivo evaluation consisted of a mouse bone marrow micronucleus study at oral doses of 500, 1000, and 2000 mg/kg (m1.4.4, M2000113).

#### **4.3.3. FTC/TDF**

No exacerbation of mutagenicity was apparent in either the bacterial reverse mutation assay (Ames assay) or the in vitro mammalian cell gene mutation assay (L5178Y/TK<sup>+/−</sup> mouse lymphoma assay) when FTC and TDF were administered together compared with each agent alone (m1.4.4, TX-164-2002; TX-164-2003).

#### **4.3.4. F/TAF**

FTC and TAF were negative in genotoxicity studies. The combination of the 2 components is therefore not expected to have an altered genotoxicity profile as compared with that of the individual agents.

### **4.4. Carcinogenicity**

Per separate agreements with the FDA ( [REDACTED] ) and with the EMA

( [REDACTED] ), [REDACTED] )  
[REDACTED] Summaries of the carcinogenicity studies with TDF are provided below.

#### **4.4.1. FTC**

In long-term carcinogenicity studies of FTC, no drug-related increases in tumor incidence were found in mice at doses up to 750 mg/kg/day (23-fold the human systemic exposure at the therapeutic dose of 200 mg/day; m1.4.4, TOX109) or in rats at doses up to 600 mg/day/day (28-fold the human systemic exposure at the therapeutic dose; m1.4.4, TOX108).

#### **4.4.2. TAF**

Long-term oral carcinogenicity studies of TDF in mice and rats were carried out at exposures up to approximately 10 times (mice) and 4 times (rats) those observed in humans at the 300 mg therapeutic dose in the EVG/COBI/FTC/TDF FDC (m1.4.4, M990205; R990204). Female mice showed a low incidence of liver adenomas at the highest dose of 600 mg/kg/day. Rats did not show any carcinogenic potential in the long-term study.

#### **4.4.3. F/TAF**

Emtricitabine and TDF/TFV have demonstrated low carcinogenic potential in conventional 2-year bioassays at exposures that exceeded (TDF) or far exceeded (FTC) human exposures at the therapeutic doses. It is considered unlikely that combination dosing would change these profiles as no exposure difference would be expected and no exacerbation of toxicity/genotoxicity is expected.

As conventional 104-week bioassays have been conducted for EVG, COBI, FTC and TDF, alternative short- or medium-term carcinogenicity studies are not necessary.

### **4.5. Reproductive Toxicity**

#### **4.5.1. FTC**

Emtricitabine did not affect fertility in male rats at approximately 140-fold or in male and female mice at approximately 60-fold higher exposures than in humans administered the recommended 200 mg daily dose (m1.4.4, TTEP/95/0028, TOX036). There were no adverse effects in embryo-fetal development studies in mice at exposures approximately 60-fold higher and in rabbits at exposures approximately 120-fold higher than human exposures (m1.4.4, TOX037, TOX038). In the pre/postnatal study in mice, F<sub>1</sub> dams at 1000 mg/kg/day had slightly longer estrous cycles than controls, but fertility was normal in the offspring exposed daily from before birth (in utero) through sexual maturity at daily exposures of approximately 60-fold higher than human exposures at the recommended 200 mg daily dose (m1.4.4, TOX039).

#### **4.5.2. TAF**

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



The TAF fumarate (GS-7340-03) oral rat fertility study (m1.4.4, TX-120-2012) data indicate dose related decreases in body weight gain in males and females, but no drug related changes occurred in male or female fertility endpoints including mating index, fertility index, sperm motility, sperm concentration, number of corpora lutea, implantation sites, early and late resorptions and live fetuses at doses up to 160 mg free base equivalents/kg/day. The TAF NOAEL for reproductive and early embryonic toxicity was 160 mg/kg/day.

There was no effect on fetal viability or fetal development in pregnant rats administered doses of GS-7340-02 up to 250 mg/kg/day (m1.4.4, TX-120-2002), or in pregnant rabbits administered GS-7340-02 up to 100 mg/kg/day (m1.4.4, TX-120-2005). The highest doses were maternally toxic. In the rat, decreased fetal body weight associated with some minor transitory delays in the rate of ossification was observed at 250 mg/kg/day, a maternally toxic dose. At the NOAEL for embryo-fetal development of approximately 100 mg/kg/day in rats, AUC<sub>tau</sub> values for TAF and TFV on Day 17 were 0.2 and 17.4 µg·h/mL, respectively. At the NOAEL for embryo-fetal development of 100 mg/kg/day in rabbits, AUC<sub>tau</sub> values for TAF and TFV on Day 20 were 11.0 and 27.3 µg·h/mL, respectively.

Tenofovir DF, but not TAF, has been tested in a perinatal/postnatal study. Per separate agreements with the FDA ( ) and with the EMA

( ). There was an alteration of the estrous cycle in female rats in the perinatal study in rats (m1.4.4, R990202). The NOEL for behavioral, reproductive, and development toxicity was 150 mg/kg/day. Maternally toxic doses (≥ 450 mg/kg/day) had effects on pup survival, pup body weights, and sexual maturation.

### **4.5.3. F/TAF**

#### **4.5.3.1. Fertility and Early Embryonic Development**

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

#### **4.5.3.2. Embryo-Fetal Development**

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

#### **4.5.3.3. Pre- and Postnatal Development**

Slightly longer estrous cycles were observed in first (F<sub>1</sub>) generation rats after exposure to high doses of FTC and a delay in sexual maturation was observed in F<sub>1</sub> generation rats after exposure to high (maternally toxic) doses of TDF. NOELs/NOAELs for FTC and TAF were at exposures above human exposures. As with the other reproductive toxicity tests, a repeat of this test with the F/TAF FDC is unlikely to add any new information.

## **4.6. Juvenile Toxicity**

### **4.6.1. FTC**

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

### **4.6.2. TAF/TFV**

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

Tenofovir administered to newborn or infant rhesus monkeys at doses of 4 to 30 mg/kg/day did not cause adverse effects in short term studies (up to 12 weeks). However, prolonged TFV treatment (generally more than 4 months of daily treatment at 30 mg/kg/day administered subcutaneously) resulted in a Fanconi-like syndrome with glucosuria, aminoaciduria, hypophosphatemia, growth restriction, and bone pathology (osteomalacia) {7311}. Clinical, biochemical, and radiographic resolution/improvement occurred with dose reduction (from 30 to  $\leq 10 \text{ mg/kg/day}$ ) or discontinuation of treatment.

Three animals (1 SIV-infected) were dosed chronically, beginning as neonates, with 10 mg/kg/day TFV administered subcutaneously. After more than 5 years of treatment, there were no clinical, radiographic, or dual-emission X-ray absorptiometry scan {7311} findings of an adverse effect on bone. The mean AUC associated with this dosage (18  $\mu\text{g}\cdot\text{h}/\text{mL}$ ) 68-fold greater than the human AUC<sub>ss</sub> following a 25 mg/day dose of TAF.

### **4.6.3. F/TAF**

Although TDF has produced effects in reproductive toxicity studies, effects on rat fetuses have only been observed at dose levels associated with significant maternal toxicity. Neither agent has shown an effect on rabbit fetuses. For FTC and TDF, NOELs and NOAELs have been clearly identified and were at exposures above human exposures.

No specific studies were conducted with the F/TAF FDC. This new drug application proposes that the F/TAF FDC tablet initially be registered for HIV-1 infected patients  $\geq 12$  years old using the safety data from the E/C/F/TAF clinical program. Refer to m2.7.3 and m.2.7.4 for results of Study GS-US-292-0106 (A Phase 2/3, Open-Label Study of the Pharmacokinetics, Safety, and Antiviral Activity of the Elvitegravir/Cobicistat/Emtricitabine/Tenofovir Alafenamide (E/C/F/TAF) Single Tablet Regimen (STR) in HIV-1 Infected Antiretroviral Treatment-Naive Adolescents).

#### **4.7. Local Tolerance**

No local tolerance studies have been conducted with FTC. Local toxicity studies concluded that TAF was predicted to be a noncorrosive/nonsevere eye irritant (m1.4.4, TX-120-2013), and nonirritating/noncorrosive to rabbit skin under semi-occluded conditions (m1.4.4, TX-120-2011).

The F/TAF tablet is intended for oral use. No local tolerance studies were conducted for the F/TAF combination. Evaluation of local tolerance to the GI tract has been conducted during the repeat-dose oral toxicity studies with each of the individual agents and with the E/C/F/TAF FDC. In the pivotal registration clinical studies (m2.5), ECF/TAF was generally well tolerated; the incidences of GI disorders were consistent with those expected in the subject population and the known safety profiles of the study drugs.

#### **4.8. Other Toxicity Studies**

##### **4.8.1. Antigenicity**

TAF showed no potential for sensitization (m1.4.4, TX-120-2014).

##### **4.8.2. Immunotoxicity**

The immunotoxicity of FTC was evaluated in a 28-day study in CD rats at doses up to 1000 mg/kg/day (m1.4.4, TOX146). There were no adverse effects of FTC during the dosing period and FTC did not affect the immunoglobulin M (IgM) antibody titers to sheep RBCs at any of the doses administered. The NOEL for immunotoxicity was 1000 mg/kg/day.

Data from repeat-dose toxicity studies with FTC or TAF (hematology, lymphoid organ weights, microscopy of lymphoid tissues, bone marrow cellularity) did not suggest immunotoxic potential. There were no notable effects of the combination of FTC/TDF on immune cells or NK cell assay values in a 4-week dog study (m1.4.4, TX-164-2004). No further studies were deemed necessary with the F/TAF FDC.

##### **4.8.3. Impurities/Degradation Products**

###### **4.8.3.1. FTC**

The process impurities and degradation products of FTC have been qualified in animal studies. The major degradation product, <sup>†</sup>treated substance A, was qualified in 2 genotoxicity studies (m1.4.4, TOX151; TOX152) using a batch of FTC that contained 1% (w/w) of the <sup>†</sup>treated substance A degradant. Both studies

were negative for genotoxicity. In addition, there was no toxicity in a 28-day mouse study at doses (FTC/<sup>related substance A</sup>) of 50/1 mg/kg/day, 150/3 mg/kg/day, and 450/9 mg/kg/day (m1.4.4, TOX153).

A 28-day mouse bridging study (m1.4.4, TX-162-2001) was performed to qualify impurities in FTC (specifically \*related substance B). There was no toxicity of FTC at doses of 50, 150, and 450 mg/kg/day.

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

#### 4.8.3.2. TAF

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

Two 1-month impurity qualification studies were conducted in rats to evaluate potential drug substance impurities. Administration of 97.7% pure and 83.1% pure GS-7340-02 by oral gavage for 14 days to male rats was well tolerated at dose levels of 5 and 50 mg/kg/day (m1.4.4, TX-120-2008). No test article-related findings were noted, and no differences were found between the 2 lots tested. The NOAEL for both lots is 50 mg kg/day (40 mg f.b.e./kg/day).

The second impurity qualification study evaluated daily administration of GS-7340-03 via oral gavage to male and female rats for at least 28 days (m1.4.4, TX-120-2021). Three lots of GS-7340-03 were each administered at 25 and 50 mg/kg/day (free base equivalents). Test article 1 was 99.3% pure GS-7340-03. Test article 2 was 98% pure GS-7340-03 containing 11 spiked known and potential impurities. Test article 3 was 97.8% pure GS-7340-03 containing 4 spiked potential impurities. Control animals were administered the vehicle control article. Administration of GS-7340-03 drug substance with 3 different impurity profiles by oral gavage for 28 days to rats was well tolerated at dose levels of 25 and 50 mg/kg/day. There were no significant clinical or histopathological differences between the 3 lots tested. The NOAEL for all 3 lots is 50 mg f.b.e./kg/day.

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

#### 4.8.3.3. FTC/TDF

Four degradation products not present in the individual drug substances have been observed in FTC/TDF tablets placed on accelerated stability at high temperature. The trivial name for these degradation products are \*related substance C, \*related substance D, \*related substance E and \*related substance F. The adducts of FTC/TDF form when [REDACTED], formed by the [REDACTED] of TDF (\*related substance G), reacts with one molecule of each TDF and FTC to form an adduct. FTC may undergo [REDACTED] and may additionally [REDACTED] group of the [REDACTED], thereby creating \*related substance H. While \*related substance H has the potential to exist as 4 diastereomers, only 2 of these diastereomers have been observed in FTC/TDF containing products. The 2 observed diastereomers of \*related substance H are \*related substance E and \*related substance F.

Two 14-day GLP oral toxicity studies have been conducted in rats to qualify impurities and degradants in the FTC/TDF tablets (m1.4.4, TX-164-2001; TX-164-2005). The second qualification study (TX-164-2005) was conducted to verify the qualification of \*related substance E and \*related substance F as these degradants were identified later in development by virtue of a new analytical assay. In these studies, rats were administered formulations prepared from crushed tablets that were experimentally degraded by humidity and high temperatures or formulations prepared from crushed tablets that were not degraded. The doses were 0/0, 20/30, 67/100, and 200/300 mg/kg/day FTC/TDF in both studies. Although there were slight differences in the findings from both studies, there were no new toxicities or exacerbation of previously defined toxicities, and there was no difference in toxicity between non-degraded and degraded material. The NOAEL in the initial study (TX-164-2001) was considered to be 67/100 mg/kg/day, and 200/300 mg/kg/day FTC/TDF in the second study (TX-164-2005).

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

#### 4.8.3.4. F/TAF

The F/TAF FDC is a monolayer tablet. The degradation products of TAF observed in the F/TAF tablets are consistent with those in the TAF drug substance. There are no unique impurities or degradants in the F/TAF tablets.

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

## **4.9. Summary of Toxicology and Target Organ Effects**

### **4.9.1. Target Organ Effects**

#### **4.9.1.1. FTC**

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

#### **4.9.1.2. TAF**

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

##### **4.9.1.2.1. Kidney**

Renal tubular karyomegaly was observed in rats (m1.4.4, R990182, TOX-120-001) and dogs (m1.4.4, D990175, TOX-120-002) orally administered TAF. Focal areas of minimal renal cortical tubular basophilia and associated minimal nuclear karyomegaly were present in rats administered 400 mg/kg/day for 4 weeks and 100 mg/kg/day for 26 weeks. Renal tubular karyomegaly and/or basophilia were observed in dogs administered 3 and 10 mg/kg/day for 4 weeks and dogs administered 6 or 18/12 mg/kg/day for at least 13 weeks.

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

##### **4.9.1.3. Bone**

Atrophy of metaphyseal cancellous bone was observed in rats administered TAF at 100 mg/kg/day for 26 weeks. TAF also increased biochemical markers of bone turnover and decrease serum 1, 25-dihydroxyvitamin D<sub>3</sub> and 25-hydroxyvitamin D<sub>3</sub> in rats ( $\geq 25$  mg/kg/day) and dogs ( $\geq 37.5$  mg/kg/day for 6 days). In the 39-week dog study, bone mineral density changes at 18/12 mg/kg/day may have been secondary to body weight loss but these changes were accompanied by a slight but significant decrease in serum 1, 25-dihydroxyvitamin D<sub>3</sub> in males only and a significant increase in 25-hydroxyvitamin D<sub>3</sub> in females only.

#### 4.9.1.4. Other

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

TAF had no discernible electrocardiographic effect at the low dose of 2 mg/kg/day. There was some evidence at 6 and 18/12 mg/kg/day for an effect to slightly prolong PR intervals (~13-24%) which was associated with significant decreases in  $T_3$  {29101}, {29104}. After the 13-week recovery period, serum  $T_3$  values returned to levels similar to the control group animals at the end of the study. No PR prolongation or any change in ECG results occurred in the safety pharmacology study that evaluated a TAF dose up to 100 mg/kg (m1.4.4, D2000006) or in the thorough QT study (m1.4.4, GS-US-120-0107).

At 18/12 mg/kg/day in dogs, the highest dose tested, a minimal infiltration of histiocytes was present in some organs (eye (choroid plexus, ciliary body), lung, and spleen) in some animals. In-life ophthalmologic examinations were normal. The histiocytic infiltration observed in multiple organs was most likely an indirect drug effect due to general debilitation and was not observed in other repeat dose toxicity studies. There were no drug-related effects on ophthalmic exams or microscopic exams of ocular tissue observed in repeat dose toxicity studies in mice (up to 13 weeks), rats (up to 26 weeks), and nonhuman primates (4 weeks) or in the 4-week dog toxicology study. The minimal to slight infiltration of mononuclear cells in the ocular posterior uvea occurred only in dogs administered the highest dose of TAF where compound-related toxicities occurred on clinical condition, weight gain, serum chemistry, BMD, and other organ histopathology. Distribution of  $^{14}\text{C}$ -TAF to eyes has been assessed in mice, rats, and dogs (m1.4.4, AD-120-2011, AD-120-2020, and D990173-BP). Melanin binding has specifically been assessed by comparing distribution in pigmented and non-pigmented mice (C57 black and CD-1, respectively) and rats (Long Evans and Sprague-Dawley, respectively). [ $^{14}\text{C}$ ]-TAF-related radioactivity distributed poorly to the eyes of rats and dogs ( $C_{\text{max}}$  in eyes  $< 8\%$  that observed in plasma). Transient exposure to low levels of [ $^{14}\text{C}$ ]-TAF-related radioactivity was observed in the eyes of rats decreasing to undetectable levels at 8 hours postdose. No difference in distribution was observed between Sprague-Dawley and Long Evans rats, including in the skin and eyes, suggesting no binding to melanin. The distribution of [ $^{14}\text{C}$ ]-TAF to eyes in mice was higher than other species studied ( $C_{\text{max}}$  in eyes 15% -20 % that observed in plasma). More persistent exposures in eye lens, eye uveal tract, and eyes were observed in C57 black mice compared to CD-1 mice. However, no difference in distribution between pigmented and nonpigmented skin was observed illustrating that [ $^{14}\text{C}$ ]-TAF-related radioactivity was not selectively associated with melanin-containing tissues. The posterior uveitis in dogs administered the highest dose of TAF occurred at 3.7- and 17-fold higher exposure to TAF and TFV, respectively, than that observed in human subjects administered a 25-mg dose of TAF and does not correlate with the tissue distribution where TAF was found to poorly penetrate across the blood brain and blood retinal barrier in dogs. Because TAF has poor penetration across the blood brain and blood retinal barrier in dogs it is unlikely that TAF directly caused the observed histiocytic infiltration in the

posterior uvea. Based on the evidence from tissue distribution and toxicology studies, Gilead concludes that the risk of posterior uveitis in humans is very low.

TAF is unlikely to cause mitochondrial toxicity. TAF did not affect the amount of mtDNA levels up to 1  $\mu\text{M}$  (approximately 2-fold higher than  $C_{\text{max}}$  after a 25 mg TAF dose), the highest concentration tested, in HepG2 cells in a 10-day assay (m1.4.4, PC-120-2006). The active metabolite of TAF, tenofovir diphosphate is highly discriminated as a substrate by mitochondrial DNA polymerase  $\gamma$  relative to the natural substrate, ATP ( $> 10,000$  fold) {4923}. No toxicity indicative of mitochondrial toxicity was observed in nonclinical or clinical studies. Therefore, TAF is unlikely to inhibit mitochondrial DNA polymerase  $\gamma$  under clinical relevant conditions.

#### 4.9.1.5. F/TAF

Administration of TAF in combination with FTC is unlikely to exacerbate known toxicities. Emtricitabine and TAF exhibit different patterns of target organ toxicity. The only toxicity observed in chronic animal studies with FTC was mild, reversible anemia in mice and minor decreases in erythrocyte counts/increases in mean corpuscular hemoglobin in monkeys at large multiples of clinical exposure (137-fold in mice; 20-fold in monkeys); therefore, these hematological findings are not considered relevant to clinical use.

Extensive nonclinical investigations of the toxicity of TAF have shown that unlike FTC, the bone marrow is not a target for this agent, and that the target organs for TAF are distinctly different (bone and kidney). Emtricitabine has not shown any potential for bone toxicity; thus, exacerbation of any TAF effects on bone is not expected. As pathological changes in the kidney have not been observed with FTC, exaggerated renal toxicity is not anticipated to be an issue with the F/TAF FDC product.

From in vitro data and clinical experience (m2.7.2), the anticipated drug-drug interaction upon administration of the 2-drug combination is the inhibition of P-gp-mediated intestinal secretion by TAF which increases the bioavailability of TAF. The inhibition of P-gp has minimal impact because of the comparatively low plasma concentrations of TAF and TFV. The two available dosage strengths of TAF in the FTC/TAF FDC allows dose modification if the concomitant ARV product markedly inhibits P-gp.

### 4.9.2. Safety Margins

Safety margins for FTC are calculated based on population PK derived from E/C/F/TDF clinical studies. In the clinical study (GS-US-311-1473) that demonstrated that F/TAF (200/25 mg) was bioequivalent to E/C/F/TAF (100/100/200/10 mg), FTC and TAF were measured in the plasma; TFV was not measured. Because TFV is a more relevant analyte for nonclinical comparisons, the TAF and TFV margins presented below are based on E/C/F/TAF (100/100/200/10mg) Phase 3 clinical data.

#### 4.9.2.1. FTC

Cross-species comparisons of FTC exposure (expressed based on  $\text{AUC}_{\text{ss}}$  levels) for the major target organs are shown in Table 4.



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

**Table 4. Estimated Safety Margins of Emtricitabine Based on AUC<sub>ss</sub> When Comparing Animal No-Effect-Level (NOEL)**

Target Organ Effect	Species	Study Duration	NOEL (mg/kg/day)	AUC <sub>ss</sub> (µg·h/mL) NOEL	Margin Relative to Human AUC <sub>ss</sub>
			<b>FTC</b>		
Anemia	Mouse	6 months	500	350	27X
	Rat	3 months	600	346	27X
	Monkey	1 year	200	98	10 X

Human AUC<sub>tau</sub> (13 µg·h/mL) following a 200 mg/day dose of FTC (m2.7.2, Table 53).

4.9.2.2. TAF

Cross-species comparisons of exposure (expressed based on AUC<sub>ss</sub> levels) for the major target organs are shown in Table 4.

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

**Table 5. Estimated Safety Margins of TAF Based on AUC<sub>ss</sub> When Comparing Animal No-Adverse-Effect-Level (NOAEL)**

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

NA = not applicable; NC = insufficient data to calculate

- a Predicted safety margin for TFV human exposure is based on pooled PK data from E/C/F/TAF Phase 3 pivotal studies GS-US-292-104 and GS-US-292-111 where the mean TFV AUC<sub>ss</sub> = 0.293 µg.h/mL; m2.7.2, Section 3.2.1.2.1
- b Predicted safety margin for TAF human exposure is based on pooled PK data from E/C/F/TAF Phase 3 pivotal studies GS-US-292-104 and GS-US-292-111 where the mean TAF AUC<sub>ss</sub> = 0.206 µg.h/mL; m2.7.2, Section 3.2.1.2.1
- c NOAEL for reproductive endpoints provided; AUC data is for maternal exposure; the peri/postnatal study was conducted with TDF not TAF

## 5. INTEGRATED OVERVIEW AND CONCLUSIONS

### 5.1. Correlation of Nonclinical and Clinical Findings

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

### 5.2. Justification for Text in Labeling

The proposed Prescribing Information for the F/TAF FDC includes all relevant nonclinical safety findings.

Based on findings in the nonclinical studies, the key safety points for consideration that are related to FTC or TAF include: (1) use in patients with severe hepatic impairment, (2) use during pregnancy and lactation, and (3) potential for carcinogenicity.

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

1. The potential for hepatotoxicity appears to be low. Emtricitabine and TFV are not metabolized, do not interact significantly with P450 enzymes and are not excreted to any significant extent by the liver. In addition, there was no substantive hepatotoxicity identified in the nonclinical studies with FTC and TAF. Administration of 25 mg TAF to non-HIV infected subjects with mild or moderate hepatic impairment resulted in TAF and TFV plasma PK exposure parameters comparable to those in subjects with normal hepatic function (m2.5 and m2.7.2).
2. Animal data indicate that FTC and TAF do not cause reproductive or embryo-fetal toxicity. Emtricitabine has been shown to cross the placenta and the ratio of FTC concentrations in plasma in pregnant mice and rabbits as compared to their fetuses was approximately 0.4. It is unknown if TAF is secreted in rat milk.
3. In long-term carcinogenicity studies of FTC, no drug-related increases in tumor incidence were found in mice or in rats. Tenofovir DF was negative in the rat carcinogenicity assay, but weakly positive at the highest dose in the mouse carcinogenicity assay (liver adenomas) at exposures 10 times those in humans. While the mechanism of this tumor formation is uncertain, the findings are unlikely to be of relevance to humans. Appropriate information regarding the results of the carcinogenicity studies is included in the 'Nonclinical Toxicology' section of the proposed Prescribing Information.

In addition to the items addressed above, which are product specific, other appropriate warnings have been included in the proposed Prescribing Information. The toxicities of potential concern outlined above are adequately highlighted and addressed in the current Prescribing Information for the individual agents and the proposed Prescribing Information for the combination tablet. The proposed dose of the combination tablet for administration to HIV-1 infected patients  $\geq 12$  years old is justified from a safety perspective based on the nonclinical data presented in this dossier.

### 5.3. Overall Conclusions

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

The pharmacokinetic and toxicologic profiles of FTC, TAF, and TFV are well characterized in multiple animal species and the findings are pertinent in consideration of the use of these agents in combination. Adverse pharmacokinetic interactions that would negatively affect safety or pharmacological efficacy are not anticipated. This is based on the well-characterized routes of elimination demonstrated for each compound and the differences in physicochemical properties between the compounds, which influence drug distribution. Emtricitabine and TAF are analogs of 2 different nucleosides, cytidine and adenosine, respectively, and do not share a common intracellular metabolism pathway for pharmacological activation through phosphorylation. In conclusion, the drug interactions between FTC and TAF are unlikely. Data from clinical studies of the E/C/F/TAF FDC demonstrated acceptable tolerability and safety profiles to support use in patients  $\geq 12$  years old.

The overall program including the data from the combination and individual agent studies is considered adequate to support the efficacy and safety of F/TAF FDC tablet based on the following considerations. Emtricitabine has antiviral activity against HIV-1, HIV-2, and HBV and has demonstrated additive to synergistic activity with a variety of other ARV drugs. TAF exhibits potent antiviral activity and limited cytotoxicity in target cell lines and PBMCs. TFV demonstrated additive to synergistic activity with a variety of other ARV drugs in vitro.

The F/TAF FDC is not anticipated to produce any new human metabolites. Because significant pharmacokinetic interactions are unlikely and that the target organ profiles are different, administration of the combination product is unlikely to exacerbate known toxicities of the individual agents. The availability of two F/TAF FDC with 10 or 25 mg TAF will allow adjustment of the TAF dose if administered with P-gp inhibitors such as COBI and RTV, which increase TAF exposure

The toxicity profiles of FTC and TAF differ substantially with no clinically significant overlapping toxicity. The only toxicity observed in chronic animal studies with FTC was mild, reversible anemia at large multiples of clinical exposure; therefore, these hematological findings are not considered relevant to clinical use. Emtricitabine has an established clinical safety profile with no significant toxicities observed. The principal target organs of toxicity in animals following oral administration of TAF were the kidney (karyomegaly, tubular degeneration) and bone.

Emtricitabine has not shown any potential for bone toxicity in chronic rat and dog toxicity studies; thus, exacerbation of any TAF effects on bone is not expected. Neither FTC nor TAF had positive findings in genotoxicity studies; the F/TAF FDC is not anticipated to alter the genotoxicity profiles of the individual agents. Emtricitabine and TDF/TFV have demonstrated low carcinogenic potential in conventional 2-year bioassays. Combination dosing would not be expected to change these profiles, and no exacerbation of toxicity is expected. Emtricitabine and TAF have not shown significant adverse effects in reproductive and developmental toxicity

studies, and the combination of FTC and TAF is not expected to have an altered reproductive toxicity profile compared with that of the individual agents.

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

The absence of nonclinical safety studies with the F/TAF combination is in accordance with the FDA Guidance for Industry, Nonclinical Safety Evaluation of Drug or Biologic Combinations, March 2006 and the CHMP Guideline on the Non-Clinical Development of Fixed Combinations of Medicinal Products (EMA/CHMP/SWP/258498/2005, January 2008). There are no anticipated clinically relevant pharmacokinetic or toxicological interactions expected in the F/TAF FDC. Because the target organ profiles are different, and there is no evidence of genotoxicity, carcinogenicity, or reproductive toxicity, administration of the 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 strongly indicate further toxicological investigations are unlikely to yield new data relevant to humans. Additionally, the clinical safety data available from the approved FTC/TDF regimens and with the E/C/F/TAF FDC supports the safety of the F/TAF FDC in combination with other ARV products for the treatment of HIV-1 infection in adult and adolescent patients 12 years of age and older.

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