2.6 NONCLINICAL SUMMARY

SECTION 2.6.4—PHARMACOKINETICS WRITTEN SUMMARY

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

Gilead Sciences

[redacted] **20**[redacted]

TABLE OF CONTENTS

SECTION 2.6.4—PHARMACOKINETICS WRITTEN SUMMARY 1
TABLE OF CONTENTS 2
LIST OF IN-TEXT TABLES 4
LIST OF IN-TEXT FIGURES 4
LIST OF ABBREVIATIONS 5
NOTE TO REVIEWER 9
1. BRIEF SUMMARY 12
2. METHODS OF ANALYSIS 16
2.1. TAF 16
2.1.1. Bioanalytical Methods Supporting Pharmacokinetic Studies 16
2.1.2. Bioanalytical Methods Supporting GLP Studies 16
2.1.3. Other In Vivo Bioanalytical Methods 17
2.1.4. In vitro methods 17
2.2. E/C/F/TAF 20
2.2.1. In Vitro Transporter Methods 20
3. ABSORPTION 21
3.1. In Vitro Absorption Studies 21
3.1.1. TAF 21
3.2. Single-Dose In Vivo Studies 21
3.2.1. TAF: Mouse, Oral 21
3.2.2. TAF: Rat, Oral 22
3.2.3. TAF: Dog, Oral, and IV 23
3.2.4. TAF: Monkey, Oral 24
3.3. Repeat-Dose In Vivo Studies 25
3.3.1. TAF: Mouse 25
3.3.2. TAF: Rat 26
3.3.3. TAF: Dog 26
3.3.4. TAF: Monkey 27
3.4. E/C/F/TAF: Absorption 27
4. DISTRIBUTION 28
4.1. Plasma Protein Binding 28
4.1.1. TAF and TFV 28
4.2. Tissue Distribution Studies 28
4.2.1. TAF: Mouse 28
4.2.2. TAF: Rat 34
4.2.3. TAF: Dog 40
4.3. Studies in Pregnant or Nursing Animals 40
4.3.1. TAF: Pregnant Rats 40
4.3.2. TAF: Pregnant Rabbits 40
4.3.3. TFV: Pregnant Monkeys 41
4.3.4. TFV: Lactating Monkeys 41
4.3.5. TFV: Immature monkeys 41
4.4. E/C/F/TAF: Distribution 41
5. METABOLISM 43
5.1. Proposed Metabolic Pathways 43

5.1.1. TAF 43
5.2. Metabolism In Vitro 45
5.2.1. TAF and TFV: Metabolism In Vitro 45
5.3. Metabolism In Vivo 46
5.3.1. TAF and TFV: Metabolism In Vivo 46
5.4. E/C/F/TAF: Metabolism 48

6. EXCRETION 50

6.1. Route and Extent of Excretion 50
6.1.1. TAF/TFV 50
6.2. Excretion into Bile 51
6.2.1. TAF and TFV: Excretion into Rat and Dog Bile 51
6.3. Excretion into Milk 52
6.3.1. TFV: Monkeys 52
6.4. E/C/F/TAF: Excretion 52

7. PHARMACOKINETIC DRUG INTERACTIONS 53

7.1. TAF and TFV: Pharmacokinetic Drug Interactions 53
7.1.1. TAF and TFV: Cytochrome P450 and UGT1A1 Inhibition 53
7.1.2. TAF: Enzymology of Metabolism 54
7.1.3. TAF: Assessment of Induction Liability 55
7.1.4. Potential for Transporter-Mediated Drug Interactions with TAF and TFV 56
7.2. E/C/F/TAF: Pharmacokinetic Drug Interactions 57

8. OTHER PHARMACOKINETIC STUDIES 62

9. DISCUSSION AND CONCLUSIONS 63

9.1. TAF 63
9.2. E/C/F/TAF 64

10. REFERENCES 66

LIST OF IN-TEXT TABLES

Table 1. Names and Structures of TFV, TAF, and Related Compounds....10
Table 2. Dose-Dependent Bidirectional Permeability of TAF in Caco-2 Cells....21
Table 3. Plasma Pharmacokinetic Parameters Following a Single Dose of GS-7340-02 and GS-7340-03 to Male CD-1 Mice....22
Table 4. Plasma Pharmacokinetic Parameters Following a Single Dose of GS-7340-03 to 001178-W Wild Type Mice....22
Table 5. Plasma Pharmacokinetic Parameters following a Single Dose of GS-7340-02 and GS-7340-03 to Male Sprague-Dawley Rats....23
Table 6. Plasma Pharmacokinetic Parameters for TAF And TFV Following a Single Dose of GS-7340-02 to Rhesus Monkeys....25
Table 7. Concentrations of TFV in PBMCs from Monkeys Dosed with GS-7340-02....25
Table 8. Comparative Tissue Concentrations of Radioactivity in Male CD-1 and C57 Black Mice After Oral Administration of [^{14}C]TAF (n = 1 per time point)....30
Table 9. Comparative Tissue Concentrations of Radioactivity in Male Sprague-Dawley and Long Evans Rats After Oral Administration of [^{14}C]TAF (n = 1 per time point)....35
Table 10. Stability of TAF in Biological Matricies from Dog and Human....45
Table 11. Relative quantification of TAF Metacolites in Plasma, Urine, Feces, and Bile as % Total Dose....47
Table 12. Steady State Pharmacokinetic Parameters for TAF and TFV....53
Table 13. Stability of TAF in Human Intestinal Subcellular Fraction in the Absence and Presence of Test Compounds....54
Table 14. Effect of TAF Treatment on CYP mRNA Levels and Activity in Cultured Human Hepatocytes (mean, n = 3 donors)....55
Table 15. Mean Plasma Pharmacokinetic Parameters for TAF and TFV Following Oral Administration of TAF to Male Beagle Dogs....56
Table 16. Transporter Substrate Assessment of E/C/F/TAF Components....59
Table 17. Transporter Inhibition Assessment of E/C/F/TAF Components....60
Table 18. Effect of COBI on the Bidirectional Permeability of TAF in Caco-2 Cells....61
Table 19. Comparison of TFV Noncompartmental Pharmacokinetic Parameters Between Species Following Intravenous Administration of TFV....65

LIST OF IN-TEXT FIGURES

Figure 1. Plasma and Liver Pharmacokinetic Following a Single Dose of TAF to Beagle Dogs....24
Figure 2. Annotated Whole-Body Autoradiograph for a Male Sprague-Dawley Rat 0.25 hours after a Single Oral Administration of ^{14}C-GS-7340 at 5 mg/kg....38
Figure 3. Annotated Whole-body Autoradiograph for a Male Long Evans Rat 0.25 hours after a Single Oral Administration of ^{14}C-GS-7340 at 5 mg/kg....39
Figure 4. Metabolites of TAF....44
Figure 5. Cumulative Percent Total Radioactive Dose Recovered in Urine, Feces, Bile, and Cage Rinse Following Oral Administration of [^{14}C]TAF at 5 mg/kg to Bile Duct-Cannulated Dogs....52

LIST OF ABBREVIATIONS

AhR	aryl hydrocarbon receptor (AHR gene product)
ATV	atazanavir (Reyataz®, Bristol-Myers Squibb)
BCRP	breast cancer resistance protein (ABCG2)
BDC	bile duct-cannulated
BNPP	bis-p-nitrophenyl phosphate
BSEP	bile salt export pump
Caco-2	human colonic adenocarcinoma cell line
CatA	cathepsin A
CEM-R1	human T-cell leukemic lymphoblast cells line
CES1	carboxylesterase 1
CHO	Chinese hamster ovary cell line
COBI; C	cobicistat (GS-9350), Tybost®, Gilead
CsA	cyclosporine A
CYP	cytochrome(s) P450
DMSO	dimethyl sulfoxide
DRE	drug/dioxin response element
DRV	darunavir (Prezista™, Tibotec)
E/C/F/TAF	elvitegravir/cobicistat/emtricitabine/tenofovir alafenamide (coformulated)
EVG; E	elvitegravir (GS-9137; JTK-303), Vitekta®, Gilead
EVG/COBI/FTC/TDF	elvitegravir/cobicistat/ emtricitabine/tenofovir disoproxil fumarate (coformulated; Stribild®, Gilead)
FDC	fixed-dose combination
FTC; F	emtricitabine (Emtriva®, Gilead)
GD	gestation day
GI	gastrointestinal
Gilead	Gilead Sciences, Inc.
GLP	good laboratory practice
GS-7340	tenofovir alafenamide (TAF) free base
GS-7340-02	tenofovir alafenamide (TAF) monofumarate
GS-7340-03	tenofovir alafenamide (TAF) hemifumarate
GS-9202	EVG metabolite M1 (JTP-71081; hydroxylation of the chlorofluorophenyl group)
HIV, HIV-1	human immunodeficiency virus, type 1
HPLC	high performance liquid chromatography
IC_{50}	concentration required to produce 50% inhibition
INSTI	integrase strand transfer inhibitor
IV	intravenous
LC	liquid chromatography
LC/MS/MS	high performance liquid chromatography coupled to tandem mass spectrometry

LIST OF ABBREVIATIONS (CONTINUED)

LLOQ	lower limit of quantification
LPV	lopinavir (Aluviran®, Abbott)
LSC	liquid scintillation counting
MATE1	multidrug and toxin extrusion protein 1 (SLC47A1)
MATE2-K	multidrug and toxin extrusion protein 2-K (SLC47A2)
MDCK II	Madin-Darby canine kidney cell line
MDR1	P-glycoprotein (P-gp, ABCB1 gene product)
mRNA	messenger ribonucleic acid
MRP1	multidrug resistance-associated protein-1 (ABCC1)
MRP2	multidrug resistance-associated protein-2 (ABCC2, cMOAT)
MRP4	multidrug resistance-associated protein-4 (ABCC4)
MS	mass spectrometry
MTT	3-[4,5-Dimethyl-2-thiazolyl]-2,5-diphenyl-2H-tetrazolium bromide (assay)
NADPH	β-nicotinamide adenine dinucleotide phosphate (reduced form)
ND	not detectable / not determined
NDA	new drug application
NRTI	nucleoside reverse transcriptase inhibitor
NtRTI	nucleotide reverse transcriptase inhibitor
OAT1	organic anion transporter 1 (SLC22A6)
OAT3	organic anion transporter 3 (SLC22A8)
OATP	organic anion transporting polypeptide (SLCO or SLC22A gene products)
OATP1B1	organic anion transporting polypeptide 1B1 (SLCO1B1)
OATP1B3	organic anion transporting polypeptide 1B3 (SLCO1B3)
OCT2	organic cation transporter 2 (SLC22A2)
OCTN1	organic cation transporter novel, type 1 (SLC22A4)
P_{app}	apparent permeability
PBMC	peripheral blood mononuclear cell
P-gp	P-glycoprotein (also see MDR1)
PI	protease inhibitor
PXR	pregnane X receptor (SXR, NR1I2 gene product)
RBC	red blood cell
RFD	radio flow-through detector
RTV	ritonavir (Norvir®, Abbott)
S9	postmitochondrial (9,000 x g) supernatant
SD	standard deviation
STB	elvitegravir/cobicistat/ emtricitabine/tenofovir disoproxil fumarate (coformulated; Stribild)
TAF	tenofovir alafenamide, formerly GS-7340

LIST OF ABBREVIATIONS (CONTINUED)

TDF	tenofovir disoproxil fumarate (Viread®, Gilead)
TFV	tenofovir
TFV-MP	tenofovir monophosphate
TFV-DP	tenofovir diphosphate
UDPGA	uridine diphosphoglucuronic acid
UGT	uridine diphosphate glucuronosyl transferase
US	United States

PHARMACOKINETIC ABBREVIATIONS

AUC	area under the plasma concentration versus time curve
$AUC_{0-\infty}$	area under the plasma concentration versus time curve extrapolated to infinite time, calculated as $AUC_{0-last} + (C_{last}/\lambda_z)$
AUC_{x-xx}	partial area under the plasma concentration versus time curve from time “x” to time “xx” (default units are hours)
CL	systemic clearance of the drug after intravenous administration
CL/F	apparent clearance after oral administration of the drug
C_{last}	last observed quantifiable concentration of the drug in plasma
C_{max}	maximum observed concentration of drug in plasma
C_x	plasma concentration at time "x" (default units are hours)
F	estimated oral bioavailability of the drug (%)
λ_z	elimination rate determined from the terminal phase of the plasma concentration versus time curve
MAT	mean absorption time
MRT	mean residence time
$t_{½}$	half-life
t_{last}	time (observed time point) of C_{last}
t_{max}	time (observed time point) of C_{max}
V_{ss}	apparent steady-state volume of distribution of the drug
V_{ss}/F	apparent steady-state volume of distribution of the drug after oral administration

NOTE TO REVIEWER

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

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

[redacted]. To assist the reviewer, [redacted]

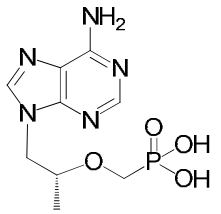
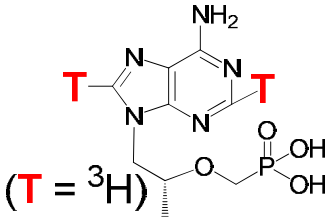
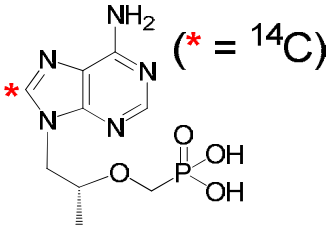
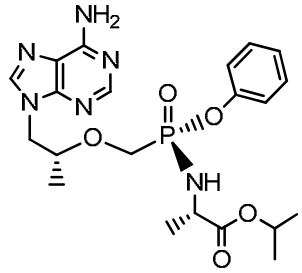
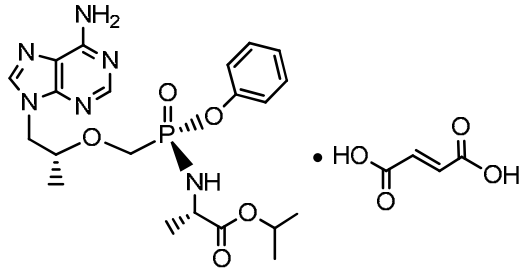
Given the lack of remarkable effects in the nonclinical studies with the individual agents, no additional pharmacokinetic studies have been conducted for the E/C/F/TAF combination.

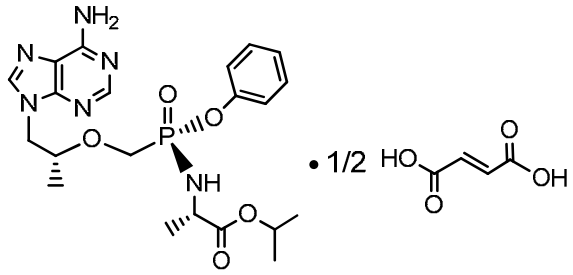
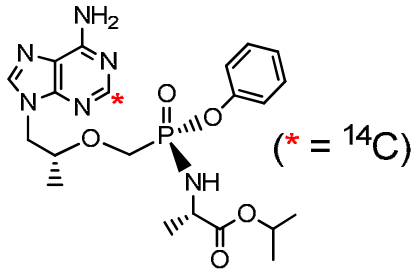
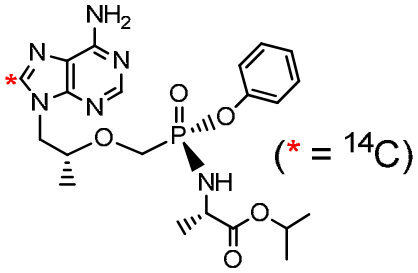
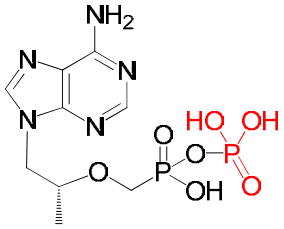
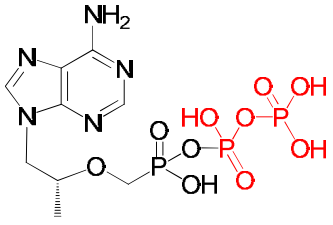
TAF

Structures of TFV, TAF, and related compounds are illustrated in Table 1. The following conversions are also provided to aid the reviewer:

1 µM TFV (GS-1278) = 0.287 µg/mL
1 ng/mL TFV = 3.48 nM
1 µM TAF (GS-7340) = 0.477 µg/mL
1 ng/mL TAF = 2.10 nM

Table 1. Names and Structures of TFV, TAF, and Related Compounds

Name	Alternative Names	Identity	Structure
TFV	Tenofovir, GS-1278, R-PMPA	Parent Compound	
[^{3}H]TFV	[Adenine-2,8-^{3}H]TFV	Radiolabeled Parent	
[^{14}C]TFV	[Adenine-8-^{14}C]TFV	Radiolabeled Parent	
TAF (free base)		Parent Compound, free base	
GS-7340-02		Parent Compound, monofumarate	

Name	Alternative Names	Identity	Structure
GS-7340-03		Parent Compound, hemifumarate	
[^{14}C]TAF	[Adenine-2-^{14}C]TAF	Radiolabeled Parent	
	[Adenine-8-^{14}C]TAF	Radiolabeled Parent	
GS-342031	GS-77389, PMPAp, TFV-MP	Monophosphorylated Anabolite of Parent	
GS-077635	PMPApp, TFV-DP	Diphosphorylated Anabolite of Parent	

1. BRIEF SUMMARY

This dossier is being submitted in support of a new drug application for a fixed-dose combination (FDC) that contains elvitegravir (EVG; E [Vitekta®]), cobicistat (COBI; C [Tybost®]), emtricitabine (FTC; F [Emtriva®]), and tenofovir alafenamide (TAF, formerly GS-7340) for use once daily for the treatment of human immunodeficiency virus-1 (HIV-1) infection in patients 12 years of age and older: the E/C/F/TAF FDC (150/150/200/10 mg).

Comprehensive studies have been completed characterizing the absorption, distribution, metabolism, and excretion profiles of EVG, COBI, FTC, TAF (and/or tenofovir [TFV]/tenofovir disoproxil fumarate [TDF]). In addition, the potential of each agent to be involved in pharmacokinetic drug-drug interactions has been characterized.

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

EVG

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

A comprehensive program of studies has been conducted to characterize the nonclinical drug absorption and disposition profile for EVG. The results of this evaluation were presented in detail in the original new drug application (NDA) and subsequent submissions for Vitekta and Stribild.

COBI

Cobicistat is a mechanism-based cytochrome P450 (CYP) 3A inhibitor (a pharmacokinetic enhancer) that increases the systemic levels of coadministered agents metabolized by CYP3A enzymes, including EVG and the HIV PIs atazanavir (ATV) and darunavir (DRV). Tybost (COBI 150 mg tablets), developed by Gilead, is approved in the US as a pharmacokinetic enhancer of ATV 300 mg once daily or DRV 800 mg once daily as part of antiretroviral combination therapy in adults with HIV-1 infection. Cobicistat is also a component of Stribild.

A comprehensive program of studies has been conducted to characterize the nonclinical drug absorption and disposition profile for COBI. The results of this evaluation were presented in detail in the original NDA and subsequent submissions for Tybost and Stribild.

FTC

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

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

TAF and TFV

Tenofovir alafenamide is an investigational prodrug of TFV, a nucleotide reverse transcriptase inhibitor (NtRTI). Tenofovir alafenamide is more stable in plasma than TDF, allowing for it to circulate for a longer period of time and interact with target cells. Tenofovir alafenamide provides enhanced delivery of TFV to the liver and HIV-target cells and tissues, resulting in higher intracellular levels of the active phosphorylated metabolite TFV-diphosphate (TFV-DP). In lymphoid cells and tissues, TAF is predominantly hydrolyzed intracellularly to TFV by cathepsin A (CatA) {13119}. Once released intracellularly, TFV is metabolized to the active metabolite, TFV-DP {1574}, a competitive inhibitor of HIV-1 reverse transcriptase that terminates the elongation of the viral DNA chain {21}, {1131}. Combined, this results in higher intracellular levels of TFV-DP and lower circulating levels of TFV relative to TDF {17137}.

Nonclinical studies to characterize the absorption and disposition of TAF have been performed in mice, rats, dogs, and monkeys. The pharmacokinetic studies are listed in the overview table (m2.6.5, Section 1), and study details are given in the individual study overview tables in m2.6.5. The tabulated summaries in m2.6.5 also provide toxicokinetic data for studies described in m2.6.6. For the pharmacokinetic studies using GS-7304-02 or GS-7340-03, all doses are presented as TAF free base equivalent.

Tenofovir alafenamide was rapidly absorbed with t_{max} values of 0.08 hours in mouse and dog and 0.5 hours in monkey (the first time point assessed). Tenofovir alafenamide was undetectable in any of the rat plasma samples. Thereafter, TAF plasma concentrations declined rapidly with a terminal half-life of less than 1 hour. Concomitant with the rapid decline in TAF concentrations in plasma, the predominant metabolite TFV was formed and persisted in plasma over the dosing interval.

Protein binding of TAF was moderate in human plasma with the percent unbound of 46.8% in vitro which was higher than values observed in multiple human ex vivo studies where the mean percent unbound of TAF ranged from 14% to 23% in all subjects. Following oral administration of [^{14}C] TAF to mouse, rat, and dog, [^{14}C]TAF-derived radioactivity was widely distributed to most of the tissues in all species studied. Consistent with high hepatic extraction, high levels of radioactivity were observed in the liver. High levels of radioactivity were also measured in the kidney. Low levels of radioactivity were observed in brain and testis in mouse. No evidence for melanin binding was observed in rats. Distribution trends in the pigmented uveal tract of the eye

and pigmented skin suggested that [^{14}C]TAF-related radioactivity was not selectively associated with melanin-containing tissues in the pigmented mouse.

Metabolite profiling of TAF in mice, rats, dogs and humans demonstrated formation of endogenous purine metabolites including hypoxanthine, xanthine, allantoin, and uric acid in all species including humans. 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. No metabolites unique to human were observed. TAF is not an inhibitor or an inducer of most CYP enzymes and uridine diphosphate glucuronosyl transferase (UGT) 1A1. However, it is a weak inhibitor of CYP3A. At clinically relevant concentrations, TAF is unlikely to affect hepatic CYP3A activity. While high levels of TAF can be achieved in the intestine, local exposure should be transient and the potential for significant drug interaction associated with intestinal CYP3A is low. Tenofovir alafenamide was not an inhibitor of any of the transporters tested in vitro indicating that TAF is unlikely to be a perpetrator of transporter-mediated drug interactions. Tenofovir alafenamide was found to be a substrate for efflux transporters P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP) and hepatic uptake transporters organic anion transporting polypeptide (OATP) 1B1 and OATP1B3. A modest increase in TAF absorption has been observed upon inhibition of the intestinal efflux transporters in vitro and in vivo. Exposure to TAF may be affected by inhibitors of OATP1B1 and OATP1B3 or by genetic polymorphisms affecting their transport activities. The effects of differences in OATP1B1 and OATP1B3 activity are, however, not expected to be clinically relevant given the high passive permeability of TAF.

Following oral dosing of mice, rats, and dogs with [^{14}C]TAF, the majority of radiolabel is recovered in the feces or urine in all species. The elimination of a large amount of radioactivity in bile of bile duct-cannulated (BDC) dogs indicates that biliary excretion is a major route of elimination of [^{14}C]TAF-derived radioactivity in dogs. Total recovery of radiolabel was high for all species. Renal excretion was identified as the primary route of elimination of TFV in all species tested, and is achieved by a combination of glomerular filtration and active tubular secretion. In vitro transport studies indicate that the active tubular secretion of TFV in humans is mediated by organic anion transporter (OAT) 1 and multidrug resistance-associated protein (MRP) 4 acting in series, as the major uptake and efflux transporters in proximal tubules, respectively. Human OAT3 may play a secondary role in the tubular uptake of TFV. Neither P-gp nor MRP1 or MRP2 are involved in the tubular efflux of TFV. As the primary transporter for the tubular uptake of TFV, OAT1 has been assessed for its potential as a target for drug-drug interactions between TFV and other renally secreted therapeutics including antibiotics, anti-inflammatory agents, and other antivirals (including COBI and PIs). Under physiologically relevant conditions, a number of renally excreted drugs showed no effect in vitro on the OAT1-mediated transport of TFV. Similarly, PIs and COBI did not exhibit any effect on the cellular elimination of TFV mediated by the MRP4 efflux pump in vitro, indicating that PIs and COBI are unlikely to exert any substantial effect on the accumulation of TFV in renal proximal tubules or renal elimination of TFV. Tenofovir did not inhibit the activity of the renal uptake transporter, organic cation transporter (OCT2), or the renal efflux transporter, multidrug and toxin extrusion protein 1 (MATE1).

E/C/F/TAF

The intended, positive pharmacokinetic interaction within the 4-drug combination causes an increase in the bioavailability and a decrease in the rate of elimination of EVG due to inhibition of CYP3A activity by COBI, and a consequent profound reduction in the formation of M1 (GS-9202), the major oxidative metabolite of EVG. This interaction has been well characterized in vitro. Animal models are inappropriate to investigate this interaction due to the lack of mechanism-based inhibition by COBI in nonhuman species. Based upon the differences in routes of elimination, EVG and COBI are unlikely to affect the pharmacokinetic profiles of FTC or TFV adversely. The other drug interactions among the 4 components includes the inhibition of intestinal efflux of TAF by COBI and the potential for interaction due to inhibition of OATP-mediated hepatic uptake of TAF by COBI and EVG. The increase in TAF exposure due to inhibition of intestinal efflux by COBI has been taken into account during the TAF clinical dose selection for E/C/F/TAF FDC. The effect of OATP inhibition by COBI and EVG on TAF exposure is unlikely to be clinically significant as only a modest increase in exposure (not considered clinically relevant) of the OATP substrate, rosuvastatin, was observed when it was codosed with both EVG and COBI.

2. METHODS OF ANALYSIS

2.1. TAF

The in vivo pharmacokinetic, toxicokinetics, distribution, and excretion of TAF were assessed in mouse, rat, dog, and monkey. The in vitro absorption, metabolism, and drug interaction characteristics of TAF were studied in appropriate model systems.

2.1.1. Bioanalytical Methods Supporting Pharmacokinetic Studies

In the early preclinical absorption studies TFV levels in rat plasma and TAF and TFV levels in dog plasma and peripheral blood mononuclear cells (PBMCs) were determined using a fluorescence derivitization/high performance liquid chromatography (HPLC) procedure (m2.6.5, Section 3, R990130; 99-DDM-1278-001-PK). Analyses of TAF and TFV in plasma and PBMCs during pharmacokinetic studies following single or multiple oral administration to rats (m2.6.5, Section 3, R2000065; AD-120-2015), mice (m2.6.5, Section 3, AD-120-2014; AD-120-2016; m2.6.7, Section 6, TX-120-2006), dogs (m2.6.5, Section 3.8, AD-120-2034 and m2.6.5, Section 14.2.8, AD-120-2035), and monkeys (m2.6.5, Section 3.9, P2000087) were performed using HPLC coupled to tandem mass spectrometry (LC/MS/MS) as described in the study reports or in the bioanalytical method reports (m2.6.5, Section 2, BA-120-2003; BA-120-2004; BA-120-2010; BA-120-2011;, BA-120-2012; BA-120-2013). Some of these methods did not strictly conform to Good Laboratory Practice (GLP) guidelines but were evaluated for appropriate selectivity, sensitivity, linearity, as well as intra-assay accuracy and precision.

2.1.2. Bioanalytical Methods Supporting GLP Studies

Validated methods of HPLC with MS detection were used for the earlier rat toxicokinetic studies (m2.6.5, Section 2, 001092/NGE; R-BA-Tox-120-001). Plasma concentrations of TAF and TFV were quantified in toxicology studies in pregnant mouse, rat, and rabbit using validated LC/MS/MS methods (m2.6.5, Section 2, BA-120-2003; BA-120-2004; BA-120-2005). In a 28-day toxicology study in dog, the plasma TAF concentrations were determined using a validated LC/MS/MS assay with a limit of quantification of 5 ng/mL and the concentrations of TFV in plasma and in PBMCs were determined using fluorescence derivitization followed by HPLC with fluorescence detection (m1.4.4, P1278-0017) and HPLC with MS detection (m2.6.5, Section 2, 993680 MYS), respectively. Analytical methods for determination of TAF in plasma and TFV in plasma and PBMCs from a 9-month dog toxicology study (m2.6.5, Section 2, TOX-120-002) are described within the appendices of this study report.

In a 28-day repeat dose study in monkeys, validated methods were used to determine TAF and TFV in plasma (m2.6.5, Section 2, 010520/PDW; 010521/PHZ) and TFV in PBMCs (m2.6.5, Section 2, AA01240-RQZ).

Two assay techniques were used to determine plasma TFV concentrations. The first was a reverse phase ion-pair HPLC method following fluorescence derivatization (m1.4.4, P4331-00008 [mouse]; P1278-00001 [rat, monkey]; P1278-00017 [dog]). This assay was validated with a lower limit of quantification (LLOQ) of 25 ng/mL, and was used in earlier

studies. The second assay utilizes LC/MS/MS and was validated with LLOQs of 1–3 ng/mL (m1.4.4, P1278-00028 [rat]; P1278-00029 [monkey]; P4331-0037 [dog]). The LC/MS/MS assay was also validated for the determination of TFV in rat milk with an LLOQ of 10 ng/mL (m1.4.4, P1278-00034).

2.1.3. Other In Vivo Bioanalytical Methods

The absorption, distribution, metabolism, and excretion of TAF were assessed in various species following a single oral administration of [^{14}C]TAF. The location of [^{14}C] label at the 2- or 8-position of the adenine base is indicated by an asterisk in the structures described in Table 1. The tissue distribution of [^{14}C]TAF-derived material was assessed using quantitative whole body autoradiography of pigmented and nonpigmented male mice and rats or a liquid scintillation counter (LSC) in dogs (m2.6.5, Section 5, AD-120-2009; AD-120-2011; AD-120-2020). The metabolism and excretion of [^{14}C]TAF has been assessed in intact male mice, and in intact and BDC rats and dogs (m2.6.5, Section 10, AD-120-2008; AD-120-2012; AD-120-2021). HPLC or LC/MS/MS coupled with radio flow-through detector (RFD) was used for metabolite profiling and identification. Tenofovir alafenamide and its metabolites were separated using reverse phase chromatography, and detected using an RFD and MS simultaneously. The retention times of the metabolites using reverse phase chromatography were determined by the peaks on radiochromatograms generated by an inline RFD, and the molecular ions of the metabolites were determined on the full scan mass spectra by tandem MS corresponding to the retention times of metabolites on the radiochromatograms. Tandem MS of the molecular ions was performed, and the structures of the metabolites were proposed based on their mass spectra. Where possible, the structures of the metabolites were confirmed by comparison of the chromatographic and mass spectral characteristics of the metabolites with authentic reference standards.

2.1.4. In vitro methods

2.1.4.1. Permeability Across Caco-2 Cell Monolayers

In vitro bidirectional permeability of TAF (incubated at 10 μM) was assessed using monolayers of the human colonic adenocarcinoma cell line Caco-2, on 12 well transwell dual chamber plates. Permeability rates were determined by quantifying the TAF levels in each chamber by LC/MS/MS. Experiments were done in the absence or in the presence of COBI, a known inhibitor of P-glycoprotein (m2.6.5, Section 14.2.3, AD-120-2013).

2.1.4.2. Stability

The stability of TAF has been assessed in plasma, intestinal S9 and hepatic S9 fractions from dog and human (m2.6.5, Section 8, AD-120-2025; AD-120-2024; AD-120-2023). Intestinal stability of TAF was also studied in the presence of HIV PIs or pharmacoenhancers using intestinal S9 fractions (m2.6.5, Section 14.2.5, AD-120-2027). The disappearance of the parent prodrug was monitored by LC/MS/MS.

The stability of [^{14}C]TFV in rat hepatic microsomal fractions and in liver S9, intestinal S9, and plasma from dog and human was determined by LC-radioprofiling (m1.4.4, 96-DDM-1278-003).

CYP-mediated metabolism was assessed with CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 by incubating TAF at 5 μM with bacterially expressed each human CYP450 enzyme preparations (Bactosomes) coexpressed with human β-nicotinamide adenine dinucleotide phosphate, reduced form (NADPH) CYP reductase in the presence of NADPH (m2.6.5, Section 11.3, AD-120-2004). The disappearance of the parent prodrug was monitored by LC/MS/MS.

2.1.4.3. Intracellular Metabolism

Intracellular activation of TAF was assessed in primary human hepatocytes. Tenofovir alafenamide was continuously incubated with primary human hepatocytes for 24 hours and cell samples were collected at select time point followed by washing and extraction. The samples were analyzed by LC/MS/MS and intracellular concentrations of GS-77389 were quantified (m2.6.5, Section 8.4, AD-120-2017 and m2.6.5, Section 14.2.7, AD-120-2031).

2.1.4.4. Plasma Protein Binding

The binding of TAF to plasma was assessed by equilibrium dialysis against phosphate buffer at 37°C using pooled plasma from Beagle dogs and humans (m2.6.5, Section 6.1, AD-120-2026). Unbound TAF was quantified by LC/MS/MS.

The binding of [^{3}H]TFV in human plasma was determined by ultrafiltration (m1.4.4, P0504-00039.1).

2.1.4.5. Inhibition of Cytochrome P450 Enzymes and UGT1A1

The inhibition of CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A was assessed by incubating TAF with human liver microsomes and NADPH in the presence of individual probe substrates. Each probe substrate metabolite was quantified by LC/MS/MS. The following positive control inhibitors for each CYP isoform were used to establish the validity of the assay; α-naphthoflavone (CYP1A2), ticlopidine (CYP2B6), montelukast (CYP2C8), sulfaphenazole (CYP2C9), tranylcypromine (CYP2C19), quinidine (CYP2D6), and ketoconazole (CYP3A4) (m2.6.5, Section 11.1, AD-120-2003). The mechanism-based inhibition was assessed using CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A following a 2-step incubation protocol. First, TAF or positive control inhibitors were incubated with human liver microsomes in the presence or absence of NADPH for 30 min at 37°C. The mixture was then diluted 10-fold with a phosphate buffer containing the respective probe substrate and fresh NADPH to initiate the second incubation for the CYP reaction. Each probe substrate metabolite was quantified by LC/MS/MS. The following positive control inhibitors for each CYP isoform were used to establish the validity of the assay: resveratrol and furafylline (CYP1A2); ticlopidine (CYP2B6); gemfibrozil glucuronide (CYP2C8); tienilic acid (CYP2C9); ticlopidine (CYP2C19); paroxetine (CYP2D6); and mibefradil and mifepristone (CYP3A4) (m2.6.5, Section 11.2, AD-120-2040).The inhibition of UGT1A1 was assessed by incubating TAF (up to 50 μM) with insect cell microsomal fraction containing expressed human UGT1A1, uridine diphosphoglucuronic acid (UDPGA), and a probe substrate, estradiol at 37°C. Formation of the

metabolite, estradiol 3-glucuronide was monitored by LC/MS/MS (m2.6.5, Section 11.6, AD-120-2006).

The effects of TFV on human CYP1A2, CYP2C9, CYP2E1, CYP2D6, and CYP3A activity were determined with human hepatic microsomal fraction and enzyme-selective activities (m1.4.4, V990172-104).

2.1.4.6. Induction Potential

The potential of TAF to induce human drug metabolizing enzymes and transporters through the activation of the aryl hydrocarbon receptor (AhR) and pregnane X receptor (PXR) was assessed in vitro in reporter cell lines (m2.6.5, Section 11.4, AD-120-2005). Briefly, assessments of induction were done using Puracyp's hepatoma-derived cell lines, DRE12.6 and DPX2. DPX2 cells are stably transformed with an expression vector for human PXR and a reporter gene vector containing the enhancer regions of CYP3A4 linked to luciferase. DRE12.6 cells are transformed with an expression vector for human AhR and the drug/dioxin response element (DRE) of the human CYP1A2 gene linked to a luciferase reporter.

The induction potential of TAF on CYP activity and CYP, P-gp, and UGT1A1 messenger RNA (mRNA) levels was assessed in primary human hepatocytes (m2.6.5, Section 11.5, AD-120-2032). Three preparations of cryopreserved human hepatocytes isolated from 3 separate livers were treated once daily for 3 consecutive days with dimethyl sulfoxide (DMSO; 0.1% v/v, vehicle control), 1 of 3 concentrations of TAF (1, 10, or 100 μM). For positive controls for induction, omeprazole (CYP1A2) at 50 μM, phenobarbital (CYP2B6 and P-gp) at 100 μM, rifampin (CYP3A4) at 10 μM, or β-naphthoflavone (UGT1A1) at 20 μM were used. After treatment, the cells were incubated with CYP probe substrate for the analysis of phenacetin O dealkylation (marker for CYP1A2), bupropion hydroxylation (marker for CYP2B6), and testosterone 6β hydroxylation (marker for CYP3A4) by LC/MS/MS. After the activity assay, RNA was analyzed by qRT PCR to assess the effect of TAF on CYP1A2, CYP2B6, CYP3A4, P-gp, and UGT1A1 mRNA levels. In addition, a test to assess toxicity potential of TAF to human hepatocytes during the course of induction treatment was conducted with the same lots of cryopreserved human hepatocytes using the 3-[4,5-Dimethyl-2-thiazolyl]-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay.

2.1.4.7. Interaction with Transporters

The potential for TAF and TFV to be a substrate for human P-gp was assessed using monolayers of Caco-2 cells, or Madin-Darby canine kidney (MDCK) II cells transfected with an expression vector for the protein, and through the use of chemical inhibitors (m2.6.5, Section 14.2.1, AD-120-2018, m1.4.4, AD-104-2002). The potential for TAF to inhibit P-gp or BCRP was assessed using MDCK II cells expressing each of the tansporters (m2.6.5, Section 14.2.2, AD-120-2019). The potential of TAF to be a substrate or an inhibitor of human OATP1B1 and OATP1B3 were evaluated using Chinese hamster ovary (CHO) cells (AD-120-2019; m2.6.5, Section 14.2.4, AD-120-2022). Interactions of TAF with human OAT1, OCT1, MATE1, and OCT2 were studied using CHO cells expressing the individual transporters (m2.6.5, Section 14.2.6, AD-120-2036). The effect of TAF on the human bile salt export pump

(BSEP) and OAT3 was determined using the transporter expressing Sf9 cell membrane vesicles and Flp-In 293 cells, respectively (AD-120-2036).

Interactions of TFV with human MRP2 were evaluated with the human ovarian carcinoma cell line, 2008, transfected with an expression vector for the protein (m1.4.4, AD-104-2001). Interactions of TFV with human MRP4 were performed with the human T-cell leukemic lymphoblast cells line, CEM-R1, overexpressing the protein, and were compared with the CEM-SS parental line. The potential for TFV to be a substrate for human OAT3, OCT1, or OCT2 was determined by examining uptake of [^{3}H]TFV by microinjected Xenopus laevis oocytes (m1.4.4, PC-103-2001). The effect of TFV on human MRP1 activity was determined using MDCK II cells overexpressing the protein (m1.4.4, PC-104-2014). The effect of TFV on human OCT2 and MATE1 activities was determined using CHO cells expressing the individual proteins (m1.4.4, AD-104-2012).

2.2. E/C/F/TAF

2.2.1. In Vitro Transporter Methods

The effects of EVG, COBI, FTC, and TFV, the major metabolite of TAF, on P-gp and BCRP were tested using MDCKII cells (m1.4.4, AD-236-2003; AD-236-2004; AD-236-2005). The potential for EVG and COBI to be substrates and for FTC and TFV to be inhibitors of OATP1B1 and OATP1B3 was tested in CHO cells expressing the individual transporters (m1.4.4, AD-236-2002; AD-236-2006). The effects of EVG, COBI, FTC, and TFV on OCT2 and MATE1 were assessed in CHO cells (m1.4.4, AD-236-2001; AD-236-2011). The effects of EVG and FTC on OAT1 and OAT3 were tested in CHO cells and on MRP4 were evaluated using vesicles containing the transporter (m1.4.4, AD-236-2007; AD-236-2010). The effects of EVG, COBI, FTC, and TFV on OCT1 were examined in CHO cells and the effects on BSEP were tested using vesicles (m1.4.4, AD-236-2008; AD-236-2009). The potential for EVG and FTC to be inhibitors of MRP2 or for FTC to be a substrate for MRP3 was assessed using vesicles (m1.4.4, AD-236-2012; AD-236-2013).

3. ABSORPTION

3.1. In Vitro Absorption Studies

3.1.1. TAF

Permeability of TAF was studied in both apical to basolateral (forward) and basolateral to apical (reverse) directions at 10, 100, and 1000 μM using Caco-2 monolayers (m2.6.5, Section 3.1, AD-120-2037). Tenofovir alafenamide showed a dose dependent increase in forward permeability and a decrease in efflux ratio indicating saturable efflux transport (Table 2). At 100 μM TAF, the apparent forward permeability rate was 0.63 x 10^{-6} cm/s and the efflux ratio was 13.6. Addition of the efflux transport inhibitor, cyclosporine A (CsA) diminished the efflux ratio and increased the forward permeability.

Table 2. Dose-Dependent Bidirectional Permeability of TAF in Caco-2 Cells

Compound	Inhibitor	Concentration (μM)	Mean P_{app} (x10^{-6} cm/s)		Efflux Ratio
			Forward	Reverse	
TAF	None	10	0.34	6.98	20.2
TAF	None	100	0.63	8.47	13.6
TAF	None	1000	1.08	5.86	6.28
TAF	10 μM CsA	10	1.51	1.34	1.00

3.2. Single-Dose In Vivo Studies

3.2.1. TAF: Mouse, Oral

The mouse plasma pharmacokinetic studies were conducted 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 (m2.6.5, Section 3.2, AD-120-2014 and m2.6.5, Section 3.3, AD-120-2016). Although TAF was observed in mouse plasma in a dose dependent manner, the concentrations were limited and $t_{½}$ could not be determined. Tenofovir exposure increased with the increase in dose and was greater than dose proportional between 10 to 100 mg/kg. Gender differences in plasma TFV levels were less than 2-fold in C_{max} and AUC_{0-t} values. The pharmacokinetic profiles for the 2 different fumarate forms were generally similar. Pharmacokinetic parameters for GS-7340-02 and GS-7340-03 are summarized in Table 3. Values for C_{max} and AUC_{0-t} for TFV were generally similar between GS-7340-02 and GS-7340-03. Together with the rat pharmacokinetics results, the 2 different forms of TAF do not affect the pharmacokinetic properties. The pharmacokinetic parameters following a single dose of GS-7340-03 to 001178-W wild type mice are summarized in Table 4. Measurable concentrations were limited for TAF. No consistent gender-based differences were observed in TAF C_{max} and AUC_{0-t} values. Tenofovir alafenamide was extensively converted to TFV in 001178-W mice following oral gavage administration of

GS-7340-03. Exposure to TFV increased with the increase in dose level from 10 to100 mg/kg. The increases in C_{max} and AUC_{0-t} were greater than dose proportional between the 10 to 100 mg/kg. Gender differences in plasma concentration of TFV were less than 2-fold in C_{max} and AUC_{0-t} values.

Table 3. Plasma Pharmacokinetic Parameters Following a Single Dose of GS-7340-02 and GS-7340-03 to Male CD-1 Mice

Test Article	GS-7340-02						GS-7340-03					
Dose (mg/kg)	10		30		100		10		30		100	
Analyte	TAF	TFV	TAF	TFV	TAF	TFV	TAF	TFV	TAF	TFV	TAF	TFV
C_{max} (µg/mL)	5.53	106	NA	440	37.1	1827	NA	85.4	10.3	383	34.7	2152
t_{max} (h)	0.083	0.50	NA	0.25	0.083	0.75	NA	0.50	4.00	0.50	0.25	1.50
$t_{½}$ (h)	NA	NA	NA	NA	NA	NA	NA	5.16	NA	10.1	NA	NA
AUC_{0-t} (ng·h/mL)	NA	455	NA	2005	26.0	10643	NA	493	NA	2477	11.3	10866

NA = not applicable
Source: AD-120-2014

Table 4. Plasma Pharmacokinetic Parameters Following a Single Dose of GS-7340-03 to 001178-W Wild Type Mice

Dose (mg/kg)	10				30				100			
Analyte	TAF		TFV		TAF		TFV		TAF		TFV	
Sex	M	F	M	F	M	F	M	F	M	F	M	F
C_{max} (ng/mL)	NA	NA	175	100	8.80	117	615	421	648	280	1988	1733
t_{max} (h)	NA	NA	0.25	0.50	0.083	0.5	0.25	0.25	0.25	0.50	0.50	0.50
$t_{½}$ (h)	NA	NA	9.78	8.20	NA	NA	9.51	10.9	NA	NA	8.04	11.0
AUC_{0-t} (ng·h/mL)	NA	NA	735	354	NA	NA	2639	2053	194	104	10026	7131

NA = not applicable
Source: AD-120-2016

3.2.2. TAF: Rat, Oral

Plasma pharmacokinetic studies following a single oral administration of TAF were performed to determine pharmacokinetic parameters for TFV, to assess potential differences between GS-7340-02 and GS-7340-03, and to compare exposure to TFV between TAF and TDF (m2.6.5, Section 3, R990130; AD-120-2015; R2000065). In all cases TAF was rapidly absorbed and converted to TFV. The plasma TFV t_{max} was less than 1 hour. Tenofovir exposure increased with the increase in dose and was greater than dose proportional between 5 to 100 mg/kg. No significant differences in pharmacokinetic profiles were observed between GS-7340-02 and

GS-7340-03 (Table 5). The plasma C_{max} and AUC for TFV were 2- to 3-fold higher when Sprague-Dawley rats were orally dosed with 400 mg/kg TAF compared to 400 mg/kg TDF.

Table 5. Plasma Pharmacokinetic Parameters following a Single Dose of GS-7340-02 and GS-7340-03 to Male Sprague-Dawley Rats

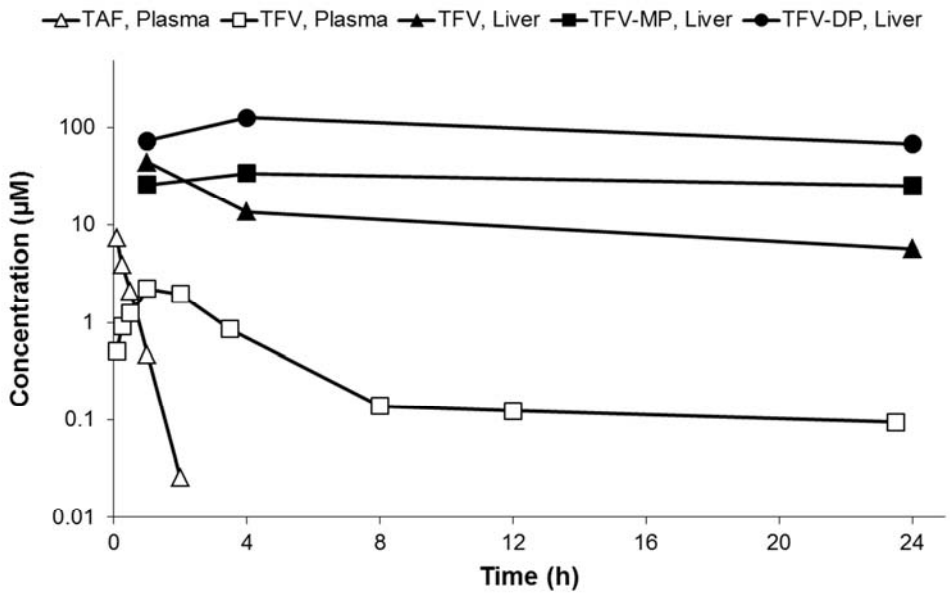
Test Article	GS-7340-02			GS-7340-03		
Dose (mg/kg)	**5**	**25**	**100**	**5**	**25**	**100**
Analyte	**TFV**	**TFV**	**TFV**	**TFV**	**TFV**	**TFV**
C_{max} (µg/mL)	32.5	199	1240	39.3	364	1670
t_{max} (h)	0.667	0.583	0.833	0.583	0.833	0.667
$t_{½}$ (h)	NA	11.2	10.3	NA	7.89	7.85
AUC_{0-t} (ng·h/mL)	122	1395	7771	88.5	1810	9759

3.2.3. TAF: Dog, Oral, and IV

In order to assess the effects of the stereo configuration, fumarate form, food, and the route of administration, the plasma and PBMC pharmacokinetic profiles were determined in Beagle dogs following intravenous (IV) bolus (GS-7340-02 [6.3 mg/kg]), or oral administration (TAF as free base [18.0 mg/kg], its diastereomer GS-7339 [18.0 mg/kg], the mixture GS-7171 [16.0 mg/kg], or GS-7340-02 [4,8, 5.0, and 20 mg/kg under fasted and 5.0 mg/kg under fed conditions]) (m2.6.5, Section 3.7, 99-DDM-1278-001-PK). Following oral administration, TAF and its diasteroisomer were rapidly absorbed and eliminated with a t_{max} of less than 0.5 h and $t_{½}$ ranging from 0.2-0.9 h. The plasma exposures to the intact prodrugs were similar when TAF or GS-7339 were dosed separately, however, when the isomeric mixture, GS-7171, was dosed, the exposure to GS-7339 was approximately 3-fold higher than TAF. The TFV exposure in plasma was similar for both diastereomers. The TFV exposure in PBMCs was approximately 4-fold higher when animals were dosed with TAF than GS-7339. All the pharmacokinetic parameters were similar for the free base and fumarate form. Plasma exposures to TAF and TFV and PBMC exposure to TFV were approximately 2.5-fold higher in fasted dogs than in fed dogs.

The plasma and liver pharmacokinetic profiles were determined following a single oral dose of 10 mg/kg TAF to male Beagle dogs (m2.6.5, Section 14.2.8, AD-120-2035). Tenofovir alafenamide was rapidly absorbed and eliminated with observed plasma t_{max} of 0.08 hours and $t_{½}$ of 0.24 hours. Tenofovir was the major metabolite found in plasma with a C_{max} value of 2.23 µM. The pharmacologically active metabolite, TFV-DP was the major metabolite in liver achieving a C_{max} of 126 µM at 4.0 hours postdose. The plasma and liver pharmacokinetic profiles are summarized in Figure 1.

Figure 1. Plasma and Liver Pharmacokinetic Following a Single Dose of TAF to Beagle Dogs



3.2.4. TAF: Monkey, Oral

The plasma pharmacokinetic profiles for TAF and TFV and TFV concentrations in PBMCs were determined in rhesus monkeys following a single oral dose of GS-7340-02 at 0.5, 5.0, and 50 mg/kg (m2.6.5, Section 3.9, P2000087). The plasma pharmacokinetic parameters are summarized in Table 6. Tenofovir alafenamide and TFV levels increased rapidly with t_{max} values of approximately 0.5 and 1 hour, respectively. The TFV levels in PBMCs were determined before and after treatment with acid phosphatase, which was used to convert the phosphorylated metabolites to TFV (Table 7). Tenofovir persisted in PBMCs up to 96 hours with an apparently slower decline in PBMCs than in plasma. The TFV levels were significantly higher in the samples treated with acid phosphatase suggesting that a significant proportion of TFV-related material in PBMCs was in phosphorylated forms.

Table 6. Plasma Pharmacokinetic Parameters for TAF And TFV Following a Single Dose of GS-7340-02 to Rhesus Monkeys

GS-7340-02 Dose (mg/kg)	0.5	5	50	0.5	5	50
Analyte	**TAF**			**TFV**		
C_{max} (ng/mL)	2.79	125	4143	7.72	161	1326
t_{max} (h)	0.38	0.8	0.5	1	1.33	1.0
$t_{½}$ (h)	0.61	0.23	0.40	4.62	9.92	17.33
$AUC_{0\text{-last}}$ (ng·h/mL)	1.22	95.1	3811	39.9	1037	9934
$AUC_{0\text{-}\infty}$ (ng·h/mL)	2.47	80.0	3846	52.7	1069	10250

Table 7. Concentrations of TFV in PBMCs from Monkeys Dosed with GS-7340-02

GS-7340-02 Dose (mg/kg)	TFV PBMC Levels (ng/10^6 Cells)			
	Without Phosphatase Treatment		With Phosphatase Treatment	
	5	50	5	50
2 h	0.47	17.0	0.73	34.2
24 h	0.06	6.82	0.62	20.1
96 h	BLQ	3.03	0.18	8.68

3.3. Repeat-Dose In Vivo Studies

3.3.1. TAF: Mouse

GS-7340-02 was administered by oral gavage for up to 14 days to male and female Crl:CD1(ICR) mice at a dose of 100, 500, or 1000 mg/kg/day (m2.6.7, Section 6, TX-120-2006). Due to early death for animals given 500 or 1000 mg/kg/day, only the 100 mg/kg/day dose group was evaluated. GS-7340 at 100 mg/kg/day corresponded to a Day 14 C_{max} of 27.1 and 2.89 ng/mL for males and females, respectively; the $AUC_{0\text{-}24}$ could not be calculated due to the lack of a distinct elimination phase. GS-7340 rapidly converted to its metabolite TFV. There were no significant differences in TFV pharmacokinetic profiles between males and females.

Following daily administration of GS-7340-02 to mice via oral gavage for at least 13 weeks at doses of 0, 10, 30, and 100 mg/kg/day, the pharmacokinetic parameters for TAF and TFV were determined (m2.6.7, Section 7.1, TX-120-2007). Blood samples were collected from up to 3 toxicokinetic animals/sex/group/timepoint on Day 1 and during Week 13 predose and at approximately 0.25, 0.5, 1, 4, 8, and 24 hours postdose. Exposure to TFV increased with the increase in GS-7340-02 dose from 10 to 100 mg/kg/day. The increases in C_{max} and $AUC_{0\text{-}t}$ were generally greater than proportional between the 10 to 100 mg/kg/day dose levels. Gender-based

differences were less than 2-fold in TFV C_{max} and AUC_{0-t} values. No unexpected accumulation of TFV was observed after multiple dosing. Tenofovir alafenamide was rapidly and extensively converted to TFV after oral administration in mice.

3.3.2. TAF: Rat

The plasma pharmacokinetic profile of TFV was determined during the course of a 28-day oral gavage toxicity study in adult male and female albino (Sprague-Dawley) rats following daily administration of either 1.5, 6.25, 25, 100 or 400 mg/kg/day GS-7340-02 (m2.6.7, Section 7.2, R990182). A nonlinear pharmacokinetic response was observed for total plasma exposure versus dose for both sexes on both Day 1 and Day 28. A greater than linear increase in plasma exposure was observed as the dose was increased. There was no observed plasma accumulation (accumulation ratio 0.96 to 1.11) over the 28-day study for any dose group.

In a 26-week toxicology study, GS-7340-02 was administered once daily at doses of 0 (vehicle only), 5, 25 and 100 mg/kg/day by oral gavage and plasma pharmacokinetic parameters of TFV were determined on Day 1 and during Weeks 13 and 26 (m2.6.7, Section 7.3, TOX-120-001). No consistent differences in plasma pharmacokinetic parameters were found between male and female rats. Mean TFV C_{max} and AUC values increased dose proportionally over the dose range of 5 to 100 mg/kg/day. Mean tenofovir AUC obtained on Day 1 was slightly lower than that measured during Weeks 13 and 26, which suggested that there was a slight accumulation of tenofovir with repeat dosing.

3.3.3. TAF: Dog

Following daily oral administration of 8.29 mg/kg TAF for 7 days to male Beagle dogs, the plasma and liver pharmacokinetic profiles were determined on Days 1 and 7 (m2.6.5, Section 4.1, AD-120-2033). Tenofovir alafenamide was rapidly absorbed and exhibited a short terminal half-life ($t_{½}$) of 0.3 hours in plasma on both Day 1 and 7. The rapid disappearance of TAF was accompanied by an increase in TFV. Tenofovir was the major metabolite detected in plasma achiving a maximal plasma concentration (C_{max}) of 1.47 and 2.12 µM on Days 1 and 7, respectively. The pharmacologically active diphosphate metabolite, TFV-DP, was efficiently formed in dog livers achieving concentrations of 242 and 153 µM at 4.0 and 24 hours postdose, respectively, on Day 7.

The plasma PK of TAF and TFV and TFV levels in PBMCs were determined during the course of a 28-day oral gavage toxicity study in adult male and female beagle dogs following daily administration of either vehicle, 0.1, 0.3, 1.0, 3.0, or 10 mg/kg/day GS-7340-02 (m2.6.5, Section 4.2, D990175-PK). Repeat dosing at 10 mg/kg/day resulted in nonlinear pharmacokinetics between Days 1 and 28 with TAF median AUC values of 0.454 and 0.985 µg·h/mL, C_{max} values of 582 and 1280 ng/mL, and $t_{½}$ λ_z values of 18 and 23 minutes, respectively. The TFV C_{max} values appeared to be linear with increasing dose as well as repeat dosing. The TFV $t_{½}$ was estimated to be 37 hours and substantial accumulation of TFV was observed after repeat dosing. The TFV levels in PBMCs were not linear with increasing dose; however, a linear correlation was observed between TFV levels in PBMCs and corresponding

trough plasma concentrations. PBMC concentrations were approximately 100-fold higher than corresponding plasma concentrations.

In a 9-month toxicology study in dog, GS-7340-02 was administered once daily at doses of 0, 2, 6, and 18 mg/kg/day (m2.6.7, Section 7.5, TOX-120-002). The dose of 18 mg/kg/day was decreased to 12 mg/kg/day on Day 2 of Week 7 for males and Day 2 of Week 8 for females due to severe clinical signs and reduced body weight and food consumption. The concentrations of GS-7340 and tenofovir in plasma samples and total TFV in Week 39/40 PBMC samples were determined. GS-7340 was rapidly absorbed and converted to tenofovir following oral dose administration, with peak plasma concentrations of GS-7340 and tenofovir occurring at 0.5 and 1 hour postdose, respectively. GS-7340 was eliminated rapidly from the plasma with a terminal phase half-life of less than 1 hour. The median $t_{½}$ of tenofovir was estimated to be in the range of 25 to 31 hours on Day 1. The plasma pharmacokinetics of GS-7340 and tenofovir were comparable between male and female dogs after oral administration. Plasma C_{max} and AUC values for TAF increased more than proportionally over the dose range of 2 to 18/12 mg/kg/day. The plasma TFV C_{max} and AUC increased roughly dose proportional. There was some accumulation of tenofovir following repeat dosing (~3-fold). Tenofovir concentrations in PBMCs were measurable at 24-hour postdose for all dose groups. The median terminal phase half-life of total tenofovir in PBMCs was estimated to be 31 hours (similar to the tenofovir plasma estimate) from the recovery animals with PBMC concentrations measured up to 72 hours. Dose-normalized PBMC mean AUC values of total tenofovir increased more than dose proportionally during Week 39/40.

3.3.4. TAF: Monkey

Following daily oral administration of GS-7340-02 at 0, 3, and 30 mg/kg/day or TFV at 15 mg/kg/day for 28 days, pharmacokinetic profiles of TAF and/or TFV were determined on Day 1, Day 14 and Day 28 (m2.6.5, Section 4.3, P2000114-PK). Concentrations of TFV in PBMCs were determined on Day 14 and Day 28. No significant differences in pharmacokinetic parameters were found between males and females. The pharmacokinetic parameters for TFV were dose linear on Day 1 but were greater than dose-linear on Day 28 after oral administration of GS-7340-02. There was no statistically significant accumulation of TFV following repeat dosing of either GS-7340-02 or TFV. The intracellular TFV concentrations in PBMCs were only determined from the 30 mg/kg/day GS-7340-02 dose group where 72.3 and 27.2 μg/mL were detected on Day 14 and Day 28, respectively.

3.4. E/C/F/TAF: Absorption

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 EVG, COBI, or TAF. Tenofovir alafenamide and EVG are both efflux substrates in the intestine; therefore, absorption is increased in the presence of COBI due to inhibition of intestinal efflux transport. Although formal nonclinical studies of the absorption kinetics of the E/C/F/TAF FDC have not been conducted, comprehensive clinical studies on the combination have been performed (m2.7.2, Section 1.2.1).

4. DISTRIBUTION

4.1. Plasma Protein Binding

4.1.1. TAF and TFV

Since TAF is highly unstable in rodent plasma due to high levels of plasma esterases expressed in some rodent species, the extent of TAF binding to plasma could only be determined in dog and human plasma in vitro (m2.6.5, Section 6.1, AD-120-2026). Protein binding of TAF was moderate in dog and human plasma with the percent unbound values of 48.0% and 46.8%, respectively. These in vitro values were higher than those observed in multiple human ex vivo studies with the mean percent unbound TAF ranging from 14% to 23% in all subjects (GS-US-120-0108 and GS-US-120-0114). Since the ex vivo results should be more clinically relevant, percent unbound TAF of 20% was used for the assessments for potential drug interactions (Section 7.1).

The protein binding of TFV has been determined in human plasma and serum using centrifugal ultrafiltration over the range of 0.01 to 25 μg/mL (m1.4.4, P0504-00039.1). The percent unbound for TFV was 99.3 ± 3.3% in human plasma, and 92.8 ± 3.6% in human serum. Tenofovir therefore showed very low protein binding in either human plasma or serum.

4.2. Tissue Distribution Studies

4.2.1. TAF: Mouse

After oral dosing of 100 mg/kg [^{14}C]TAF to male CD-1 mice, [^{14}C]TAF-derived radioactivity was widely distributed to most of the tissues by the first collection time point (0.5 hours postdose) (m2.6.5, Section 5.1, AD-120-2011). Most tissues reached maximum concentration by 1 hour postdose. The tissues showing the highest maximum concentrations of radioactivity, excluding gastrointestinal (GI) tract, included liver, gall bladder, urinary bladder, diaphragm, kidney cortex, kidneys, and kidney medulla. The tissues with the lowest C_{max} values were testis, brain cerebrum, fat (abdominal), spinal cord, and brain medulla.

In male C57 Black mice, [^{14}C]TAF-derived radioactivity was widely distributed to most of the tissues by the first collection time point (0.5 hours postdose), similar to CD-1 mice. Most tissues reached maximum concentration by 0.5 hours postdose. The tissues showing the highest maximum concentrations of radioactivity, excluding the GI tract, included liver, gall bladder, urinary bladder, kidney cortex, kidneys, kidney medulla, and diaphragm. The tissues with the lowest C_{max} values were testis, spinal cord, brain cerebrum, brain medulla, and brain cerebellum.

Low levels of radioactivity were detected in brain in mice suggesting [^{14}C]TAF-derived radioactivity poorly crossed the blood:brain barrier. Low levels of radioactivity were also measured in testis in mice, suggesting [^{14}C]TAF-derived radioactivity poorly crossed the blood:testis barrier.

More persistent exposures in eye lens, eye uveal tract, and eyes were observed in CD57 black mice compared to CD-1 mice. However, no difference in distribution between pigmented and nonpigmented skin was observed illustrating that ^{14}C-TAF-related radioactivity was not selectively associated with melanin-containing tissues. Comparative quantification data for selected time points are provided in Table 8.

Table 8. Comparative Tissue Concentrations of Radioactivity in Male CD-1 and C57 Black Mice After Oral Administration of [^{14}C]TAF (n = 1 per time point)

Organ	Radioactivity (µg equivalents ^{14}C-TAF/g tissue)									
Time point	**0. 5 h**		**1 h**		**3 h**		**12 h**		**24 h**	
Rat Strain	**CD-1**	**C57 Black**	**CD-1**	**C57 Black**	**CD-1**	**C57 Black**	**CD-1**	**C57 Black**	**CD-1**	**C57 Black**
Adrenal gland(s)	9.92	34[a]	27.5	26.4	9.45	20.4	3.01	7.75[a]	3.6	10.4[a]
Bile	60.3	198	127	136	115	150	65.9	40.3	12.9	26.6
Blood	14.6	16.7	14.5	9.27	5.28	7.91	4.38	4.17	6.1	3.78
Bone	1.3	11.2	2.42	4.49	1.23	4.9	1.01	2.43	0.525	1.77
Bone marrow	4.73	24.3	6.61	19	2.03	16.1	2.04	6.44	2.12	6.47
Brain cerebellum	0.569	0.611	1.94	0.59	0.395	BLQ	BLQ	ND	BLQ	ND
Brain cerebrum	0.768	1.24	1.43	0.661	BLQ	BLQ	0.379	ND	BLQ	ND
Brain medulla	0.42	0.886	0.833	BLQ	BLQ	BLQ	BLQ	ND	BLQ	ND
Brain olfactory lobe	1.12	3.79	2.89	1.73	0.464	1.64	0.461	0.688	BLQ	0.707
Cecum	3.44	25.4	6.96	95.4	90.5	ND[b]	19.1	50.5	8.31	10.5
Diaphragm	40.6	60.3	167	103	46.6	114	26.6	27.5	28.3	25.9
Epididymis	3.6	10.8[a]	22.3	4.19	1.31	4.99	0.54	2.12	0.864	2.15[a]
Esophagus	38.4	76.1	90.8	81.3	57.3	48.9	20.1	27.9	8.66	8.54
Exorbital lacrimal gland	3.6	18.1	5.46	12	1.4	11.8	1.25	4.91	1.29	3.53
Eye lens	0.967	4.31	2.98	1.87	0.508	1.11	BLQ	0.527	BLQ	BLQ
Eye uveal tract	4.18	13.4	3.69	12.2	0.754	11.6	0.947	4.74	0.779	6.14

Organ	Radioactivity (µg equivalents ^{14}C-TAF/g tissue)									
Time point	**0. 5 h**		**1 h**		**3 h**		**12 h**		**24 h**	
Rat Strain	**CD-1**	**C57 Black**	**CD-1**	**C57 Black**	**CD-1**	**C57 Black**	**CD-1**	**C57 Black**	**CD-1**	**C57 Black**
Eye(s)	1.88	5	2.66	2.92	0.539	2.44	0.428	1.04	0.363	1.06
Fat (abdominal)	1.25	5.46	1.17	6.82	0.968	2.14	0.512	4.18	0.787	1.7
Fat (brown)	3.07	19.2	5.59	18.7	2.53	14.5	2.06	10.8	3.03	8.66
Gall bladder	335	163	216	379	108	275	68.1	94	37.1	39.8
Harderian gland	4.24	18.5	6.5	14.6	1.53	13.6	2.17	6.2	1.39	4.58
Intra-orbital lacrimal gland	7.63	NR	3.58	NR	1.9	NR	2.28	NR	1.5	3.76
Kidney cortex	92.3	137	89	125	74	104	30	58	23.1	34.5
Kidney medulla	65	125	70	94.7	54.8	80	18.1	40.2	12.8	19.4
Kidney(s)	84.8	132	86.1	107	68.9	89.6	25.9	47.5	19.9	29.5
Large intestine	6.68	25.7	8.09	17.3	7.73	32.3	22.5	74.2	6.77	35
Liver	282	488	447	490	290	385	197	282	164	118
Lung(s)	13.2	32.3	23.9	32.5	4	26.5	10	18.1	11.5	13.5
Lymph node(s)	5.19	17.9	7.75	14.6	1.68	12.6	1.76	8.02	2.28	10.9
Muscle	1.68	8.78	2.1	4.14	0.466	3.19	0.768	2.01	0.579	3.9
Myocardium	7.57	23.2	11.7	13.5	2.38	13.6	4.6	8.47	4.62	9.51
Nasal turbinates	2.72	10.6	6.44	2.98	1.29	3.55	0.983	2.39	1.19	1.92
Pancreas	6.13	34.9	14.5	26.9	2.95	31.5	3.54	15.4	2.88	13.9
Pituitary gland	2.91	17.4	7.44	8.44	1.28	6.59	2.51	0.85	1.3	ND

Organ	Radioactivity (µg equivalents ^{14}C-TAF/g tissue)									
Time point	**0. 5 h**		**1 h**		**3 h**		**12 h**		**24 h**	
Rat Strain	**CD-1**	**C57 Black**	**CD-1**	**C57 Black**	**CD-1**	**C57 Black**	**CD-1**	**C57 Black**	**CD-1**	**C57 Black**
Preputial gland	1.9	11.5	2.58	6.39	0.635	6.13	0.708	2.24	0.503	1.97
Prostate gland	5.5	ND[c]	2.88	8.63	2.15	4.84	3.06	8.62	2.59	ND
Salivary gland(s)	5.39	36.2	11.5	24.2	1.88	27.2	2.42	9.91	2.16	12.2
Seminal vesicle(s)	1.79	5.54	3.41	3.53	0.957	2.47	0.852	2.15	0.62	0.825
Skin (nonpigmented)	8.52	NA	5.58	NA	1.51	NA	0.674	NA	0.715	NA
Skin (pigmented)	NA	11.6	NA	5.93	NA	3.35	NA	1.18	NA	1.34
Small intestine	8.77	26.9	74.5	56.1	88.1	28	14.1	14.1	4.04	11.3
Spinal cord	0.757	1.95	1.21	0.742	BLQ	BLQ	BLQ	BLQ	BLQ	ND
Spleen	6.1	35.3	12.9	29.6	3.85	29.1	5.46	16.6	6.27	12.2
Stomach	77.6	60.5	39.3	70.6	26.6	29.2	5.46	7.9	5.94	6.73
Stomach mucosa	96.9	ND	46.9	ND	19.8	ND	5.28	ND	3.66	ND
Stomach wall	48.6	ND	24.5	ND	26.6	ND	7.69	ND	22.7	ND
Testis(es)	1.16	2.64	1.49	1.76	0.774	1.07	BLQ	0.512	0.319	0.752
Thymus	2.51	12.4	6.14	8.19	0.914	7.19	0.903	3.44	1.19	3.41
Thyroid	7.12	40.7	12.1	32.7	2.83	29	1.2	NR	4.6	10.9
Urinary bladder	ND[c]	ND[c]	174	138	85.6	49.1	5.8	12	10.3	6.29
Urine	1790	1170	413	626	200	128	36.8	135	24.7	18.4

BLQ = below the limit of quantitation [<311 (CD-1) or <490 (C57 Black) µg equivalents ^{14}C-TAF/g]; h = hours; NA = not applicable; ND = not detectable (sample shape not discernable from background or surrounding tissue); NR = not represented (tissue not present in section).

a Tissue appeared to be fat-soaked.
b Not detectable due to flare from cecum contents.
c Not detectable due to flare from urine.

4.2.2. TAF: Rat

After oral dosing of 5 mg/kg [^{14}C]TAF to male Sprague-Dawley or Long Evans rats, [^{14}C]TAF-derived radioactivity was widely distributed to most tissues by the first collection time point (0.25 hours postdose) (m2.6.5, Section 5.2, AD-120-2020). Representative autoradiographic images from animals terminated at 0.25 hours postdose are provided in Figure 2 (nonpigmented) and Figure 3 (pigmented). Comparative quantification data for selected time points are provided in Table 9. A full listing of tissue concentrations of radioactivity is provided in tabular form in m2.6.5 (Section 5.2 for albino animals and pigmented animals). In both Sprague-Dawley and Long Evans rats, most tissues reached maximum concentrations by the first collection time point. The tissues showing the highest maximum concentrations of radioactivity included kidney cortex, kidney(s), kidney medulla, and liver.

The tissues with the lowest C_{max} values were brain olfactory lobe, seminal vesicle(s), eye vitreous humor, thymus, eyes, testis(es), and harderian gland for Sprague-Dawley rats and bone, brain olfactory lobe, seminal vesicle(s), fat (abdominal), muscle, eye vitreous humor, and eye(s) for Long Evans rats. Transient exposure to low levels of ^{14}C[TAF]-related radioactivity was observed in the eyes of rats decreasing to undetectable levels at 8 hours postdose. No difference in distribution was observed between Sprague-Dawley and Long Evans rats, including in the skin and eyes, suggesting no binding to melanin.

Table 9. Comparative Tissue Concentrations of Radioactivity in Male Sprague-Dawley and Long Evans Rats After Oral Administration of [^{14}C]TAF (n = 1 per time point)

Organ	Radioactivity (ng equivalents ^{14}C-TAF/g tissue)									
Time point	0.25 h		1 h		4 h		12 h		24 h	
Rat Strain	SD	LE	SD	LE	SD	LE	SD	LE	SD	LE
Adrenal gland(s)	181[a]	353[a]	129[a]	115	BLQ[a]	48.5[a]	ND	ND	ND	ND
Arterial wall	817	1350	299	270	118	90.4	ND	ND	ND	ND
Bile	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Blood	1070	1260	334	221	138	116	83.1	117	ND	ND
Bone	BLQ	50.4	ND	BLQ	ND	ND	ND	ND	ND	ND
Bone marrow	233	311	125	84.9	72.6	BLQ	BLQ	BLQ	ND	ND
Brain cerebellum	BLQ	BLQ	ND	ND	ND	ND	ND	ND	ND	ND
Brain cerebrum	BLQ	BLQ	ND	ND	ND	ND	ND	ND	ND	ND
Brain medulla	BLQ	BLQ	ND	ND	ND	ND	ND	ND	ND	ND
Brain olfactory lobe	45.7	57.2	BLQ	51.0	ND	ND	ND	ND	ND	ND
Bulbo-urethral gland	396	831	177	209	236	ND	ND	ND	ND	ND
Cecum	218	603	132	118	541	889	NR	362	323	494
Diaphragm	210	353	145	124	52.9	55.8	ND	ND	ND	ND
Epididymis	249[a]	516	101[a]	79.3	BLQ	BLQ	ND	ND	ND	ND
Esophagus	341	923	222	218	187	186	58.9	65.8	ND	ND
Exorbital lacrimal gland	234	353	101	56.1	51.2	BLQ	ND	ND	ND	ND
Eye lens	BLQ	BLQ	ND	BLQ	ND	ND	ND	ND	ND	ND

Organ	Radioactivity (ng equivalents ^{14}C-TAF/g tissue)									
Time point	**0.25 h**		**1 h**		**4 h**		**12 h**		**24 h**	
Rat Strain	**SD**	**LE**	**SD**	**LE**	**SD**	**LE**	**SD**	**LE**	**SD**	**LE**
Eye uveal tract	409	555	187	89.5	78.4	59.4	ND	ND	ND	ND
Eye vitreous humor	84.9	139	89.3	BLQ	BLQ	BLQ	ND	ND	ND	ND
Eye(s)	86.1	150	92.7	BLQ	BLQ	BLQ	ND	ND	ND	ND
Fat (abdominal)	BLQ	70.0	BLQ	BLQ	ND	ND	ND	ND	ND	ND
Fat (brown)	200	232	97.1	BLQ	48.2	ND	55.7	ND	ND	ND
Harderian gland	98.6	209	52.4	BLQ	BLQ	ND	ND	ND	ND	ND
Intra-orbital lacrimal gland	250	402	118	53.4	BLQ	49.1	ND	ND	ND	ND
Kidney cortex	10700	8000	12400	8890	11800	6980	8300	5150	2010	2440
Kidney medulla	8240	6900	5040	3670	2800	655	1010	757	317	367
Kidney(s)	9520	7750	8710	7570	8250	5160	4590	3000	1380	1310
Large intestine	364	548	140	91.5	55.8	474	ND	133	ND	132
Liver	6730	10300	6730	7800	4010	7710	3570	5610	1090	1380
Lung(s)	592	854	211	145	81.1	67.2	ND	66.3	ND	BLQ
Lymph node(s)	318[a]	422[a]	ND	94.9[a]	ND	ND	ND	ND	ND	ND
Muscle	101	115	BLQ	BLQ	ND	ND	ND	ND	ND	ND
Myocardium	361	512	136	46.6	58.6	BLQ	ND	BLQ	ND	ND
Nasal turbinates	123	201	83.8	71.9	52.8	BLQ	ND	ND	ND	ND
Pancreas	215	274	120	82.1	68.9	53.5	52.0	ND	ND	ND
Pituitary gland	335	434	116	53.1	50.5	ND	76.2	ND	ND	ND

Organ	Radioactivity (ng equivalents ^{14}C-TAF/g tissue)									
Time point	**0.25 h**		**1 h**		**4 h**		**12 h**		**24 h**	
Rat Strain	**SD**	**LE**	**SD**	**LE**	**SD**	**LE**	**SD**	**LE**	**SD**	**LE**
Preputial gland	140[a]	247	60.1[a]	67.9	BLQ	ND	ND	ND	ND	ND
Prostate gland	134	247	88.6	BLQ	117	49.9	BLQ	ND	ND	ND
Salivary gland(s)	292	368	119	85.3	BLQ	BLQ	ND	BLQ	ND	ND
Seminal vesicle(s)	62.7	67.7	ND	BLQ	ND	ND	ND	ND	ND	ND
Skin (nonpigmented)	393	526	123	71.8	BLQ	BLQ	ND	ND	ND	ND
Skin (pigmented)	NA	623	NA	78.9	NA	BLQ	NA	ND	NA	ND
Small intestine	479	530	500	376	364	122	86.5	220	171	98.6
Spinal cord	BLQ	BLQ	ND	BLQ	ND	ND	ND	ND	ND	ND
Spleen	147	231	105	83.1	59.0	58.3	62.8	60.7	BLQ	BLQ
Stomach	475	682	126	113	66.6	71.4	ND	159	ND	BLQ
Testis(es)	98.2	157	50.9	BLQ	BLQ	BLQ	ND	BLQ	ND	ND
Thymus	91.2	181	68.1	BLQ	BLQ	BLQ	ND	ND	ND	ND
Thyroid	324	412	147	59.1	63.6	ND	ND	ND	ND	ND
Tooth pulp	646	793	228	194	84.4	78.7	ND	57.0	ND	NR
Urinary bladder	925	352[a]	205	155	ND[b]	125	1050	45.9	BLQ	48.3
Urine	112000	50200	34400	61000	51100	2280	14500	1140	988	1500

BLQ = below the limit of quantitation (<45.6 ng equivalents ^{14}C-GS-7340/g); h = hours; NA = not applicable; ND = not detectable (sample shape not discernable from background or surrounding tissue); SD = standard deviation

a Tissue appeared to be fat-soaked.

b Not detectable due to flare from urine.

Figure 2. Annotated Whole-Body Autoradiograph for a Male Sprague-Dawley Rat 0.25 hours after a Single Oral Administration of ^{14}C-GS-7340 at 5 mg/kg

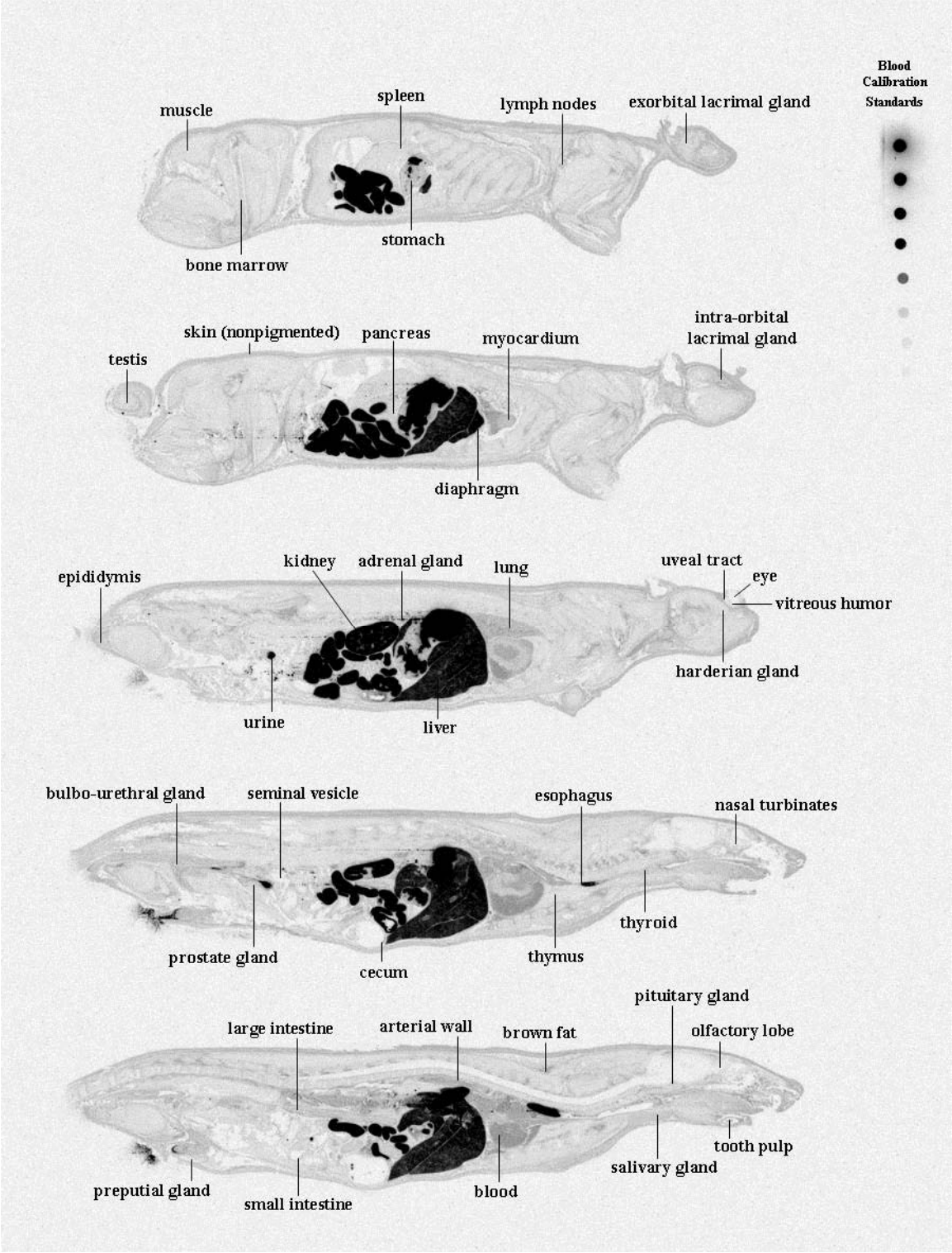
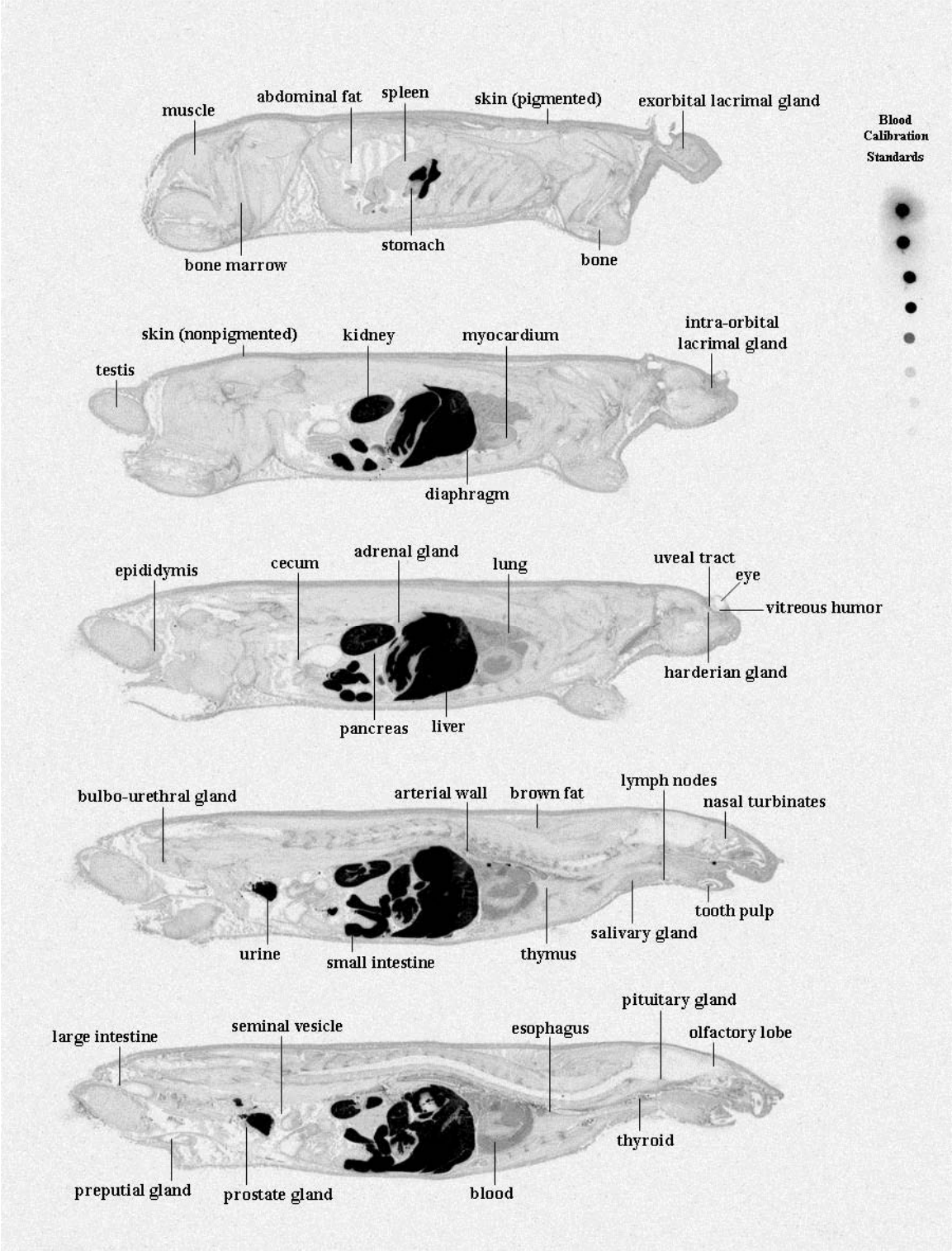


Figure 3. Annotated Whole-body Autoradiograph for a Male Long Evans Rat 0.25 hours after a Single Oral Administration of ^{14}C-GS-7340 at 5 mg/kg



4.2.3. TAF: Dog

The absorption and distribution of [^{14}C]TAF were determined following multiple 15-mg/kg oral doses of GS-7340 and a single oral dose of [^{14}C]TAF to male dogs at 15-mg/kg or at 18.1-mg/kg (m2.6.5, Section 5.3, AD-120-2009 and m2.6.5, Section 5.4, D990173-BP). Radioactivity was widely distributed but not detected in brain, eye, and cerebrospinal fluid. High concentrations were observed in kidney, liver, GI tract, spleen, lymph nodes and PBMCs. Following multiple or single oral administrations of TAF or [^{14}C]TAF to male dogs, the highest concentrations of radioactivity were observed in the liver and kidney through 24 hours postdose. Concentrations of radioactivity in tissues after multiple doses of unlabeled GS-7340 followed by a single dose of [^{14}C]TAF were higher compared to tissues from animals dosed with a single dose of [^{14}C]TAF only.

4.3. Studies in Pregnant or Nursing Animals

4.3.1. TAF: Pregnant Rats

Tenofovir concentrations were determined in plasma from pregnant female rats dosed with GS-7340-02 by oral gavage for at least 12 days (gestation days [GD] 6 to 17) at 5, 100, and 200 mg/kg as an oral range-finding study (m2.6.7, Section 7.3, TX-120-2001) or at 25, 100, and 250 mg/kg as an embryo-fetal development study (m2.6.7, Section 7.5, TX-120-2002). Blood samples were collected on GD 6 and 17. Following oral gavage of GS-7340, concentrations of TFV readily appeared in plasma. Exposure to TFV increased with the increase in dose. The increases in C_{max} and AUC_{0-t} were generally greater than dose proportional between the 5 to 200 mg/kg/day dose levels in the range-finding study. In the embryo-fetal development study, C_{max} and AUC_{0-t} were inconsistently proportional between the 25 to 250 mg/kg/day dose levels. While accumulation of TFV was observed after multiple dosing of GS-7340-02 in pregnant rats in the range-finding study, no accumulation of TAF and TFV was observed in the embryo-fetal development study.

4.3.2. TAF: Pregnant Rabbits

The toxicokinetics of TAF and TFV were determined in plasma from pregnant female rabbits following administration of GS-7340-02 via oral gavage once daily on GD 7 through 20. GS-7340-02 was administered at dose levels of 5, 25, 50, and 100 mg/kg/day as an oral range-finding study (m2.6.7, Section 11, TX-120-2004) or at 10, 30, and 100 mg/kg/day as an embryo-fetal development study (m2.6.7, Section 13.2, TX-120-2005). Blood samples were collected from all animals on GD 7 and 20 predose and approximately 30 minutes and 2, 4, 8, and 24 hours postdose. Tenofovir alafenamide increased with the increase in GS-7340-02 dose level among all groups. The increases in C_{max} and AUC_{0-t} were greater than dose proportional in all dose levels in both studies. Exposure to TFV increased with the increase in GS-7340-02 dose level and the increases in C_{max} and AUC_{0-t} were approximately proportional in all dose levels. No unexpected accumulation of TFV was observed after repeat dosing of GS-7340-02 in rabbits. GS-7340 was extensively converted to TFV in rabbits following oral administration of GS-7340-02.

4.3.3. TFV: Pregnant Monkeys

Placental transfer of TFV following subcutaneous administration to a pregnant rhesus monkey was determined (m1.4.4, 96-DDM-1278-005). One rhesus monkey received daily subcutaneous injections of 30-mg/kg/day TFV, beginning at Day 111 of gestation. Maternal and fetal blood samples were drawn at Days 115, 127, 134, 140, and 151 of gestation. Placental transfer of TFV appeared to be significant with a fetal/maternal serum concentration ratio of 0.17 ± 0.07 (mean ± SD) at approximately 30 minutes postdose.

4.3.4. TFV: Lactating Monkeys

The pharmacokinetic parameters of TFV were investigated in 2 healthy adult lactating rhesus monkeys which were administered a single 30-mg/kg subcutaneous dose of TFV (m1.4.4, P2000116). Following dosing, serum TFV C_{max} values of 18.3 and 30.2 μg /mL were observed in the 2 monkeys. Absorption was rapid, with t_{max} occurring at 0.5 hour. As observed in other species, elimination was biphasic, with apparent half-lives of 3.97 and 2.85 hours for the 2 animals. While this appears shorter than the approximately 9-hour terminal half-life observed in male and nonlactating female monkeys (m1.4.4, P2000031) it may have resulted from the more limited period of sampling (24 vs. 48 hours) in the present experiment.

Serum $AUC_{0-\infty}$ values in this study were 68.9 and 56.2 μg·h/mL for the 2 animals. In comparison to AUC values obtained following 30 mg/kg IV doses of TFV (P2000031), these data suggest essentially complete absorption of TFV after subcutaneous administration.

4.3.5. TFV: Immature monkeys

The pharmacokinetic parameters of TFV have been determined in infant rhesus monkeys following subcutaneous administration (m1.4.4, 96-DDM-1278-005). Tenofovir was formulated as an aqueous solution and was evaluated in monkeys in 4 age groups (newborn, 1, 3, and 12 months old; n = 2 per group). Tenofovir was administered as a 30-mg/kg injection into the dorsal subcutis region. Plasma samples were obtained over the course of 24 hours and concentrations of TFV were determined by HPLC following fluorescence derivatization. The mean TFV C_{max} values in newborn, 1, 3, and 12-month-old monkeys were 51.8, 30.7, 34.6, and 18.8 μg/mL, respectively; with a t_{max} of 0.5 hour for all age groups. The corresponding plasma clearance (CL/F) of TFV was 0.18, 0.54, 0.41, and 1.02 L/h/kg, respectively, showing an increase in the clearance from birth through 1 year. These results suggest that, at an equivalent dose, younger monkeys received greater TFV exposure. The clearance of TFV was dependent on both the weight and the age of the infant monkeys. It is likely that newborn monkeys lack the anion transport system responsible for tubular secretion of TFV.

4.4. E/C/F/TAF: Distribution

Drug interactions, within the 4-drug combination, that affect distribution would not be expected from the data available. Although plasma protein binding is high for EVG and moderate for COBI and TAF, the binding is very low for FTC and TFV. As a result, interactions through binding displacement would not be anticipated. An in vivo study with [^{14}C]EVG and codosed

RTV revealed no change in the tissue distribution of EVG, and notably no increase in central nervous system penetration of EVG. Because COBI displays transporter inhibition potencies similar to, or weaker than RTV, COBI would not be expected to affect the tissue distribution of the other agents.

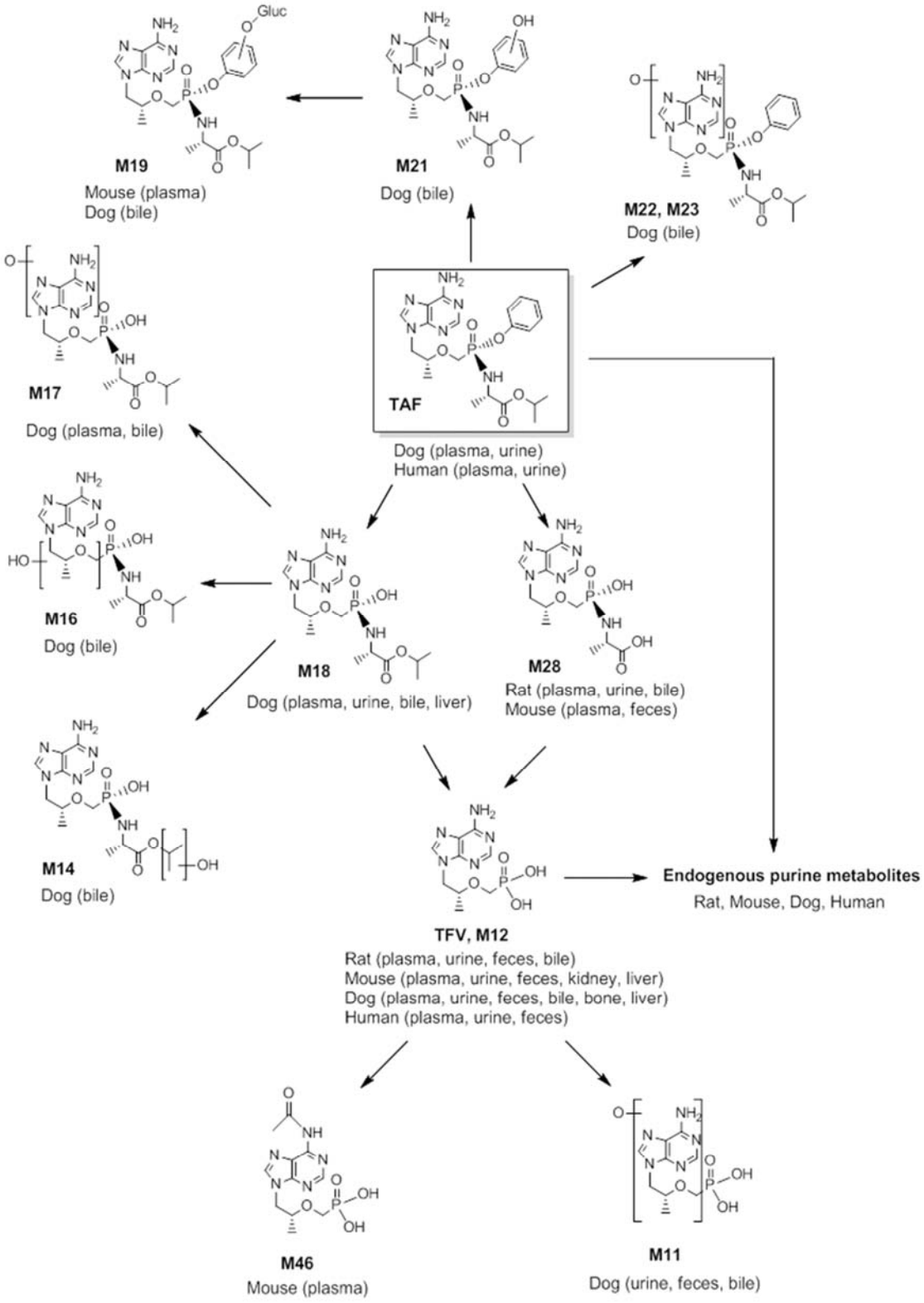
5. METABOLISM

5.1. Proposed Metabolic Pathways

5.1.1. TAF

The metabolic profiles of TAF were determined in plasma, urine, feces, kidney, liver, and nasal turbinate from mice (m2.6.5, Section 10.1, AD-120-2012), in plasma, urine, bile, and feces from rats (m2.6.5, Section 10.2, AD-120-2021), and in plasma, urine, bile, feces, bone, and liver from dogs (m2.6.5, Section 10.3, AD-120-2008). The metabolite profiles were also determined in human plasma, urine, and feces following administration of a single oral dose of [^{14}C]TAF (GS-US-120-0109). Based on the results from mouse, rat, dog, and human, a proposed biotransformation pathway is summarized (Figure 4). Tenofovir alafenamide is also subject to intracellular metabolism to TFV, which is further phosphorylated to the anabolites, tenofovir-monophosphate and TFV-DP (see Table 1) with TFV-DP being the pharmacologically active form.

Figure 4. Metabolites of TAF



5.2. Metabolism In Vitro

5.2.1. TAF and TFV: Metabolism In Vitro

Stability of TAF was assessed in plasma, intestinal S9, and hepatic S9 fractions from dogs and humans (m2.6.5, Section 8, AD-120-2023; AD-120-2024; AD-120-2025; and m2.6.5, Section 14.2.5, AD-120-2027). Tenofovir alafenamide was moderately stable in plasma and intestinal S9 with half-lives of 74.7 and 58.3 minutes for human and 69.5 and 47.1 minutes for dog, respectively (m2.6.5, Section 8.1, AD-120-2025 and m2.6.5, Section 8.3, AD-120-2024). The stability of TAF in human intestinal S9 fractions was also determined in a separate study assessing the effect of HIV-PIs on TAF stability in intestinal S9 (discussed in Section 5.2.1) and a somewhat lower but similar half-life for TAF was observed (24.5 minutes; AD-120-2027). Relative to plasma or intestinal S9, TAF was somewhat less stable in human and dog hepatic S9 fractions with half-lives of 20.6 and 31.1 minutes, respectively (Table 10). Based on these data, predicted hepatic extraction ratios for human and dog were calculated to be 76.2% and 60.5%, respectively (m2.6.5, Section 8.2, AD-120-2023).

Table 10. Stability of TAF in Biological Matricies from Dog and Human

	TAF Stability, $t_{½}$ (min)		
Species	**Plasma**	**Intestinal S9**	**Hepatic S9**
Human	74.7	24.5-58.3	20.6
Beagle Dog	69.5	47.1	31.1

The potential for CYP enzymes to metabolize TAF was assessed by incubating TAF with 6 individual bacterially expressed human CYP enzyme preparations (Bactosomes) coexpressed with human NADPH CYP reductase (m2.6.5, Section 11.3, AD-120-2004). Metabolism of TAF was not detected by CYP1A2, CYP2C8, CYP2C9, CYP2C19 or CYP 2D6. Tenofovir alafenamide was slowly metabolized by CYP3A4 at a rate of 1.9 min^{-1} which was 26.6% of the positive control, testosterone.

Intracellular metabolic activation of TAF in PBMCs or other lymphatic tissues involves conversion to TFV by CatA (m2.6.2, Section 2.1.1) {10427}, {13119}. In contrast to PBMCs, TAF was primarily hydrolyzed by carboxylesterase 1 (CES1) in primary hepatocytes as described in Section 7.1.2. Tenofovir is then further phosphorylated to TFV-DP by cellular nucleotide kinases. These steps are high capacity and low affinity and are not readily inhibited by other xenobiotics.

The in vitro activation of TAF in human primary hepatocytes was evaluated and compared with that of TDF and TFV (m2.6.5, Section 8.4, AD-120-2017). Following a 24-hour continuous incubation of primary hepatocytes with 5 μM TAF, TDF, or TFV, the levels of GS-77938 increased to 1,470, 302, and 12.1 pmol/million cells illustrating that incubation with TAF resulted in 5- and 120-fold higher intracellular levels of GS-77938 compared to TDF and TFV, respectively.

The in vitro metabolism of [^{14}C]TFV was studied in dog plasma, in control and induced (Aroclor™ 1254) rat liver microsomes, and also in dog liver and intestinal S9 fractions (m1.4.4, 96-DDM-1278-003). Potential isomerization of TFV was determined using a chiral HPLC assay with radioactive flow detection. Radioactivity associated with the protein pellet was also determined by sample oxidation and liquid scintillation (< 0.1% of radioactivity). Tenofovir was recovered unchanged under all conditions: no metabolites were detected in either rat microsomal preparation, with or without the addition of NADPH cofactor. There was no evidence of chiral inversion. Similarly, there was no apparent loss of TFV following incubation with dog plasma, liver, or intestinal S9 fractions, and no metabolites were detected.

5.3. Metabolism In Vivo

5.3.1. TAF and TFV: Metabolism In Vivo

Based on the studies from mouse, rat, dog and human (m2.6.5, Section 10, AD-120-2008; AD-120-2012; AD-120-2021; m5.3.3.1, GS-US-120-0109), relative quantification of TAF metabolites in plasma as percent total AUC and in urine, feces, and bile as percent total dose recovered in different species is summarized in Table 11. 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 (m2.6.5, Section 10.1, 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 (m2.6.5, Section 10.3, 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}. It is possible that M28 may be formed transiently at low levels. M18 was detected as a minor metabolite in plasma, urine, and liver. Relatively high levels of M18 were observed in bile. Low levels of M28 were observed in rat and mouse plasma with relatively high levels in rat bile.

Table 11. Relative quantification of TAF Metacolites in Plasma, Urine, Feces, and Bile as % Total Dose

		Plasma[a]	Urine	Feces	Bile
Mouse	TFV (M12)	54.8	18.1	30.7	NA[b]
	M28	1.02	0	0.7	NA
	Allantoin (M27A)	12.2	2.6	0.4	NA
	Uric acid (M27B)	19.4	0	0	NA
	Unknown metabolites	12.3	3.2	0	NA
	Total	100	23.9	31.8	NA
Rat	TFV (M12)	66.7	17.1	63.6	NA
	M28	5.8	0	0	NA
	Allantoin (M27A)	23.2	0.1	0	NA
	Unknown metabolites	4.3	1.6	0	NA
	Total	100	18.8	63.3	NA
Rat (BDC[c])	TFV (M12)	NA	17.1	61.7	0.66
	M28	NA	0.4	0	1.17
	Allantoin (M27A)	NA	0.2	0	0
	Uric acid (M27B)	NA	0	0	0.02
	Unknown metabolites	NA	1.7	0	0
	Total	NA	19.4	61.7	1.85
Dog	TAF	1.3	1.3	0	NA
	TFV (M12)	68.3	24.2	20.8	NA
	M11	0	0.4	0.4	NA
	M17	0.44	0	0	NA
	M18	17.6	0.2	0	NA
	M20	0.2	0	0	NA
	Unknown metabolites	12.2	3.0	0.2	NA
	Total	100	29.1	21.4	NA
Dog (BDC)	TAF	NA	1.3	0	0.2
	TFV (M12)	NA	16.8	26.4	1.0
	M11	NA	0.4	0.7	0.1
	M14	NA	0	0	0.2
	M16	NA	0	0	4.4
	M17	NA	0	0	0.5
	M18	NA	0	0	3.4
	M19	NA	0	0	0.1

		Plasma[a]	Urine	Feces	Bile
	M21	NA	0	0	0.4
	M22	NA	0	0	0.2
	M23	NA	0.2	0	0.2
	Unknown metabolites	NA	2.9	0	0.9
	Total	NA	21.6	27.1	11.6
Human	TAF	1.8	1.41	0	NA
	TFV (M12)	1.5	22.2	31.4	NA
	Uric acid (M27B)	73.9	1.93	0	NA
	Xanthine/hypoxantine (M7.M8)	0	0.26	0	NA
	Unknown metabolites	22.8	0	0.29	NA
	Total	100	25.8	31.7	NA

a Plasma data represent % of total AUC.
b NA = not applicable
c BDC = bile duct cannulated

5.3.1.1. TFV: Monkeys

The kinetics of intracellular TFV anabolism in PBMCs, red blood cells (RBCs), and lymph nodes were studied in monkeys that received a single dose of either 15, 30, or 60 mg/kg of [^{14}C]TFV subcutaneously (m2.7.2, Section 4.1.1.1 [P2001025]). Tenofovir was efficiently taken up by PBMCs, with intracellular concentrations of the active antiviral anabolite reaching 1.6 μM (60 mg/kg dose group). The half-life of TFV-DP in this experiment was > 50 hours. Similar concentrations of TFV anabolites were observed in RBCs. Significant intracellular concentrations of TFV and its anabolites were observed in axillary, inguinal, and mesenteric lymph nodes. This long intracellular half-life of the active diphosphate form observed both in vitro and in vivo supports the proposed once daily clinical dosing regimen.

5.4. E/C/F/TAF: Metabolism

Emtricitabine does not interact with drug metabolizing enzymes as a substrate, inhibitor, or inducer while TAF is a weak substrate and inhibitor of CYP3A. The effect of CYP3A inhibition by TAF should be minimal as COBI is a potent inhibitor of CYP3A. Therefore, metabolic drug interactions between these agents and EVG or COBI are very unlikely. The intended pharmacokinetic drug interaction of inhibition of the CYP3A-dependent metabolism of EVG by COBI has been studied extensively in vitro and in humans in vivo (m2.7.2).

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

FTC to FTC-triphosphate, was not negatively affected by the presence of TFV (m2.7.2, Section 4.1.1.7.2 [PC-164-2002]). Also, because the 2 drugs form analogs of different nucleotides, there should be no competition for incorporation by HIV-1 RT and subsequent chain termination. This was confirmed in vitro in antiviral assays where strong synergy between the 2 compounds was observed (m2.7.2, Section 4.1.3.2.2 [PC-183-2004]). Similarly, because of the highly restricted substrate specificity of the enzymes catalyzing the phosphorylation of FTC and TFV, inhibition of pharmacological activation by EVG or COBI is unlikely and, again, there is no evidence for antagonism in antiviral assays in vitro.

6. EXCRETION

6.1. Route and Extent of Excretion

6.1.1. TAF/TFV

6.1.1.1. Excretion of Radioactivity after Administration of [^{14}C]TAF to Mice

After oral administration of 100 mg/kg [^{14}C]TAF to CD-1 mice, most of the radioactivity was eliminated by 48 hours postdose (m2.6.5, Section 5.1, AD-120-2011). An average of approximately 61% of the radioactive dose was recovered in urine and feces from CD-1 mice through 48 hours postdose. A large amount of radioactivity (average of 6.65% of the dose) was recovered in the cage rinse. An average of 41.3 and 27.7% of the administered radioactivity were excreted in feces and urine, respectively, by 168 hours postdose. An average overall recovery of radioactivity after oral dosing to CD-1 mice was 83.2%.

6.1.1.2. Excretion of Radioactivity after Administration of [^{14}C]TAF or [^{14}C]TFV to Rats

The excretion of [^{14}C]TAF was determined after administration of a single 5-mg/kg oral dose of [^{14}C]TAF to bile duct-intact and BDC male Sprague-Dawley rats (m2.6.5, Section 5.2, AD-120-2020). The results from BDC rats are discussed in Section 6.2.1. Most of radioactivity derived from [^{14}C]TAF was rapidly excreted within 24 hours after oral dosing. The mean values of 71.9 and 22.2% of the administered radioactivity were excreted in feces and urine, respectively, by 168 hours postdose. The mean overall recovery of radioactivity was 96.7%.

The effect of dose on excretion of [^{14}C]TFV was evaluated in Sprague-Dawley rats following IV administration at doses of 10 or 50 mg/kg (400 µCi/kg) (m1.4.4, 96-DDM-1278-001). Following dosing at 10 mg/kg, the mean cumulative recovery in the urine/cage wash was 85.2% ± 7.63% by 24 hours and 92.7% ± 6.77 % by 7 days postdose. The mean terminal elimination half-life calculated from urine data was 15.82 ± 1.79 hours. The mean recovery of the administered dose in the feces was 3.18% ± 1.85% by 24 hours, and 4.48% ± 1.89% by 7 days postdose. Similar results were seen following dosing at 50 mg/kg. Tenofovir was the only species present in the urine and feces; no metabolites were detected. These results indicate that TFV is primarily excreted by renal clearance of the unchanged drug.

6.1.1.3. Excretion of Radioactivity after Administration of [^{14}C]TAF or [^{14}C]TFV to Dogs

The excretion of [^{14}C]TAF was determined after administration of a single 15-mg/kg oral dose of ^{14}C-TAF to bile duct-intact and BDC male dogs (m2.6.5, Section 13.1, AD-120-2007). The results from BDC dogs are discussed in Section 6.2.1. Radioactivity derived from [^{14}C]TAF was readily excreted mostly within 48 hours after oral dosing. The mean values of 37.4% and 35.9% of the administered radioactivity were excreted in feces and urine, respectively, by 168 hours postdose. Overall mean recovery of radioactivity was 80.4%.

Tenofovir excretion was also evaluated following IV administration of [^{14}C]TFV (m1.4.4, 96-DDM-1278-002). The primary route of elimination was via the kidneys, as 70.03% of the

total radioactive dose was recovered in the urine during the first 48 hours following dosing. Total fecal recovery of radioactivity was 0.42% of the total dose.

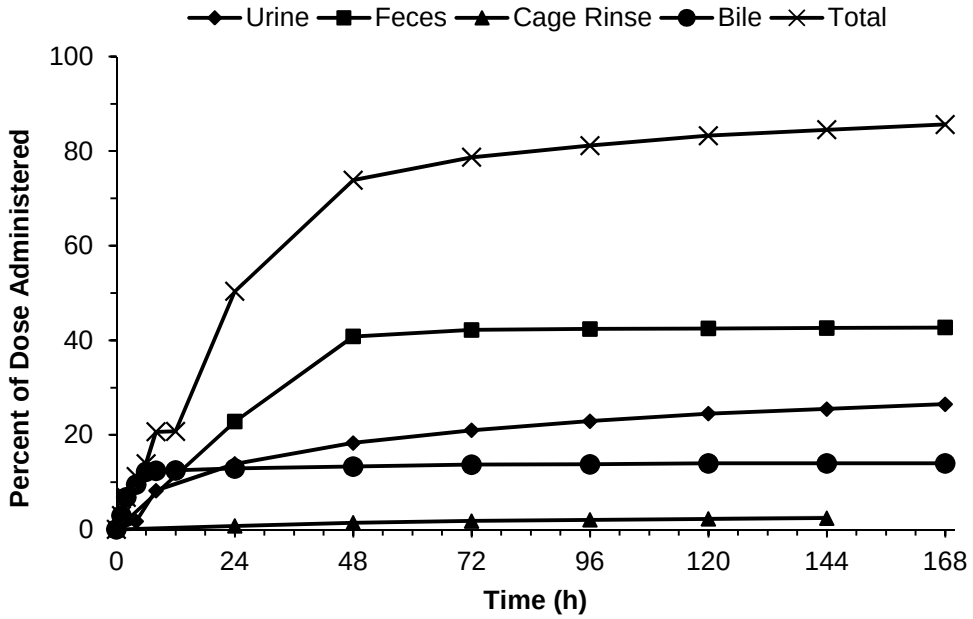
6.2. Excretion into Bile

6.2.1. TAF and TFV: Excretion into Rat and Dog Bile

The excretion of [^{14}C]TAF was determined after administration of a single 5-mg/kg oral dose of [^{14}C]TAF to BDC male Sprague-Dawley rats (m2.6.5, Section 5.2, AD-120-2020). Mean values of 72.6%, 23.2%, and 2.11% of the administered radioactivity were excreted in feces, urine, and bile, respectively, by 168 hours postdose. Recoveries of radioactivity in bile and urine from BDC rats indicated that at least 25% of the dose was absorbed. The mean overall recovery of radioactivity after oral dosing to BDC rats was 99.9%.

The excretion of [^{14}C]TAF was determined following oral administration of a single 15-mg/kg dose of [^{14}C]TAF to male BDC dogs (m2.6.5, Section 13.1, AD-120-2007). Mean values of 42.7%, 26.5%, and 14.0% of the administered radioactivity were excreted in feces, urine, and bile, respectively, through 168 hours postdose (Figure 5). Based on the radioactivity excreted in urine and bile, a minimum of approximately 41% of the orally administered dose was absorbed. The elimination of a large amount of radioactivity in bile of BDC dogs indicates that biliary excretion is a major route of elimination of [^{14}C]TAF-derived radioactivity in dogs. The overall recovery of radioactivity in BDC dogs was 86.2%. Radioactivity was measurable in urine and feces at 168 hours postdose, indicating low recoveries were probably due to radioactivity retained in the carcasses.

Figure 5. Cumulative Percent Total Radioactive Dose Recovered in Urine, Feces, Bile, and Cage Rinse Following Oral Administration of [^{14}C]TAF at 5 mg/kg to Bile Duct-Cannulated Dogs



The extent of biliary excretion of radioactivity following a single IV administration of 10 mg/kg [^{14}C]TFV to a beagle dog was evaluated (m1.4.4, 96-DDM-1278-002). Total biliary recovery of radioactivity through 48 hours postdose was 0.26% of the total dose.

6.3. Excretion into Milk

6.3.1. TFV: Monkeys

Milk was obtained from 2 lactating adult female rhesus monkeys following a single 30-mg/kg subcutaneous dose of TFV (m1.4.4, P2000116). Both milk and serum samples were collected over time up to 24 hours postdose. Concentrations of TFV in milk reached an apparent maximum at 4 hours for 1 animal and at 1 hour for the second animal. Tenofovir C_{max} in milk was 4.04% and 2.02% of the observed C_{max} in plasma for the 2 animals, respectively, and declined with apparent half-lives of 10.3 and 10.9 hours. The tenofovir $AUC_{0-\infty}$ in milk was 18.6% and 21.5% of the observed $AUC_{0-\infty}$ in plasma for the 2 animals, respectively.

6.4. E/C/F/TAF: Excretion

Since FTC and TFV are almost exclusively eliminated by renal excretion, while very little EVG or COBI is excreted in the urine, interactions between the compounds during excretion are unlikely. Cobicistat has also been shown to have no inhibitory effect on OAT1 and only weak inhibition of MRP4, the transporters responsible for renal excretion of TFV.

7. PHARMACOKINETIC DRUG INTERACTIONS

Discussions of drug interaction liability are made by reference to current industry and US and European regulatory guidelines {11006}, {15555}, {18022}, {18670}.

7.1. TAF and TFV: Pharmacokinetic Drug Interactions

The pharmacokinetic profiles of TAF and TFV following oral administration of the E/C/F/TAF FDC are described in Table 12. The unbound C_{max} of TAF was calculated based on the percent unbound value of 20% obtained from multiple human ex vivo studies which should be more clinically relevant than the value determined in vitro, which had somewhat higher percent unbound TAF.

Table 12. Steady State Pharmacokinetic Parameters for TAF and TFV

	TAF	TFV
Dose (mg)	10	-
Total C_{max} (μM)	0.34	0.053
Unbound C_{max} (μM)	0.068	0.053
Intestinal (μM)	84	-

Source: m2.7.2, Section 3.2.1.3.1 [TAF Population PK Report]

The potential of TAF to be a victim or perpetrator of drug-drug interactions was assessed in various in vitro systems. The potential of TAF or its metabolites to inhibit or induce CYP enzymes and serve as substrates or inhibitors of xenobiotic transporters was assessed. The effect of other drugs, including other antiviral agents that may be coadministered with TAF, on intestinal stability and the absorption potential was also determined.

7.1.1. TAF and TFV: Cytochrome P450 and UGT1A1 Inhibition

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 (m2.6.5, Section 11.1, 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_{50}) values calculated for CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, and CYP2D6 were greater than 25 μM. Tenofovir alafenamide weakly inhibited CYP3A-mediated oxidation of midazolam or testosterone with IC_{50} of 7.6 or 7.4 μM, respectively. 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 a TAF concentration of 50 μM (m2.6.5, Section 11.2, AD-120-2040). There was no evidence for time- or cofactor-

dependent inhibition of any enzyme by GS-7340, with the maximum change in activity of 17.4% with CYP2C8 reletive to control.

Tenofovir alafenamide was assessed for inhibition of estradiol 3-glucuronide formation in insect cell microsomal fractions containing baculovirus-expressed human UGT1A1 (m2.6.5, Section 11.6, AD-120-2006). Tenofovir alafenamide did not inhibit UGT1A1 up to 50 μM (IC_{50} > 50 μM).

7.1.2. TAF: Enzymology of Metabolism

Orally administered TAF undergoes intestinal absorption. During intestinal absorption, TAF may be metabolized by intestinal esterases and/or CYP enzymes. The effects of other HIV-1 PIs and CYP inhibitors on the stability of TAF in intestinal subcellular fractions were determined (m2.6.5, Section 14.2.5, AD-120-2027). Incubation of TAF with the HIV-1 PIs atazanavir or darunavir, or the CYP inhibitors, RTV or COBI did not markedly affect the stability of TAF in intestinal subcellular fractions at concentrations up to 100 μM (Table 13).

Table 13. Stability of TAF in Human Intestinal Subcellular Fraction in the Absence and Presence of Test Compounds

Inhibitor	Concentration (μM)	Intestinal S9 Stability[a] T½ (min)
Vehicle control	0	24.5 ± 4.1
Atazanavir	25	28.9 ± 5.2
	100	38.9 ± 5.3
Darunavir	25	32.2 ± 5.1
	100	30.8 ± 5.0
Ritonavir	25	19.0 ± 2.8
	100	18.9 ± 1.5
Cobicistat	25	30.1 ± 5.1
	100	32.9 ± 4.8
Dichlorvos	500	> 789[b]

a Data are mean ± SD, n = 2 (6 data points per replicate)
b Less than 10% loss of substrate in 120 minutes

To understand the enzymes involved in the activation of TAF in primary human hepatocytes, cells were incubated with TAF together with known CatA inhibitors (approved HCV NS3 inhibitors, telaprevir and boceprevir), CES1 inhibitor (bis-p-nitrophenyl phosphate, BNPP), a CYP3A4 and P-gp inhibitor (COBI), or telaprevir and BNPP together (m2.6.5, Section 14.2.7, AD-120-2031). The metabolism of TAF was inhibited by BNPP in a dose-dependent manner. Little or no effect on TFV-DP formation was observed with telaprevir, boceprevir, or COBI. When telaprevir and BNPP were combined a greater inhibition was seen at higher concentrations of the 2 compounds. These results indicated that TAF is primarily hydrolyzed by CES1 and that

CatA also contributed to hydrolysis in primary human hepatocytes. Several CES1 genetic variants that are associated with the enzyme activity have been identified at very low frequency; G143E (heterozygous: 2%-4% and homozygous: 0.05%) and D260fs (very rare) {26538}, {26712}. In a study of hepatitis B virus-infected subjects, a total of 42/51 (82.4%) patients were genotyped for the rs71647871 (G143E) variant of CES1. All patients were found to carry the reference homozygous genotype, ie, there were no carriers of the minor allele of this variant genotype. In addition, since TAF can be activated by both CES1 and CatA in liver, the genetic polymorphisms causing a marked effect on TAF activation should be extremely rare.

7.1.3. TAF: Assessment of Induction Liability

The induction of CYP, P-gp, and UGT1A1 mRNA and CYP activity by TAF was assessed in cultured human hepatocytes from 3 separate donors treated with 1, 10, and 100 μM TAF once daily for 3 consecutive days (m2.6.5, Section 11.5, AD-120-2032). CYP induction data as mean fold increase in mRNA levels and activity upon treatment with TAF and corresponding positive controls are summarized in Table 14. Due to cytotoxicity, the cell viability was significantly affected at 100 μM TAF and mixed responses to TAF with increased mRNA levels and reduced CYP activities were observed. At noncytotoxic concentrations of TAF (1 and 10 μM), no significant increases in the mRNA levels and the CYP activities were observed. After treatment with 10 μM TAF, the mRNA levels of CYP1A2 and CYP3A4 increased by 3.0- and 8.3-fold which correspond to 3% and 6% of the induction levels observed with the respective positive controls. Therefore, TAF showed little or no potential for CYP induction at clinically relevant concentration (1 μM). No significant induction of P-gp and UGT1A1 mRNA was observed (less than 2-fold).

Table 14. Effect of TAF Treatment on CYP mRNA Levels and Activity in Cultured Human Hepatocytes (mean, n = 3 donors)

	Mean Fold Increase (% Positive Control)					
	mRNA			Activity[c]		
Concentration	CYP1A2	CYP2B6	CYP3A	CYP1A2	CYP2B6	CYP3A
1 μM TAF	1.2 (<1%)	0.95 (<1%)	0.92 (<1%)	1.0 (<1%)	1.1 (<1%)	0.97 (<1%)
10 μM TAF	3.0 (3%)	1.6 (4%)	8.3 (6%)	1.4 (1%)	0.85 (<1%)	0.99 (<1%)
100 μM TAF[a]	6.9 (8%)	2.5 (10%)	44 (36%)	0.84 (<1%)	0.42 (<1%)	0.37 (<1%)
Positive control[b]	72	16	120	28	13	29

a The viability of the hepatocytes was affected at this concentration of TAF and therefore caution should be taken when interpreting the corresponding induction data.
b Positive controls 50 μM omeprazole, 1000 μM phenobarbital, and 10 μM rifampin for CYP1A2, CYP2B6, and CYP3A, respectively.
c Phenacetin, bupropion, and testosterone were used as probe substrates for CYP1A2, 2B6, and 3A, respectively.

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 (m2.6.5, Section 11.4, AD-120-2005). For PXR activation, at 50 μM TAF the extent of activation of PXR was only 23% of the maximal effect of rifampicin and 15 μM TAF

demonstrated activation of < 5% of the maximal induction elicited by rifampicin. Tenofovir alafenamide did not activate AhR up to 50 μM, the highest concentration tested. Therefore, TAF is unlikely to activate either of these human xenobiotic receptors supporting the in vitro induction results in human hepatocytes.

7.1.4. Potential for Transporter-Mediated Drug Interactions with TAF and TFV

The potential for TAF and TFV to inhibit or to act as substrates for drug transporters has been assessed in vitro. Inhibition constants and substrate assessments for tested transporters are presented Table 16 and Table 17 in the following section. Tenofovir alafenamide showed little or no inhibition of the transporter of model substrates by P-gp, BCRP, OAT1, OAT3, and OCT2 (m2.6.5, Section 14.2.2, AD-120-2019 and m2.6.5, Section 14.2.6, AD-120-2036). Weak inhibition of OATP1B1, OATP1B3, BSEP, OCT1, and MATE1 was observed but none of these transporters was inhibited by 50% at 100 μM TAF which is approximately 200-fold over the total maximal plasma concentrations. Therefore, TAF is unlikely to be a perpetrator 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 (m2.6.5, Section 3.1, AD-120-2037 and m2.6.5, Section 14.2.3, AD-120-2013). The effect of CsA on TAF oral bioavailability was also assessed in vivo in dogs (m2.6.5, Section 14.2.8, AD-120-2035). As described in Table 15, plasma pharmacokinetic parameters were determined in dogs following oral administration of TAF at 2 mg/kg to untreated or pretreated animals with 75 mg CsA. The CsA pretreatment increased the plasma exposure to TAF and oral bioavailability by approximately 10-fold, while the pharmacokinetic 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 efficacy by increasing the TFV-DP levels in PBMCs.

Table 15. Mean Plasma Pharmacokinetic Parameters for TAF and TFV Following Oral Administration of TAF to Male Beagle Dogs

	No CsA Pretreatment		Pretreatment with 75 mg CsA	
Parameter	**TAF**	**TFV**	**TAF**	**TFV**
AUC_{0-t} (nM•h)	31.4	1140	312	1330
t_{max} (h)	0.14	0.667	0.24	1.0
C_{max} (nM)	109	342	813	187
$t_{½}$ (h)	0.21	>24	0.15	>24
%F	1.67	NA	16.6	NA

NA = Not applicable

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 genetic polymorphisms that affect the transport activities. Unlike TFV, TAF was 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 human OAT1 (basolateral uptake) and MRP4 (apical efflux) transporters acting in series in proximal tubules (m1.4.4, PC-103-2001; AD-104-2001; AD-104-2002), {2520}, {7299}, {8418}. Human OAT3 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, 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 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; PC-104-2011), {2520}. Cobicistat also shows no detectable inhibition of human OAT1 or OAT3. Similarly, PIs ATV, lopinavir (LPV), and RTV did not exhibit any effect on the active cellular elimination of TFV mediated by the MRP4 efflux pump {8418}, and COBI is a very weak inhibitor of MRP4. The results of in vitro drug interaction studies indicate that PIs or COBI are unlikely to exert any substantial effect on the renal elimination of TFV in general or result in 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.

Tenofovir did not inhibit the activity of human OCT2 or MATE1 (IC_{50} > 300 μM) so TFV is unlikely to cause drug interactions through inhibition of these transporters (m1.4.4, AD-104-2012).

7.2. E/C/F/TAF: Pharmacokinetic Drug Interactions

In addition to the studies described for the individual component of E/C/F/TAF FDC, a number of transporter studies were conducted with the STB components, EVG, COBI, FTC, and TFV. Since TFV is the major circulating metabolite for both TDF and TAF, these results are relevant to evaluate the potential for transporter-mediated drug-drug interactions among these components.

Results from all the transporter substrate and inhibition studies are summarized in Table 16 and Table 17. 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.

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, EVG, or COBI as they show weak or undetectable inhibition of OAT1, OAT3, and MRP4 in vitro. Consistently, transport of TFV by OAT1, OAT, and MRP4 was not meaningfully inhibited by COBI under physiological conditions and clinically relevant concentrations (m1.4.4, PC-236-2008; PC-236-2009). In addition, COBI had no effect on the accumulation of TFV in human renal tissue slices at clinically relevant concentrations (m1.4.4, PC-236-2007). Cobicistat is a weak to moderate inhibitor of OCT2, MATE2-K, and organic cation transporter novel, type 1 (OCTN1), while COBI and EVG are more potent inhibitors of MATE1. However, TAF is not an inhibitor of any of these transporters (m1.4.4, AD-104-2012); therefore, there is low potential to further decrease the activity of the transporters relative to STB. The inhibition of BSEP by COBI is unlikely to be clinically meaningful as the IC_{50} (6.5 μM) is in excess of the total plasma C_{max} of COBI. Cobicistat was found to be a substrate of OCT2 in vitro (m2.6.5, Section 14.1.1, AD-216-2112). However, the OCT2-mediated transport of COBI is unlikely to be affected by other components, EVG, FTC, TAF, and TFV as they are not inhibitors of OCT2.

Cobicistat is a weak inhibitor of intestinal efflux transporters, but high concentrations of COBI in the intestinal lumen, achievable briefly during absorption, may inhibit P-gp and result in increase in TAF exposure. As shown in Table 18, 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 (m2.6.5, Section 14.2.3, AD-120-2013).

Since both EVG and COBI are inhibitors and TAF is a substrate of OATP transporters in vitro, the exposure to TAF may be affected by EVG and COBI via inhibition of hepatic uptake. However, only a modest increase in exposure (not considered clinically relevant) of the OATP substrate, rosuvastatin, was observed when it was codosed with both EVG and COBI (m2.7.2, Section 3.1.3.4.2 [GS-US-216-0123]).

Table 16. Transporter Substrate Assessment of E/C/F/TAF Components

Transporter	Substrate Assessment (y/n)					Module, Section, Report
	EVG	COBI	FTC	TAF	TFV	
MDR1	y	y	n	y	n	m1.4.4, AD-236-2004 m2.6.5, Section 14.2.1, AD-120-2018
BCRP	y	y	n	y	n	m1.4.4, AD-236-2005 m2.6.5, Section 14.2.1, AD-120-2018
OATP1B1	y	y	ND	y	ND	m1.4.4, AD-236-2002 m2.6.5, Section 14.2.4, AD-120-2022
OATP1B3	y	y	ND	y	ND	m1.4.4, AD-236-2002 m2.6.5, Section 14.2.4, AD-120-2022
OAT1	ND	ND	n	n	y	m1.4.4, AD-236-2010; PC-104-2010 m2.6.3, Section 1.2, PC-120-2018
OAT3	ND	ND	y	n	y	m1.4.4, AD-236-2010; PC-103-2001 m2.6.3, Section 1.2, PC-120-2018
OCT1	n	n	ND	n	n	m1.4.4, AD-236-2009; PC-103-2001; m2.6.5, Section 14.2.6, AD-120-2036
OCT2	ND	y	n	ND	n	m2.6.5, Section 14.1.1, AD-216-2112 m1.4.4, PC-103-2001; AD-236-2011
MRP1	ND	ND	ND	ND	n	m1.4.4, PC-104-2014
MRP2	ND	ND	ND	ND	n	m1.4.4, AD-104-2001
MRP3	ND	ND	n	ND	ND	m1.4.4, AD-236-2013
MRP4	ND	ND	ND	ND	y	m1.4.4, AD-104-2001

BCRP = breast cancer resistance protein; MDR1 = multidrug resistance protein 1; 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; y = yes

Table 17. Transporter Inhibition Assessment of E/C/F/TAF Components

Transporter	IC_{50} (μM)					Module, Section, Report
	EVG	COBI	FTC	TAF	TFV	
MDR1	69.7	36	>100	>100	>1000	m1.4.4, AD-236-2003; AD-216-2030 m2.6.5, Section 14.2.2, AD-120-2019
BCRP	88.9	59	>100	>100	>100	m1.4.4, AD-236-2003; AD-216-2099 m2.6.5, Section 14.2.2, AD-120-2019
BSEP	>20	6.5	>100	>100	>100	m1.4.4, AD-236-2008 m2.6.5, Section 14.2.6, AD-120-2036
OATP1B1	>2	3.50	>100	>100	>100	m1.4.4, AD-183-2030; AD-216-2100; AD-236-2006 m2.6.5, Section 14.2.2, AD-120-2019
OATP1B3	0.44	1.88	>100	>100	>100	m1.4.4, AD-183-2030; AD-216-2100; AD-236-2006 m2.6.5, Section 14.2.2, AD-120-2019
MATE1	2.0	1.87	>100	>100	>300	m1.4.4, AD-236-2001; AD-104-2012 m2.6.5, Section 14.2.6, AD-120-2036
MATE2-K	ND	33.5	ND	ND	ND	m1.4.4, AD-216-2094
OAT1	>20	>100	>100	>100	33.8[a]	m1.4.4, AD-236-2007; AD-216-2105 m2.6.5, Section 14.2.6, AD-120-2036
OAT3	>20	>100	>100	>100	>1000	m1.4.4, AD-236-2007; AD-216-2105; PC-103-2001 m2.6.5, Section 14.2.6, AD-120-2036
OCT1	>20	14.7	>100	>100	>100	m1.4.4, AD-236-2008; PC-103-2001 m2.6.5, Section 14.2.6, AD-120-2036
OCT2	>20	14.4	>100	>100	>300	m1.4.4, AD-236-2001 m2.6.5, Section 14.2.6, AD-120-2036
OCTN1	ND	2.49	ND	ND	ND	m1.4.4, AD-216-2098
MRP1	ND	45-90	ND	ND	>500	m1.4.4, AD-216-2030; PC-104-2014
MRP2	>20	45-90	>100	ND	>100	m1.4.4, AD-236-2012; AD-216-2030 AD-104-2001
MRP4	>20	20.7	>100	ND	>1000[b]	m1.4.4, AD-236-2007; AD-216-2105

BCRP = breast cancer resistance protein; BSEP = bile salt excretory pump; MATE1 or 2-K = multidrug and toxin extrusion protein 1 or 2-K; MDR1 = multidrug resistance protein 1; 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

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

b Imaoka et al 2007{10260}

Table 18. Effect of COBI on the Bidirectional Permeability of TAF in Caco-2 Cells

Inhibitor	Direction	TAF Initial Concentration (μM)	Recovery (%)	P_{app} ($x10^{-6}$ cm/s)	Efflux Ratio
None	Cell-Free	9.61	119	30.8	—
	Forward	9.92	64	0.74	20
	Reverse	8.71	102	15.1	
COBI (90 μM)	Forward	11.0	101	3.4	1.6
	Reverse	11.4	115	4.9	

For the E/C/F/TAF FDC, the clinical pharmacokinetic interaction studies with the components should be given the greatest consideration. Complete details are described in m2.5 and m2.7.2.

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 EVG or COBI. Tenofovir alafenamide was a weak inhibitor of CYP3A but the TAF-mediated CYP3A inhibition is unlikely to play any role since COBI is a specific and potent inhibitor of CYP3A. As described above (Section 5.4), TFV and FTC do not inhibit each other's pharmacological activation through phosphorylation.

Thus, the intended positive drug interaction within the 4-drug combination is the pharmacokinetic enhancement of EVG by COBI, due to inhibition of the oxidative metabolism of EVG.

8. OTHER PHARMACOKINETIC STUDIES

There are no additional studies to report.

9. DISCUSSION AND CONCLUSIONS

Tenofovir alafenamide has been assessed individually in comprehensive nonclinical absorption, distribution, metabolism and excretion studies. Results from these studies are summarized below.

9.1. TAF

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}. Following a 15-mg/kg oral dose of [^{14}C]TAF to BDC dog, the fraction absorbed was at least 41% based on excretion in urine and bile. Therefore, hepatic extraction was calculated to be approximately 65%, which was consistent with that estimated from the in vitro stability study in dog hepatic S9 fractions (60.5%). A summary of the pharmacokinetic parameters obtained with TFV is presented in Table 19.

Following oral administration of [^{14}C]TAF to mouse, rat, and dog, [^{14}C]TAF-derived radioactivity was widely distributed to most of the tissues in all species. Consistent with high hepatic extraction, high levels of radioactivity were observed in the liver; high levels of radioactivity were also measured in the kidney. Low levels of radioactivity were observed in brain and testis in mouse. Distribution trends in the pigmented uveal tract of the eye and pigmented skin suggested that [^{14}C]TAF-related radioactivity was not selectively associated with melanin-containing tissues in the pigmented mouse. No melanin binding was observed in rats.

The biotransformation of TAF was studied in mice, rats, and dogs and compared with that in humans. Endogenous purine metabolites including hypoxanthine, xanthine, allantoin, and uric acid were observed in all species including humans. 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. No metabolites unique to human were observed. In addition, none of the intermediate metabolites formed during intracellular conversion of TAF to TFV (eg, M18 [isopropylalaninyl TFV] and M28 [alaninyl TFV]), were observed in humans. The major enzymes involved in intracellular conversion of TAF to TFV in PBMCs and primary human hepatocytes are CatA and CES1, respectively. Tenofovir is further phosphorylated to pharmacologically active TFV-DP by cellular nucleotide kinases. These steps are usually high capacity and low affinity and are not readily inhibited by other xenobiotics.

Following oral dosing of mice, rats, and dogs with [^{14}C]TAF, the majority of radiolabel is recovered in the feces or urine in all species. The elimination of a large amount of radioactivity in bile of BDC dogs indicates that biliary excretion is a major route of elimination of [^{14}C]TAF-derived radioactivity in dogs. Total recovery of radiolabel is high for all species.

Tenofovir alafenamide is not an inhibitor or an inducer of most of CYP enzymes and UGT1A1; however, it is a weak inhibitor of CYP3A. At clinically relevant concentrations, TAF is unlikely to affect hepatic CYP3A activity. While CYP3A activity may be affected in the intestine, where

high levels of TAF can be achieved, the exposure to TAF in intestine should be transient and the potential for significant drug interaction is unlikely. In addition, since TAF is intended to be used in combination with COBI, a potent and specific CYP3A inhibitor, the effect caused by TAF, if any, should be minimal. 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/or 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 suggesting that TAF is not contributing to renal tubular cell loading of TFV; as a result, intracellular TFV concentrations in renal cells are likely to correlate with plasma TFV levels, which are lower following the administration of TAF than of TDF.

9.2. E/C/F/TAF

Based on the clinical data with EVG, COBI, FTC, and TAF in Phase 2 and 3 studies, 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. Pharmacokinetic enhancement of EVG exposure by COBI has been studied in vitro and in humans in vivo. A modest increase in TAF absorption, due to inhibition of intestinal P-gp by COBI, is observed in vitro. Since TAF was found to be a substrate for OATP1B1 and OATP1B3, TAF exposure may be affected by EVG and COBI which can inhibit OATP transporters in vitro. However, the combination of codosed EVG and COBI has only a modest effect on the exposure of the OATP substrate, rosuvastatin. 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. Pharmacological activation of FTC and TFV is by phosphorylation by enzymes with highly restricted substrate specificities, so inhibition by EVG or COBI is very unlikely. This is supported by antiviral assays where no evidence for antagonistic interactions was observed. In conclusion, the primary drug interaction within the 4-drug combination, predicted in vitro and confirmed in vivo, is the intended enhancement of the pharmacokinetic profile of EVG due to inhibition of its CYP3A-dependent metabolism by COBI. The pharmacokinetic assessment of EVG, COBI, FTC, and TAF supports the safe use of these agents in combination.

Table 19. Comparison of TFV Noncompartmental Pharmacokinetic Parameters Between Species Following Intravenous Administration of TFV

Species	Rat R2000075		Dog D2000076, 97-DDM-4331-001		Rhesus P4331-00033, P2000031		Human	
Dose (mg/kg)	**10**	**50**	**1**	**10**	**5**	**30**	**1**	**3**
N (sex)	4M	4M	4M	4M	3M/3F	3M/3F	8M	8M
C_{max} (µg/mL)	22.0	162	2.99	31.0	13.8	79.0	2.71	8.52
$AUC_{0-\infty}$ (µg·h/mL)	5.86	53.7	4.48	41.2	5.12	38.4	4.41	15.2
Terminal t½ (h)	4.02	5.41	45.3	38.6	5.37	8.79	5.27	7.80
CL (mL/h/kg)	1706	931	226	244	1031	888	236	229
C_{last} (µg/mL)	0.0309	0.0428	0.0017	0.0103	0.0042	0.0062	0.0426	0.0508
T_{last} (h)	12.0	24.0	120	120	24.0	38.0	12.0	24.0
V_{ss} (mL/kg)	2810	1120	3090	2510	1280	969	763	975

10. REFERENCES

References are available upon request.

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

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

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

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

7299 Cihlar T, Bleasby K, Roy A, Pritchard J. Antiviral acyclic nucleotide analogs tenofovir and adefovir are substrates for human kidney organic anion, but not cation transporters: implications for potential renal drug interactions [poster A-443]. 44th Interscience Conference on Antimicrobial Agents and Chemotherapy; 2004 October 30-November 2; Washington, DC, USA.

8418 Ray AS, Vela JE, Robinson KL, Cihlar T, Rhodes GR. Efflux of Tenofovir by the Multidrug Resistance-Associated Protein 4 (MRP4) is not Affected by HIV Protease Inhibitors [poster]. 7th International Workshop on Adverse Drug Reactions and Lipodystrophy in HIV; 2005 November 13-16; Dublin, Ireland. Poster Number 91.

10260 Imaoka T, Kusuhara H, Adachi M, Schuetz JD, Takeuchi K, Sugiyama Y. Functional involvement of multidrug resistance associated protein 4 (MRP4/ABCC4) in the renal elimination of the anti-viral drugs, adefovir and tenofovir. Mol Pharmacol 2007;71 (2):619-27.

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

11006 Bjornsson TD, Callaghan JT, Einolf HJ, Fischer V, Gan L, Grimm S, et al. The conduct of in vitro and in vivo drug-drug interaction studies: a Pharmaceutical Research and Manufacturers of America (PhRMA) perspective. Drug Metab Dispos 2003;31 (7):815-32.

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

15555 Giacomini KM, Huang SM, Tweedie DJ, Benet LZ, Brouwer KL, Chu X, et al. Membrane transporters in drug development. Nat Rev Drug Discov 2010;9 (3):215-36.

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

18022 Industry GF. Drug interaction studies - study design, data analysis, and implications for dosing and labeling. US department of health and human services 2006:1-55.

18670 European Medicines Agency (EMEA). Committee for for Human Medicinal Products (CHMP). Guideline on the investigation of drug interactions [EMA/CHMP/EWP/125211/2010]. April 22, 2010.

23907 Babusis D, Phan TK, Lee WA, Watkins WJ, Ray AS. Mechanism for Effective Lymphoid Cell and Tissue Loading Following Oral Administration of Nucleotide Prodrug GS-7340. Mol Pharm 2013;10 (2):459-66.

26538 Zhu HJ, Patrick KS, Yuan HJ, Wang JS, Donovan JL, DeVane CL, et al. Two CES1 gene mutations lead to dysfunctional carboxylesterase 1 activity in man: clinical significance and molecular basis. American journal of human genetics 2008;82 (6):1241-8.

26712 Tarkiainen EK, Backman JT, Neuvonen M, Neuvonen PJ, Schwab M, Niemi M. Carboxylesterase 1 polymorphism impairs oseltamivir bioactivation in humans. Clin Pharmacol Ther 2012;92 (1):68-71.

2.6 NONCLINICAL SUMMARY

SECTION 2.6.5—PHARMACOKINETICS TABULATED SUMMARY

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

Gilead Sciences

[redacted] **20**[redacted]

CONFIDENTIAL AND PROPRIETARY INFORMATION

TABLE OF CONTENTS

SECTION 2.6.5—PHARMACOKINETICS TABULATED SUMMARY 1

TABLE OF CONTENTS 2

NOTE TO REVIEWER 4

1. PHARMACOKINETICS: OVERVIEW 5

2. PHARMACOKINETICS: ANALYTICAL METHODS AND VALIDATION REPORTS 10

3. PHARMACOKINETICS: ABSORPTION AFTER A SINGLE DOSE 11

   3.1. AD-120-2037: Caco-2 Permeability of TAF (In Vitro) 11
   3.2. AD-120-2014: Pharmacokinetics of TAF in Mice 12
   3.3. AD-120-2016: Pharmacokinetics of TAF in Mice 13
   3.4. AD-120-2015: Pharmacokinetics of TAF in Rats 14
   3.5. R990130: Pharmacokinetics of TAF in Rats 15
   3.6. R2000065: Pharmacokinetics of TAF in Rats 16
   3.7. 99-DDM-1278-001-PK: Pharmacokinetics of TAF in Dogs 17
   3.8. AD-120-2034: Pharmacokinetics of TAF in Dogs 21
   3.9. P2000087: Pharmacokinetics of TAF in Rhesus Monkeys 22

4. PHARMACOKINETICS: ABSORPTION AFTER REPEATED DOSES 24

   4.1. AD-120-2033: 7-Day Repeated Dose Study of TAF in Dogs – Pharmacokinetics 24
   4.2. D990175-PK: 28-Day Toxicity Study of TAF in Dogs – Toxicokinetics 25
   4.3. P2000114-PK: 28-Day Toxicity Study of TAF in Monkeys – Toxicokinetics 28

5. PHARMACOKINETICS: ORGAN DISTRIBUTION STUDIES 31

   5.1. AD-120-2011: Pharmacokinetics, Absorption, Distribution, and Excretion of [^{14}C]TAF in Mouse Following Oral Administration 31
   5.2. AD-120-2020: Pharmacokinetics, Absorption, Distribution, and Excretion of [^{14}C]TAF in Rat Following Oral Administration 40
   5.3. AD-120-2009: Distribution of [^{14}C]TAF in Dog Following Oral Administration 51
   5.4. D990173-BP: Distribution of [^{14}C]TAF in Dog Following Oral Administration 57

6. PHARMACOKINETICS: PLASMA PROTEIN BINDING 59

   6.1. AD-120-2026: Plasma Protein Binding of TAF In Vitro 59

7. PHARMACOKINETICS: STUDY IN PREGNANT OR NURSING ANIMALS 60

8. PHARMACOKINETICS: METABOLISM IN VITRO 61

   8.1. AD-120-2025: Metabolism of TAF In Vitro (Plasma Stability) 61
   8.2. AD-120-2023: Metabolism of TAF In Vitro in Hepatic S9 62
   8.3. AD-120-2024: Metabolism of TAF In Vitro in Intestine S9 63
   8.4. AD-120-2017: Metabolism of TAF In Vitro in Primary Human Hepatocytes 64

9. PHARMACOKINETICS: POSSIBLE METABOLIC PATHWAYS 65

10. PHARMACOKINETICS: METABOLISM IN VIVO 66

   10.1. AD-120-2012: Metabolism of TAF in Mouse 66
   10.2. AD-120-2021: Metabolism of TAF in Rat 71
   10.3. AD-120-2008: Metabolism of TAF in Dog 74

11. PHARMACOKINETICS: INDUCTION/INHIBITION OF DRUG METABOLIZING ENZYMES 81

   11.1. AD-120-2003: Human Cytochrome P450 Inhibition Potential of TAF 81
   11.2. AD-120-2040: Human CYP Mechanism-Based Inhibition of TAF 82
   11.3. AD-120-2004: Human Cytochrome P450 Metabolic Reaction Phenotyping of TAF 83

11.4. AD-120-2005: Induction of Metabolizing Enzymes by TAF In Vitro 84
11.5. AD-120-2032: Assessment of Induction Potential of TAF in Human Hepatocyte In Vitro 86
11.6. AD-120-2006: In Vitro Assessment of Human UGT1A1 Inhibition Potential of TAF 88

12. PHARMACOKINETICS: EXCRETION 89

13. PHARMACOKINETICS: EXCRETION INTO BILE 90

13.1. AD-120-2007: Excretion of [^{14}C]TAF Following Single oral Administration in Dog 90

14. PHARMACOKINETICS: DRUG-DRUG INTERACTIONS 93

14.1. COBI 93
14.1.1. AD-216-2112: In Vitro Assessment of COBI as a Substrate for Human OCT2 Transporter 93
14.2. TAF 94
14.2.1. AD-120-2018: Bidirectional Permeability of TAF Through Monolayers of P-glycoprotein and BCRP Overexpressing Cells 94
14.2.2. AD-120-2019: In Vitro Assessment of TAF Inhibition of Human OATP1B1, OATP1B3, P-gp, and BCRP 96
14.2.3. AD-120-2013: Effect of GS-9350 on the Bidirectional Permeability of TAF Through Caco-2 Cells 97
14.2.4. AD-120-2022: In Vitro Assessment of TAF as a Substrate for Human OATP1B1 and OATP1B3 98
14.2.5. AD-120-2027: Effects of HIV Protease Inhibitors and Pharmacokinetic Enhancers on TAF Metabolism In Vitro 99
14.2.6. AD-120-2036: In Vitro Assessment of TAF as an Inhibitor of OAT1, OAT3, OCT1, OCT2, MATE1, and BSEP or as a Substrate for OCT1 100
14.2.7. AD-120-2031: Effect of Inhibitors of CatA, CES1, and CYP3A4 on TAF Metabolism In Vitro 103
14.2.8. AD-120-2035: Effect of Cyclosporin A Pretreatment on Pharmacokinetics of TAF in Dogs 104

15. PHARMACOKINETICS: OTHER 107

NOTE TO REVIEWER

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

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

. To assist the reviewer,

Given the lack of remarkable effects in the nonclinical studies with the individual agents, no additional pharmacokinetic studies have been conducted for the E/C/F/TAF combination.

The following conversions are also provided to aid the reviewer:

- COBI (GS-9350) 1 μM = 0.776 μg/mL
- TAF (GS-7340) 1 μM = 0.477 μg/mL
- TFV (GS-1278) 1 μM = 0.287 μg/mL

1. PHARMACOKINETICS: OVERVIEW

Test Article: TAF, COBI

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Gilead Study No. (CRO Study No.)
Analytical Methods and Validation (TAF)					
Mouse plasma	No	NA	In Vitro	[redacted], USA	BA-120-2004
Rat plasma	No	NA	In Vitro	[redacted], USA	BA-120-2003
Rabbit plasma	No	NA	In Vitro	[redacted], USA	BA-120-2005
Dog PBMC	No	NA	In Vitro	[redacted], Canada	993680 MYS (D990175)
Dog, monkey, human plasma	No	NA	In Vitro	[redacted], Canada	TOX-120-002 Appendix 32
Monkey plasma	No	NA	In Vitro	[redacted], Canada	BA-120-2010
Monkey plasma	No	NA	In Vitro	[redacted], Canada	BA-120-2011
Monkey PBMC	No	NA	In Vitro	[redacted], Canada	BA-120-2012
Monkey PBMC	No	NA	In Vitro	[redacted], Canada	BA-120-2013
Monkey plasma	No	NA	In Vitro	[redacted], Canada	010520/PDW
Monkey plasma	No	NA	In Vitro	[redacted], Canada	010521/PHZ
Monkey PBMC	No	NA	In Vitro	[redacted], Canada	AA01240-RQZ (P2000114)

Test Article: TAF, COBI

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Gilead Study No. (CRO Study No.)
Absorption After a Single Dose (TAF)					
Permeability across Caco-2 cell monolayers	No	In Vitro	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	AD-120-2037
Absorption	Yes	Mouse	Oral	[redacted], USA	AD-120-2014
Absorption	No	Mouse	Oral	[redacted], USA	AD-120-2016
Absorption	Yes	Rat	Oral	[redacted], USA	AD-120-2015
Absorption	No	Rat	Oral	Gilead Sciences, Inc., Boulder, CO, USA (In-life phase); Gilead Sciences, Inc., Foster City, CA, USA (Analysis)	R990130
Formulation comparison	No	Rat	Oral	Gilead Sciences, Inc., Boulder, CO, USA (in-life phase); [redacted], Canada (Analysis)	R2000065
Absorption, Bioavailability	No	Dog	IV, Oral	[redacted], USA (In-life phase), Gilead Sciences, Inc., Foster City, CA, USA (Analysis)	99-DDM-1278-001-PK
Absorption	No	Dog	Oral	[redacted], USA (In-life phase), Gilead Sciences, Inc., Foster City, CA, USA (Analysis)	AD-120-2034
Absorption, Bioavailability	No	Rhesus Monkey	Oral	[redacted], USA, (In-life phase), [redacted] Canada (Analysis)	P2000087

Test Article: TAF, COBI

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Gilead Study No. (CRO Study No.)
Absorption After Repeated Dose (TAF)					
Repeated dose pharmacokinetics	No	Dog	Oral	[redacted], USA (In-life phase) Gilead Sciences, Inc., Foster City, CA, USA (Analysis)	AD-120-2033
Toxicokinetics	Yes	Dog	Oral	[redacted] Canada (In-life phase), [redacted], USA (Analysis); [redacted], USA (Analysis); [redacted], Canada (Analysis)	D990175-PK
Toxicokinetics	Yes	Monkey	Oral	[redacted], (In-life phase), [redacted], Canada (Analysis)	P2000114-PK
Distribution (TAF)					
Plasma protein binding in vitro	No	In Vitro	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	AD-120-2026
Tissue distribution of radioactivity	No	Mouse	Oral	[redacted], USA	AD-120-2011
Tissue distribution of radioactivity	No	Rat	Oral	[redacted], USA	AD-120-2020
Absorption	No	Dog	Oral	[redacted], USA	AD-120-2009
Single dose tissue distribution	Yes	Dog	Oral	[redacted], USA	D990173-BP

Test Article: TAF, COBI

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Gilead Study No. (CRO Study No.)
Metabolism (TAF)					
In vitro metabolism, plasma stability	No	In Vitro	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	AD-120-2025
In vitro metabolism, hepatic S9 stability	No	In Vitro	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	AD-120-2023
In vitro metabolism, intestinal S9 stability	No	In Vitro	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	AD-120-2024
Metabolism in vitro	No	In Vitro	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	AD-120-2017
Metabolite identification, mouse	No	Mouse	Oral	[redacted], USA	AD-120-2012
Metabolite identification, rat	No	Rat	Oral	[redacted], USA	AD-120-2021
Metabolite identification, dog	No	Dog	Oral	[redacted], USA	AD-120-2008
Excretion (TAF)					
Absorption and excretion	No	Dog	Oral	[redacted], USA	AD-120-2007
Pharmacokinetic Drug Interactions (COBI)					
Effect on uptake in human OCT2	No	In Vitro	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	AD-216-2112
Pharmacokinetic Drug Interactions (TAF)					
In vitro cytochrome P450 inhibition	No	In Vitro	In Vitro	[redacted], UK	AD-120-2003
In vitro cytochrome P450 mechanism-based inhibition	No	In Vitro	In Vitro	[redacted], UK	AD-120-2040
Permeability across Caco-2 cell monolayers	No	In Vitro	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	AD-120-2013
In Vitro cytochrome P450 phenotyping	No	In Vitro	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	AD-120-2004

Test Article: TAF, COBI

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Gilead Study No. (CRO Study No.)
In Vitro cytochrome P450 induction	No	In Vitro	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	AD-120-2005
Human UGT1A1 inhibition potential	No	In Vitro	In Vitro	[redacted], UK	AD-120-2006
Permeability in P-gp and BCRP overexpressing cells	No	In Vitro	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	AD-120-2018
Inhibition of human OATP1B1, OATP1B3, P-gp, and BCRP	No	In Vitro	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	AD-120-2019
Effect on uptake in human OATP1B1 and OATP1B3	No	In Vitro	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	AD-120-2022
Effect of pharmacoenhancer on intestinal stability	No	In Vitro	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	AD-120-2027
Inhibition of human OAT1, OAT3, OCT1, OCT2. MATE1 and BSEP	No	In Vitro	In Vitro	[redacted], Hungary	AD-120-2036
Effect of cathepsin A, carboxylesterase 1, CYP3A4 on primary human hepatocytes	No	In Vitro	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	AD-120-2031
Human hepatocyte induction potential	No	In Vitro	In Vitro	[redacted], USA	AD-120-2032
Effect of CsA pretreatment on pharmacokinetics	No	Dog	IV, Oral	Gilead Sciences, Inc., Foster City, CA, USA	AD-120-2035

Other Pharmacokinetic Studies: None

BCRP = breast cancer resistance protein; BSEP = bile salt export protein; CsA = cyclosporin A; CYP = cytochrome P450; MATE1 = multidrug and toxin extrusion protein 1; OAT = organic anion transporter; OATP = organic anion transporting polypeptide; OCT = organic cation transporter; PBMC = peripheral blood mononuclear cell; P-gP = human P-glycoprotein; TAF = tenofovir alafenamide; UGT = uridine diphosphate glucuronosyl transferase

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

b Study included tenofovir as the test article

2. PHARMACOKINETICS: ANALYTICAL METHODS AND VALIDATION REPORTS

Test Article: TAF

Type of Study:	**Analytical Method Validation**		
Study No.	**Matrices**	**Analyte**	**Analytical Method**
001092/NGE	Rat Plasma	TFV	LC/MS/MS
R-BA-Tox-120-001	Rat Plasma	TFV	LC/MS/MS
BA-120-2004	Mouse Plasma	TAF, TFV	LC/MS/MS
BA-120-2003	Rat Plasma	TAF, TFV	LC/MS/MS
BA-120-2005	Rabbit Plasma	TAF, TFV	LC/MS/MS
993680 MYS	Dog PBMC	TFV	LC/MS/MS
TOX-120-002 Appendix 32	Dog, Monkey, Human Plasma	TAF, TFV	LC/MS/MS
BA-120-2010	Monkey Plasma	TAF	LC/MS/MS
BA-120-2011	Monkey Plasma	TFV	LC/MS/MS
BA-120-2012	Monkey PBMC	TFV	LC/MS/MS
BA-120-2013	Monkey PBMC	TFV	LC/MS/MS
010520/PDW	Monkey Plasma	TFV	LC/MS/MS
010521/PHZ	Monkey Plasma	TAF	LC/MS/MS
AA01240-RQZ	Monkey PBMC	TFV	LC/MS/MS

LC/MS/MS = high performance liquid chromatography coupled to tandem mass spectrometry; PBMC = peripheral blood mononuclear cell; TAF = tenofovir alafenamide; TFV = tenofovir

3. PHARMACOKINETICS: ABSORPTION AFTER A SINGLE DOSE

3.1. AD-120-2037: Caco-2 Permeability of TAF (In Vitro)

Report Title	Study Type	Test Article	Report Number
Concentration Dependent Permeability of Tenofovir Alafenamide through Caco-2 Cell Monolayers	Absorption (in vitro)	TAF	AD-120-2037

Bidirectional Permeability of TAF Through Caco-2 Cells

Inhibitor	Direction	Target Concentration (μM)	Initial Conc. (μM)	Recovery (%)	P_{app} (10^{-6} cm/s) Average	Efflux Ratio
–	Cell-Free	10	11.3	106	42.9	20.2
	Forward		10.9	42.7	0.34	
	Reverse		10.0	85.3	6.98	
–	Cell-Free	100	104	112	53.2	13.6
	Forward		92.0	40.4	0.63	
	Reverse		98.1	85.2	8.47	
–	Cell-Free	1000	969	109	46.1	6.28
	Forward		933	77.4	1.08	
	Reverse		1038	88.8	5.86	
CsA	Cell-Free	10	9.85	113	43.5	1.00
	Forward		10.4	42.2	1.51	
	Reverse		10.8	70.5	1.34	

Caco-2 = human colonic adenocarcinoma cell line; TAF = tenofovir alafenamide; P_{app} = apparent permeability; CsA = cyclosporine A

3.2. AD-120-2014: Pharmacokinetics of TAF in Mice

Report Title	Study Type	Test Article	Report Number
Collection of Samples for Determination of the Pharmacokinetics of GS-7340-02 and GS-7340-03 After a Single Oral Dose to Mice	Absorption	TAF	AD-120-2014

Species	CD-1 mice
Feeding Condition	Not fasted
Vehicle Formulation	0.1% (v/v) tween 20 and 0.1% (w/v) hydroxypropylmethylcellulose (HPMC) K100LV prepared in reverse osmosis water
Method of Administration	Oral gavage
Sample	Plasma
Assay	LC/MS/MS

Test Article	GS-7340-02						GS-7340-03					
Sex (M/F) / N of Animals	M/36						M/36					
Dose (mg/kg)	10		30		100		10		30		100	
Analyte	TAF	TFV	TAF	TFV	TAF	TFV	TAF	TFV	TAF	TFV	TAF	TFV
PK Parameters												
t_{max} (h)	0.08	0.50	NA	0.25	0.08	0.75	NA	0.50	4.00	0.50	0.25	1.50
C_{max} (ng/mL)	5.53	106	NA	440	37.1	1827	NA	85.4	10.3	383	34.7	2152
$t_{½}$ (h)	NA	NA	NA	NA	NA	NA	NA	5.16	NA	10.1	NA	NA
AUC_{0-t} (ng•h/mL)	NA	455	NA	2005	26.0	10643	NA	493	NA	2477	11.3	10866
t_{last} (h)	NA	12.0	NA	24.0	1.50	24.0	NA	12.0	NA	24.0	0.50	24.0
C_{last} (ng/mL)	NA	21.6	NA	35.9	12.8	157	NA	20.5	NA	34.4	24.9	205

F = female; M = male; NA = not applicable; TAF = tenofovir alafenamide; TFV = tenofovir

3.3. AD-120-2016: Pharmacokinetics of TAF in Mice

Report Title	Study Type	Test Article	Report Number
Collection of Samples for Determination of the Pharmacokinetics of GS-7340-03 After a Single Oral Gavage Dose to Male and Female 001178-W (wild type) Mice	Absorption	TAF	AD-120-2016

Species	Model 001178-W [Wild, CByB6F1-Tg(HRAS)2Jic] mice
Feeding Condition	Not fasted
Vehicle Formulation	0.1:0.1:99.8 (w/w/w) hydroxypropyl methylcellulose K100LV (HPMC)/polysorbate (tween) 20/reverse osmosis water
Method of Administration	Oral gavage
Sample	Plasma
Assay	LC/MS/MS

Test Article	GS-7340-03											
Sex (M/F) / N of Animals	M/44		F/44		M/44		F/44		M/44		F/44	
Dose (mg/kg)	10				30				100			
Analyte	TAF	TFV	TAF	TFV	TAF	TFV	TAF	TFV	TAF	TFV	TAF	TFV
PK Parameters												
t_{max} (h)	NA	0.25	NA	0.50	0.08	0.25	0.50	0.25	0.25	0.50	0.50	0.50
C_{max} (ng/mL)	NA	175	NA	100	8.80	615	117	421	648	1988	280	1733
$t_{½}$ (h)	NA	9.78	NA	8.20	NA	9.51	NA	10.9	NA	8.04	NA	11.0
AUC_{0-t} (ng•h/mL)	NA	735	NA	354	NA	2639	NA	2053	194	10026	104	7131
t_{last} (h)	NA	24.0	NA	12.0	0.25	24.0	NA	24.0	0.50	24.0	0.50	24.0
C_{last} (ng/mL)	NA	13.5	NA	16.5	5.40	31.1	NA	36.3	61.4	99.9	280	113

F = female; M = male; NA = not applicable; TAF = tenofovir alafenamide; TFV = tenofovir

3.4. AD-120-2015: Pharmacokinetics of TAF in Rats

Report Title	Study Type	Test Article	Report Number
Collection of Samples for Determination of the Pharmacokinetics of GS-7340-02 and GS-7340-03 After a Single Oral Dose to Rats	Absorption	TAF	AD-120-2015

Species	SD rats
Feeding Condition	Not fasted
Vehicle Formulation	0.1% (v/v) tween 20 and 0.1% (w/v) hydroxypropylmethylcellulose (HPMC) K100LV prepared in reverse osmosis water
Method of Administration	Oral gavage
Sample	Plasma
Assay	LC/MS/MS

Test Article	GS-7340-02						GS-7340-03					
Sex (M/F) / N of Animals	M/3						M/3					
Dose (mg/kg)	5		25		100		5		25		100	
Analyte	TAF	TFV	TAF	TFV	TAF	TFV	TAF	TFV	TAF	TFV	TAF	TFV
PK Parameters												
t_{max} (h)	NA	0.67	NA	0.58	NA	0.83	NA	0.58	NA	0.83	NA	0.67
C_{max} (ng/mL)	NA	32.5	NA	199	NA	1240	NA	39.3	NA	364	NA	1670
$t_{½}$(h)	NA	NA	NA	11.2	NA	10.3	NA	NA	NA	7.89	NA	7.85
AUC_{0-t} (ng•h/mL)	NA	122	NA	1395	NA	7771	NA	88.5	NA	1810	NA	9759
t_{last} (h)	NA	8.00	NA	24.0	NA	24.0	NA	4.67	NA	24.0	NA	24.0
C_{last} (ng/mL)	NA	10.5	NA	25.1	NA	156	NA	12.6	NA	19.4	NA	113

F = female; M = male; NA = not applicable; TAF = tenofovir alafenamide; TFV = tenofovir

3.5. R990130: Pharmacokinetics of TAF in Rats

Report Title	Study Type	Test Article	Report Number
Tenofovir (GS-1278) Plasma Pharmacokinetics Following a Single Oral Dose of GS-7340-02 in the Male Albino Rat	Absorption	TAF	R990130

Species	Sprague-Dawley rats
Feeding Condition	Fasted
Vehicle Formulation	50 mM citric acid
Method of Administration	Oral gavage
Sample	Plasma
Assay	LC/Fluorescence

Test Article	GS-7340-02			
Sex (M/F) / N of Animals	M/4			
Dose (mg/kg)	6.25	25	100	400
Analyte	TFV			
PK Parameters				
t_{max} (h)	0.26	0.50	0.50	0.25
C_{max} (ng/mL)	104	1220	3870	15800
$t_{½}$ (h)	NA	14.5	16.2	17.3
AUC_{0-t} (ng•h/mL)	160	3270	12200	48300
t_{last} (h)	4.00	20.0	20.0	24.0
$MRT_{0-\infty}$ (h)	NA	20.8	18.9	20.6

F = female; M = male; NA = not applicable; TAF = tenofovir alafenamide; TFV = tenofovir

3.6. R2000065: Pharmacokinetics of TAF in Rats

Report Title	Study Type	Test Article	Report Number
Comparison of Plasma Pharmacokinetics in Rats of Tenofovir Following Oral Administration of GS-7340-02 or Tenofovir DF as Either a suspension in CMC or a Solution in Citric Acid	Absorption	TAF	R2000065

Species	Sprague-Dawley rats
Feeding Condition	Fasted
Dose (mg/kg)	400
Method of Administration	Oral gavage
Sample	Plasma
Assay	LC/MS/MS

Test Article	GS-7340-02		TDF	
Sex (M/F) / N of Animals	M/3			
Vehicle Formulation	CMC suspension	50 mM citric acid	CMC suspension	50 mM citric acid
Analyte	TFV			
PK Parameters				
t_{max} (h)	0.50	0.25	0.25	0.50
C_{max} (ng/mL)	14229	8418	8101	2699
$t_{½}$ (h)	11.3	11.4	7.21	8.31
AUC_{0-t} (ng•h/mL)	36288	33067	15774	11403
$AUC_{0-\infty}$ (ng•h/mL)	36795	33638	15848	11581
t_{last} (h)	55.0	55.0	55.0	48.0

CMC = carboxymethylcellulose; F = female; M = male; TAF = tenofovir alafenamide; TFV = tenofovir

3.7. 99-DDM-1278-001-PK: Pharmacokinetics of TAF in Dogs

Report Title	Study Type	Test Article	Report Number
Analysis of Data from SRI Bioavailability Study M059-98 of GS-7340 in Dogs	Absorption, Bioavailability	TAF and its Diastereoisomers Mixture	99-DDM-1278-001-PK

Species	Beagle dogs
Feeding Condition	Fasted
Vehicle Formulation	Sterile Saline
Method of Administration	IV bolus
Assay	LC/Fluorescence

Test Article	GS-7340-2		
Sex (M/F) / N of Animals	M/5		
Dose (mg/kg)	6.20		
Sample	Plasma		PBMC
Analyte	TAF	TFV	TFV
PK Parameters			
t_{max} (h)	0.00*	0.04	7.6
C_{max} (µg/mL)	8.88*	2.09	26.2
$t_{½}$ (h)	0.13	13.7	>24
AUC_{0-t} (µg•h/mL)	1.46	2.01	448
$AUC_{0-\infty}$ (µg•h/mL)	1.52	2.69	NC
V_z (L/kg)	0.82	NA	NA
CL (L/h/kg)	4.48	NA	NA

F = female; IV = intravenous; M = male; NA = not applicable; NC = not calculated; PBMC = peripheral blood mononuclear cells; TAF = tenofovir alafenamide; TFV = tenofovir; TFV-MP = tenofovir monophosphate; TFV-DP = tenofovir diphosphate
* Extrapolated to time zero

Report Title	Study Type	Test Article	Report Number
Analysis of Data from SRI Bioavailability Study M059-98of GS-7340 in Dogs	Absorption, Bioavailability	TAF and its Diastereoisomers Mixture	99-DDM-1278-001-PK

Species	Beagle dogs
Feeding Condition	Fasted
Vehicle Formulation	50 mM citric acid
Method of Administration	Oral gavage
Assay	LC/Fluorescence

Test Article	GS-7340			GS-7340-2								
Sex (M/F) / N of Animals	M/5											
Dose (mg/kg)	18.0			20.0			4.80			5.00		
Sample	Plasma		PBMC	Plasma		PBMC	Plasma		PBMC	Plasma		PBMC
Analyte	TAF	TFV	TFV	TAF	TFV	TFV	TAF	TFV	TFV	TAF	TFV	TFV
PK Parameters												
t_{max} (h)	0.17	1.00	10.0	0.18	0.60	4.40	0.14	0.63	10.0	0.32	1.65	NA
C_{max} (µg/mL)	6.39	0.83	46.0	5.82	0.97	59.7	1.12	0.26	8.30	1.01	0.32	NA
$t_{½}$ (h)	0.32	23.1	NA	0.33	22.0	NA	0.24	20.1	NA	0.19	11.7	NA
AUC_{0-t} (µg•h/mL)	2.79	4.61	706	3.25	5.19	805	0.44	1.26	120	0.48	1.43	NA
$AUC_{0-\infty}$ (µg•h/mL)	2.93	7.89	NC	3.73	8.38	NC	0.50	2.08	NC	0.50	1.78	NA
F (%)[a]	53.4	16.9	NA	76.3	20.1	NA	42.4	20.7	NA	41.2	17.1	NA

F = female; M = male; NC = not calculated; TAF = tenofovir alafenamide; TFV = tenofovir; TFV-DP = tenofovir diphosphate; TFV-MP = tenofovir monophosphate

a Oral bioavailability (F%) for TAF was calculated based on $AUC_{0-\infty}$ value obtained from intravenous administration of GS-7340-2 in dogs (Report #99-DDM-1278-001-PK); Oral bioavailability for tenofovir (TFV) was calculated based on historical $AUC_{0-\infty}$ value (4.30 µg•h/mL) from intravenous administration of TFV in dogs

Report Title	Study Type	Test Article	Report Number
Analysis of Data from SRI Bioavailability Study M059-98of GS-7340 in Dogs	Absorption, Bioavailability	TAF and its Diastereoisomers Mixture	99-DDM-1278-001-PK

Species	Beagle dogs		
Feeding Condition	Fed		
Vehicle Formulation	50 mM citric acid		
Method of Administration	Oral gavage		
Assay	LC/Fluorescence		
Test Article	GS-7340-2		
Sex (M/F) / N of Animals	M/5		
Dose (mg/kg)	5.00		
Sample	Plasma		PBMC
Analyte	TAF	TFV	TFV
PK Parameters			
t_{max} (h)	0.30	1.90	NA
C_{max} (µg/mL)	0.24	0.07	NA
$t_{½}$ (h)	0.91	6.14	NA
AUC_{0-t} (µg•h/mL)	0.16	0.43	NA
$AUC_{0-\infty}$ (µg•h/mL)	0.20	0.53	NA
F (%)[a]	16.5	5.08	NA

F = female; M = male; TAF = tenofovir alafenamide; TFV = tenofovir; TFV-MP = tenofovir monophosphate; TFV-DP = tenofovir diphosphate; PBMC = peripheral blood mononuclear cells; NA = not applicable

a Oral bioavailability (F%) for TAF was calculated based on $AUC_{0-\infty}$ value obtained from intravenous administration of GS-7340-2 in dogs (Report #99-DDM-1278-001-PK). Oral bioavailability for tenofovir (TFV) was calculated based on historical $AUC_{0-\infty}$ value (4.30 µg•h/mL) from intravenous administration of TFV in dogs

Report Title	Study Type	Test Article	Report Number
Analysis of Data from SRI Bioavailability Study M059-98of GS-7340 in Dogs	Absorption, Bioavailability	TAF and its Diastereoisomers Mixture	99-DDM-1278-001-PK

Species	Beagle dogs
Feeding Condition	Fasted
Vehicle Formulation	50 mM citric acid
Method of Administration	Oral gavage
Assay	LC/Fluorescence

Test Article	GS-7171				GS-7339		
Sex (M/F) / N of Animals	M/5				M/5		
Dose (mg/kg)	16.0				18.0		
Sample	Plasma			PBMC	Plasma		PBMC
Analyte	TAF	GS-7339	TFV	TFV	GS-7339	TFV	TFV
PK Parameters							
t_{max} (h)	0.17	0.23	0.94	8.00	0.21	1.25	5.60
C_{max} (µg/mL)	2.23	5.62	0.91	32.0	4.77	0.94	19.8
$t_{½}$ (h)	0.58	0.57	20.0	NA	0.49	10.4	NA
AUC_{0-t} (µg•h/mL)	1.11	4.63	5.31	707	2.77	5.08	199
$AUC_{0-\infty}$ (µg•h/mL)	1.63	4.89	8.17	NC	3.20	6.14	NC
F (%)[a]	NA	NA	19.7	NA	NA	13.1	NA

F = female; M = male; NA = not applicable; NC = not calculated; PBMC = peripheral blood mononuclear cells; TAF = tenofovir alafenamide; TFV = tenofovir; TFV-DP = tenofovir diphosphate; TFV-MP = tenofovir monophosphate
Note: GS-7171 contains 1:1 mixture of diastereoisomers TAF and GS-7339
a Oral bioavailability for tenofovir (TFV) was calculated based on historical $AUC_{0-\infty}$ value (4.30 µg•h/mL) from intravenous administration of TFV in dogs

3.8. AD-120-2034: Pharmacokinetics of TAF in Dogs

Report Title	Study Type	Test Article	Report Number
Plasma and Liver Pharmacokinetics of Tenofovir Alafenamide (TAF) Following Single Oral Administration in Male Beagle Dogs	Absorption	TAF	AD-120-2034

Species	Beagle dogs
Feeding Condition	Fasted
Vehicle Formulation	0.1% (w/w) hydroxypropylmethylcellulose K100LV (HPMC) K100LV, 0.1% polysorbate 20 in water
Method of Administration	Oral gavage[a]
Dose	10 mg/kg
Assay	LC/MS/MS

Test Article	GS-7340-02				
Sex (M/F) / N of Animals	M/6				
Sample	Plasma		Liver		
Analyte	TAF	TFV	TFV	TFV-MP	TFV-DP
PK Parameters					
t_{max} (h)	0.08	1.00	1.00	4.00	4.00
C_{max} (µg/mL)	3.49	0.64	12.7	12.6	56.4
$t_{½}$ (h)	0.24	>24	15.9	>24	22.3
AUC_{0-t} (µg•h/mL)	1.38	2.72	86.8	258	1017

F = female; M = male; TAF = tenofovir alafenamide; TFV = tenofovir; TFV-DP = tenofovir diphosphate; TFV-MP = tenofovir monophosphate
a Animals were pretreated with pentagastrin 20 minutes prior to the dose administration

3.9. P2000087: Pharmacokinetics of TAF in Rhesus Monkeys

Report Title	Study Type	Test Article	Report Number
A Single Dose Pharmacokinetic and Oral Bioavailability Study ofGS-7340-02 in Rhesus Monkeys	Absorption, bioavailability	TAF	P2000087

Species	Rhesus monkeys
Feeding Condition	Fasted
Vehicle Formulation	50 mM citric acid
Method of Administration	Nasogastric gavage
Sample	Plasma
Assay	LC/MS/MS

Test Article	GS-7340-02											
Dose (mg/kg)	0.5				5				50			
Sex (M/F) / N of Animals	M/3		F/3		M/3		F/3		M/3		F/3	
Analyte	TAF	TFV	TAF	TFV	TAF	TFV	TAF	TFV	TAF	TFV	TAF	TFV
PK Parameters												
t_{max} (h)	0.40	1.00	0.33	1.00	0.80	1.33	0.80	1.30	0.40	1.00	0.70	1.00
C_{max} (ng/mL)	3.92	8.50	1.65	6.94	161	169	88.6	152	5388	1576	2897	1076
$t_{½}$ (h)	NC	4.24	0.61	7.18	0.27	10.0	0.19	12.6	0.22	23.9	0.57	14.8
AUC_{0-t} (ng•h/mL)	1.85	34.0	0.60	45.9	131	1151	59.5	924	5038	13119	2583	6749
$AUC_{0-\infty}$ (ng•h/mL)	NC	43.5	2.47	62.0	77.3	1186	82.0	951	5109	13585	2584	6914
MRT (h)	0.43	6.72	0.59	11.0	0.98	11.8	0.88	11.8	0.77	20.0	0.90	15.9

F = female; M = male; TAF = tenofovir alafenamide; TFV = tenofovir

Report Title	Study Type	Test Article	Report Number
A Single Dose Pharmacokinetic and Oral Bioavailability Study ofGS-7340-02 in Rhesus Monkeys	Absorption, bioavailability	TAF	P2000087

Species	Rhesus monkeys
Feeding Condition	Fasted
Vehicle Formulation	50 mM citric acid
Method of Administration	Nasogastric gavage
Sample	PBMC
Assay	LC/MS/MS

Test Article	GS-7340-02							
	TFV Extract Concentration				Total Converted TFV Extract Concentration[a]			
Dose (mg/kg)	5		50		5		50	
Sex (M/F) / N of Animals	M/3	F/3	M/3	F/3	M/3	F/3	M/3	F/3
2 h	4.03	3.33	167	60.9	5.37	7.2	311	130
24 h	BLQ	2.18	53.3	57.2	4.46	7.57	137	134
96 h	BLQ	BLQ	21.7	18.2	4.03	4.92	66.7	40.6

BLQ = below the limit of quantitation (2 ng/mL); F = female; M = male; PBMC = peripheral blood mononuclear cell; TAF = tenofovir alafenamide; TFV = tenofovir
a Phosphorylated metabolites of TFV were converted to TFV by treatment with acid phosphatase for 30 minutes at 40ºC

4. PHARMACOKINETICS: ABSORPTION AFTER REPEATED DOSES

4.1. AD-120-2033: 7-Day Repeated Dose Study of TAF in Dogs – Pharmacokinetics

Report Title	Study Type	Test Article	Report Number
Plasma and Liver Pharmacokinetics of Tenofovir Alafenamide (GS-7340) Following 7-Day Oral Administration in Male Beagle Dogs	Absorption	TAF	AD-120-2033

Species	Beagle dogs
Feeding Condition	Not fasted
Vehicle Formulation	0.1% (w/w) hydroxypropylmethylcellulose K100LV (HPMC) K100LV, 0.1% polysorbate 20 in water
Method of Administration	Oral Gavage
Dose	8.29 mg/kg/Day
Assay	LC/MS/MS

Test Article	GS-7340-02						
Sex (M/F) / N of Animals	M/4						
Sample	Plasma				Liver		
Sample Collection Time	Day 1		Day 7		Day 7		
Analyte	TAF	TFV	TAF	TFV	TFV	TFV-MP	TFV-DP
PK Parameters							
t_{max} (h)	0.17	0.75	0.17	0.75	4.00	4.00	4.00
C_{max} (µg/mL)	2.15	0.42	1.12	0.61	4.34	27.3	108
$t_{½}$ (h)	0.30	15.7	0.28	19.2	NA	NA	NA
AUC_{0-t} (µg•h/mL)	0.88	1.96	0.37	3.39	NA	NA	NA

F = female; M = male; NA = not applicable; TAF = tenofovir alafenamide; TFV = tenofovir; TFV-DP = tenofovir diphosphate; TFV-MP = tenofovir monophosphate

4.2. D990175-PK: 28-Day Toxicity Study of TAF in Dogs – Toxicokinetics

Report Title	Study Type	Test Article	Report Number
Toxicokinetics of a 28-Day Oral Gavage Toxicity Study of GS-7340-02 in the Beagle Dog	Absorption	TAF	D990175-PK

Species	Beagle dogs
Feeding Condition	Not fasted
Vehicle Formulation	50 mM citric acid
Method of Administration	Oral Gavage
Sample	Plasma
Analyte	TAF
Assay	LC/MS/MS

Test Article	GS-7340-02											
Dose (mg/kg/day)	1.0				3.0				10.0			
Sex (M/F) / N of Animals	M/4		F/4		M/4		F/4		M/4		F/4	
Sample Collection Time	Day 1	Day 28	Day 1	Day 28	Day 1	Day 28	Day 1	Day 28	Day 1	Day 28	Day 1	Day 28
PK Parameters												
t_{max} (h)	0.33	0.42	0.25	0.33	0.31	0.31	0.50	0.50	0.44	0.44	0.38	0.38
C_{max} (µg/mL)	0.02	0.03	0.02	0.05	0.05	0.11	0.06	0.10	0.86	2.07	0.75	1.01
$t_{½}$ (h)	NA	NA	NA	NA	NA	NA	NA	NA	0.31	0.65	0.33	0.56
AUC_{0-t} (µg•h/mL)	NA	NA	NA	NA	NA	NA	NA	NA	0.60	NA	0.41	NA
$AUC_{0-\tau}$(µg•h/mL)	NA	NA	NA	NA	NA	NA	NA	NA	NA	1.30	NA	0.86
T_{last} (h)	NA	NA	NA	NA	NA	NA	NA	NA	1.50	3.00	1.00	3.00
C_{last} (ng/mL)	NA	NA	NA	NA	NA	NA	NA	NA	0.08	0.07	0.18	0.05

F = female; M = male; NA = not applicable; TAF = tenofovir alafenamide; TFV = tenofovir

Report Title	Study Type	Test Article	Report Number
Toxicokinetics of a 28-Day Oral Gavage Toxicity Study of GS-7340-02 in the Beagle Dog	Absorption	TAF	D990175-PK

Species	Beagle dogs
Feeding Condition	Not fasted
Vehicle Formulation	50 mM citric acid
Method of Administration	Oral Gavage
Sample	Plasma
Analyte	TFV
Assay	LC/MS/MS

Test Article	GS-7340-02											
Dose (mg/kg/Day)	1.0				3.0				10.0			
Sex (M/F) / N of Animals	M/4		F/4		M/4		F/4		M/4		F/4	
Sample Collection Time	Day 1	Day 28	Day 1	Day 28	Day 1	Day 28	Day 1	Day 28	Day 1	Day 28	Day 1	Day 28
PK Parameters												
t_{max} (h)	NA	3.50	NA	4.75	1.13	1.25	1.75	0.88	1.25	0.81	0.75	0.63
C_{max} (µg/mL)	NA	0.06	NA	0.07	0.12	0.09	0.15	0.13	0.38	0.55	0.40	0.43
$t_{½}$ (h)	NA	NA	NA	NA	NA	NA	NA	NA	16.3	>24	16.6	>24
AUC_{0-t} (µg•h/mL)	NA	NA	NA	NA	NA	NA	NA	NA	1.85	NA	1.78	NA
$AUC_{0-\tau}$(µg•h/mL)	NA	NA	NA	NA	NA	NA	NA	NA	NA	5.45	NA	5.13
T_{last} (h)	NA	NA	NA	NA	NA	NA	NA	NA	21.0	24.0	18.0	24.0
C_{last} (ng/mL)	NA	NA	NA	NA	NA	NA	NA	NA	0.03	0.15	0.05	0.16

F = female; M = male; NA = not applicable; TAF = tenofovir alafenamide; TFV = tenofovir

Report Title	Study Type	Test Article	Report Number
Toxicokinetics of a 28-Day Oral Gavage Toxicity Study of GS-7340-02 in the Beagle Dog	Absorption	TAF	D990175-PK

Species	Beagle dogs
Feeding Condition	Not fasted
Vehicle Formulation	50 mM citric acid
Method of Administration	Oral Gavage
Sample	PBMC
Analyte	TFV
Assay	LC/MS/MS

Test Article	GS-7340-02					
Dose (mg/kg/Day)	1.0		3.0		10.0	
Sex (M/F) / N of Animals	M/4	F/4	M/4	F/4	M/4	F/4
Intracellular Concentration						
$C_{Day\ 1}$ (µg/mL)	NA	NA	NA	NA	NA	NA
$C_{Day\ 8}$ (µg/mL)	NA	NA	NA	1.80	6.92	7.85
$C_{Day\ 22}$ (µg/mL)	NA	NA	1.70	NA	10.6	10.2
$C_{Day\ 28}$ (µg/mL)	NA	NA	2.44	3.81	19.4	17.5

F = female; M = male; NA = not applicable; PBMC = peripheral blood mononuclear cells; TAF = tenofovir alafenamide; TFV = tenofovir

4.3. P2000114-PK: 28-Day Toxicity Study of TAF in Monkeys – Toxicokinetics

Report Title	Study Type	Test Article	Report Number
Toxicokinetics from a 28 Day Toxicity Study of GS-7340-02 and Tenofovir (GS-1278) Administered Orally to Rhesus Monkeys	Absorption	TAF	P2000114-PK

Species	Rhesus monkeys
Feeding Condition	Fasted
Vehicle Formulation	50 mM citric acid
Method of Administration	Nasogastric gavage
Sample	Plasma
Assay	LC/MS/MS

Test Article	GS-7340-02															
Dose (mg/kg/Day)	3								30							
Sex (M/F) / N of Animals	M/3				F/3				M/3				F/3			
Sample Collection Time	Day 1		Day 28		Day 1		Day 28		Day 1		Day 28		Day 1		Day 28	
Analyte	TAF	TFV	TAF	TFV	TAF	TFV	TAF	TFV	TAF	TFV	TAF	TFV	TAF	TFV	TAF	TFV
PK Parameters																
t_{max} (h)	1.00	1.00	1.00	2.30	1.00	1.00	0.83	1.00	1.00	1.00	0.50	0.75	1.00	1.00	0.50	0.67
C_{max} (µg/mL)	0.003	0.09	0.01	0.05	0.005	0.09	0.02	0.05	0.49	0.96	2.21	1.24	2.29	0.85	0.82	0.78
$t_{½}$ (h)	NA	7.77	NA	12.9	NA	8.41	NA	14.2	NA	14.8	0.18	15.6	NA	10.3	0.43	16.4
$AUC_{0-\infty}$ (µg•h/mL)	NA	0.45	NA	NA	NA	0.49	NA	NA	NA	8.70	NA	NA	NA	4.63	NA	NA
$AUC_{0-\tau}$ (µg•h/mL)	NA	NA	NA	0.32	NA	NA	NA	0.38	NA	NA	1.53	7.36	NA	NA	0.70	3.89
$MRT_{0-\infty}$ (h)	NA	9.38	NA	16.6	NA	10.8	NA	17.4	NA	19.9	0.71	17.7	NA	12.9	2.61	20.2

F = female; M = male; NA = not applicable; PBMC = peripheral blood mononuclear cells; TAF = tenofovir alafenamide; TFV = tenofovir

Report Title	Study Type	Test Article	Report Number
Toxicokinetics from a 28 Day Toxicity Study of GS-7340-02 and Tenofovir (GS-1278) Administered Orally to Rhesus Monkeys	Absorption	TAF	P2000114-PK

Species	Rhesus monkeys
Feeding Condition	Fasted
Vehicle Formulation	50 mM citric acid
Method of Administration	Nasogastric gavage
Sample	Plasma
Analyte	TFV
Assay	LC/MS/MS

Test Article	GS-1278			
Dose (mg/kg/Day)	15			
Sex (M/F) / N of Animals	M/3		F/3	
Sample Collection Time	Day 1	Day 28	Day 1	Day 28
PK Parameters				
t_{max} (h)	1.00	1.5	2.00	2.17
C_{max} (µg/mL)	0.16	0.15	0.12	0.18
$t_{½}$ (h)	6.79	17.6	11.2	12.6
$AUC_{0-\infty}$ (µg•h/mL)	1.01	NA	1.01	NA
$AUC_{0-\tau}$ (µg•h/mL)	NA	1.19	NA	1.46
$MRT_{0-\infty}$ (h)	8.83	21.8	16.0	16.8

F = female; M = male; NA = not applicable; TAF = tenofovir alafenamide; TFV = tenofovir

Report Title	Study Type	Test Article	Report Number
Toxicokinetics from a 28 Day Toxicity Study of GS-7340-02 and Tenofovir (GS-1278) Administered Orally to Rhesus Monkeys	Absorption	TAF	P2000114-PK

Species	Beagle dogs
Feeding Condition	Fasted
Vehicle Formulation	50 mM citric acid
Method of Administration	Nasogastric gavage
Sample	PBMC
Analyte	TFV
Assay	LC/MS/MS

Test Article	GS-7340-02				GS-1278	
Dose (mg/kg/Day)	3		30		15	
Sex (M/F) / N of Animals	M/3	F/3	M/3	F/3	M/3	F/3
Intracellular concentrations						
C_{Day14} (µg/mL)	NA	NA	70.9	73.6	NA	NA
C_{Day28} (µg/mL)	NA	NA	31.1	24.6	NA	NA

F = female; M = male; NA = not applicable; PBMC = peripheral blood mononuclear cells; TAF = tenofovir alafenamide; TFV = tenofovir

5. PHARMACOKINETICS: ORGAN DISTRIBUTION STUDIES

5.1. AD-120-2011: Pharmacokinetics, Absorption, Distribution, and Excretion of [^{14}C]TAF in Mouse Following Oral Administration

Report Title	Study Type	Test Article	Report Number
Pharmacokinetics, Absorption, Distribution, and Excretion of [^{14}C]GS-7340 Following Oral Administration to Mice	Distribution	[^{14}C]TAF	AD-120-2011

Species	CD-1 Mice
Sex (M/F)/No. of Animals	M/30
Method of Administration	Oral gavage
Dose (mg/kg/day)	100
Feeding Condition	Not fasted
Specific Activity	300 µCi/kg
Radionuclide	Carbon-14
Vehicle Formulation	water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)
Sample	Plasma/Blood
Analyte/Assay:	[^{14}C]TAF/Liquid Scintillation Counter

Tabulated PK Results (Mean):

Sample Type	Plasma	Blood
T_{max} (h)	0.25	0.50
C_{max} (ng eq/g)	24500	23100
$t_{½}$ (h)	15.8	45.0
AUC_{0-t} (ng eq•h/g)	108675	514848
$AUC_{0-\infty}$ (ng eq•h/g)	111574	NA

F = female; M = male; NA = not applicable; TAF = tenofovir alafenamide

Report Title	Study Type	Test Article	Report Number
Pharmacokinetics, Absorption, Distribution, and Excretion of [^{14}C]GS-7340 Following Oral Administration to Mice	Distribution	[^{14}C]TAF	AD-120-2011

Species	CD-1 Mice
Sex (M/F) No. of Animals	M/30
Method of Administration	Oral gavage
Dose (mg/kg)	100
Feeding Condition	Not fasted
Radionuclide	Carbon-14
Specific Activity	300 μCi/kg
Vehicle Formulation	water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)
Analyte/Assay:	[^{14}C]TAF/Liquid Scintillation Counter

	Mean Concentrations Postdosing (ng Equivalents [^{14}C]TAF/g)				
Time Point	**Brain**	**Heart**	**Kidney**	**Liver**	**Nasal turbinates**
1 h	604	7660	106000	519000	5680
4 h	183	2440	67500	394000	1750
12 h	198	4290	47900	296000	1870
24 h	219	4870	26900	194000	1540
48 h	159	3550	13400	83600	1170
72 h	88.9	1460	5820	55700	546

F = female; M = male; TAF = tenofovir alafenamide

Report Title	Study Type	Test Article	Report Number
Pharmacokinetics, Absorption, Distribution, and Excretion of [^{14}C]GS-7340 Following Oral Administration to Mice	Distribution	[^{14}C]TAF	AD-120-2011

Species	CD-1 Mice
Sex (M/F) / No. of Animals	M/9
Method of Administration:	Oral gavage
Dose (mg/kg):	100
Feeding Condition:	Not fasted
Radionuclide:	Carbon-14
Specific Activity:	300 μCi/kg
Vehicle / Formulation:	water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)
Sampling Time:	0.5, 1, 3, 8, 12, 24, 48, 96, and 168 hours postdose
Analyte/Assay:	[^{14}C]TAF/Quantitative whole body autoradiography

Report Title	**Study Type**	**Test Article**	**Report Number**
Pharmacokinetics, Absorption, Distribution, and Excretion of [^{14}C]GS-7340 Following Oral Administration to Mice	Distribution	[^{14}C]TAF	AD-120-2011

Blood and Tissue radioactivity:	**Concentration of Radioactivity (ng Equivalents [^{14}C]TAF/g)**								
	Animal Number (Sacrifice Time)								
Tissue	**A18225 0.5h**	**A18226 1 h**	**A18227 3 h**	**A18228 8 h**	**A18229 12 h**	**A18230 24 h**	**A18231 48 h**	**A18232 96 h**	**A18233 168 h**
Adrenal gland(s)	9920	27500	6450	5420	3010	3600	2550	1530	669
Bile	60300	127000	115000	NR	65900	12900	12200	5940	NR
Blood	14600	14500	5280	8640	4380	6100	4100	2710	1000
Bone	1300	2420	1230	1140	1010	525	BLQ	372	ND
Bone marrow	4730	6610	2030	1670	2040	2120	1190	1140	513
Brain cerebellum	569	1940	395	BLQ	BLQ	BLQ	BLQ	ND	ND
Brain cerebrum	768	1430	BLQ	BLQ	379	BLQ	BLQ	ND	ND
Brain medulla	420	833	BLQ	BLQ	BLQ	BLQ	BLQ	ND	ND
Brain olfactory lobe	1120	2890	464	398	461	BLQ	BLQ	ND	ND
Cecum	3440	6960	90500	63200	19100	8310	2290	2540	1310
Diaphragm	40600	167000	46600	82900	26600	28300	19000	10300	3320
Epididymis	3600	22300	1310	508	540	864	BLQ	ND	ND
Esophagus	38400	90800	57300	114000	20100	8660	8660	5860	933
Exorbital lacrimal gland	3600	5460	1400	1840	1250	1290	748	729	364
Eye lens	967	2980	508	BLQ	BLQ	BLQ	BLQ	BLQ	ND
Eye uveal tract	4180	3690	754	488	947	779	BLQ	ND	ND
Eye(s)	1880	2660	539	312	418	363	BLQ	BLQ	ND
Fat (abdominal)	1250	1170	968	757	512	787	BLQ	ND	ND
Fat (brown)	3070	5590	2530	3790	2060	3030	1920	1060	BLQ
Gall bladder	335000	216000	108000	NR	68100	37100	20900	29200	NR
Harderian gland	4240	6500	1530	2050	2170	1390	764	1210	487
Intra-orbital lacrimal gland	7630	3580	1900	1190	2280	1500	NR	ND	442
Kidney cortex	82300	89000	74000	37400	30000	23100	10500	7300	2830
Kidney medulla	65000	70000	54800	29600	18100	12800	7070	4430	1670

Report Title	Study Type	Test Article	Report Number
Pharmacokinetics, Absorption, Distribution, and Excretion of [^{14}C]GS-7340 Following Oral Administration to Mice	Distribution	[^{14}C]TAF	AD-120-2011

Blood and Tissue radioactivity:	**Concentration of Radioactivity (ng Equivalents [^{14}C]TAF/g)**								
	Animal Number (Sacrifice Time)								
Tissue	**A18225 0.5h**	**A18226 1 h**	**A18227 3 h**	**A18228 8 h**	**A18229 12 h**	**A18230 24 h**	**A18231 48 h**	**A18232 96 h**	**A18233 168 h**
Kidney(s)	84800	86100	68900	3370	25900	19900	9090	6080	2300
Large intestine	6680	8090	7730	96100	22500	6770	3100	2200	607
Liver	282000	447000	290000	295000	197000	164000	77000	44600	19000
Lung(s)	13200	23900	4000	8900	10000	11500	6890	5220	1820
Lymph node(s)	2190	7750	1680	1690	1760	2280	1470	2090	468
Muscle	1680	2100	466	522	768	579	363	366	BLQ
Myocardium	7570	11700	2380	4100	4600	4620	3430	3500	905
Nasal turbinates	2720	6440	1290	1170	983	1190	519	709	370
Pancreas	6130	14500	2950	2970	3540	2880	1760	1970	917
Pituitary gland	2910	7440	1280	1390	2510	1300	663	718	BLQ
Preputial gland	1900	2580	635	390	708	503	BLQ	ND	ND
Prostate gland	5500	2880	2150	16500	3060	2590	427	440	ND
Salivary gland(s)	5390	11500	1880	2210	2420	2160	1130	907	511
Seminal vesicle(s)	1790	3410	957	27000	852	620	462	BLQ	ND
Skin (pigmented)	8520	5580	1510	939	674	715	368	BLQ	ND
Small intestine	8770	74500	88100	54900	14100	4040	3400	3520	1610
Spinal cord	757	1210	BLQ	437	BLQ	BLQ	BLQ	ND	ND
Spleen	6100	12900	3850	4700	5460	6270	4020	3040	1430
Stomach	77600	39300	26600	49700	5460	5940	5870	1760	571
Stomach mucosa	96900	46900	19800	44000	5280	3660	5910	2550	638
Stomach wall	48600	24500	26600	24700	7690	22700	4230	3940	378
Testis(es)	1160	1490	774	BLQ	BLQ	319	ND	ND	ND
Thymus	2510	6140	914	1300	903	1190	808	789	BLQ
Thyroid	7120	12100	2830	2290	1200	4600	3200	3500	1530
Urinary bladder	ND	174000	85600	ND	5800	10300	9240	2800	924
Urine	1790000	413000	200000	351000	36800	24700	14600	327	BLQ

BLQ = below the limit of quantitation (< 311 ng equivalents [^{14}C]GS-7340/g); F = female; M = male; ND = not detectable; NR = not represented; TAF = tenofovir alafenamide

Report Title	Study Type	Test Article	Report Number
Pharmacokinetics, Absorption, Distribution, and Excretion of [^{14}C]GS-7340 Following Oral Administration to Mice	Distribution	[^{14}C]TAF	AD-120-2011

Methods:

Species	C57 Black Mice
Sex (M/F) / No. of Animals	M/9
Method of Administration:	Oral gavage
Dose (mg/kg):	100
Feeding Condition:	Not fasted
Radionuclide:	Carbon-14
Specific Activity:	300 μCi/kg
Vehicle / Formulation:	water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)
Sampling Time:	0.5, 1, 3, 12, 24, 96, 168, 240, and 336 hours postdose
Analyte/Assay:	[^{14}C]TAF/Quantitative whole body autoradiography

Report Title	Study Type	Test Article	Report Number
Pharmacokinetics, Absorption, Distribution, and Excretion of [^{14}C]GS-7340 Following Oral Administration to Mice	Distribution	[^{14}C]TAF	AD-120-2011

Blood and Tissue radioactivity:	**Concentration of Radioactivity (ng Equivalents [^{14}C]TAF/g) Animal Number (Sacrifice Time)**								
Tissue	**A19068 0.5h**	**A19069 1 h**	**A19070 3 h**	**A19071 12 h**	**A19072 24 h**	**A19073 96 h**	**A19074 168 h**	**A19075 240 h**	**A19076 336 h**
Adrenal gland(s)	34000	26400	20400	7750	10400	2710	2310	1610	1430
Bile	198000	136000	150000	40300	26600	3620	1020	2360	2160
Blood	16700	9270	7910	4170	3780	1100	788	675	BLQ
Bone	11200	4490	4900	2430	1770	ND	ND	ND	ND
Bone marrow	24300	19000	16100	6440	6470	ND	ND	ND	ND
Brain cerebellum	611	590	BLQ	ND	ND	ND	ND	ND	ND
Brain cerebrum	1240	661	BLQ	ND	ND	ND	ND	ND	ND
Brain medulla	886	BLQ	BLQ	ND	ND	ND	ND	ND	ND
Brain olfactory lobe	3790	1730	1640	688	707	ND	ND	ND	ND
Cecum	25400	95400	ND	50500	10500	BLQ	ND	ND	ND
Diaphragm	60300	103000	114000	27500	25900	2340	2360	1830	1410
Epididymis	10800	4190	4990	2120	2150	ND	ND	ND	ND
Esophagus	76100	81300	48900	27900	8540	1540	1660	723	ND
Exorbital lacrimal gland	18100	12000	11800	4910	3530	1050	1010	940	ND
Eye lens	4310	1870	1110	527	BLQ	ND	ND	ND	ND
Eye uveal tract	13400	12200	11600	4740	6140	ND	ND	ND	ND
Eye(s)	5000	2920	2440	1040	1060	ND	ND	ND	ND
Fat (abdominal)	5460	6820	2140	4180	1700	6410	BLQ	ND	ND
Fat (brown)	19200	18700	14500	10800	8660	2420	1190	812	569
Gall bladder	163000	379000	275000	94000	39800	6580	4130	ND	ND
Harderian gland	18500	14600	13600	6200	4580	861	ND	ND	ND
Intra-orbital lacrimal gland	NR	NR	NR	NR	3760	ND	ND	ND	ND
Kidney cortex	137000	125000	104000	58000	34500	5660	5350	4300	2530
Kidney medulla	125000	94700	80000	40200	19400	3640	2980	2620	1820
Kidney(s)	132000	107000	89600	47500	29500	4820	4540	3550	2240
Large intestine	25700	17300	32300	74200	35000	2930	1530	785	BLQ

Report Title	Study Type	Test Article	Report Number
Pharmacokinetics, Absorption, Distribution, and Excretion of [^{14}C]GS-7340 Following Oral Administration to Mice	Distribution	[^{14}C]TAF	AD-120-2011

Blood and Tissue radioactivity:	**Concentration of Radioactivity (ng Equivalents [^{14}C]TAF/g) Animal Number (Sacrifice Time)**								
Tissue	**A19068 0.5h**	**A19069 1 h**	**A19070 3 h**	**A19071 12 h**	**A19072 24 h**	**A19073 96 h**	**A19074 168 h**	**A19075 240 h**	**A19076 336 h**
Liver	488000	490000	385000	282000	118000	21900	14200	9840	7510
Lung(s)	32300	32500	26500	18100	13500	4440	4260	3330	2120
Lymph node(s)	17900	14600	12600	8020	10900	ND	ND	ND	ND
Muscle	8780	4140	3190	2010	3900	753	982	650	491
Myocardium	23200	13500	13600	8470	9510	2490	2170	2070	1540
Nasal turbinates	10600	2980	3550	2390	1920	799	ND	ND	ND
Pancreas	34900	26900	31500	15400	13900	1780	1940	1650	1220
Pituitary gland	17400	8440	6590	850	ND	ND	ND	ND	ND
Preputial gland	11500	6390	6130	2240	1970	ND	ND	ND	ND
Prostate gland	ND	8630	4840	8620	ND	ND	ND	ND	ND
Salivary gland(s)	36200	24200	27200	9910	12200	1150	1080	899	522
Seminal vesicle(s)	5540	3530	2470	2150	825	BLQ	ND	ND	ND
Skin (pigmented)	11600	5930	3350	1180	1340	ND	ND	ND	ND
Small intestine	26900	56100	28000	14100	11300	5030	ND	ND	ND
Spinal cord	1950	742	BLQ	BLQ	ND	ND	ND	ND	ND
Spleen	35300	29600	29100	16600	12200	2570	2560	1550	978
Stomach	60500	70600	29200	7900	6730	1610	1500	1230	859
Stomach mucosa	ND	ND	ND	ND	ND	ND	ND	ND	ND
Stomach wall	ND	ND	ND	ND	ND	ND	ND	ND	ND
Testis(es)	2640	1760	1070	512	752	ND	ND	ND	ND
Thymus	12400	8190	7190	3440	3410	BLQ	ND	ND	ND
Thyroid	40700	32700	29000	NR	10900	4840	4150	3160	2620
Urinary bladder	ND	138000	49100	12000	6290	1290	ND	ND	ND
Urine	1170000	626000	128000	135000	18400	495	ND	ND	ND

BLQ = below the limit of quantitation (< 490 ng equivalents [^{14}C]GS-7340/g); F = female; M = male; ND = not detectable; NR = not represented; TAF = tenofovir alafenamide

Report Title	Study Type	Test Article	Report Number
Pharmacokinetics, Absorption, Distribution, and Excretion of [^{14}C]GS-7340 Following Oral Administration to Mice	Distribution	[^{14}C]TAF	AD-120-2011

Species	CD-1 Mice
Sex (M/F) / No. of Animals	M/4
Method of Administration:	Oral gavage
Dose (mg/kg):	100
Feeding Condition:	Not fasted
Radionuclide:	Carbon-14
Specific Activity:	300 μCi/kg
Vehicle / Formulation:	water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)
Analyte/Assay:	[^{14}C]TAF/ Liquid Scintillation Counter

	Cumulative Excretion of Radioactivity (% of dose)			
Time Point	**Urine**	**Feces**	**Cage Rinse**	**Total**
0-12 h	9.75	NA	NA	9.75
0-24 h	19.3	31.1	3.95	54.4
0-48 h	24.6	36.1	4.91	65.6
0-72 h	25.9	37.6	5.50	69.0
0-96 h	26.7	39.4	6.06	72.2
0-120 h	27.1	40.4	6.40	73.9
0-144 h	27.4	41.0	6.65	75.1
0-168 h	27.7	41.3	NA	69.0

F = female; M = male; NA = not applicable; TAF = tenofovir alafenamide

5.2. AD-120-2020: Pharmacokinetics, Absorption, Distribution, and Excretion of [^{14}C]TAF in Rat Following Oral Administration

Report Title	Study Type	Test Article	Report Number
Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]GS-7340 Following Single Oral Administration to Rats	Distribution	[^{14}C]TAF	AD-120-2020

Methods:

Species	Sprague-Dawley Rats
Sex (M/F)/No. of Animals	M/15
Method of Administration	Oral gavage
Dose (mg/kg/day)	5
Feeding Condition	Fasted
Specific Activity	100 µCi/mg
Radionuclide	Carbon-14
Vehicle Formulation	water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)
Sample	Plasma/Blood
Analyte/Assay:	[^{14}C]TAF/Liquid Scintillation Counter

Tabulated PK Results (Mean):

Sample Type	Plasma	Blood
T_{max} (h)	0.25	0.25
C_{max} (ng eq/g)	1110	603
t½ (h)	14.1	21.5
AUC_{0-t} (ng eq•h/g)	3591	2432
$AUC_{0-\infty}$ (ng eq•h/g)	3870	2588

F = female; M = male; TAF = tenofovir alafenamide

Report Title	Study Type	Test Article	Report Number
Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]GS-7340 Following Single Oral Administration to Rats	Distribution	[^{14}C]TAF	AD-120-2020

Methods:

Species	Sprague-Dawley Rats
Sex (M/F) / No. of Animals	M/9
Method of Administration:	Oral gavage
Dose (mg/kg):	5
Feeding Condition:	Fasted
Radionuclide:	Carbon-14
Specific Activity:	100 μCi/kg
Vehicle / Formulation:	water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)
Sampling Time:	0.25, 1, 4, 8, 12, 24, 48, 96, and 168 hours postdose
Analyte/Assay:	[^{14}C]TAF/Quantitative whole body autoradiography

Report Title	Study Type	Test Article	Report Number
Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]GS-7340 Following Single Oral Administration to Rats	Distribution	[^{14}C]TAF	AD-120-2020

Blood and Tissue radioactivity:	**Concentration of Radioactivity (ng Equivalents [^{14}C]TAF/g)**								
	Animal Number (Sacrifice Time)								
Tissue	**B35426 0.25h**	**B35427 1 h**	**B35428 4 h**	**B35429 8 h**	**B35430 12 h**	**B35431 24 h**	**B35432 48 h**	**B35433 96 h**	**B35434 168 h**
Adrenal gland(s)	181	129	BLQ	ND	ND	ND	ND	ND	ND
Arterial wall	817	299	118	ND	ND	ND	ND	ND	ND
Bile	ND	ND	ND	ND	ND	ND	ND	ND	ND
Blood	1070	334	138	76.6	83.1	ND	ND	ND	ND
Bone	BLQ	ND	ND	ND	ND	ND	ND	ND	ND
Bone marrow	233	125	72.6	BLQ	BLQ	ND	ND	ND	ND
Brain cerebellum	BLQ	ND	ND	ND	ND	ND	ND	ND	ND
Brain cerebrum	BLQ	ND	ND	ND	ND	ND	ND	ND	ND
Brain medulla	BLQ	ND	ND	ND	ND	ND	ND	ND	ND
Brain olfactory lobe	45.7	BLQ	ND	ND	ND	ND	ND	ND	ND
Bulbo-urethral gland	396	177	236	BLQ	ND	ND	ND	ND	ND
Cecum	218	132	541	313	NR	323	261	ND	ND
Diaphragm	210	145	52.9	BLQ	ND	ND	ND	ND	ND
Epididymis	249	101	BLQ	BLQ	ND	ND	ND	ND	ND
Esophagus	341	222	187	79.8	58.9	ND	ND	ND	ND
Exorbital lacrimal gland	234	101	51.2	ND	ND	ND	ND	ND	ND
Eye lens	BLQ	ND	ND	ND	ND	ND	ND	ND	ND
Eye uveal tract	409	187	78.4	ND	ND	ND	ND	ND	ND
Eye vitreous humor	84.9	89.3	BLQ	ND	ND	ND	ND	ND	ND
Eye(s)	86.1	92.7	BLQ	ND	ND	ND	ND	ND	ND
Fat (abdominal)	BLQ	BLQ	ND	ND	ND	ND	ND	ND	ND
Fat (brown)	200	97.1	48.2	BLQ	55.7	ND	ND	ND	ND
Harderian gland	98.6	52.4	BLQ	ND	ND	ND	ND	ND	ND
Intra-orbital lacrimal gland	250	118	BLQ	ND	ND	ND	ND	ND	ND
Kidney cortex	10700	12400	11800	6410	8300	2010	677	70.3	ND

Report Title	Study Type	Test Article	Report Number
Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]GS-7340 Following Single Oral Administration to Rats	Distribution	[^{14}C]TAF	AD-120-2020

Blood and Tissue radioactivity:	**Concentration of Radioactivity (ng Equivalents [^{14}C]TAF/g)**								
	Animal Number (Sacrifice Time)								
Tissue	**B35426 0.25h**	**B35427 1 h**	**B35428 4 h**	**B35429 8 h**	**B35430 12 h**	**B35431 24 h**	**B35432 48 h**	**B35433 96 h**	**B35434 168 h**
Kidney medulla	8240	5040	2800	1210	1010	317	161	BLQ	ND
Kidney(s)	9520	8710	8250	4720	4590	1380	511	52.0	ND
Large intestine	364	140	55.8	BLQ	ND	ND	ND	ND	ND
Liver	6730	6730	4010	2410	3570	1090	751	145	195
Lung(s)	592	211	81.1	ND	ND	ND	ND	ND	ND
Lymph node(s)	318	ND	ND	ND	ND	ND	ND	ND	ND
Muscle	101	BLQ	ND	ND	ND	ND	ND	ND	ND
Myocardium	361	136	58.6	BLQ	ND	ND	ND	ND	ND
Nasal turbinates	123	83.8	52.8	ND	ND	ND	ND	ND	ND
Pancreas	215	120	68.9	46.1	52.0	ND	ND	ND	ND
Pituitary gland	335	116	50.5	BLQ	76.2	ND	ND	ND	ND
Preputial gland	140	60.1	BLQ	ND	ND	ND	ND	ND	ND
Prostate gland	134	88.6	117	BLQ	BLQ	ND	ND	ND	ND
Salivary gland(s)	292	119	BLQ	ND	ND	ND	ND	ND	ND
Seminal vesicle(s)	62.7	ND	ND	ND	ND	ND	ND	ND	ND
Skin (nonpigmented)	393	123	BLQ	ND	ND	ND	ND	ND	ND
Small intestine	479	500	364	284	86.5	171	58.3	ND	ND
Spinal cord	BLQ	ND	ND	ND	ND	ND	ND	ND	ND
Spleen	147	105	59.0	BLQ	62.8	BLQ	ND	ND	ND
Stomach	475	126	66.6	ND	ND	ND	ND	ND	ND
Testis(es)	98.2	50.9	BLQ	ND	ND	ND	ND	ND	ND

Report Title	Study Type	Test Article	Report Number
Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]GS-7340 Following Single Oral Administration to Rats	Distribution	[^{14}C]TAF	AD-120-2020

Blood and Tissue radioactivity:

	Concentration of Radioactivity (ng Equivalents [^{14}C]TAF/g)								
	Animal Number (Sacrifice Time)								
Tissue	**B35426 0.25h**	**B35427 1 h**	**B35428 4 h**	**B35429 8 h**	**B35430 12 h**	**B35431 24 h**	**B35432 48 h**	**B35433 96 h**	**B35434 168 h**
Thymus	91.2	68.1	BLQ	ND	ND	ND	ND	ND	ND
Thyroid	324	147	63.6	ND	ND	ND	ND	ND	ND
Tooth pulp	646	228	84.4	ND	ND	ND	ND	ND	ND
Urinary bladder	925	205	ND	BLQ	1050	BLQ	ND	ND	ND
Urine	112000	34400	51100	3260	14500	988	645	88.3	ND

BLQ = below the limit of quantitation (< 45.6 ng equivalents [^{14}C]GS-7340/g); F = female; M = male; ND = not detectable; NR = not represented (tissue not present in section); TAF = tenofovir alafenamide

Report Title	Study Type	Test Article	Report Number
Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]GS-7340 Following Single Oral Administration to Rats	Distribution	[^{14}C]TAF	AD-120-2020

Methods:

Species	Long Evans rats
Sex (M/F) / No. of Animals	M/9
Method of Administration:	Oral gavage
Dose (mg/kg):	5
Feeding Condition:	Fasted
Radionuclide:	Carbon-14
Specific Activity:	100 μCi/kg
Vehicle / Formulation:	water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)
Sampling Time:	0.25, 1, 4, 8, 12, 24, 48, 96, and 168 hours postdose
Analyte/Assay:	[^{14}C]TAF/Quantitative whole body autoradiography

Report Title	Study Type	Test Article	Report Number
Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]GS-7340 Following Single Oral Administration to Rats	Distribution	[^{14}C]TAF	AD-120-2020

Blood and Tissue radioactivity:

Tissue	Concentration of Radioactivity (ng Equivalents [^{14}C]TAF/g) Animal Number (Sacrifice Time)								
	B35435 0.25h	B35436 1 h	B35437 4 h	B35438 8 h	B35439 12 h	B35440 24 h	B35441 48 h	B35442 96 h	B35443 168 h
Adrenal gland(s)	353	115	48.5	ND	ND	ND	ND	ND	ND
Arterial wall	1350	270	90.4	93.8	ND	ND	ND	ND	ND
Bile	ND	ND	ND	ND	ND	ND	ND	ND	ND
Blood	1260	221	116	125	117	ND	ND	ND	ND
Bone	50.4	BLQ	ND	ND	ND	ND	ND	ND	ND
Bone marrow	311	84.9	BLQ	62.4	BLQ	ND	ND	ND	ND
Brain cerebellum	BLQ	ND	ND	ND	ND	ND	ND	ND	ND
Brain cerebrum	BLQ	ND	ND	ND	ND	ND	ND	ND	ND
Brain medulla	BLQ	ND	ND	ND	ND	ND	ND	ND	ND
Brain olfactory lobe	57.2	51.0	ND	ND	ND	ND	ND	ND	ND
Bulbo-urethral gland	831	209	ND	ND	ND	ND	ND	ND	ND
Cecum	603	118	889	462	362	494	101	BLQ	ND
Diaphragm	353	124	55.8	BLQ	ND	ND	ND	ND	ND
Epididymis	516	79.3	BLQ	58.4	ND	ND	ND	ND	ND
Esophagus	923	218	186	637	65.8	ND	ND	ND	ND
Exorbital lacrimal gland	353	56.1	BLQ	BLQ	ND	ND	ND	ND	ND
Eye lens	BLQ	BLQ	ND	ND	ND	ND	ND	ND	ND
Eye uveal tract	555	89.5	59.4	BLQ	ND	ND	ND	ND	ND
Eye vitreous humor	139	BLQ	BLQ	ND	ND	ND	ND	ND	ND
Eye(s)	150	BLQ	BLQ	BLQ	ND	ND	ND	ND	ND
Fat (abdominal)	70.0	BLQ	ND	ND	ND	ND	ND	ND	ND

Blood and Tissue radioactivity:	**Concentration of Radioactivity (ng Equivalents [^{14}C]TAF/g) Animal Number (Sacrifice Time)**								
Tissue	**B35435 0.25h**	**B35436 1 h**	**B35437 4 h**	**B35438 8 h**	**B35439 12 h**	**B35440 24 h**	**B35441 48 h**	**B35442 96 h**	**B35443 168 h**
Fat (brown)	232	BLQ	ND	ND	ND	ND	ND	ND	ND
Harderian gland	209	BLQ	ND	ND	ND	ND	ND	ND	ND
Intra-orbital lacrimal gland	402	53.4	49.1	ND	ND	ND	ND	ND	ND
Kidney cortex	8000	8890	6980	7360	5150	2440	570	65.6	ND
Kidney medulla	6900	3670	655	1920	757	367	117	BLQ	ND
Kidney(s)	7750	7570	5160	5260	3000	1310	390	48.1	ND
Large intestine	548	91.5	474	97.5	133	132	575	166	ND
Liver	10300	7800	7710	6670	5610	1380	671	221	139
Lung(s)	854	145	67.2	76.5	66.3	BLQ	ND	ND	ND
Lymph node(s)	422	94.9	ND	ND	ND	ND	ND	ND	ND
Muscle	115	BLQ	ND	ND	ND	ND	ND	ND	ND
Myocardium	512	46.6	BLQ	72.7	BLQ	ND	ND	ND	ND
Nasal turbinates	201	71.9	BLQ	ND	ND	ND	ND	ND	ND
Pancreas	274	82.1	53.5	71.0	ND	ND	ND	ND	ND
Pituitary gland	434	53.1	ND	ND	ND	ND	ND	ND	ND
Preputial gland	247	67.9	ND	ND	ND	ND	ND	ND	ND
Prostate gland	247	BLQ	49.9	ND	ND	ND	ND	ND	ND
Salivary gland(s)	368	85.3	BLQ	BLQ	BLQ	ND	ND	ND	ND
Seminal vesicle(s)	67.7	BLQ	ND	ND	ND	ND	ND	ND	ND
Skin (nonpigmented)	526	71.8	BLQ	ND	ND	ND	ND	ND	ND
Skin (pigmented)	623	78.9	BLQ	ND	ND	ND	ND	ND	ND
Small intestine	530	379	122	278	220	98.6	BLQ	ND	ND
Spinal cord	BLQ	BLQ	ND	ND	ND	ND	ND	ND	ND
Spleen	231	83.1	58.3	75.8	60.7	BLQ	BLQ	ND	ND
Stomach	682	113	71.4	69.9	159	BLQ	BLQ	ND	ND
Testis(es)	157	BLQ	BLQ	BLQ	BLQ	ND	ND	ND	ND
Thymus	181	BLQ	BLQ	BLQ	ND	ND	ND	ND	ND

Blood and Tissue radioactivity:	**Concentration of Radioactivity (ng Equivalents [^{14}C]TAF/g) Animal Number (Sacrifice Time)**								
Tissue	**B35435 0.25h**	**B35436 1 h**	**B35437 4 h**	**B35438 8 h**	**B35439 12 h**	**B35440 24 h**	**B35441 48 h**	**B35442 96 h**	**B35443 168 h**
Thyroid	412	59.1	ND	ND	ND	ND	ND	ND	ND
Tooth pulp	793	194	78.7	89.3	57.0	NR	ND	ND	ND
Urinary bladder	352	155	125	89.1	45.9	48.3	BLQ	ND	ND
Urine	50200	61000	2280	6540	1140	1500	109	BLQ	ND

BLQ = below the limit of quantitation (< 45.6 ng equivalents [^{14}C]GS-7340/g); F = female; M = male; ND = not detectable; NR = not represented (tissue not present in section); TAF = tenofovir alafenamide

Report Title	Study Type	Test Article	Report Number
Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]GS-7340 Following Single Oral Administration to Rats	Distribution	[^{14}C]TAF	AD-120-2020

Methods:

Species	Sprague-Dawley rats
Sex (M/F) / No. of Animals	M/3
Method of Administration:	Oral gavage
Dose (mg/kg):	5
Feeding Condition:	Fasted
Radionuclide:	Carbon-14
Specific Activity:	100 μCi/kg
Vehicle / Formulation:	water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)
Analyte/Assay:	[^{14}C]TAF/ Liquid Scintillation Counter

	Cumulative Excretion of Radioactivity (% of dose)			
Time Point	**Urine**	**Feces**	**Cage Rinse**	**Total**
0-8 h	14.6 ± 3.78	NA	NA	14.6 ± 3.78
0-24 h	19.8 ± 4.96	66.8 ± 6.14	1.22 ± 0.16	87.9 ± 1.06
0-48 h	21.5 ± 5.45	71.1 ± 4.73	1.46 ± 0.08	94.1 ± 0.71
0-72 h	21.8 ± 5.45	71.6 ± 4.50	1.56 ± 0.15	95.0 ± 1.01
0-96 h	22.0 ± 5.48	71.8 ± 4.52	1.67 ± 0.26	95.4 ± 0.97
0-120 h	22.1 ± 5.47	71.8 ± 4.51	1.70 ± 0.28	95.6 ± 0.90
0-144 h	22.1 ± 5.47	71.9 ± 4.51	1.71 ± 0.28	95.7 ± 0.95
0-168 h	22.2 ± 5.42	71.9 ± 4.49	NA	94.1 ± 0.93

F = female; M = male; NA = not applicable; TAF = tenofovir alafenamide

Report Title	Study Type	Test Article	Report Number
Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]GS-7340 Following Single Oral Administration to Rats	Distribution	[^{14}C]TAF	AD-120-2020

Methods:

Species	Bile Duct-Cannulated SD rats
Sex (M/F) / No. of Animals	M/5
Method of Administration:	Oral gavage
Dose (mg/kg):	5
Feeding Condition:	Fasted
Radionuclide:	Carbon-14
Specific Activity:	100 μCi/kg
Vehicle / Formulation:	water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)
Analyte/Assay:	[^{14}C]TAF/ Liquid Scintillation Counter

	Cumulative Excretion of Radioactivity (% of dose)				
Time Point	**Urine**	**Feces**	**Bile**	**Cage Rinse**	**Total**
0-2 h	NA	NA	1.81 ± 0.76	NA	1.81 ± 0.76
0-4 h	NA	NA	1.93 ± 0.76	NA	1.93 ± 0.76
0-6 h	NA	NA	1.97 ± 0.77	NA	1.97 ± 0.77
0-8 h	15.3 ± 1.82	NA	2.00 ± 0.78	NA	17.3 ± 2.18
0-12 h	NA	NA	2.04 ± 0.79	NA	2.04 ± 0.80
0-24 h	21.1 ± 3.42	56.7 ± 15.3	2.09 ± 0.81	0.91 ± 0.17	80.7 ± 11.4
0-48 h	22.7 ± 3.95	70.9 ± 7.10	2.11 ± 0.82	1.14 ± 0.21	96.8 ± 2.58
0-72 h	23.0 ± 4.06	72.3 ± 6.02	2.11 ± 0.82	1.28 ± 0.27	98.6 ± 1.49
0-96 h	23.1 ± 4.11	72.4 ± 5.95	2.11 ± 0.82	1.33 ± 0.29	99.0 ± 1.40
0-120 h	23.2 ± 4.12	72.5 ± 5.96	2.11 ± 0.82	1.35 ± 0.29	99.1 ± 1.40
0-144 h	23.2 ± 4.13	72.5 ± 5.96	2.11 ± 0.82	1.37 ± 0.30	99.2 ± 1.49
0-168 h	23.2 ± 4.15	72.6 ± 5.96	2.11 ± 0.82	NA	97.9 ± 1.35

F = female; M = male; NA = not applicable; TAF = tenofovir alafenamide

5.3. AD-120-2009: Distribution of [^{14}C]TAF in Dog Following Oral Administration

Report Title	Study Type	Test Article	Report Number
Absorption and Distribution of [^{14}C]GS-7340 Following Single and Multiple Oral Doses to Dogs	Distribution	[^{14}C]TAF	AD-120-2009

Methods:

Species	Beagle dogs
Sex (M/F) / No. of Animals	M/10
Method of Administration:	Oral gavage
Dose Administration:	Nonradiolabeled test article was dosed for 4 days followed by a single dose of radiolabeled test article on Day 5
Dose (mg/kg/day):	15
Feeding Condition:	Fasted
Radionuclide:	Carbon-14
Specific Activity:	10 μCi/kg
Vehicle / Formulation:	water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)
Analyte/Assay:	[^{14}C]TAF/ Liquid Scintillation Counter

	Mean Concentration (ng equivalents [^{14}C]GS-7340/g)	
Time Point	**Blood**	**Plasma**
1 h	2220	3180
2 h	1290	1750
6 h	811	783
12 h	449	363
24 h	340	277

F = female; M = male; NA = not applicable; TAF = tenofovir alafenamide
Note: Mean concentrations were calculated from 2 animals/timepoint

Report Title	Study Type	Test Article	Report Number
Absorption and Distribution of [^{14}C]GS-7340 Following Single and Multiple Oral Doses to Dogs	Distribution	[^{14}C]TAF	AD-120-2009

Methods:

Species	Beagle dogs
Sex (M/F) / No. of Animals	M/10
Method of Administration:	Oral gavage
Dose (mg/kg):	15
Feeding Condition:	Fasted
Radionuclide:	Carbon-14
Specific Activity:	10 μCi/kg
Vehicle / Formulation:	water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)
Analyte/Assay:	[^{14}C]TAF/ Liquid Scintillation Counter

	Mean Concentration (ng equivalents [^{14}C]GS-7340/g)	
Time Point	**Blood**	**Plasma**
1 h	485	778
2 h	1280	1890
6 h	401	355
12 h	295	262
24 h	201	172

F = female; M = male; NA = not applicable; TAF = tenofovir alafenamide
Note: Mean concentrations were calculated from 2 animals/timepoint

Report Title	Study Type	Test Article	Report Number
Absorption and Distribution of [^{14}C]GS-7340 Following Single and Multiple Oral Doses to Dogs	Distribution	[^{14}C]TAF	AD-120-2009

Methods:

Species	Beagle dogs
Sex (M/F) / No. of Animals	M/10
Method of Administration:	Oral gavage
Dose Administration:	Nonradiolabeled test article was dosed for 4 days followed by a single dose of radiolabeled test article on Day 5
Dose (mg/kg/day):	15
Feeding Condition:	Fasted
Radionuclide:	Carbon-14
Specific Activity:	10 μCi/kg
Vehicle Formulation	water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)
Analyte/Assay:	[^{14}C]TAF/Liquid Scintillation Counter

	Concentrations (ng Equivalents [^{14}C]GS-7340/g)					
Tint Point	**Bone**	**Heart**	**Kidney(s)**	**Liver**	**Lung**	**Thyroid**
1 h	2390	3450	45500	74900	8660	15200
2 h	4410	4420	89300	105000	13000	26500
6 h	5600	5310	162000	109000	15000	30500
12 h	4350	4330	144000	84600	9640	21600
24 h	2990	3970	107000	78100	8610	16800

F = female; M = male; TAF = tenofovir alafenamide

Report Title	Study Type	Test Article	Report Number
Absorption and Distribution of [^{14}C]GS-7340 Following Single and Multiple Oral Doses to Dogs	Distribution	[^{14}C]TAF	AD-120-2009

Methods:

Species	Beagle dogs
Sex (M/F) / No. of Animals	M/10
Method of Administration:	Oral gavage
Dose (mg/kg):	15
Feeding Condition:	Fasted
Radionuclide:	Carbon-14
Specific Activity:	10 μCi/kg
Vehicle Formulation	water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)
Analyte/Assay:	[^{14}C]TAF/Liquid Scintillation Counter

	Concentrations (ng Equivalents [^{14}C]GS-7340/g)					
Time Point	**Bone**	**Heart**	**Kidney(s)**	**Liver**	**Lung**	**Thyroid**
1 h	476	776	17000	47900	2480	2960
2 h	2440	2750	82500	97600	10200	12700
6 h	2600	3070	99700	97000	8870	15600
12 h	2220	2820	91800	78300	6970	16800
24 h	1100	1990	88500	64100	5260	4900

F = female; M = male; TAF = tenofovir alafenamide

Report Title	Study Type	Test Article	Report Number
Absorption and Distribution of [^{14}C]GS-7340 Following Single and Multiple Oral Doses to Dogs	Distribution	[^{14}C]TAF	AD-120-2009

Methods:

Species Beagle dogs
Sex (M/F) / No. of Animals M/10
Method of Administration: Oral gavage
Dose Administration: Nonradiolabeled test article was dosed for 4 days followed by a single dose of radiolabeled test article on Day 5
Dose (mg/kg/day): 15
Feeding Condition: Fasted
Radionuclide: Carbon-14
Specific Activity: 10 μCi/kg
Vehicle Formulation water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)
Analyte/Assay: [^{14}C]TAF/Liquid Scintillation Counter

	Percent of Radioactive Dose (% of Dose)						
Time Point	**Blood**	**Bone**	**Heart**	**Kidney(s)**	**Liver**	**Lung**	**Thyroid**
1 h	0.64	1.48	0.19	1.39	15.4	0.50	0.013
2 h	0.37	2.70	0.24	2.78	23.1	0.75	0.019
6 h	0.23	3.45	0.26	4.17	19.4	0.87	0.020
12 h	0.13	2.69	0.24	3.93	18.3	0.60	0.011
24 h	0.10	1.85	0.22	2.76	15.0	0.50	0.008

F = female; M = male; TAF = tenofovir alafenamide

Report Title	Study Type	Test Article	Report Number
Absorption and Distribution of [^{14}C]GS-7340 Following Single and Multiple Oral Doses to Dogs	Distribution	[^{14}C]TAF	AD-120-2009

Methods:

Species	Beagle dogs
Sex (M/F) / No. of Animals	M/10
Method of Administration:	Oral gavage
Dose (mg/kg):	15
Feeding Condition:	Fasted
Radionuclide:	Carbon-14
Specific Activity:	10 μCi/kg
Vehicle Formulation	water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)
Analyte/Assay:	[^{14}C]TAF/Liquid Scintillation Counter

	Percent of Radioactive Dose (% of Dose)						
Tint Point	**Blood**	**Bone**	**Heart**	**Kidney(s)**	**Liver**	**Lung**	**Thyroid**
1 h	0.14	0.30	0.05	0.60	8.78	0.19	0.002
2 h	0.37	1.51	0.17	2.84	19.6	0.62	0.009
6 h	0.12	1.61	0.17	3.46	18.6	0.50	0.009
12 h	0.08	1.37	0.15	3.02	18.2	0.44	0.012
24 h	0.06	0.67	0.10	2.79	13.9	0.34	0.003

F = female; M = male; TAF = tenofovir alafenamide

5.4. D990173-BP: Distribution of [^{14}C]TAF in Dog Following Oral Administration

Report Title	Study Type	Test Article	Report Number
Analysis of Data from Oread Biosafety Study G545 "Tissue Distribution of [^{14}C]GS-7340 in Beagle Dogs Following Oral Administration"	Distribution	[^{14}C]TAF	D990173-BP

Methods:

Species	Beagle dogs
Sex (M/F) / No. of Animals	M/2
Method of Administration:	Oral gavage
Dose (mg/kg):	18.1
Feeding Condition:	Fasted
Radionuclide:	Carbon-14
Specific Activity:	31.7 μCi/kg
Vehicle Formulation	50 mM citric acid
Sampling Time:	24 hours postdose
Analyte/Assay:	[^{14}C]TAF/Liquid Scintillation Counter

Report Title	Study Type	Test Article	Report Number
Analysis of Data from Oread Biosafety Study G545 "Tissue Distribution of [^{14}C]GS-7340 in Beagle Dogs Following Oral Administration"	Distribution	[^{14}C]TAF	D990173-BP

Distribution of Radioactivity

Tissue/Fluid	Percent of Total Dose (%)	Percent of Recovered Dose[a] (%)	Concentration (μg eq TFV/g)
Liver	16.5	26.0	52.9
Kidney	3.78	5.98	80.2
Mesenteric Lymph Nodes	0.04	0.06	6.88
Spleen	0.17	0.27	8.13
Femoral Bone	0.00	0.00	0.28
Bone Marrow	0.00	0.00	2.05
Stomach	0.26	0.41	2.68
Jejunum	0.79	1.26	4.16
Duodenum	0.44	0.70	8.77
Ileum	0.16	0.26	4.61
large Intestine	2.66	4.28	47.2
Bile	0.23	0.35	40.5
Feces	0.19	0.15	480
Stomach Content	0.04	0.06	0.91
Small Intestine Content	1.25	2.00	11.3
Large Intestine Content	20.4	32.4	155
Urine	14.7	23.1	25.2
Plasma	0.00	0.00	0.20
PBMCs	0.09	0.14	63.2[b]

F = female; M = male; TAF = tenofovir alafenamide; TFV = tenofovir
a Selected tissues reported; b Estimated based on the mean reported single cell volume of 0.2 picoliters

6. PHARMACOKINETICS: PLASMA PROTEIN BINDING

6.1. AD-120-2026: Plasma Protein Binding of TAF In Vitro

Report Title	Study Type	Test Article	Report Number
Plasma Protein Binding of GS-7340	Plasma protein binding, in vitro	TAF	AD-120-2026

Study System: Plasma from Dog and Human

Target Entry, Test System, and Methods: Equilibrium dialysis for 3 hours at 37°C against 0.133 M phosphate buffer, pH 7.4. Analysis by LC/MS/MS.

Matrix	Fraction Unbound (%)
Dog plasma	48.0 ± 6.2
Human plasma	46.8 ± 2.3

TAF = tenofovir alafenamide
Note: Incubation concentration of TAF was 2 μM

7. PHARMACOKINETICS: STUDY IN PREGNANT OR NURSING ANIMALS

Studies of TAF in pregnant animals are presented in m2.6.7, Sections 11.4 and 12.6.

8. PHARMACOKINETICS: METABOLISM IN VITRO

8.1. AD-120-2025: Metabolism of TAF In Vitro (Plasma Stability)

Report Title	Study Type	Test Article	Report Number
In Vitro Metabolism of GS-7340 in Plasma from Dog and Human	Metabolism, In Vitro	TAF	AD-120-2025

Study Systems	Duplicate aliquots of 3 µM TAF were incubated with pooled plasma from beagle dog and human at 37ºC up to 4 hours. Rates of metabolism (in vitro half-life values) were determined. Analysis was done by LC/MS/MS.
Species	**Half-Life (min)**
Dog	69.5 ± 3.20
Human	74.7 ± 6.40

TAF = tenofovir alafenamide

8.2. AD-120-2023: Metabolism of TAF In Vitro in Hepatic S9

Report Title	Study Type	Test Article	Report Number
In Vitro Metabolism of GS-7340 in Hepatic Subcellular Fractions from Dog and Human	Metabolism, In Vitro	TAF	AD-120-2023

Study Systems	Duplicate aliquots of 3 µM TAF were incubated with pooled hepatic S9 fractions from beagle dog and human at 37ºC up to 90 minutes. Rates of metabolism (in vitro half-life values) were determined and hepatic extraction was predicted using the well-stirred liver model. Analysis was done by LC/MS/MS.

Species	Half-Life (min)	Predicted Hepatic Extraction (%)
Dog	31.1 ± 3.40	60.5
Human	20.6 ± 0.70	76.2

TAF = tenofovir alafenamide

8.3. AD-120-2024: Metabolism of TAF In Vitro in Intestine S9

Report Title	**Study Type**	**Test Article**	**Report Number**
In Vitro Metabolism of GS-7340 in Intestinal Subcellular Fractions from Dog and Human	Metabolism, In Vitro	TAF	AD-120-2024

Study Systems	Duplicate aliquots of 3 µM TAF were incubated with pooled intestinal S9 fractions from beagle dog and human at 37ºC up to 120 minutes. Rates of metabolism (in vitro half-life values) were determined. Analysis was done by LC/MS/MS.
Species	**Half-Life (min)**
Dog	47.1 ± 2.60
Human	58.3 ± 4.40

TAF = tenofovir alafenamide

8.4. AD-120-2017: Metabolism of TAF In Vitro in Primary Human Hepatocytes

Report Title	**Study Type**	**Test Article**	**Report Number**
In Vitro Activation of GS-1278, GS-4331 and GS-7340 in Primary Human Hepatocytes	Metabolism, In Vitro	TFV, TDF, TAF	AD-120-2017

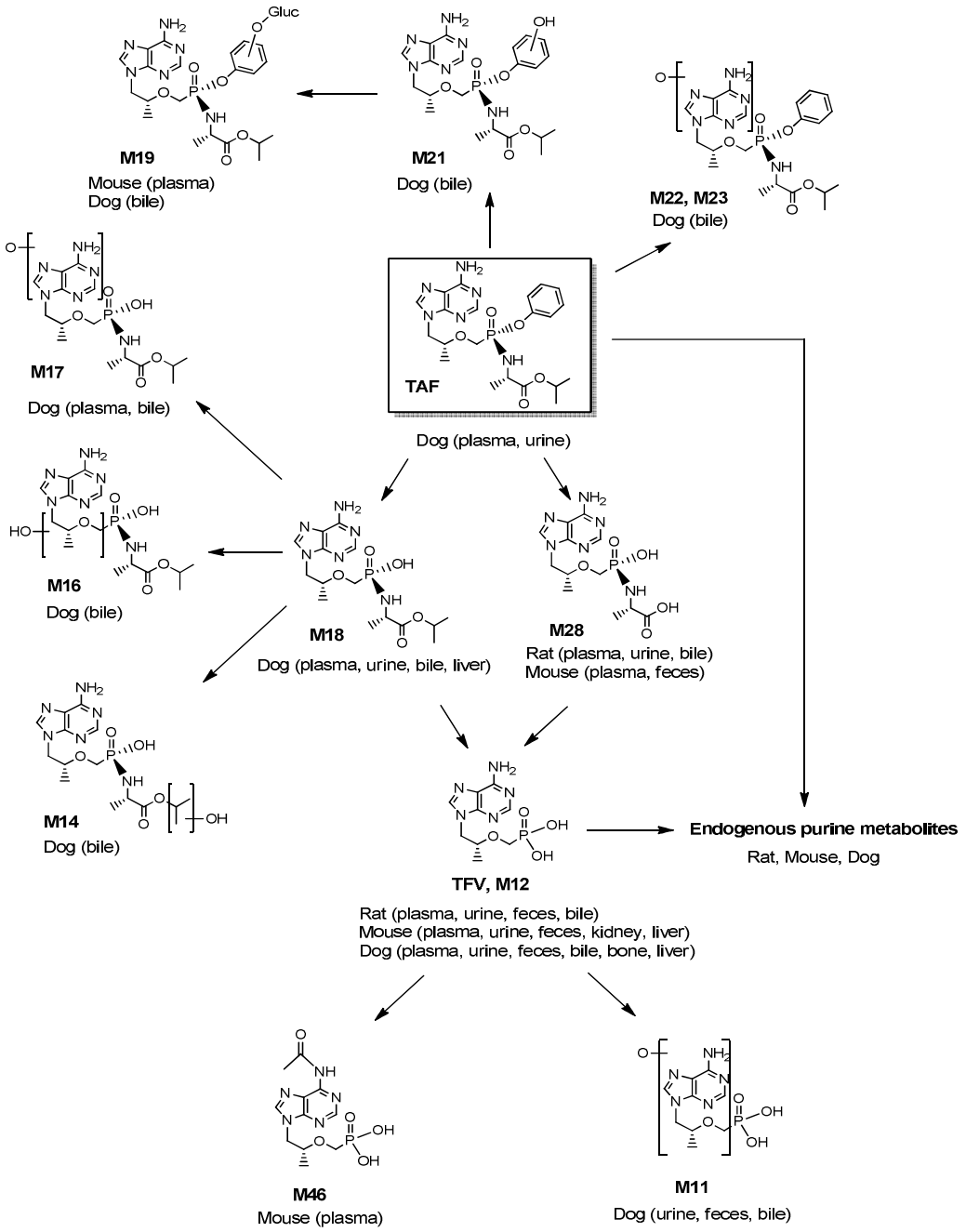
Study Systems	Duplicate wells of 5 µM test compounds were incubated with primary human hepatocytes for 24 hours. Intracellular concentrations of TFV-DP were determined. Analysis was done by LC/MS/MS.
Sampling Time	24 hours

	TFV-DP Concentration (pmol/million cell)
TFV	12.1
TDF	302
TAF	1,470

TAF = tenofovir alafenamide; TDF = tenofovir disoproxil; TFV = tenofovir; TFV-DP = tenofovir diphosphate

9. PHARMACOKINETICS: POSSIBLE METABOLIC PATHWAYS

The diagram shows TAF metabolites observed in rats, mice, and dogs after a single oral dose of [^{14}C]TAF.



10. PHARMACOKINETICS: METABOLISM IN VIVO

10.1. AD-120-2012: Metabolism of TAF in Mouse

Report Title	Study Type	Test Article	Report Number
Profiling and Identification of Metabolites in Selected Plasma, Urine, Feces, Kidney, Liver, and Nasal Turbinate Samples from Mice after Oral Administration of [^{14}C]GS-7340 and Stability of [^{14}C]GS-7340 in vitro using CD-1 Mouse Hepatic Microsomes and Plasma	Metabolism	[^{14}C]TAF	AD-120-2012

Methods:

Species	CD-1 mice
Sex (M/F) / No. of Animals	Male/30
Method of Administration:	Oral gavage
Dose (mg/kg):	100
Feeding Condition:	Not fasted
Vehicle Formulation	water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)
Sample	Plasma
Analyte/Assay:	[^{14}C]TAF/Liquid Scintillation Counter

	Concentrations (ng Equivalents [^{14}C]GS-7340/g)					AUC_{0-24h}	% AUC of Total
Final Metabolite Designation	**0.25 h**	**1 h**	**2 h**	**12 h**	**24 h**	**(ng eq•h/g)**	**Radioactivity**
M27A (Allantoin)	549	420	1820	143	121	10530	12.2
M27B (Uric acid)	259	1150	1420	390	371	16670	19.4
M5	1050	239	277	73.4	ND	2411	2.80
M6	280	86.4	160	ND	ND	NA	NA
M28	1690	425	169	ND	ND	878	1.02
M12	13600	4740	5030	1340	385	47167	54.8
M45	2260	667	354	ND	ND	3177	3.69
M46	2740	688	326	ND	ND	2738	3.18
M18	ND	ND	201	ND	ND	NA	NA
M47	ND	ND	166	ND	ND	NA	NA
M19	332	20.9	765	ND	ND	1533	1.78
M23	ND	ND	125	ND	ND	NA	NA

NA = not applicable; ND = peak was not detected or below the established limit of quantitation; TAF = tenofovir alafenamide

Report Title	Study Type	Test Article	Report Number
Profiling and Identification of Metabolites in Selected Plasma, Urine, Feces, Kidney, Liver, and Nasal Turbinate Samples from Mice after Oral Administration of [^{14}C]GS-7340 and Stability of [^{14}C]GS-7340 in vitro using CD-1 Mouse Hepatic Microsomes and Plasma	Metabolism	[^{14}C]TAF	AD-120-2012

Methods:

Species CD-1 mice

Sex (M/F) / No. of Animals Male/30

Method of Administration: Oral gavage

Dose (mg/kg): 100

Feeding Condition: Not fasted

Vehicle Formulation water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)

Analyte/Assay: [^{14}C]TAF/Liquid Scintillation Counter

	Urine (% Radioactive Dose)				Feces (% Radioactive Dose)			
	Collection Interval (h)				Collection Interval (h)			
Final Metabolite Designation	0-24	24-48	48-72	Total	0-24	24-48	48-72	Total
M27A (Allantoin)	1.56	0.70	0.32	2.57	0.42	ND	0.02	0.43
M5	1.91	0.23	0.02	2.16	NA	NA	NA	NA
M12	13.0	4.12	0.99	18.1	25.3	4.14	1.26	30.7
M28	NA	NA	NA	NA	0.67	ND	ND	0.67
M32	0.36	ND	ND	0.36	NA	NA	NA	NA
M35	0.66	ND	ND	0.66	NA	NA	NA	NA

NA = not applicable; ND = peak was not detected or below the established limit of quantitation; TAF = tenofovir alafenamide

Report Title	Study Type	Test Article	Report Number
Profiling and Identification of Metabolites in Selected Plasma, Urine, Feces, Kidney, Liver, and Nasal Turbinate Samples from Mice after Oral Administration of [^{14}C]GS-7340 and Stability of [^{14}C]GS-7340 in vitro using CD-1 Mouse Hepatic Microsomes and Plasma	Metabolism	[^{14}C]TAF	AD-120-2012

Methods:

Species CD-1 mice

Sex (M/F) / No. of Animals Male/30

Method of Administration: Oral gavage

Dose (mg/kg): 100

Feeding Condition: Not fasted

Vehicle Formulation water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)

Analyte/Assay: [^{14}C]TAF/Liquid Scintillation Counter

	Kidney Concentration (ng equivalents [^{14}C]GS-7340/g)			**Liver Concentration (ng equivalents [^{14}C]GS-7340/g)**		
	Collection Time (h)			**Collection Time (h)**		
Final Metabolite Designation	**1**	**24**	**48**	**1**	**24**	**48**
M27A (Allantoin)	NA	NA	NA	107000	63800	35900
M27B (Uric acid)	4250	3050	2180	NA	NA	NA
M7 (Xanthine)	2830	2070	2460	NA	NA	NA
M8 (Hypoxanthine)	1010	731	1190	NA	NA	NA
M12	90300	18000	4620	356000	93200	18500

NA = not applicable; TAF = tenofovir alafenamide

Report Title	Study Type	Test Article	Report Number
Profiling and Identification of Metabolites in Selected Plasma, Urine, Feces, Kidney, Liver, and Nasal Turbinate Samples from Mice after Oral Administration of [^{14}C]GS-7340 and Stability of [^{14}C]GS-7340 in vitro using CD-1 Mouse Hepatic Microsomes and Plasma	Metabolism	[^{14}C]TAF	AD-120-2012

Methods:

Species CD-1 mice

Sex (M/F) / No. of Animals Male/30

Method of Administration: Oral gavage

Dose (mg/kg): 100

Feeding Condition: Not fasted

Vehicle Formulation water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)

Analyte/Assay: [^{14}C]TAF/Liquid Scintillation Counter

	Concentration (ng equivalents [^{14}C]GS-7340/g)				
	Collection Time (h)				
Final Metabolite Designation	**1**	**4**	**12**	**24**	**48**
M1	392	214	250	325	399
M2	794	326	364	ND	ND
M5	ND	ND	ND	90.6	ND
M7 (Xanthine)	953	ND	ND	46.8	ND
M8 (Hypoxanthine)	254	121	342	298	373
M29	2060	737	599	254	ND
M30	159	ND	164	213	136
M34	ND	ND	ND	70.1	106

ND = peak was not detected or below the established limit of quantitation; TAF = tenofovir alafenamide

Report Title	Study Type	Test Article	Report Number
Profiling and Identification of Metabolites in Selected Plasma, Urine, Feces, Kidney, Liver, and Nasal Turbinate Samples from Mice after Oral Administration of [^{14}C]GS-7340 and Stability of [^{14}C]GS-7340 in vitro using CD-1 Mouse Hepatic Microsomes and Plasma	Metabolism	[^{14}C]TAF	AD-120-2012

Study Systems [^{14}C]GS-7340 (5 µM) was incubated at 37°C with either pooled plasma or hepatic microsomes (with 1 mMNADPH) from male CD-1 mice for 0 and 60 minutes. The extracted samples were analyzed by HPLC with in-line radioactivity detection to determine the metabolite profiles.

Final Metabolite Designation	Plasma		Hepatic Microsomes (1 mg/mL protein)	
	Percent of Radioactivity Injected (% of Run)		Percent of Radioactivity Injected (% of Run)	
	0 minute	60 minute	0 minute	60 minute
[^{14}C]M1	NA	NA	ND	0.77
[^{14}C]M2	ND	43.0	3.00	11.1
[^{14}C]M5	NA	NA	ND	ND
[^{14}C]M6	NA	NA	ND	ND
[^{14}C]M28	ND	46.0	2.24	3.98
[^{14}C]M29	NA	NA	ND	0.72
[^{14}C]M30	NA	NA	ND	0.82
[^{14}C]M31	NA	NA	ND	0.35
[^{14}C]M32	ND	5.70	NA	NA
[^{14}C]M33 ([^{14}C]Adenine)	NA	NA	ND	41.0
[^{14}C]M18	43.6	0.87	1.63	0.89
[^{14}C]M20	28.5	ND	ND	ND
[^{14}C]M36	NA	NA	3.35	1.38
[^{14}C]M37	NA	NA	ND	0.70
[^{14}C]M38	NA	NA	ND	3.08
[^{14}C]M39	NA	NA	ND	1.87
[^{14}C]M40	NA	NA	ND	2.96
[^{14}C]M41	NA	NA	ND	0.41
[^{14}C]M42	NA	NA	ND	0.50
[^{14}C]GS-7340	24.1	ND	84.1	16.3

NA = not applicable; ND = peak was not detected or below the established limit of quantitation; TAF = tenofovir alafenamide

10.2. AD-120-2021: Metabolism of TAF in Rat

Report Title	Study Type	Test Article	Report Number
Profiling and Identification of Metabolites in Selected Plasma, Urine, Bile, and Feces Samples from Rats after Oral Administration of [^{14}C]GS-7340	Metabolism	[^{14}C]TAF	AD-120-2021

Methods:

Species Sprague-Dawley rats

Sex (M/F) / No. of Animals Male/15

Method of Administration: Oral gavage

Dose (mg/kg): 5

Feeding Condition: Not fasted

Vehicle Formulation water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)

Sample Plasma

Analyte/Assay: [^{14}C]TAF/Liquid Scintillation Counter

Final Metabolite Designation	Plasma Concentrations (ng Equivalents [^{14}C]GS-7340/g)						$AUC_{0\text{-}24h}$ (ng eq•h/g)	% AUC of Total Radioactivity
	0.25 h	0.5 h	1 h	2 h	12 h	24 h		
M27A (Allantoin)	425	259	42.4	8.55	ND	ND	634	23.2
M28	221	135	15.0	ND	ND	ND	159	5.80
M12	246	264	99.7	58.8	19.4	ND	1153	66.7

ND = peak was not detected or below the established limit of quantitation; TAF = tenofovir alafenamide

Report Title	Study Type	Test Article	Report Number
Profiling and Identification of Metabolites in Selected Plasma, Urine, Bile, and Feces Samples from Rats after Oral Administration of [^{14}C]GS-7340	Metabolism	[^{14}C]TAF	AD-120-2021

Methods:

Species Sprague-Dawley rats
Sex (M/F) / No. of Animals Male/3
Method of Administration: Oral gavage
Dose (mg/kg): 5
Feeding Condition: Not fasted
Vehicle Formulation water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)
Sample Urine
Analyte/Assay: [^{14}C]TAF/Liquid Scintillation Counter

	Urine (Percent of Radioactive Dose)	Feces (Percent of Radioactive Dose)		
	Collection Interval (h)	Collection Interval (h)		
Final Metabolite Designation	0-24	0-24	24-48	Total
M27A (Allantoin)	0.14	NA	NA	NA
M44	1.41	NA	NA	NA
M28	ND	NA	NA	NA
M12	17.1	59.8	3.56	63.3
M32	0.19	NA	NA	NA

NA = not applicable; ND = peak was not detected or below the established limit of quantitation; TAF = tenofovir alafenamide

Report Title	Study Type	Test Article	Report Number
Profiling and Identification of Metabolites in Selected Plasma, Urine, Bile, and Feces Samples from Rats after Oral Administration of [^{14}C]GS-7340	Metabolism	[^{14}C]TAF	AD-120-2021

Methods:

Species Bile Duct-Cannulated Sprague-Dawley rats
Sex (M/F) / No. of Animals Male/3
Method of Administration: Oral gavage
Dose (mg/kg): 5
Feeding Condition: Not fasted
Vehicle Formulation water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)
Analyte/Assay: [^{14}C]TAF/Liquid Scintillation Counter

	Urine (Percent of Radioactive Dose)	Bile (Percent of Radioactive Dose)	Feces (Percent of Radioactive Dose)		
	Collection Interval (h)		Collection Interval (h)		
Final Metabolite Designation	**0-24**	**0-4**	**0-24**	**24-48**	**Total**
M27A (Allantoin)	0.23	NA	NA	NA	NA
M27B (Uric acid)	NA	0.02	NA	NA	NA
M44	1.38	NA	NA	NA	NA
M28	0.38	1.17	NA	NA	NA
M12	17.1	0.67	49.3	12.4	61.7
M32	0.30	NA	NA	NA	NA

NA = not applicable; TAF = tenofovir alafenamide

10.3. AD-120-2008: Metabolism of TAF in Dog

Report Title	Study Type	Test Article	Report Number
Profiling and Identification of Metabolites in Selected Plasma, Urine, Bile, Feces, Bone, and Liver Samples from Dogs after Oral Administration of [^{14}C]GS-7340	Metabolism	**[^{14}C]TAF**	AD-120-2008

Methods:

Species	Beagle dogs
Sex (M/F) / No. of Animals	Male/10
Method of Administration:	Oral gavage
Dose (mg/kg):	15
Feeding Condition:	Not fasted
Vehicle Formulation	water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)
Sample	Plasma
Analyte/Assay:	[^{14}C]TAF/Liquid Scintillation Counter

Final Metabolite Designation	Plasma Concentrations (ng Equivalents [^{14}C]GS-7340/g)						$AUC_{0\text{-}24h}$ (ng eq•h/g)	% AUC of Total Radioactivity
	0.25 h	0.5 h	2 h	4 h	12 h	24 h		
M2	ND	45.7	58.5	69.1	ND	30.4	1212	7.61
M3	ND	49.0	85.2	67.3	ND	ND	265	1.67
M5	ND	ND	36.9	ND	ND	ND	36.9	0.23
M9	ND	ND	22.2	20.8	ND	ND	65.2	0.41
M10	ND	ND	23.7	ND	ND	ND	23.7	0.15
M12	2760	2070	1050	541	278	175	10874	68.3
M17	213	132	ND	ND	ND	ND	69.8	0.44
M18	4640	1840	38.4	ND	ND	ND	2799	17.6
M20	92.4	76.3	ND	ND	ND	ND	32.6	0.20
TAF	616	420	ND	ND	ND	ND	207	1.30

NA = not applicable; ND = peak was not detected or below the established limit of quantitation; TAF = tenofovir alafenamide

Report Title	Study Type	Test Article	Report Number
Profiling and Identification of Metabolites in Selected Plasma, Urine, Bile, Feces, Bone, and Liver Samples from Dogs after Oral Administration of [^{14}C]GS-7340	Metabolism	**[^{14}C]TAF**	AD-120-2008

Methods:

Species	Beagle dogs
Sex (M/F) / No. of Animals	Male/10
Method of Administration:	Oral gavage
Dose (mg/kg):	15
Feeding Condition:	Not fasted
Vehicle Formulation	water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)
Sample	Urine
Analyte/Assay:	[^{14}C]TAF/Liquid Scintillation Counter

	Urine (Percent of Radioactive Dose)				
	Collection Interval (h)				
Final Metabolite Designation	**0-24**	**24-48**	**48-72**	**72-96**	**Total**
M1	ND	ND	ND	ND	ND
M2	0.88	0.28	0.16	0.19	1.49
M4	0.69	0.06	0.03	0.02	0.79
M5	0.47	ND	ND	ND	0.47
M11	0.43	ND	ND	ND	0.43
M12	13.3	5.08	3.43	2.35	24.2
M15	0.22	0.02	ND	ND	0.23
M18	ND	0.12	0.07	0.04	0.23
M23	ND	ND	ND	ND	ND
TAF	1.32	ND	ND	ND	1.32

TAF = tenofovir alafenamide; ND = peak was not detected or below the established limit of quantitation

Report Title	Study Type	Test Article	Report Number
Profiling and Identification of Metabolites in Selected Plasma, Urine, Bile, Feces, Bone, and Liver Samples from Dogs after Oral Administration of [^{14}C]GS-7340	Metabolism	**[^{14}C]TAF**	AD-120-2008

Methods:

Species	Bile Duct-Cannulated Beagle dogs
Sex (M/F) / No. of Animals	Male/10
Method of Administration:	Oral gavage
Dose (mg/kg):	15
Feeding Condition:	Not fasted
Vehicle Formulation	water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)
Sample	Urine
Analyte/Assay:	[^{14}C]TAF/Liquid Scintillation Counter

	Urine (Percent of Radioactive Dose)				
	Collection Interval (h)				
Final Metabolite Designation	**0-24**	**24-48**	**48-72**	**72-96**	**Total**
M1	0.12	0.04	0.04	ND	0.19
M2	1.06	0.35	0.14	0.15	1.70
M4	0.36	0.06	0.05	ND	0.48
M5	0.32	0.06	0.03	0.04	0.44
M11	0.39	0.04	ND	ND	0.42
M12	8.97	3.75	2.41	1.68	16.8
M15	ND	ND	ND	ND	ND
M18	ND	ND	ND	ND	ND
M23	0.24	ND	ND	ND	0.24
TAF	1.30	ND	ND	ND	1.30

TAF = tenofovir alafenamide; ND = peak was not detected or below the established limit of quantitation

Report Title	Study Type	Test Article	Report Number
Profiling and Identification of Metabolites in Selected Plasma, Urine, Bile, Feces, Bone, and Liver Samples from Dogs after Oral Administration of [^{14}C]GS-7340	Metabolism	**[^{14}C]TAF**	AD-120-2008

Methods:

Species Bile Duct-Cannulated Beagle dogs
Sex (M/F) / No. of Animals Male/10
Method of Administration: Oral gavage
Dose (mg/kg): 15
Feeding Condition: Not fasted
Vehicle Formulation water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)
Analyte/Assay: [^{14}C]TAF/Liquid Scintillation Counter

	Bile (Percent of Radioactive Dose)			
	Collection Interval (h)			
Final Metabolite Designation	**0-2**	**2-4**	**4-6**	**Total**
M2	0.08	0.02	0.02	0.12
M4	0.23	0.05	0.04	0.32
M5	0.23	0.10	0.10	0.43
M6	0.07	0.02	0.01	0.10
M11	0.04	0.02	0.03	0.09
M12	0.42	0.23	0.32	0.96
M14	0.10	0.06	0.06	0.22
M16	2.72	0.84	0.82	4.38
M17	0.29	0.09	0.10	0.48
M18	2.07	0.64	0.66	3.37
M19	0.01	0.04	0.04	0.09
M21	0.14	0.13	0.11	0.38
M22	0.07	0.09	0.07	0.23
M23	0.02	0.15	0.08	0.24
TAF	0.06	0.09	0.05	0.19

TAF = tenofovir alafenamide; ND = peak was not detected or below the established limit of quantitation

Report Title	Study Type	Test Article	Report Number
Profiling and Identification of Metabolites in Selected Plasma, Urine, Bile, Feces, Bone, and Liver Samples from Dogs after Oral Administration of [^{14}C]GS-7340	Metabolism	**[^{14}C]TAF**	AD-120-2008

Methods:

Species Beagle dogs

Sex (M/F) / No. of Animals Male/10

Method of Administration: Oral gavage

Dose (mg/kg): 15

Feeding Condition: Not fasted

Vehicle Formulation water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)

Sample Feces

Analyte/Assay: [^{14}C]TAF/Liquid Scintillation Counter

	Intact Dog (Feces, Percent of Radioactive Dose)			BDC Dog (Feces, Percent of Radioactive Dose)		
	Collection Interval (h)			Collection Interval (h)		
Final Metabolite Designation	0-24	24-48	Total	0-24	24-48	Total
M2	0.21	0.04	0.25	ND	ND	ND
M11	0.35	ND	0.35	0.50	0.20	0.70
M12	18.3	2.49	20.8	16.0	10.4	26.4

TAF = tenofovir alafenamide; ND = peak not detected or below the established limit of quantitation; BDC = bile duct-cannulated

Report Title	Study Type	Test Article	Report Number
Profiling and Identification of Metabolites in Selected Plasma, Urine, Bile, Feces, Bone, and Liver Samples from Dogs after Oral Administration of [^{14}C]GS-7340	Metabolism	**[^{14}C]TAF**	AD-120-2008

Methods:

Species	Beagle dogs
Sex (M/F) / No. of Animals	Male/10
Method of Administration:	Oral gavage
Dose (mg/kg):	15
Feeding Condition:	Not fasted
Vehicle Formulation	water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)
Sample	Bone
Analyte/Assay:	[^{14}C]TAF/Liquid Scintillation Counter

	Single Dose		**Multiple Dose**	
	Bone Concentration (ng equivalents [^{14}C]GS-7340/g)			
	Collection Time (h)			
Final Metabolite Designation	**2**	**24**	**2**	**24**
M2	ND	ND	52.9	ND
M6	ND	ND	45.1	ND
M12	2190	1070	4000	2820

TAF = tenofovir alafenamide; ND = peak not detected or below the established limit of quantitation

Report Title	Study Type	Test Article	Report Number
Profiling and Identification of Metabolites in Selected Plasma, Urine, Bile, Feces, Bone, and Liver Samples from Dogs after Oral Administration of [^{14}C]GS-7340	Metabolism	**[^{14}C]TAF**	AD-120-2008

Methods:

Species	Beagle dogs
Sex (M/F) / No. of Animals	Male/10
Method of Administration:	Oral gavage
Dose (mg/kg):	15
Feeding Condition:	Not fasted
Vehicle Formulation	water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)
Sample	Liver
Analyte/Assay:	[^{14}C]TAF/Liquid Scintillation Counter

	Single Dose		**Multiple Dose**	
	Liver Concentration (ng equivalents [^{14}C]GS-7340/g)			
	Collection Time (h)			
Final Metabolite Designation	**2**	**24**	**2**	**24**
M2	3120	1230	4440	2610
M7	3840	1160	ND	3260
M8	7760	2680	11700	7340
M12	58200	43300	68100	49400
M13	ND	336	723	716
M18	1570	1040	556	294

TAF = tenofovir alafenamide; ND = peak not detected or below the established limit of quantitation

11. PHARMACOKINETICS: INDUCTION/INHIBITION OF DRUG METABOLIZING ENZYMES

11.1. AD-120-2003: Human Cytochrome P450 Inhibition Potential of TAF

Report Title	Study Type	Test Article	Report Number
In Vitro Assessment of Human Liver Cytochrome P450 Inhibition Potential of GS-7340	Metabolism	TAF	AD-120-2003

Methods The inhibitory effect of TAF on human P450 enzymes (CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4) was investigated using human liver microsomes in the presence of NADPH at concentrations of TAF up to 25 µM

Enzyme	Activity	Calculated IC_{50} (µg/mL)	
		TAF	Control Inhibitor[a]
CYP1A2	Phenacetin O-deethylase	>25	0.05
CYP2B6	Bupropion 4-hydroxylase	>25	0.72
CYP2C8	Paclitaxel 6α-hydroxylase	>25	0.34
CYP2C9	Tolbutamide 4-hydroxylase	>25	0.64
CYP2C19	(S) Mephenytoin 4'-hydroxylase	>25	7.65
CYP2D6	Dextromethorphan O-demethylase	>25	0.05
CYP3A	Midazolam 1'-hydroxylase	7.6	0.03
	Testosterone 6β-hydroxylase	7.4	0.11

CYP = cytochrome P450 enzyme; TAF = tenofovir alafenamide

a Control inhibitors: CYP1A2, α Naphthoflavone (0-3 µM); CYP2B6, Ticlopidine (0-10 µM); CYP2C8, Montelukast (0-3 µM); CYP2C9, Sulfaphenazole (0-10 µM); CYP2C19, Tranylcypromine (0-50 µM); CYP2D6, Quinidine (0-3 µM); CYP3A, Ketoconazole (0-3 µM)

11.2. AD-120-2040: Human CYP Mechanism-Based Inhibition of TAF

Report Title	Study Type	Test Article	Report Number
In Vitro Assessment of Human Hepatic Microsomal Cytochrome P450 Mechanism Based Inhibition Potential of GS 7340	Metabolism	TAF	AD-120-2040

Methods The potential for test compound to act as a mechanism based inhibitor of human hepatic microsomal cytochromes P450 drug metabolizing enzymes, CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19 and CYP2D6, was assessed. Two-stage incubation protocol was used, with the first stage allowing inactivation of the enzyme in the absence of substrate, and the second stage being used to assay remaining enzyme activity. A 10-fold dilution was performed between the two stages to reduce the direct inhibitory effects of the test compounds. The enzyme-specific metabolites were quantified by LC MS/MS, except for resorufin (CYP1A2 metabolite), which was quantified fluorometrically.

Test Compound	Probe Activity	Calculated % Change	
		Control Inhibitor[a]	TAF
CYP1A2	Ethoxyresorufin O-deethylase	71.2 ± 1.9	-5.61 ± 9.16
		57.9 ± 3.1	
CYP2B6	Bupropion 4-hydroxylase	85.4 ± 0.8	-5.29 ± 3.52
CYP2C8	Paclitaxel 6α-hydroxylase	60.3 ± 4.8	17.4 ± 14.2
CYP2C9	Diclofenac 4'-hydroxylase	79.6 ± 2.5	-7.36 ± 17.2
CYP2C19	S-Mephenytoin 4'-hydroxylase	62.3 ± 0.9	5.30 ± 4.10
CYP2D6	Dextromethorphan O-demethylase	80.1 ± 3.6	3.94 ± 16.1
CYP3A	Midazolam 1'-hydroxylase	60.1 ± 1.8	16.4 ± 5.87
		80.2 ± 1.2	
	Testosterone 6β-hydroxylase	91.3 ± 0.5	10.3 ± 4.66
		70.7 ± 1.2	

CYP = cytochrome P450 enzyme; TAF = tenofovir alafenamide
a CYP1A2, furafylline and resveratrol; CYP2B6, ticlopidine; CYP2C8, gemfibrozil glucuronide; CYP2C9, tienilic acid; CYP2C19, ticlopidine; CYP2D6, paroxetine; CYP3A, mibefradril and mifepristone

11.3. AD-120-2004: Human Cytochrome P450 Metabolic Reaction Phenotyping of TAF

Report Title	Study Type	Test Article	Report Number
Cytochrome P450 Metabolic Reaction Phenotyping of GS-7340	Metabolism	TAF	AD-120-2004

Methods Rates of metabolism of TAF (5 µM) catalyzed by cDNA expressed major human cytochrome P450 enzyme preparations co-expressed with NADPH CYP450 reductase

	Metabolism Rate (min^{-1})					
Test Compound	**CYP1A2**	**CYP2C8**	**CYP2C9**	**CYP2C19**	**CYP2D6**	**CYP3A4**
GS-7340 (% Positive Control)	< 0.12 (< 0.9%)	0.23 (< 0.8%)	< 0.47 (< 1.0%)	< 0.12 (< 40%)	< 0.23 (< 0.9%)	1.9 (26.6%)
Ethoxycoumarin	13.2	-	-	-	-	-
Amodiaquine	-	29.3	-	-	-	-
Diclofenac	-	-	47.3	-	-	-
Diazepam	-	-	-	0.29[a]	-	-
Dextromethorphan	-	-	-	-	25.4	-
Testosterone	-	-	-	-	-	7.2

TAF = tenofovir alafenamide; NA = not applicable
a Diazepam is a selective substrate for CYP2C19 but is metabolized slowly

11.4. AD-120-2005: Induction of Metabolizing Enzymes by TAF In Vitro

Report Title	Study Type	Test Article	Report Number
In Vitro Assessment of Induction Potential of GS-7340 in Humans	Metabolism	TAF	AD-120-2005

Methods Assessments of induction were done using Puracyp's hepatoma-derived cell line DPX2. DPX2 cells are stably transformed with an expression vector for human PXR and a reporter gene vector containing the enhancer regions of CYP3A4 linked to luciferase. Following 24 hours of exposure to the test articles, the luciferase substrate was added and the luminescence was read in a luminometer. The average luminescent units for the three replicates were divided by the average for the DMSO solvent control to determine the fold induction

	Fold Induction Over 0.1% DMSO Control (PXR Activiation)	
Concentration	**TAF**	**Rifampicin**
0.1 μM	NA	1.56
0.15 μM	0.81	NA
0.5 μM	0.92	4.59
1.0 μM	NA	6.36
1.5 μM	0.88	NA
5 μM	1.03	12.5
10 μM	NA	13.4
15 μM	1.58	NA
20 μM	NA	12.6
50 μM	3.89	NA

TAF = tenofovir alafenamide; NA = not applicable

Report Title	Study Type	Test Article	Report Number
In Vitro Assessment of Induction Potential of GS-7340 in Humans	Metabolism	TAF	AD-120-2005

	Fold Induction Over 0.1% DMSO Control (AhR Activiation)	
Test Compound	**TAF**	**β-Naphthoflavone**
0.1 μM	NA	2.56
0.15 μM	1.09	NA
0.5 μM	1.04	6.37
1.0 μM	NA	11.1
1.5 μM	0.97	NA
5 μM	0.91	47.5
10 μM	NA	40.0
15 μM	0.87	NA
20 μM	NA	27.7
50 μM	0.90	NA

TAF = tenofovir alafenamide; NA = not applicable

11.5. AD-120-2032: Assessment of Induction Potential of TAF in Human Hepatocyte In Vitro

Report Title	Study Type	Test Article	Report Number
Evaluation of Induction Potential of GS-7340 in Cultured Human Hepatocytes	Drug-drug interaction (in vitro)	TAF	AD-120-2032

Methods The induction potential of cytochrome P450 (CYP) isoforms, P-glycoprotein (P-gp), and UDP glucuronosyl transferase (UGT) 1A1 by TAF was assessed in cultured human hepatocytes. GS-7340 was incubated in three preparations of cryopreserved human hepatocyte cultures at concentrations of 1, 10, and 100 µM with vehicle control and appropriate positive controls. Following 3 days of exposure, induction was determined in *situ* by catalytic activity assays, and mRNA expression was determined using real-time PCR (RT-PCR). Additionally, the cytotoxic potential of GS-7340 was assessed using the MTT assay.

Test Compound Concentration (µM)	Fold Induction							
	mRNA Expression in Human Hepatocytes					Human Hepatocytes		
	CYP1A2	CYP2B6	CYP3A4	P-gp	UGT1A1	CYP1A2	CYP2B6	CYP3A
1	1.2 ± 0.14	0.95 ± 0.17	0.92 ± 0.098	1.0 ± 0.037	0.94 ± 0.074	1.0 ± 0.20	1.1 ± 0.047	0.97 ± 0.083
10	3.0 ± 0.47	1.6 ± 0.26	8.3 ± 1.4	1.1 ± 0.11	1.7 ± 0.15	1.4 ± 0.15	0.85 ± 0.085	0.99 ± 0.057
100	6.9 ± 0.86	2.5 ± 0.21	44 ± 3.6	0.87 ± 0.093	3.9 ± 0.47	0.84 ± 0.065	0.42 ± 0.095	0.37 ± 0.025

TAF = tenofovir alafenamide
Note: Positive control for CYP1A2 was omeprazole, for CYP2B6 and P-gp was phenobarbital, for CYP3A was rifampicin, and for UGT1A1 was β-naphthoflavone.

Report Title	Study Type	Test Article	Report Number
Evaluation of Induction Potential of GS-7340 in Cultured Human hepatocytes	Drug-drug interaction (in vitro)	TAF	AD-120-2032

Methods The induction potential of cytochrome P450 (CYP) isoforms, P-glycoprotein (P-gp), and UDP glucuronosyl transferase (UGT) 1A1 by TAF was assessed in cultured human hepatocytes. GS-7340 was incubated in three preparations of cryopreserved human hepatocyte cultures at concentrations of 1, 10, and 100 µM with vehicle control and appropriate positive controls. Following 3 days of exposure, induction was determined in *situ* by catalytic activity assays, and mRNA expression was determined using real-time PCR (RT-PCR). Additionally, the cytotoxic potential of GS-7340 was assessed using the MTT assay.

Test Compound Concentration (µM)	Cell Viability (% Control Incubations)[a]			
	Hepatocyte Lot 228	Hepatocyte Lot 307	Hepatocyte Lot 321	Mean
0	100 ± 6	100 ± 14	100 ± 7	100 ± 9
1	104 ± 3	120 ± 10	104 ± 6	109 ± 6
10	58 ± 2	90 ± 13	81 ± 2	76 ± 6
100	47 ± 1	73 ± 8	54 ± 2	58 ± 4

TAF = tenofovir alafenamide
a Values are the mean ± standard deviation of triplicate determinations and represent percentage viability relatice to solvent vehicle-only treated cells

11.6. AD-120-2006: In Vitro Assessment of Human UGT1A1 Inhibition Potential of TAF

Report Title	Study Type	Test Article	Report Number
In Vitro Assessment of Human UGT1A1 Inhibition Potential of GS-7340	Metabolism	TAF	AD-120-2006

Methods The potential for TAF to inhibit the catalytic activity of human UGT1A1 was assessed. The rates of formation of β-estradiol-3-glucuronide from estradiol substrate by hepatic microsomal fractions were determined in the presence and absence of TAF, and, where possible, IC_{50} values were determined. Silybin was used as positive control.

Enzyme	Activity	Calculated IC_{50} (µM)	
		TAF	Silybin
UGT1A1	β-estradiol-3-glucuronidation	>50	1.69

TAF = tenofovir alafenamide; UGT = uridine diphosphate glucuronosyl transferase

12. PHARMACOKINETICS: EXCRETION

Results from the TAF excretion studies are described in Sections 5.1, 5.2, and 13.1 of this document.

13. PHARMACOKINETICS: EXCRETION INTO BILE

13.1. AD-120-2007: Excretion of [^{14}C]TAF Following Single oral Administration in Dog

Report Title	Study Type	Test Article	Report Number
Pharmacokinetics, Absorption, and Excretion of [^{14}C]GS-7340 Following Oral Administration to Intact and Bile Duct-Cannulated Dogs	Excretion	[^{14}C]TAF	AD-120-2007

Methods:

Species	Beagle dogs
Sex (M/F)/No. of Animals	M/3
Method of Administration	Oral gavage
Dose (mg/kg)	15
Feeding Condition	Fasted
Specific Activity	10 µCi/mg
Radionuclide	Carbon-14
Vehicle Formulation	water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)
Sample	Plasma/Blood
Analyte/Assay:	[^{14}C]TAF/Liquid Scintillation Counter

Tabulated PK Results :

Sample Type	Plasma	Blood
T_{max} (h)	0.25 ± 0.00	0.25 ± 0.00
C_{max} (ng eq/g)	8830 ± 774	7320 ± 895
t½ (h)	30.8 ± 4.34	107 ± 30.0
AUC_{0-t} (ng eq•h/g)	18500 ± 2020	44600 ± 11900

F = female; M = male; TAF = tenofovir alafenamide; NA = not applicable

Report Title	Study Type	Test Article	Report Number
Pharmacokinetics, Absorption, and Excretion of [^{14}C]GS-7340 Following Oral Administration to Intact and Bile Duct-Cannulated Dogs	Excretion	[^{14}C]TAF	AD-120-2007

Methods:

Species	Beagle dogs
Sex (M/F) / No. of Animals	M/3
Method of Administration:	Oral gavage
Dose (mg/kg):	15
Feeding Condition:	Fasted
Radionuclide:	Carbon-14
Specific Activity:	10 μCi/kg
Vehicle / Formulation:	water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)
Analyte/Assay:	[^{14}C]TAF/ Liquid Scintillation Counter

	Percent of Radioactive Dose (%)			
Time Point	**Urine**	**Feces**	**Cage Rinse**	**Total**
0-8 h	10.2 ± 4.17	NA	NA	10.3 ± 4.17
0-24 h	18.6 ± 4.23	29.5 ± 7.03	2.46 ± 2.33	50.6 ± 6.40
0-48 h	24.5 ± 4.30	35.0 ± 2.58	3.69 ± 2.72	63.2 ± 2.78
0-72 h	28.4 ± 4.41	35.9 ± 2.55	4.63 ± 3.29	68.9 ± 2.21
0-96 h	31.1 ± 4.46	36.4 ± 2.46	5.28 ± 3.34	72.8 ± 2.09
0-120 h	33.1 ± 4.56	36.9 ± 2.34	5.82 ± 3.48	75.8 ± 1.85
0-144 h	34.6 ± 4.63	37.2 ± 2.29	6.26 ± 3.44	78.1 ± 1.97
0-168 h	35.9 ± 4.69	37.4 ± 2.21	NA	73.3 ± 5.27

F = female; M = male; TAF = tenofovir alafenamide; NA = not applicable

Report Title	Study Type	Test Article	Report Number
Pharmacokinetics, Absorption, and Excretion of [^{14}C]GS-7340 Following Oral Administration to Intact and Bile Duct-Cannulated Dogs	Excretion	[^{14}C]TAF	AD-120-2007

Methods:

Species	Bile Duct-Cannulated Beagle dogs
Sex (M/F) / No. of Animals	M/3
Method of Administration:	Oral gavage
Dose (mg/kg):	15
Feeding Condition:	Fasted
Radionuclide:	Carbon-14
Specific Activity:	10 μCi/kg
Vehicle / Formulation:	water:hydroxypropyl methyl cellulose (HPMC):tween 80 (99.8:0.1:0.1, v:v:v)
Analyte/Assay:	[^{14}C]TAF/ Liquid Scintillation Counter

	Cumulative Excretion of Radioactivity (% of dose)				
Time Point	**Urine**	**Feces**	**Cage Rinse**	**Bile**	**Total**
0-1 h	NA	NA	NA	2.94 ± 2.56	2.94 ± 2.56
0-2 h	NA	NA	NA	6.80 ± 4.14	6.80 ± 4.13
0-4 h	1.72 ± 2.98	NA	NA	9.48 ± 2.24	11.2 ± 5.13
0-6 h	NA	NA	NA	12.2 ± 2.45	12.2 ± 2.43
0-8 h	8.25 ± 0.90	NA	NA	12.4 ± 2.53	20.6 ± 3.30
0-12 h	NA	NA	NA	12.5 ± 2.58	12.5 ± 2.58
0-24 h	13.9 ± 1.24	22.8 ± 19.6	0.77 ± 0.12	12.9 ± 2.70	50.3 ± 22.5
0-48 h	18.3 ± 1.50	40.8 ± 2.91	1.45 ± 0.38	13.3 ± 2.90	73.9 ± 0.90
0-72 h	21.0 ± 1.90	42.2 ± 3.61	1.81 ± 0.60	13.7 ± 3.04	78.7 ± 0.83
0-96 h	22.9 ± 1.84	42.4 ± 3.64	2.05 ± 0.63	13.8 ± 3.11	81.2 ± 0.49
0-120 h	24.5 ± 2.14	42.5 ± 3.58	2.25 ± 0.62	14.0 ± 3.16	83.3 ± 0.81
0-144 h	25.5 ± 2.29	42.6 ± 3.63	2.42 ± 0.62	14.0 ± 3.21	84.6 ± 1.02
0-168 h	26.5 ± 2.44	42.7 ± 3.64	NA	14.0 ± 3.21	83.3 ± 1.77

F = female; M = male; TAF = tenofovir alafenamide; NA = not applicable

14. PHARMACOKINETICS: DRUG-DRUG INTERACTIONS

14.1. COBI

14.1.1. AD-216-2112: In Vitro Assessment of COBI as a Substrate for Human OCT2 Transporter

Report Title	Study Type	Test Article	Report Number
In Vitro Assessment of Cobicistat as a Substrate for Human OCT2 Transporter	Metabolism	COBI	AD-216-2112

Methods: The study was designed to assess whether cobicistat (COBI) is a substrate of the organic cation transporter 2 (OCT2; SLC22A2). OCT2 is primarily localized on the basolateral membrane of the renal proximal tubule. Cobicistat was evaluated in nontransfected and OCT2-transfected MDCKII cells in the presence and absence of cimetidine, a known OCT2 inhibitor. Cobicistat (1.0 µM) uptake into the cells was determined in the presence and absence of 600 µM cimetidine incubated for 15, 30, 45, 60, and 90 seconds in both nontransfected and OCT2-transfected cells. Collected cell lysates were analyzed by LC/MS/MS. Percent of cobicistat uptake was calculated relative to nontransfected and untreated controls.

Incubation Time (Second)	COBI in OCT2-Transfected Cells	COBI + Cimetidine in OCT2-Transfected Cells	COBI in Nontransfected Cells	COBI + Cimetidine in Nontransfected Cells
15	184 ± 33	132 ± 72	65 ± 15	115 ± 97
30	254 ± 108	115 ± 35	189 ± 109	148 ± 63
45	294 ± 80	127 ± 33	130 ± 66	151 ± 58
60	303 ± 58	102 ± 26	151 ± 77	119 ± 32
90	321 ± 135	148 ± 68	100 ± 0	150 ± 77

COBI = cobicistat; OCT = organic cation transporter

14.2. TAF

14.2.1. AD-120-2018: Bidirectional Permeability of TAF Through Monolayers of P-glycoprotein and BCRP Overexpressing Cells

Report Title	Study Type	Test Article	Report Number
Bidirectional Permeability of GS-7340 Through Monolayers of P-glycoprotein and BCRP Over-expressing Cells	Drug-drug interaction (in vitro)	TAF	AD-120-2018

Methods The potential for TAF to act as a substrate for P-gp (MDR1) and BCRP was tested in monolayers of either wild type, MDR1-transfected or BCRP-transfected Madin-Darby canine kidney (MDCK II) cells (MDCK II-WT, MDCK II-MDR1 and MDCK II-BCRP, respectively). The effects of transporter-selective inhibitors were also assessed.

Wild Type and MDR1 Transfected MDCKII Cells

Cell Type	Direction	Initial Conc. (µM)	Recovery (%)	P_{app} (x 10^{-6} cm/s)			Efflux Ratio
				R1	R2	Average	
MDCK II-WT	Cell-Free	7.9	112	45.7	NA	**45.7**	NA
	Forward	7.9	103	1.4	1.5	**1.5**	4.8
	Reverse	7.6	99	6.7	7.4	**7.1**	
MDCK II-MDR1	Forward	7.4	92	0.1	0.3	**0.2**	66.2
	Reverse	7.7	96	12.8	11.3	**12.1**	
MDCK II-MDR1 (10 µM Cyclosporin A)	Forward	9.4	91	1.3	1.2	**1.3**	5.6
	Reverse	8.4	90	6.8	7.4	**7.1**	

TAF = tenofovir alafenamide; MDR1 = P-glycoprotein (P-gp, ABCB1); NA = not applicable

Report Title	Study Type	Test Article	Report Number
Bidirectional Permeability of GS-7340 Through Monolayers of P-glycoprotein and BCRP Over-expressing Cells	Drug-drug interaction (in vitro)	TAF	AD-120-2018

Wild Type and BCRP Transfected MDCK II Cells

Cell Type	Direction	Initial Conc. (µM)	Recovery (%)	P_{app} (x 10^{-6} cm/s)			Efflux Ratio
				R1	R2	Average	
MDCK II-WT	Cell-Free	7.9	112	45.7	NA	**45.7**	NA
	Forward	7.9	103	1.4	1.5	**1.5**	4.8
	Reverse	7.6	99	6.7	7.4	**7.1**	
MDCK II-BCRP	Forward	7.8	79	2.1	2.2	**2.1**	6.2
	Reverse	7.8	104	13.5	13.3	**13.4**	
MDCK II-BCRP (10 µM Ko134)	Forward	8.9	103	3.8	5.8	**4.8**	1.4
	Reverse	10.3	96	5.9	8.0	**6.9**	

TAF = tenofovir alafenamide; NA = not applicable

14.2.2. AD-120-2019: In Vitro Assessment of TAF Inhibition of Human OATP1B1, OATP1B3, P-gp, and BCRP

Report Title	Study Type	Test Article	Report Number
In Vitro Assessment of GS-7340 Inhibition of Human OATP1B1, OATP1B3, P-gp, and BCRP	Drug-drug interaction (in vitro)	TAF	AD-120-2019

Methods The inhibition potential of TAF of human OATP1B1 and OATP1B3 was assessed in Chinese Hamster Ovary (CHO) cells, either wild type or transfected with the genes encoding human OATP1B1 or OATP1B3. TAF and positive control compound were diluted in assay buffer containing 2 µM Fluo 3. Following removal of media containing Fluo 3, the cells were immediately analyzed for Fluo 3 fluorescence at an excitation of 485 nm and emission of 530 nm.

The inhibition potential of TAF of human P-gp and BCRP was assessed in Madin Darby Canine Kidney (MDCKII) cells, either wild type or transfected with the genes encoding human P-gp or BCRP. The incubation was carried out in cell culture medium (without FBS) containing 10 µM Calcein AM (P-gp) or 1 µM pheophorbide a (PhA) (BCRP). Following removal of media containing calcein AM or PhA, the cells were analyzed immediately for calcein fluorescence at an excitation of 494 nm and an emission of 517 nm or PhA fluorescence at an excitation of 415 nm and an emission of 675 nm.

	Uptake Transporters IC_{50} (µM)		Efflux Transporters IC_{50} (µM)	
Test Compound	**OATP1B1**	**OATP1B3**	**P-gp**	**BCRP**
TAF	>100	>100	>100	>100
Rifampicin	2.4 ± 1.1	1.7 ± 0.4	NA	NA
Verapamil	NA	NA	3.7 ± 3.1	NA
Fumitremorgin C	NA	NA	NA	0.32 ± 0.03

BCRP = breast cancer resistance protein; NA = not applicable; P-gp = P-glycoprotein; TAF = tenofovir alafenamide

14.2.3. AD-120-2013: Effect of GS-9350 on the Bidirectional Permeability of TAF Through Caco-2 Cells

Report Title	Study Type	Test Article	Report Number
Effect of GS-9350 on the Bidirectional Permeability of GS-7340 through Caco-2 Cells	Absorption (in vitro)	TAF	AD-120-2013

Bidirectional Permeability of TAF Through Caco-2 Cells

Inhibitor	Direction	Target Concentration (μM)	Initial Conc. (μM)	Recovery (%)	P_{app} (10^{-6} cm/s)					Efflux Ratio
					Replicate 1	Replicate 2	Replicate 3	Replicate 4	Average	
–	Cell-Free	10	9.61	119	31.6	–	30.1	–	30.8	20
	Forward		9.92	64	0.59	0.69	0.82	0.88	0.74	
	Reverse		8.71	102	18.0	15.1	12.6	14.8	15.1	
GS-9350	Forward	10	11.0	101	3.98	3.56	2.47	2.55	3.14	1.6
	Reverse		11.4	115	6.71	6.18	3.33	3.33	4.89	

Caco-2 = human colonic adenocarcinoma cell line; TAF = tenofovir alafenamide; P_{app} = apparent permeability

14.2.4. AD-120-2022: In Vitro Assessment of TAF as a Substrate for Human OATP1B1 and OATP1B3

Report Title	Study Type	Test Article	Report Number
In Vitro Assessment of GS-7340 as a Substrate for Human OATP1B1 and OATP1B3	Drug-drug interaction (in vitro)	TAF	AD-120-2022

Methods: The potential of TAF as a substrate in human OATP1B1 and OATP1B3 was assessed in Chinese Hamster Ovary (CHO) cells, either wild type or transfected with the genes encoding human OATP1B1 or OATP1B3 in the presence and absence of 40 µM rifampicin (OATP inhibitor). Following removal of media, the cell extracts were analyzed by LC/MS/MS.

	Uptake Rate (pmole/min/million cells)		
Test Compound	**TAF**	**TAF + Rifampicin**	**Uptake Ratio**
CHO-WT	9.0	6.0	1.5
CHO-OATP1B1	12.0	6.2	1.9
CHO-OATP1B3	24.1	5.8	4.2
OATP1B1/WT Ratio	1.3	NA	NA
OATP1B3/WT Ratio	2.7	NA	NA

NA = not applicable; OATP = organic anion transporting polypeptide (SLCO or SLC22A gene products); TAF = tenofovir alafenamide;

14.2.5. AD-120-2027: Effects of HIV Protease Inhibitors and Pharmacokinetic Enhancers on TAF Metabolism In Vitro

Report Title	Study Type	Test Article	Report Number
Effects of HIV Protease Inhibitors and Pharmacokinetic Enhancers on the In Vitro Metabolism of GS-7340 in Human Intestinal Subcellular Fraction	Drug-drug interaction (in vitro)	TAF	AD-120-2027

Methods: Duplicate aliquots of 2 µM TAF were incubated with HIV protease inhibitors, atazanavir and darunavir, and the pharmacokinetic enhancers, ritonavir and cobicistat in pooled human intestinal S9 fractions at 37ºC up to 120 minutes. Rates of metabolism (in vitro half-life values) were determined. Analysis was done by LC/MS/MS.

Inhibitor or Enhancer	Concentration (µM)	TAF Intestinal S9 Stability, $t_{½}$ (min)
Vehicle Control	0	24.5 ± 4.1
Atazanavir	25	28.9 ± 5.2
	100	38.9 ± 5.3
Darunavir	25	32.2 ± 5.1
	100	30.8 ± 5.0
Ritonavir	25	19.0 ± 2.8
	100	18.9 ± 1.5
Cobicistat	25	30.1 ± 5.1
	100	32.9 ± 4.8
Dichlorvos (control)	500	>789[a]

TAF = tenofovir alafenamide
a Less than 10% loss of substrate in 120 min

14.2.6. AD-120-2036: In Vitro Assessment of TAF as an Inhibitor of OAT1, OAT3, OCT1, OCT2, MATE1, and BSEP or as a Substrate for OCT1

Report Title	Study Type	Test Article	Report Number
Studies to Determine if Tenofovir Alafenamide (GS-7340) is an Inhibitor of OAT1, OAT3, OCT1, OCT2, MATE1, and BSEP or a Substrate for OCT1	Drug-drug interaction (in vitro)	TAF	AD-120-2036

Methods: The potential for TAF to inhibit the human organic anion and cation uptake transporters, and multidrug and toxin extrusion transporter MATE1 was assessed in vitro using transfected Chinese Hamster Ovary (CHO) cells (for OAT1, OCT1, OCT2, and MATE1), transfected Flp-In 293 cells (for OAT3). The amount of substrate inside the cells was determined by liquid scintillation/fluorescence reader.

Inhibitor or Enhancer	IC_{50} (µM)	Maximum inhibition at 100 µM (% of control)
OCT1	>100	26
OCT2	>100	NA
OAT1	>100	8
OAT3	>100	16
MATE1	>100	34

BSEP = bile salt export protein; MATE1 = multidrug and toxin extrusion protein 1; NA = not applicable; OAT = organic anion transporter; OCT = organic cation transporter; TAF = tenofovir alafenamide;

Report Title	Study Type	Test Article	Report Number
Studies to Determine if Tenofovir Alafenamide (GS-7340) is an Inhibitor of OAT1, OAT3, OCT1, OCT2, MATE1, and BSEP or a Substrate for OCT1	Drug-drug interaction (in vitro)	TAF	AD-120-2036

Methods The potential for TAF to inhibit the bile salt export pump BSEP was assessed in vitro using transfected Chinese hamster ovary (CHO) cells. TAF was incubated with membrane vesicle preparations (total protein: 50 µg/well) and probe substrate, taurocholate (2 µM) in the absence or presence of ATP. The amount of substrate inside the filtered vesicles was determined by liquid scintillation reader.

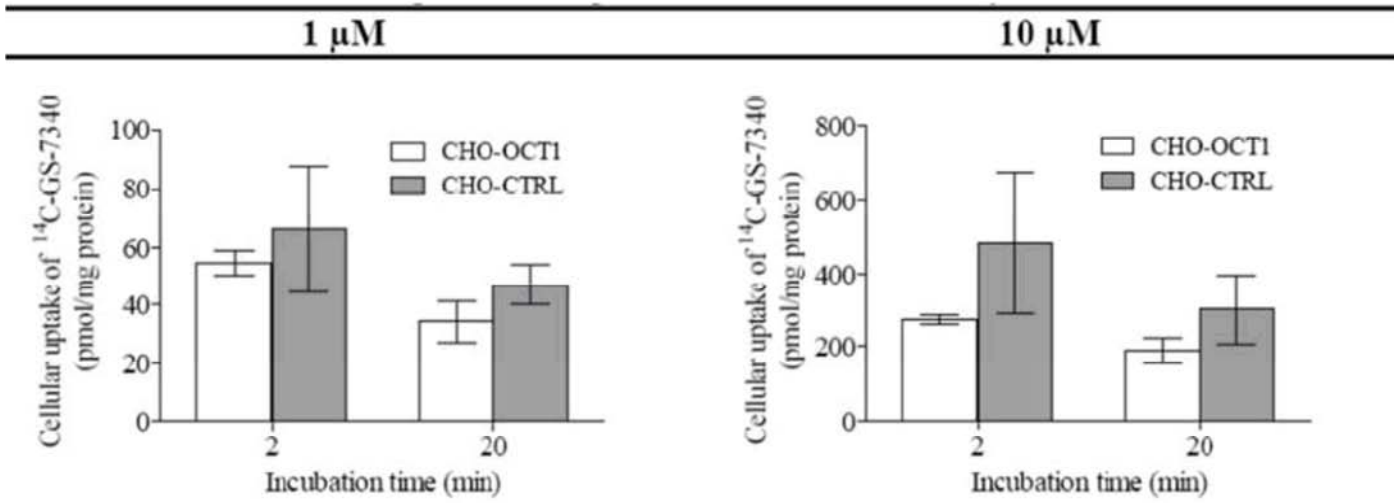
Inhibitor or Enhancer	IC_{50} (µM)	Maximum inhibition at 100 µM (% of control)
BSEP	>100	43

BSEP = bile salt export protein; TAF = tenofovir alafenamide

Report Title	Study Type	Test Article	Report Number
Studies to Determine if Tenofovir Alafenamide (GS-7340) is an Inhibitor of OAT1, OAT3, OCT1, OCT2, MATE1, and BSEP or a Substrate for OCT1	Drug-drug interaction (in vitro)	[^{14}C]TAF	AD-120-2036

Methods The interactions of TAF with human cation transporters OCT1 was assessed in vitro using OCT1 transporter-expressed Chinese hamster ovary (CHO) cells. Transporter specific accumulation into OCT1 transporter-expressing cells was investigated at two concentrations (1 and 10 µM) and time points (2 and 20 minutes) to determine if TAF is a substrate for this transporter.

Results: TAF was found not to be a substrate for OCT1 based on no transporter specific accumulation of TAF in the OCT1 transporter-expressing cells.



14.2.7. AD-120-2031: Effect of Inhibitors of CatA, CES1, and CYP3A4 on TAF Metabolism In Vitro

Report Title	Study Type	Test Article	Report Number
Effect of Inhibitors of Cathepsin A, Carboxylesterase1, and CYP3A4 on Metabolism of Tenofovir Alafenamide Fumarate (GS-7340) in Primary Human Hepatocytes	Drug-drug interaction (in vitro)	TAF	AD-120-2031

Methods The effect of inhibitors of cathepsin A (CatA), carboxylesterase 1 (CES1), and CYP3A on TAF activation to the pharmacologically active nucleotide analog diphosphate, tenofovir diphosphate (TFV-DP), was assessed in primary human hepatocytes. The duplicate wells were incubated with 0.5 µM TAF in the presence and absence of the inhibitors (concentrations at 0, 0.08, 0.4, 2, 10, and 50 µM). The amount of TFV-DP formation was analyzed by LC/MS/MS.

Inhibitor Concentration (µM)	Intracellular TFV-DP (% Control)				
	Telaprevir	Boceprevir	BNPP	COBI	Telaprevir + BNPP
0	100	100	100	100	100
0.08	109	91.2	124	123	132
0.4	93.8	91.1	94.1	98.3	103
2	103	91.7	63.4	100	51.9
10	106	87.2	70.2	88.2	16.1
50	984	100	33.6	87.9	5.30

CatA = cathepsin A; CES1 = carboxylesterase 1; CYP = cytochrome P450 enzyme; TAF = tenofovir alafenamide
Note: Telaprevir and boceprevir are CatA inhibitors; cobicistat is a CYP3A inhibitor; bis(p-nitrophenyl) phosphate (BNPP) is a CES1 inhibitor

14.2.8. AD-120-2035: Effect of Cyclosporin A Pretreatment on Pharmacokinetics of TAF in Dogs

Report Title	Study Type	Test Article	Report Number
Effect of Cyclosporin A pretreatment on Pharmacokinetics of Tenofovir Alafenamide in dogs	Drug-drug interaction (in vivo)	TAF	AD-120-2035

Methods:

Species Beagle dogs

Sex (M/F) / No. of Animals M/3

Method of Administration: Intravenous infusion

Dose (mg/kg): 0.5

Sample Plasma/PBMC

Assay: LC/MS/MS

	Plasma		PBMC		
			- Phosphatase	+ Phosphatase	–
Parameter	**TAF**	**TFV**	**TFV**	**TFV**	**TFV-DP**
T_{max} (h)	0.41	0.90	0.48	2.32	6.00
C_{max} (µg/mL)	0.47	0.03	2.96	5.25	7.24
$t_{½}$ (h)	0.12	>24	NC	>24	>24
AUC_{0-t} (µg•h/mL)	0.23	0.15	12.3	78.7	137
CL (L/h/kg)	2.23	NA	NA	NA	NA

F = female; M = male; NA = not applicable; NC = not calculable; PBMC = peripheral blood mononuclear cell; TAF = tenofovir alafenamide; TFV = tenofovir; TFV-DP = tenofovir diphosphate

Report Title	Study Type	Test Article	Report Number
Effect of Cyclosporin A pretreatment on pharmacokinetics of Tenofovir Alafenamide in dogs	Drug-drug interaction (in vivo)	TAF	AD-120-2035

Methods:

Species	Beagle dogs
Sex (M/F) / No. of Animals	M/3
Method of Administration:	Oral gavage
Dose (mg/kg):	2
Feeding Condition:	Fasted
Vehicle / Formulation:	0.9% sodium chloride in 50 mM ammonium acetate, pH 5.5
Assay:	LC/MS/MS

	Plasma		PBMC		
			- Phosphatase	+ Phosphatase	–
	TAF	**TFV**	**TFV**	**TFV**	**TFV-DP**
T_{max} (h)	0.14	0.67	1.00	1.00	8.70
C_{max} (µg/mL)	0.05	0.10	0.12	0.75	1.81
$t_{½}$ (h)	0.21	>24	NC	>24	>24
AUC_{0-t} (µg•h/mL)	0.01	0.33	NA	10.8	31.2
F%	1.67	NA	NA	NA	NA

F = female; M = male; NA = not applicable; NC = not calculable; PBMC = peripheral blood mononuclear cell; TAF = tenofovir alafenamide; TFV = tenofovir; TFV-DP = tenofovir diphosphate

Report Title	Study Type	Test Article	Report Number
Effect of Cyclosporin A pretreatment on pharmacokinetics of Tenofovir Alafenamide in dogs	Drug-drug interaction (in vivo)	TAF	AD-120-2035

Methods:

Species	Beagle dogs
Sex (M/F) / No. of Animals	M/3
Method of Administration:	Oral gavage
Dose (mg/kg):	2
Co-administered CsA Dose (mg/kg):	75
Feeding Condition:	Fasted
Vehicle / Formulation:	50 mM citrate, pH 5
Assay:	LC/MS/MS

	Plasma (Pretreated with 75 mg Cyclosporin A)		PBMC (Pretreated with 75 mg Cyclosporin A)		
			- Phosphatase	+ Phosphatase	–
	TAF	TFV	TFV	TFV	TFV-DP
T_{max} (h)	0.25	1.00	1.00	1.00	3.00
C_{max} (µg/mL)	0.39	0.05	2.10	4.91	3.15
$t_{½}$ (h)	0.15	>24	>24	>24	>24
AUC_{0-t} (µg•h/mL)	0.15	0.38	9.39	75.8	59.5
F%	16.6	NA	NA	NA	NA

F = female; M = male; NA = not applicable; PBMC = peripheral blood mononuclear cell; TAF = tenofovir alafenamide; TFV = tenofovir; TFV-DP = tenofovir diphosphate

15. PHARMACOKINETICS: OTHER

There are no additional studies to report under this heading.