SECTION 2.4 - NONCLINICAL OVERVIEW

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

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AAG	α1 acid glycoprotein
AhR	aryl hydrocarbon receptor (AHR gene product)
ALT	alanine aminotransferase
APD	action potential duration
APTT	activated partial thromboplastin time
ARV	antiretroviral
AST	aspartate aminotransferase
ATP	adenosine triphosphate
ATV	atazanavir (Reyataz®, Bristol-Myers Squibb)
AUC	area under the curve
AUC_{ss}	area under the plasma concentration curve at steady state
AUC_{tau}	the area under the plasma concentration-time curve from time zero to time tau over a dosing interval at steady state (AUC0-tau), where tau is the length of the dosing interval.
BCRP	breast cancer resistance protein (ABCG2)
BDC	bile-duct cannulated
BSEP	bile salt excretory pump
BUN	blood urea nitrogen
Caco-2	human colon carcinoma cell line
CatA	cathepsin A
CC_{50}	drug concentration that results in a 50% reduction in cell viability
CD4	cluster determinant 4
cDNA	complimentary deoxyribonucleic acid
CHL	Chinese hamster lung
CHMP	Committee for Medicinal Products for Human Use
C_{max}	maximum observed concentration of drug in serum, plasma, or peripheral blood mononuclear cells
$C_{max,u}$	unbound concentration of drug at C_{max}
CNS	central nervous system
COBI	cobicistat (GS-9350, Tybost®, Gilead)
COX II	cytochrome c oxidase II
CsA	cyclosporine A
CYP	cytochrome P450
dATP	deoxyadenosine triphosphate
dCTP	deoxycytidine triphosphate
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DRV	darunavir (Prezista®, Tibotec)
EAD	early after depolarization
EC_{50}	concentration of a compound inhibiting virus replication by 50%

EC_{95}	concentration of a compound inhibiting virus replication by 95%
E/C/F/TAF	elvitegravir/cobicistat/emtricitabine/tenofovir alafenamide (coformulated)
ECG	electrocardiograph, electrocardiogram
EFV	efavirenz (Sustiva®, Bristol-Myers Squibb)
EMA	European Medicines Agency
EVG	elvitegravir (Vitekta®, Gilead)
EVG/COBI/FTC/TDF	elvitegravir/cobicistat/emtricitabine/tenofovir DF (coformulated); STB
EU	European Union
F1	first generation
FDA	Food and Drug Administration
FDC	fixed-dose combination
FMO	flavin monooxygenase
FTC	emtricitabine (Emtriva®, Gilead)
FTC/TDF	emtricitabine/tenofovir DF, TVD (Truvada®, Gilead)
FTC-TP	emtricitabine triphosphate
GD	gestation day
GGT	gamma glutamyltransferase
GI	gastrointestinal
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
GS-7340	tenofovir alafenamide (TAF) free base
GS-7340-02	tenofovir alafenamide (TAF) monofumarate
GS-7340-03	tenofovir alafenamide (TAF) hemifumarate
GS-9200	EVG metabolite M4 (JTP-65386 and JTP-71051; glucuronide conjugate of the carboxylic acid)
GS-9202	EVG metabolite M1 (JTP-71081; hydroxylation of the chlorofluorophenyl group)
GSI	Gilead Sciences, Inc. (Gilead)
HBV	hepatitis B virus
HEK	human embryonic kidney (cell line)
hERG	human ether-à-go-go related gene
HIV-1	human immunodeficiency virus type 1
HPMC	hydroxypropylmethylcellulose
HS	human serum
HSA	human serum albumin
$[I]_1$	inhibitor concentration corresponding to steady state C_{max}
$[I]_2$	inhibitor concentration corresponding to theoretical maximum concentration in the intestinal lumen
IC_{50}	concentration resulting in 50% of maximum inhibition
ICH	International Conference on Harmonization (of Technical Requirements for Registration of Pharmaceuticals for Human Use)
IgG	immunoglobulin G

IgM	immunoglobulin M
IHC	immunohistochemical
IL-2	interleukin-2
IN	integrase
INSTI	integrase strand transfer inhibitor
IV	intravenous
K_i	kinetic inhibition constant
K_I	affinity constant for enzyme inactivation
k_{inact}	theoretical maximum enzyme inactivation rate
KLH	keyhole limpet hemocyanin
LD	lactation day
LD_{50}	the estimated dose that results in lethality in 50 percent of a group
LPV	lopinovir
LV	left ventricular
MAPD	monophasic action potential duration
MATE1	multidrug and toxin extrusion protein 1 (SLC47A1)
MATE2-K	multidrug and toxin extrusion protein 2-K (SLC47A2)
MRP1, 2, or 4	multidrug resistance related protein 1, 2, or 4
MTD	maximal tolerated dose
mtDNA	mitochondrial DNA
NDA	new drug application
NK	natural killer
NNRTI	nonnucleoside reverse transcriptase inhibitor
NOAEL	no observed adverse effect level
NOEL	no observed effect level
NRTI	nucleoside reverse transcriptase inhibitor
N(t)RTI	nucleoside or nucleotide reverse transcriptase inhibitor
OAT1	organic anion transporter 1 (SLC22A6)
OAT3	organic anion transporter 3 (SLC22A8)
OATP1B1	organic anion transporting polypeptide 1B1 (SLCO1B1)
OATP1B3	organic anion transporting polypeptide 1B3 (SLCO1B3)
OCT1	organic cation transporter 1
OCT2	organic cation transporter 2 (SLC22A2)
OCTN1	organic cation transporter novel, type 1 (SLC22A4)
PBMC	peripheral blood mononuclear cell
P-gp	permeability glycoprotein
PI	protease inhibitor
PNA	peptide nucleic acid
PND	postnatal day
PXR	pregnane X receptor
QT	interval between the start of the Q wave and the end of the T wave on ECG

QTc	QT interval duration corrected for heart rate
QTcF	QT interval duration corrected for heart rate according to Fridericia
RBC	red blood cell
RNA	ribonucleic acid
RPTECs	renal proximal tubule epithelial cells
RT	reverse transcriptase
RTV	ritonavir (Norvir®, Abbott)
S9	tissue post-mitochondrial (9,000 x g) supernatant
SI	selectivity index
SIV	simian immunodeficiency virus
SkMCs	skeletal muscle cells
STB	E/C/F/TAF (Stribild®, Gilead)
STR	single-tablet regimen
T4	thyroxine
TAF	tenofovir alafenamide (GS-7340)
TDAR	T cell dependent antibody response
TDF	tenofovir disoproxil fumarate, tenofovir DF (Viread®, Gilead)
TdP	Torsades de Pointes
TFV	tenofovir, PMPA
TFV DP	tenofovir diphosphate
TSH	thyroid stimulating hormone
TVD	emtricitabine/tenofovir DF, FTC/TDF (Truvada®, Gilead)
UGT	uridine diphosphate glucuronosyltransferase
US	United States
ZDV	zidovudine, AZT (Retrovir®, GlaxoSmithKline)

NOTE TO REVIEWER

This document contains a summary of nonclinical studies conducted in support of a fixed-dose combination (FDC) that contains the active substances elvitegravir (EVG), cobicistat (COBI), emtricitabine (FTC), and tenofovir alafenamide (TAF, GS-7340) fumarate (GS-7340-03); the EVG/COBI/FTC/TAF (E/C/F/TAF, 150/150/200/10 mg) tablet.

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

The efficacy and safety of EVG, COBI, FTC, TDF, TVD, and STB as part of regimens for the treatment of HIV-1 infection have been established through comprehensive programs of clinical and nonclinical studies with these approved medicinal products. In addition, labeling and safety supplements have been submitted post approval to update the prescribing information with emergent data. Periodic safety update reports and annual reports have been submitted in accordance with local requirements. Supplementary information, either in accordance with specific post marketing commitments or based on request by Health Authorities, has been provided as appropriate. To assist the reviewer, [redacted] Links to all study reports included in the application are highlighted in blue text.

Given the lack of remarkable effects in the nonclinical studies with the individual agents, no additional studies have been conducted for the E/C/F/TAF combination. In the development of TAF, 3 forms of the active drug substance have been used: GS-7340, synonym for GS-7340 as the free base; GS-7340-02, synonym for GS-7340 as the monofumarate (1:1 ratio of free base to fumarate), and GS-7340-03, synonym for the hemifumarate (2:1 ratio of free base to fumarate). The hemifumarate, GS-7340-03 (TAF fumarate) was selected as the form for final development. GS-7340-03 is considered comparable to GS-7340-02 based on physical/chemical properties and pharmacokinetic data.

In order to simplify the review, the order of presentation in each section follows the general format: EVG, followed by COBI, EVG/COBI combination, FTC, TAF, FTC/TDF combination, and E/C/F/TAF combination studies.

The following conversions are also provided to aid the reviewer:

- EVG (GS-9137, JTK-303) 1 μM = 0.448 μg/mL
- COBI (GS-9350) 1 μM = 0.776 μg/mL

- FTC (GS-9019) 1 µM = 0.247 µg/mL
- TAF (GS-7340) 1 µM = 0.477 µg/mL
- TFV (GS-1278) 1 µM = 0.287 µg/mL

1. NONCLINICAL OVERVIEW

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

Gilead has coformulated TAF with the integrase strand transfer inhibitor (INSTI) EVG, the pharmacokinetic enhancer COBI, and the nucleoside reverse transcriptase inhibitor FTC into an FDC. The E/C/F/TAF tablet contains the same dosages of EVG (150 mg), COBI (150 mg), and FTC (200 mg) that are currently approved within Emtriva, Truvada, and Stribild for use in adults.

A comprehensive nonclinical pharmacology/virology, pharmacokinetic, and toxicology program was conducted to support of the registration of EVG, COBI, FTC and TAF. Information from all nonclinical studies with EVG, COBI, FTC, TAF, and TDF/tenofovir (TFV) should be considered in the context of the substantial clinical experience with each component and with STB within antiretroviral combination therapy for the treatment of HIV-1 infection.

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

Results of EVG, COBI, FTC, TDF, and FTC/TDF studies are incorporated in m2.6 to describe the presence or absence of overlapping findings; a listing of all the previously conducted and submitted EVG, COBI, FTC, TDF, and FTC/TDF studies are detailed in the cross-reference tables in m1.4.4.

The nonclinical data discussed within this document support the favorable benefit/risk profile for the proposed use of E/C/F/TAF for the treatment of HIV-1 infection in adult and pediatric patients 12 years of age and older without any known mutations associated with resistance to the individual components of E/C/F/TAF. All information from nonclinical studies that is relevant to the prescriber and patient has been included in the proposed Prescribing Information and Patient Labeling.

1.1. Overview of the Nonclinical Testing Strategy

This document provides an overview of the nonclinical information that is relevant to the assessment of the E/C/F/TAF FDC. This document is structured as an overview of the studies in the various disciplines, including primary pharmacodynamics, secondary pharmacodynamics, safety pharmacology, pharmacokinetics, and toxicology. A critical assessment of the completeness and relevance of the nonclinical testing program and the key findings are included. An integrated safety assessment of E/C/F/TAF for the treatment of HIV-1 infection in patients

≥ 12 years old is included in Section 5, "Integrated Overview and Conclusions," of this document. Specific cross-disciplinary topics and proposals for the inclusion of nonclinical items in the product labeling are discussed throughout the text, as appropriate, and summarized at the end of the document.

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

1.1.1. EVG

Elvitegravir (Vitekta®) is an INSTI that is approved in the US and European Union (EU) as 85- and 150-mg tablets to be co-administered with a ritonavir (RTV)-boosted protease inhibitor and with other antiretroviral (ARV) agents for the treatment of HIV-1 infection in adults. Elvitegravir is also a component of Stribild (EVG/COBI/FTC/TDF 150/150/200/300 mg), which is marketed in the United States (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. To facilitate the evaluation of the E/C/F/TAF FDC, nonclinical virology studies of EVG are described in detail in the integrated virology summary contained in m2.7.2, Section 4.2, together with the clinical virology data.

All nonclinical studies required to support chronic use have been performed as part of the safety assessment of this ARV product. These included the following: a comprehensive set of primary and secondary pharmacodynamics studies; complete core battery of safety pharmacology studies; complete pharmacokinetic evaluation; single-dose and repeat-dose oral toxicity studies in rats and dogs; mechanistic studies to clarify the relevance of the cecal and upper small intestinal effects; genotoxicity studies; carcinogenicity studies; assessment of fertility, early embryonic development, prenatal and postnatal development and juvenile toxicity; evaluation of antigenicity, immunotoxicity, phototoxicity, and skin and eye irritation; and qualification of impurities. Studies were also conducted using the combination of EVG and COBI, and EVG and RTV.

The rat and dog were the appropriate species for the repeat-dose toxicology studies because of the similar disposition of EVG in these species compared to humans and the ability to achieve high systemic exposure. The nonclinical toxicity studies demonstrated that EVG was well tolerated for up to 26 weeks in the rat and up to 39 weeks in the dog at doses producing systemic exposure levels in animals 2.3- to 36-fold greater than those in patients treated with the recommended clinical dose.

1.1.2. COBI

Cobicistat (Tybost®) is a strong mechanism-based cytochrome P450 (CYP) 3A inhibitor (a pharmacokinetic enhancer) that increases the systemic levels of co-administered agents

metabolized by CYP3A enzymes, including EVG and the HIV protease inhibitors (PIs) atazanavir (ATV) and darunavir (DRV). Tybost (COBI 150 mg tablets) is approved in the US and EU 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.

For COBI, all nonclinical studies required to support chronic use have been performed as part of the safety assessment of this novel pharmacoenhancer. These included the following: a comprehensive set of primary and secondary pharmacodynamics studies; complete core safety pharmacology studies with appropriate follow-up in vitro studies to elucidate the most important effects; complete pharmacokinetic evaluation; single-dose oral toxicity studies in mice and rats; repeat dose toxicity studies in up to 26 weeks in rats and up to 39 weeks in dogs; genotoxicity; carcinogenicity; assessment of fertility, early embryonic development, prenatal and postnatal development and juvenile toxicity; evaluation of antigenicity, immunotoxicity, and skin and eye irritation; and qualification of impurities. A number of key studies were also conducted using the combination of COBI and EVG, and COBI and ATV.

The primary pharmacodynamic effect of COBI is mechanism-based inhibition of human CYP3A enzymes. There is a species difference in the mode of inhibition, as COBI is a reversible inhibitor in rodents, dogs, and monkeys. There is also a species difference in induction liability as COBI activates rat pregnane X receptor (PXR) and displays autoinduction in rodents, but is a very weak inducer in human hepatocytes. Despite the different mechanisms of CYP inhibition, the rat and dog were considered appropriate species for the toxicology studies because of similarities between the metabolite profiles of COBI in these species and humans and the ability to achieve high systemic exposures. The nonclinical toxicity studies demonstrate that COBI is well tolerated for up to 6 months in the rat and 9 months in the dog. While the safety margins are not large (< 7), effects above the no-observed-adverse-effect levels (NOAELs) were minimal and some effects were species-specific.

1.1.3. FTC

Emtricitabine is a nucleoside reverse transcriptase inhibitor (NRTI). It is the active ingredient in Emtriva 200 mg capsules and 10 mg/mL oral solution that have been approved in the US, the EU, and other countries worldwide for use in combination with other ARV agents for the treatment of HIV-1 infection.

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

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

1.1.4. TAF

Comprehensive nonclinical pharmacology/virology, pharmacokinetic, and toxicology programs were undertaken in support of the registration of tenofovir alafenamide (TAF, GS-7340). Tenofovir alafenamide is a prodrug of TFV. After absorption, TAF is converted to TFV intracellularly, which is phosphorylated to the active metabolite, tenofovir diphosphate (TFV-DP) {1574}, that competes with natural 2'-deoxyadenosine triphosphate (dATP) for incorporation by the HIV-1 or HBV reverse transcriptase (RT) and, once incorporated, results in chain-termination {21}, {1131}. Tenofovir diphosphate is a very weak inhibitor of mammalian DNA polymerases α, β, δ, ε, and mitochondrial DNA polymerase γ.

Tenofovir alafenamide is metabolized by hydrolases including carboxyl esterase 1 and cathepsin A (CatA) and has minimal interaction with typical xenobiotic metabolizing enzymes. Unlike tenofovir disoproxil fumarate (TDF, Viread), TAF is relatively stable in human plasma ($t_{½}$ ~75 minutes), but rapidly converts to TFV inside cells {7415}. Because TAF is more stable in plasma than the TDF, higher levels are achieved in HIV-target cells. In HIV-target cells, including lymphocytes and macrophages, TAF is metabolized by CatA, providing enhanced delivery of TFV. During clinical studies, administration of TAF resulted in subsequent formation of 4-fold (3-7-fold at 90% confidence interval) higher intracellular levels of TFV-DP in peripheral blood mononucleated cells (PBMCs) and 90% lower circulating levels of TFV relative to TDF {7415}, {13119}, {22029}. The higher TFV-DP levels lead to more effective suppression of viral replication in clinical studies. The lower circulating level of TFV is expected to result in reduced off-target effects of TFV and an improved safety profile as compared to TDF {21762}, {22031}.

Tenofovir alafenamide is well absorbed, generating sufficient exposure in animal species chosen for toxicity assessment. Tenofovir alafenamide was evaluated in mouse, rat, dog, and monkey repeat-dose toxicity studies up to 39 weeks in duration. In vitro and in vivo genotoxicity studies were conducted. The mouse was used for the in vivo genetic toxicity study and local lymph node assay. The rat was used for fertility and developmental toxicity studies and the rabbit was used for developmental and reproductive toxicity studies and local irritation. All in vivo studies utilized oral administration, the clinical route of administration, with the exception of the sensitization and dermal irritation studies. The rat and dog were demonstrated to have similar in vitro and in vivo metabolic profiles to humans (m2.6.4, Section 5.1.1). The vehicle for toxicity studies used was 1) 25mM citric acid or 2) 0.5% polysorbate 20, 0.5% carboxymethylcellulose, 0.9% benzyl alcohol or 3) 0.1% (v/v) Tween 20 and 0.1% (v/v) hydroxypropylmethylcellulose (HPMC).

Per separate agreements with the Food and Drug Administration (FDA) and European Medicines Agency (EMA), carcinogenicity studies (FDA Reference ID: 3161161, EMA/CHMP/SAWP/629722/2012, EMEA H/SA/2410/1/2012/1), and a perinatal and postnatal study (FDA Reference ID: 3244145, EMA/CHMP/SAWP/214541/2013, EMEA H/SA/2410/FU/1/2013/1) are not required for TAF registration due to the lack of TAF exposure in rats and TgRasH2 mice and lower TFV exposure in rats and mice compared to the same studies in which TDF was administered.

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

1.1.5. E/C/F/TAF

Comprehensive nonclinical pharmacology/virology, pharmacokinetic, and toxicology programs were conducted with EVG, COBI, FTC, and TAF. A small number of key studies were conducted using the combination of EVG and COBI, FTC and TDF, and EVG/COBI/FTC/TFV. The overall program, including the data from the combination and individual agent studies, is considered sufficient to support the safety of the E/C/F/TAF FDC tablet.

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

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

The toxicity profiles of the 4 agents differ substantially with no clinically significant overlapping toxicity. Because the target organ profiles are different, and there is no evidence of genotoxicity, carcinogenicity, or reproductive toxicity, administration of the EVG/COBI/FTC/TAF combination product is unlikely to introduce new toxicities or to exacerbate known toxicities of the individual agents. The ample nonclinical safety databases on these drugs, including combination toxicity studies with EVG and COBI, and with FTC and TDF, strongly indicates further toxicological investigations are unlikely to yield new data relevant to humans. Additionally, the extensive clinical safety data available from the clinical trials with the EVG/COBI/FTC/TDF FDC and with the EVG/COBI/FTC/TAF FDC support the safety of the new combination product for the treatment of HIV-1 infection.

The absence of nonclinical safety studies with the E/C/F/TAF combination is in accordance with the FDA Guidance for Industry, Nonclinical Safety Evaluation of Drug or Biologic Combinations, March 2006 and the Committee for Medicinal Products for Human Use (CHMP) Guideline on the Non-Clinical Development of Fixed Combinations of Medicinal Products (EMEA/CHMP/SWP/258498/2005, January 2008). At the [redacted] meeting, the FDA agreed that [redacted] (Reference ID: [redacted]). This was confirmed in the [redacted] meeting minutes ([redacted]). Extensive clinical safety data are available for the approved drugs FTC, TDF, the FTC/TDF FDC product, Truvada, and the EVG/COBI/FTC/TDF FDC product, Stribild, and support the overall risk/benefit of this new E/C/F/TAF FDC product for the treatment of HIV-1 infection.

2. PHARMACOLOGY/VIROLOGY

2.1. Primary Pharmacodynamics

The E/C/F/TAF FDC contains 3 active antiviral agents (EVG, FTC, and TAF) and COBI, a pharmacoenhancer that lacks antiviral activity. Nonclinical virology studies of EVG, COBI, FTC, TAF, the EVG/FTC/TFV 3-drug combination, and the EVG/COBI/FTC/TFV 4-drug combination, are described in detail in the virology summary contained in m2.7.2, Section 4.2, together with the clinical virology data.

Mechanism of Action

During HIV-1 infection, HIV integrase (IN) removes 2 terminal nucleotides from the 3'-end of viral DNA (3'-processing) and then ligates the processed viral DNA ends into the host cell DNA (strand transfer reaction). Elvitegravir inhibited laboratory strains and various clinical isolates of HIV-1 with mean EC_{50} (concentration of a compound inhibiting virus replication by 50%) values of 0.38 nM against wild type HIV-1 in T-cell lines, 0.35 nM against HIV-1 macrophage-tropic virus in monocyte/macrophage cells, and 0.62 nM against clinical HIV-1 isolates in peripheral blood mononuclear cells (PBMCs) in vitro. Elvitegravir showed similar activity against HIV-2 in cell lines and primary cells. The calculated EC_{95} value (concentration of a compound inhibiting virus replication by 95%) for EVG was 1.25 nM (0.61 ng/mL) in the absence of human serum (HS) components and 100 nM (44.8 ng/mL) in the presence of the HS components, human serum albumin (HSA) and α1 acid glycoprotein (AAG), in HIV-1 infected human PBMC cultures (m2.7.2, Section 4.2).

Cobicistat is an efficient mechanism-based inhibitor of human CYP3A enzyme activity that lacks HIV-1 activity. Studies confirmed the specificity of CYP3A inhibition (m1.4.4, AD-216-2029 and AD-216-2070), the mechanism of inhibition, and also determined the enzyme inactivation parameters of COBI (m1.4.4, AD-216-2028). Cobicistat and RTV were both shown to be potent inhibitors of all tested human hepatic microsomal CYP3A activities and enzyme inactivation kinetic studies showed COBI to be an efficient inactivator of human hepatic microsomal CYP3A activity, with kinetic parameters (k_{inact} = 0.47 min^{-1}, K_I = 1.1 μM) similar to those of RTV (k_{inact} = 0.23 min^{-1}, K_I = 0.26 μM; m1.4.4, AD-216-2028). In the context of the FDC, COBI increases the bioavailability of EVG and reduces its metabolic clearance by preventing the formation of the EVG oxidative metabolite (M1, GS-9202), a reaction that is catalyzed by human CYP3A (m1.4.4, JTK303-AD-017). Further information is provided in the drug-drug interaction section below (Section 3.7). More specific details are provided in m2.6.4, Section 7 and in m2.7.2, Section 4.2 to facilitate the presentation and interpretation of data relevant to the overall drug-drug interaction profile.

Emtricitabine, a NRTI, is a synthetic analogue of the naturally occurring pyrimidine nucleoside, 2′-deoxycytidine. Intracellularly, FTC is converted through 3 phosphorylation reactions to its active tri-phosphorylated anabolite FTC-TP {4527}, {4535}. Emtricitabine triphosphate inhibits viral polymerases, including HIV-1 reverse transcriptase (RT) by direct binding competition with the natural deoxyribonucleotide substrate (deoxycytidine triphosphate; dCTP), and after

incorporation into DNA, by DNA chain termination {4249}. The EC_{50} of FTC against laboratory adapted strains of HIV-1 ranged from 0.001 to 0.62 μM depending on cell type and virus strain used in the assay {4534}, {4541}, {4526}. With clinical isolates of HIV-1, EC_{50} values ranged from 0.002 to 0.028 μM {4534}.

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

Unlike TDF, TAF is relatively stable in human plasma ($t_{½}$ ~90 minutes), but rapidly converts to TFV inside cells. Assessment of the intracellular metabolism of TAF in various types of immune cells including cluster determinant 4 (CD4)+ T-cells, lymphocytes, and monocytes showed efficient conversion of the prodrug to the active metabolite TFV-DP
(GSI Memo: 19 February 2003).

TAF exhibits potent anti-HIV activity in lymphoid T-cells, primary human PBMCs, and macrophages with EC_{50} values ranging from 3 to 14 nM. The in vitro activity of TAF against HIV-1 is 100- to 600-fold greater than TFV and 4- to 6-fold greater than TDF {1574}. In MT-2 cells, TAF shows low cytotoxicity with a selectivity index (SI) of > 10,000). Based on data generated with the parent nucleotide TFV, TAF is expected to be active against a wide range of HIV-1 subtypes and also against HIV-2 {1574}, {1649}, {39}. The in vitro HIV-1 resistance profile of TAF is defined by the resistance profile of the parent nucleotide TFV. In addition, TAF is a potent inhibitor of HBV replication, exhibiting in vitro activity comparable to that of TDF with an EC_{50} value of 18 nM (GSI Memo: 08DEC2000).

Additive to synergistic effects were observed in in vitro interaction studies of TFV, the active metabolite of TAF, with NRTIs (abacavir, FTC, lamivudine, stavudine, zalcitabine, zidovudine [ZDV]), nonnucleoside reverse transcriptase inhibitors (NNRTIs) (delavirdine, efavirenz [EFV], nevirapine), PIs (amprenavir, indinavir, nelfinavir, RTV, saquinavir), and the IN inhibitor EVG {1649}. No antagonistic interactions were observed for any of these 2-drug combinations in a T lymphoblastoid cell line. Data show similar results for the in vitro interactions of TAF with several commonly coadministered ARVs (m2.7.2).

In Vivo Efficacy in Animal Models

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

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

In summary, EVG, FTC, and TAF/TFV are potent and selective inhibitors of HIV-1. All 3 drugs show potent antiretroviral activity against diverse subtypes of HIV-1 in vitro. Elvitegravir does not require modification for activity. Emtricitabine and TAF/TFV are phosphorylated intracellularly through nonoverlapping pathways, and in combination show no antagonism for the formation of their active metabolites. Triple-drug combinations (EVG, FTC, and TFV) and 4-drug combinations (EVG/FTC/TFV and COBI) show synergistic anti-HIV-1 activity in vitro with no evidence for antiviral antagonism observed.

2.2. Secondary Pharmacodynamics

The antiviral activity of EVG against other viruses and data showing that COBI does not affect the antiviral activity of antiretrovirals are described in detail in m2.7.2, Section 4.2.

2.2.1. In Vitro Cytotoxicity

The cytotoxicity of EVG was evaluated in a number of human cell lines and primary cells. Elvitegravir showed weak cytotoxicity in primary PBMCs (drug concentration that results in a 50% reduction in cell viability [CC_{50}] > 100 μM) (m1.4.4, JTK303-PH-010), primary T-lymphocytes (CC_{50} 40 μM), primary monocytes/macrophages (CC_{50} > 500 μM), and macrophages (CC_{50} 26 μM) (m1.4.4, PC-186-2004). Using a [^{3}H]thymidine incorporation assay, EVG cytotoxicity was observed in a dose-dependent manner after 7 days of culture with PBMCs, with a CC_{50} value of 9.7 μM (SI of > 48,000) in the absence of HS and 170 μM in the presence of 50% HS (SI of > 100,000; m1.4.4, JTK303-PH-006). No difference in the cytotoxicity of EVG was detected in unstimulated versus stimulated PBMCs in the absence of HS (mean CC_{50} values of 10.8 and 16.6 μM, respectively; m1.4.4, PC-183-2001).

Cobicistat in vitro cytotoxicity has been evaluated in MT-2 lymphoblastoid T-cells following 5-day incubation and in HepG2 hepatoma cells following 3-day incubation (m1.4.4, PC-216-2003). Cobicistat did not show significant cytotoxicity in MT-2 and HepG2 cells (CC_{50} of 88 and 44 μM, respectively).

For FTC, no cytotoxicity was observed in vitro in human PBMC, MT-2, HepG2, CEM, MOLT-4, and Vero cells at concentrations up to 100 μM {4531}, {4534}. Emtricitabine was also found to be nontoxic to human bone marrow progenitor cells in vitro. For TFV in quiescent human PBMCs, no cytotoxic effect was detected at concentrations as high as 100 μM {1574}. Low in vitro cytotoxicity of TFV was also demonstrated in human liver cells (HepG2), proliferating human skeletal muscle cells, or quiescent renal tubular epithelial cells (m2.6.3, Section 1.2, P4331-00037). In addition, TFV showed no toxicity to myeloid and erythroid hematopoietic progenitor cells in vitro {4077}. Thus, FTC and TFV have a low order of cytotoxicity and a large therapeutic ratio in vitro.

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

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

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

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

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

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

2.2.2. Mitochondrial Toxicity

A variety of clinical symptoms observed in HIV patients treated with prolonged NRTI therapy appear to be linked to mitochondrial toxicity {2522}. Several representatives of this class of HIV drugs inhibit mitochondrial DNA polymerase γ, by direct binding and competition with the natural deoxyribonucleotide substrate, incorporation into DNA, leading to DNA chain termination. Elvitegravir and COBI are not nucleoside analogs and do not share chemical structure with DNA polymerase γ natural substrate, and therefore are not expected to have this activity. Elvitegravir induced no measurable change in the content of mitochondrial DNA (mtDNA) levels in HepG2 liver cells after a 14-day treatment at 10 μM (m1.4.4, TX-183-2009). Although COBI has not been specifically investigated for mitochondrial toxicity, no effect is anticipated based on its structure and its mechanism of action as a CYP3A inhibitor.

A variety of in vitro studies have been conducted to evaluate the potential of FTC, TFV and TAF to exert mitochondrial toxicity. Results from these studies suggest that FTC and TFV have

limited capability to inhibit human DNA polymerases or to mediate cytotoxicity or mitochondrial damage ({4541}, {6053}, {1131}, {2516}). In vitro combination studies have also been conducted in HepG2 cells to further evaluate the potential mitochondrial toxicity of FTC and TFV (as well as other nucleosides, m1.4.4, TX-104-2001).

HepG2 cells were exposed to FTC and TFV (as well as other nucleosides), either alone or in combination. Assay endpoints included cell growth; extracellular production of lactic acid; relative cellular content of mtDNA and mtDNA-encoded cytochrome c oxidase II (COX II); and intracellular lipid accumulation. Tenofovir and FTC alone or in combination with each other or other nucleosides generally had no time- or concentration-dependent effects on cytotoxicity (cell counts) or mitochondrial parameters in HepG2 liver cells. The dual combination of high-dose FTC+ZDV, with or without TFV, appeared to have greater cytotoxicity than the agents alone, but showed no increase in mitochondrial effects.

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

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

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

2.2.3. Off Target Activity

Elvitegravir showed no significant inhibition of or increased binding to a series of 22 receptors, 7 enzymes, and 3 cell-based assay systems, including the immune cell functions of cell adhesion (ICAM-1/VCAM-1 mediated), interleukin-2 (IL-2) secretion, and mixed lymphocyte reaction (splenic lymphocytes; m1.4.4, JTK303-PH-008).

While there is no functional equivalent to IN activity in host cells, the viral IN and the host topoisomerases display analogous activities of DNA binding, DNA cleavage, and transesterification reactions. Elvitegravir did not inhibit the activity of human topoisomerase I and II enzymes at concentrations up to 50 and 150 μM, respectively (m1.4.4, JTK303-PH-004).

The HIV-1 IN catalytic core is composed of a DDE active site motif and may be structurally related to others members of the superfamily of polynucleotidyl transferases known as DDE recombinases (including transposases, recombinases, and RNAses). One cellular member of this family is the RAG1/2 recombinase that plays an essential role in V(D)J recombination resulting in the assembly and generation of functional genes coding for the T-cell receptor and

immunoglobulins. Early generation INSTIs (5CITEP and L-708,906) were reported to inhibit RAG1/2 recombinase activity, albeit at a high micromolar range (20 and 200 μM) {12118}. However, more recently, the effects of an INSTI (dolutegravir) on the developing immune system in juvenile rats was reported {18823}. In this study, dolutegravir was administered to juvenile rats from Day 4 to 66 postpartum at dose levels up to 75 mg/kg/day. Results indicated that there were no alterations in T and B cell numbers, the diversity of T cell repertoire (evaluated by flow cytometry) and no effects on immune responsiveness (evaluated by the T cell dependent antibody response [TDAR]) suggesting no risk for developmental immunotoxicity. For EVG, no evidence of impairment to the immune system has been identified in an immunotoxicity study in rats (m1.4.4, JTK303-TX-011) and in repeat-dose toxicity studies in mice, rats (including a juvenile toxicity study), and dogs at doses up to 2000 mg/kg (m1.4.4, TX-183-2006, TX-183-2004, JTK303-TX-022, JTK303-TX-023). Further, 2-year carcinogenicity studies in mice and rats (m1.4.4, TX-183-2011 and TX-183-2012) also showed no significant decrease in lymphocytes or lymphoid organ changes, no increase in opportunistic infections in treated animals, and no increase in tumors. Based on these data, the immunotoxic potential for EVG is considered low.

Potential molecular targets for COBI were screened using radioligand binding assays against a panel of 67 mammalian ion channels and receptors (m1.4.4, TX-168-2007 and TX-168-2011). At 10 μM, COBI demonstrated significant binding at calcium, potassium, and sodium ion channels.

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

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

2.2.4. COBI Metabolic Toxicities (Proteosome/Insulin/Lipids)

Chronic treatment of HIV-infected patients with RTV is known to induce changes in body fat distribution (lipodystrophy), elevate blood levels of cholesterol (hypercholesterolemia) and triglycerides (hypertriglyceridemia), and cause insulin resistance {5117}. Some of these effects appear to be due to the direct effects of RTV on glucose transporter activity in adipocytes {11024}. Ritonavir has been reported to inhibit proteasome activity, known to contribute to the regulation of several proteins involved in lipid metabolism {11991}, {9456}. In vitro, RTV has been shown to affect adipocyte functions such as differentiation-associated lipid accumulation and insulin-stimulated glucose uptake {5495}. As COBI is an analog of RTV, its effects on these potential targets were evaluated using in vitro models. Compared to RTV, COBI showed slightly reduced inhibition of host proteasome activity, with a 50% inhibitory concentration (IC_{50}) value of 12.8 μM (versus 7.9 μM for RTV) and no effects on host protease cathepsin D (IC_{50} of > 30 μM versus 0.87 μM for RTV (m2.6.2, Section 2.1.2, PC-216-2001). Additionally, COBI exhibited no effect on lipid accumulation at 30 μM (EC_{50} 16 μM for RTV) and a less

pronounced effect on glucose uptake (9.5% inhibition at 10 μM versus 55% inhibition for RTV at 10 μM), suggesting a lower potential of COBI for metabolism-related toxicities compared to RTV (m1.4.4; PC-216-2004). The noted effects of COBI are unlikely to be significant at a maximal unbound clinical exposure of 0.09 μM (m2.7.2).

2.3. Safety Pharmacology

2.3.1. EVG

In a battery of safety pharmacology studies, the effects of EVG on the central nervous, cardiovascular, respiratory, gastrointestinal (GI), and renal/urinary systems were examined. There were no adverse effects of EVG on the central nervous system (CNS) (m1.4.4, JTK303-SP-001), intestinal transport (m1.4.4, JTK303-SP-006), and renal/urinary systems (m1.4.4, JTK303-SP-007) at doses up to 2000 mg/kg in the rat. Elvitegravir had no adverse effects on the cardiovascular and respiratory systems in dogs at doses up to 100 mg/kg (m1.4.4, JTK303-SP-002). Elvitegravir at concentrations of 0.1 and 1 μM had no effect on the human ether-à-go-go related gene (hERG) tail current in vitro (m1.4.4, JTK303-SP-003). A slight reduction (24.3%) in the hERG tail current was observed at the highest feasible concentration of 10 μM that is not considered clinically significant (m1.4.4, JTK303-SP-004). No effect on action potential was observed in isolated guinea pig papillary muscle at concentrations up to 1.0 μM. The cardiovascular risk of EVG is considered minimal.

2.3.2. COBI

Safety pharmacology studies were conducted to determine the potential effects of COBI on the central nervous, respiratory and cardiovascular systems. In the rat CNS study, changes were limited to salivation, decreases in arousal, locomotor and motor activities, and decreases in body temperature at doses of 150 mg/kg and above (m1.4.4, TX-216-2006). The NOAEL was 50 mg/kg. Decreases in body temperature are commonly observed in rodents after xenobiotic exposure, and most likely represent an adaptive thermoregulatory response unique to rodents, rather than a direct effect on the CNS {11868}, {11869}, {11870}. Similarly, decreases in arousal and motor activity may represent a general toxicity response rather than a direct CNS response. Of note, tissue distribution studies in rats showed low levels of COBI-derived radioactivity in brain suggesting minimal transport across the blood:brain barrier (Section 3.4.2.2). No adverse effects were observed in the rat respiratory study (NOAEL 500 mg/kg) (m1.4.4, TX-216-2007).

Patch clamp studies indicated that COBI inhibited the hERG potassium current (IC_{50} 1.8 μM) and the $hCa_v1.2$ L-type calcium channel (IC_{50} 6 μM), but was a weak inhibitor of the $hNa_v1.5$ sodium channel (IC_{50} 86.5 μM) (m1.4.4, TX-216-2009 and TX-216-2015). In rabbit Purkinje fibers (protein-free environment), which are considered more sensitive to drug-induced action potential duration (APD) prolongation and early after depolarizations (EADs) than fibers isolated from dog and several other species {11871}, COBI caused a shortening of the APD at ≥ 1 μM; there was no evidence of triangulation, instability, or alternans predictive of prolongation of the QT interval (m1.4.4, TX-168-2012).

In a Langendorff study in rabbit hearts (protein-free environment) conducted with COBI alone, negative inotropic effects and shortening of the APD was noted at ≥ 1 μM (m1.4.4, PC-216-2007). In a second Langendorff study in rabbit hearts, COBI produced similar negative inotropic effects (PR interval prolongation, and produced decreases in left ventricular [LV] function) at concentrations ≥ 1.5 μM. When hearts were exposed to COBI in combination with ATV, effects on PR interval and LV function were similar to the decreases noted with COBI alone. Cobicistat had no notable effects alone, or in combination with ATV, on QRS and QT intervals, monophasic action potential duration (MAPD), or triangulation; and there were no EADs (m1.4.4, PC-216-2009).

In conscious telemetered dogs, there were no adverse effects on hemodynamic and electrocardiograph (ECG) parameters up to 45 mg/kg, the highest dose administered (m1.4.4, TX-216-2008). Cobicistat plasma levels 1 hour after dose administration at 45 mg/kg were between 2530 and 8950 ng/mL (3.3 to 11.5 μM; 2.3- to 8-fold above the clinical C_{max} at the 150 mg dose (COBI Population PK Report). Compared to vehicle control values, mild prolongation in PR intervals were noted primarily from 1 to 6 hours postdose, although mean PR intervals never exceeded the upper limits of normal for canines at any time point {11872}, {11874}. Further, based on the results of the Japanese QT PRODACT studies and others, the mild increases in QTc (< 4%) noted from 13 to 24 hours postdose at 45 mg/kg are unlikely to be biologically significant {6959}, {11875}.

Although COBI inhibits the L-type calcium ion channel and potassium hERG-current at low micromolar concentrations, data from the Purkinje fiber assay, the cardiovascular dog study, and ECG evaluations in the repeat-dose toxicity studies in dogs up to 39 weeks duration (m1.4.4, [TX-216-2002, TX-216-2005, and TX-216-2016]) suggest that COBI has a low potential for QT prolongation, but may have a tendency to slightly prolong the PR interval. These effects, including the shortening of the APD in rabbit Purkinje fibers, the negative inotropic effects in isolated rabbit hearts and the mild delay in the PR interval in dogs, may be a consequence of interaction with cardiac calcium channels {11876}, {11873}. Of note, in the 39-week dog toxicity study (m1.4.4, TX-216-2016), there were no remarkable effects on the QT and PR intervals at dose levels up to 20 mg/kg/day. Mean COBI C_{max} values during Week 39 at 20 mg/kg/day were between 7090 to 8405 ng/mL (9.1 to 10.8 μM; 6.4- to 7.6-fold above the clinical C_{max} at the 150 mg dose (m2.7.2, Appendix 6.1.5). In a thorough QT clinical study (m2.7.2, Appendix 6.1.5 [GS-US-216-0107]), COBI demonstrated a lack of prolongation effects on the QTcF interval in healthy adult subjects at therapeutic and supratherapeutic exposures. A small but statistically significant negative association between COBI plasma concentration and QTc interval, and a modest, dose-related increase in PR interval, were observed in the QT/QTc study, which are not considered to be clinically significant. Further, echocardiograms performed in healthy subjects in Study GS-US-216-0116 at baseline and after receiving 150 mg COBI for at least 15 days indicated no clinically significant change in LV function (m2.7.2, Appendix 6.1.5).

2.3.3. FTC

A comprehensive range of safety pharmacology studies revealed no treatment-related adverse effects on any organ system at systemic exposure levels much higher than those anticipated in patients at the recommended clinical dose (10- to more than 50-fold) (m1.4.4,

477, TPZZ/92/0056; TPZZ/93/0001; TPZZ/93/0119; TPZZ/92/0057). No effects on the cardiovascular system were reported in anesthetized dogs administered a cumulative dose of 38.5 mg/kg of FTC intravenously over a 1-hour period (m1.4.4, TPZZ/92/0076). In addition, there were no abnormalities reported on the ECG data obtained from the repeated-dose toxicity studies in monkeys, where AUC exposures were up to 26-fold higher than in humans administered the 200 mg dose (m1.4.4, TOX600; TOX627; TOX032).

2.3.4. TAF

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

The IC_{50} for the inhibitory effect of TAF on hERG potassium current was estimated to be greater than 10 μM, far above human exposure (m2.6.3, Section 4.1, PC-120-2005).There were no adverse effects detected in the CNS in rats dosed at 1000 mg/kg (m2.6.3, Section 4.2, R990188), in the renal system in rats administered 1000 mg/kg (m2.6.3 Section 4.2, R990186) or in the cardiovascular system of dogs dosed at 100 mg/kg (80 mg free base equivalents/kg) (m2.6.3, Section 4.2, D2000006). There was reduced gastric emptying in rats dosed at 1000 mg/kg, but not at 100 mg/kg (m2.6.3, Section 4.2, R990187).

2.3.5. E/C/F/TAF

A comprehensive safety pharmacology program has been conducted for the 4 individual agents. While the study designs for these studies varied between the agents, the major organ systems were comprehensively evaluated. Elvitegravir, FTC, and TAF had little effect on vital organ systems in safety pharmacology studies. Although COBI has shown the potential for PR prolongation and to decrease LV function, no clinically-relevant cardiovascular changes have been observed with COBI administered as an individual agent, within the E/C/F/TDF FDC or within the E/C/F/TAF FDC (m2.7.4, Section 4.2). Although TAF showed some potential to prolong the PR interval in the 39-week dog study at 18/12 mg/kg/day, the slight change was associated with decreased weight gain, bone and renal toxicity, and significant decreases in triiodothyronine (T_3) {29101}, {29104}. No PR prolongation or any change in ECG results occurred in the safety pharmacology study that evaluated a TAF dose up to 100 mg/kg (m2.6.3, Section 4.2, D-TX-D2000006) or in the thorough QT study (m2.7.2, Appendix 6.1.3, [GS-US-120-0107]). Overall, the pharmacological assessment of EVG, COBI, FTC, and TAF supports the effective use of these 4 agents together in combination therapy for the treatment of HIV-1 infection. Additional safety pharmacology studies on the E/C/F/TAF FDC are considered unwarranted.

2.4. Pharmacodynamic Drug Interactions

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

2.5. Summary of Pharmacology

The HIV-1 INSTI, EVG, and the nucleoside or nucleotide reverse transcriptase inhibitors (N[t]RTIs), FTC and TFV, have potent antiretroviral activity against wild-type and many drug-resistant strains of HIV-1 in vitro and in vivo. The combination of EVG, FTC, and TFV in 3-drug combination experiments showed additive to synergistic anti-HIV-1 activity, and synergistic anti-HIV-1 activity in 4-drug combination experiments with COBI. The nonclinical virology studies of EVG, COBI, FTC, TAF, and TFV/TDF are summarized and described in detail in the virology summary contained in m2.7.2, Section 4.1, together with the clinical virology data.

Elvitegravir does not inhibit the activity of human topoisomerase I and II enzymes, cellular enzymes that display analogous activities to the viral IN activity. Emtricitabine and TDF have a high selectivity for HIV RT and are very weak inhibitors of mammalian DNA polymerases α, β, δ, ε, and mitochondrial DNA polymerase γ.

Elvitegravir, FTC, and TDF have no pharmacologically significant off-target binding affinity to the receptors tested, while COBI had significant binding to 3 ion channels (calcium channel L-type, potassium channel, and sodium channel site-2).

Elvitegravir, COBI, FTC, and TAF have low in vitro cytotoxicity in a variety of human cell types.

Nucleoside reverse transcriptase inhibitors currently carry a class labeling for mitochondrial toxicity; however, both FTC and TAF have shown a low potential for mitochondrial toxicity in long-term toxicity studies. The potential for mitochondrial toxicity of EVG was considered low based on assessment of the mtDNA levels in HepG2 liver cells. The potential for mitochondrial toxicity by COBI is also considered low. As EVG and COBI are not anticipated to significantly increase the exposures of FTC and TAF, the potential for mitochondrial toxicity with the E/C/F/TAF FDC is low.

Elvitegravir, FTC, and TAF had little effect on vital organ systems in safety pharmacology studies. Cobicistat showed the potential to decrease LV function and prolong the PR interval in the isolated rabbit heart at concentrations approximately 11-fold above the anticipated clinical exposure at the 150-mg COBI dose within the FDC (assuming 6.3% fraction unbound from equilibrium dialysis experiments; m1.4.4, [AD-216-2026]); these effects are likely due to interactions with cardiac calcium channels {11876}, {11873}. However, as the fraction of unbound COBI has been found to be lower (2.49%–3.23%) in normal subjects and in subjects with moderate hepatic impairment or severe renal impairment in ex vivo measurements compared to the value obtained from in vitro equilibrium dialysis experiments (m2.7.2, Appendix 6.1.6 [GS-US-183-0133 and GS-US-216-0124]), the calculated margin may be an underestimate. Moreover, clinical studies, including the thorough QT study and the echocardiogram assessments have shown no clinically significant changes in ECGs or LV function. Hence, the potential of COBI to decrease LV function and prolong PR is low in HIV-1 infected patients. Although TAF showed some potential to prolong the PR interval in the 39-week dog study at 18/12 mg/kg/day, the slight change was associated with decreased weight

gain, bone and renal toxicity, and significant decreases in T3 {29101}, {29104}. No PR prolongation or any change in ECG results occurred in the safety pharmacology study that evaluated a TAF dose up to 100 mg/kg (m2.6.3, Section 4.2, D-TX-D2000006) or in the thorough QT study (m2.7.2, Appendix 6.1.5 [Study GS-US-120-0107]). Overall, the pharmacological assessment of EVG, COBI, FTC, and TAF supports the effective use of these 4 agents together in combination therapy for the treatment of HIV-1 infection. Additional safety pharmacology studies on the E/C/F/TAF FDC are not warranted.

Overall, the pharmacodynamic and pharmacological safety assessment of EVG, COBI, FTC, and TAF supports the effective and safe use of these 4 agents together in combination for the treatment of HIV-1 infection.

3. PHARMACOKINETICS

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

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

3.1. Analytical Methods

For all 4 agents (and their metabolites, where appropriate), bioanalytical methods for toxicokinetic analysis supporting GLP safety studies were validated. Some of these methods for early nonclinical studies did not strictly conform to GLP guidelines but were evaluated for appropriate selectivity, sensitivity, linearity, as well as intra-assay accuracy and precision. All bioanalytical methods were conducted using appropriate protocols and documentation to assure data integrity (m2.6.4, Pharmacokinetics Written Summary, Section 2).

3.2. In Vitro Absorption and Single Dose Pharmacokinetics

3.2.1. EVG

The permeability of EVG, studied in monolayers of LLC-PK1 porcine kidney cells, was high compared to mannitol, the low permeability control (m1.4.4, JTK303-AD-026). While polarized transport of EVG could be demonstrated in p-glycoprotein (P-gp)-expressing cells in vitro, there was no evidence for efflux-limited bioavailability in rats or dogs in vivo.

Single-dose pharmacokinetics of EVG and [^{14}C]EVG were studied in rats and dogs. In the rat, clearance of EVG was low relative to hepatic blood flow and the volume of distribution was lower than total body water (m1.4.4, JTK303-AD-009 and JTK303-AD-011). Absorption after oral dosing was rapid and bioavailability was moderate (30%–35%) and close to that for total radioactivity (41%; m1.4.4, JTK303-AD-005 and JTK303-AD-007). In the dog, clearance was intermediate relative to hepatic blood flow and the volume of distribution was ~3-fold higher than total body water (m1.4.4, JTK303-AD-010 and JTK303-AD-012). As with the rat, absorption after oral dosing was rapid; bioavailability was moderate (26%–33%) and similar to that for total radioactivity (41%; m1.4.4, JTK303-AD-006 and JTK303-AD-008).

3.2.2. COBI

In human colon carcinoma (Caco-2) cell monolayers, COBI showed high forward permeability and no evidence for efflux (m1.4.4, AD-216-2023). Single-dose pharmacokinetics were studied in rats (m1.4.4, AD-216-2020), dogs (m1.4.4, AD-216-2021), and monkeys (m1.4.4, AD-216-2022). Clearance values were high relative to hepatic blood flow (likely due to the lack of self-limiting enzyme inactivation in nonclinical species) and volumes of distribution were

similar to those for total body water. After oral dosing, bioavailability was low or low/moderate, likely due to high first-pass elimination.

3.2.3. FTC

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

3.2.4. TAF

In Caco-2 cell monolayers, TAF showed a dose-dependent increase in forward permeability and a decrease in efflux ratio indicating saturable efflux transport. Addition of the efflux transport inhibitor, cyclosporin A (CsA) diminished the efflux ratio and increased the forward permeability (m2.6.5, Section 3.1, AD-120-2037).

Single-dose plasma pharmacokinetics of TFV and/or TAF were evaluated following administration of TAF by dosing either GS-7340-02 or GS-7340-03 to male CD-1 mice or GS-7340-03 to both male and female 001178-W mice via oral gavage (m2.6.5, Section 3.2, AD-120-2014 and m2.6.5, Section 3.3, AD-120-2016), to rats via oral gavage (m2.6.5, Section 3.5, R990130; m2.6.5, Section 3.4, AD-120-2015; and m2.6.5, Section 3.6, R2000065), to dogs via intravenous (IV) bolus of GS-7340-02 or oral administration of TAF as free base, its diastereomer GS-7339, the mixture GS-7171, or GS-7340-02 under fasted and under fed conditions (m2.6.5, Section 3.7, 99-DDM-1278-001-PK). Tenofovir alafenamide was not detected in any of the rat studies. Additionally, the plasma PK profiles for TAF and TFV and TFV concentrations in PBMCs were determined in rhesus monkeys following a single oral dose of GS-7340-02 (m2.6.5, Section 3.9, P2000087). TAF is generated at sufficient exposures in nonclinical species chosen for assessment of toxicology. Consistent with dose-dependent permeability observed in vitro, the oral bioavailability of TAF increased with increasing dose in dogs and the observed oral bioavailability was 14.3% at the 10 mg/kg dose {23907}. Following an oral dose of [^{14}C]TAF to a bile-duct cannulated (BDC) dog, the fraction absorbed was at least 41% based on excretion in urine and bile. Since 41% of the total dose was absorbed and 14.3% was found in systemic circulation, approximately 27% was extracted by liver. Therefore, approximately 65% of the absorbed drug was hepatically extracted. This was consistent with hepatic extraction estimated from the in vitro stability study in dog hepatic tissue post-mitochondrial (9,000 x g) supernatant (S9) fractions (60.5%).

3.2.5. E/C/F/TAF

With respect to potential drug interactions within the combination that could affect absorption, FTC shows high passive permeability and so is unlikely to be affected when administered with EVG, COBI, or TAF. Cobicistat is a weak inhibitor of intestinal efflux transporters, but high concentrations of COBI in the intestinal lumen, achievable briefly during absorption, inhibit P-gp. 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. 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). Although formal nonclinical studies of the single dose pharmacokinetics of the E/C/F/TAF FDC have not been conducted, comprehensive pharmacokinetic clinical studies with the E/C/F/TAF FDC have been performed (m2.7.2).

3.3. Repeat Dose Pharmacokinetics

3.3.1. EVG

[^{14}C]Elvitegravir was administered orally to rats daily for 7 days at a dose of 3 mg/kg/day. There was no change in exposure between Day 1 and Day 7 (m1.4.4, JTK303-AD-022 and JTK303-AD-028), indicating that multiple dosing with EVG did not appreciably affect the fraction of total radioactivity absorbed.

The multiple-dose pharmacokinetic parameters for EVG were derived as part of the repeat-dose GLP toxicity studies in mice (100 to 2000 mg/kg/day; m1.4.4, TX-183-2004 and TX-183-2011), rats (100 to 2000 mg/kg/day; m1.4.4, JTK303-TX-003, JTK303-TX-021, JTK303-TX-022, TX-183-2012), and dogs (10 to 100 mg/kg/day; m1.4.4, JTK303-TX-004, JTK303-TX-023) dosed for periods of 4 weeks to 104 weeks. In general, there were no significant differences in pharmacokinetics following single and multiple dosing. Exposure did not change during repeat dosing, indicating no autoinduction of elimination pathways (confirmed by analysis of hepatic microsomal fractions from mice treated with EVG for up to 26 weeks). In rodents, EVG exposures were generally higher in females than males likely due to higher expression of CYP3A in males. In dogs, there was no clear sex difference, consistent with the lack of gender difference in CYP3A in this species.

Multiple dose toxicology studies were also performed with EVG in combination with RTV in mice (m1.4.4, TX-183-2011) and rats (m1.4.4, TX-183-2007) and in combination with COBI in rats (m1.4.4, TX-236-2001, TX-236-2002). EVG exposures were modestly higher when codosed with RTV or COBI on Day 1 of dosing. The PK enhancement effect of COBI on EVG levels is reduced upon chronic treatment, due to COBI's ability to act as an inducer in rodents.

3.3.2. COBI

The multiple-dose pharmacokinetic parameters for COBI were derived as part of the repeat-dose GLP toxicity studies in mice (10 to 100 mg/kg/day; m1.4.4, TX-216-2032, TX-216-2041, TX-216-2026), rats (10 to 100 mg/kg/day; m1.4.4,, TX-216-2004,TX-216-2017), and dogs (5 to 45 mg/kg/day; m1.4.4, TX-216-2005,TX-216-2016) dosed for periods of 4 weeks to 39 weeks. Toxicokinetic parameters are presented in m2.6.6.

There were species differences in autoinduction during these studies, with hepatic microsomal fractions from treated mice and rats showing higher levels of CYP3A, but with no increases in treated dogs. This is consistent with the species differences observed in induction studies in vitro where COBI was found to activate rat PXR (m1.4.4, AD-216-2039) but did not activate human PXR or induce human drug metabolizing enzymes or P-gp in hepatocytes (m1.4.4, AD-216-2027, AD-216-2071).

Multiple-dose toxicology studies in rats were also performed with COBI in combination with EVG (m1.4.4, TX-236-2001, TX-236-2002) and ATV (m1.4.4, TX-216-2024). In general, COBI only modestly increased EVG and ATV steady state exposures compared to EVG and ATV when dosed alone, consistent with its dual action as a reversible CYP3A inhibitor and a P450 inducer in rodents (Section 4.2.3).

3.3.3. FTC

The multiple-dose pharmacokinetic parameters for FTC were derived as part of the repeat-dose toxicity studies in mice (80 to 3000 mg/kg/day; m1.4.4, TOX109; TOX001; TOX599; TOX628), rats (60 to 3000 mg/kg/day; m1.4.4, TOX108; TOX097), and monkeys (40 to 2000 mg/kg/day; m1.4.4, TOX600; TOX627; TOX032) dosed for periods of 3 days to 104 weeks. In general, there were no significant differences in pharmacokinetics following single and multiple dosing. Systemic exposure to FTC (C_{max} and AUC) increased approximately proportionally with dose and was similar between males and females.

3.3.4. TAF

The multiple-dose pharmacokinetics of TFV were characterized in a pharmacokinetic study in dogs orally administered TAF (m2.6.5, Section 4.1, AD-120-2033) and in toxicokinetic studies following oral administration of TAF in mice (m2.6.7, Section 7.1, TX-120-2007), rats (m2.6.7, Section 7.2, R990182, m2.6.7, Section 7.3, TOX-120-001), dogs, (m2.6.5, Section 4.2, D990175-PK, m2.6.7, Section 7.5, TOX-120-002 and monkeys (m2.6.5, Section 4.3, P2000114-PK). After repeat dosing in mice or monkeys for up to 13 weeks or 4 weeks, respectively, no accumulation of TFV occurred; slight accumulation (up to ~3-fold) of TFV occurred in rats and dogs dosed for up to 26 and 39 weeks, respectively.

3.3.5. E/C/F/TAF

Although formal nonclinical studies of the repeat dose pharmacokinetics of the E/C/F/TAF FDC have not been conducted, comprehensive clinical studies on the combination have been performed (m2.7.2).

3.4. Distribution

3.4.1. Protein Binding

3.4.1.1. EVG

Binding of EVG was high, and concentration-independent in plasma from rats, dogs, monkeys, and humans (m1.4.4, JTK303-AD-014). The fraction unbound varied from 0.1% in rats to 1.2% in monkey. The fraction unbound in human plasma, or in a physiological concentration of HSA, averaged 0.7%. Binding to human AAG was low and addition of AAG to a solution of HSA did not affect the fraction of EVG unbound. Approximately the same extent of binding of EVG was found in human plasma samples from clinical studies in which subjects were treated daily with 150 mg EVG plus 150 mg COBI (m2.7.2, Appendix 6.1.6 [GS-US-183-0133, GS-US-216-0124). Elvitegravir does not distribute well into the cellular fraction of blood from rat, dog, monkey, or

human in vitro (m1.4.4, JTK303-AD-013). The whole blood/plasma ratio for human blood was 0.7 and this value was confirmed in an in vivo study (m2.7.2, Appendix 6.1.4, [GS-US-183-0126]).

3.4.1.2. COBI

Binding of COBI in plasma was moderately high, yielding a fraction unbound of 6.3% in human plasma at 1 μM COBI (m1.4.4, AD-216-2026; AD-216-2076). Binding to mouse, rat, and monkey plasma was similar and showed modest concentration-dependence, but binding in dog and human plasma was largely unchanged over the range 1–30 μM. Fraction unbound values were similar in nonclinical species (mean values 4.75%–6.54%) to humans. Moderately high binding of COBI in human plasma was also found in ex vivo samples from clinical studies in which subjects were treated daily with 150 mg EVG plus 150 mg COBI, although absolute values for the free fraction were slightly lower than those determined in vitro (m2.7.2, Appendix 6.1.6 [GS-US-183-0133,GS-US-216-0124]). Analysis of blood and plasma concentrations of COBI from in vivo studies revealed that COBI does not distribute well into the cellular fraction of blood from mouse, rat, dog, or human.

3.4.1.3. FTC

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

3.4.1.4. TAF and TFV

Since TAF is highly unstable in rodent plasma due to hydrolytic cleavage by plasma esterases, the extent of TAF binding to plasma was determined in dog and human plasma in vitro (m2.6.5, Section 6.1, AD-120-2026). In vitro protein binding of TAF was moderate in dog and human plasma with the percent unbound values of 48.0% and 46.8%, respectively. These in vitro values were higher than those observed in multiple human ex vivo studies with the mean percent unbound TAF ranging from 14% to 23% in all subjects (GS-US-120-0108 and GS-US-120-0114). Since the ex vivo results should be more clinically relevant than the in vitro values, percent unbound TAF of 20% was used for the assessments for potential drug interactions (m2.6.4, Section 7.1).

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

3.4.1.5. E/C/F/TAF

Although plasma protein (primarily to albumin) binding of EVG is high and moderate for COBI and TAF, protein binding for FTC and TFV is very low. As a result, interactions through binding displacement would not be anticipated, and no studies with the E/C/F/TAF FDC were considered necessary.

3.4.2. Tissue Distribution

3.4.2.1. EVG

After oral administration of [^{14}C]EVG to male or female albino rats, there was rapid distribution of radioactivity to highly perfused organs (liver, adrenal gland, kidney, heart, lung, and pancreas), with relative exclusion from the eye and brain (m1.4.4, JTK303-AD-005). Concentrations of radioactivity in tissue declined largely in parallel with those in plasma, reaching undetectable or trace levels by 96 hours postdose. Tissue/plasma concentration ratios were generally < 1 apart from liver and GI tract. Pretreatment of rats with RTV (20 mg/kg orally, 20 and 2 hours prior to EVG) raised tissue concentrations of EVG-derived radioactivity in parallel with those in plasma, but did not change the pattern of distribution (notably no entry of EVG to the brain; m1.4.4, 60N-0518).

3.4.2.2. COBI

After oral administration of [^{14}C]COBI to albino and pigmented rats (m1.4.4, AD-216-2034 and AD-216-2060, respectively), radioactivity was rapidly and widely distributed to most tissues. Generally, the radioactivity was preferentially distributed into glandular tissues and organs of elimination. The tissues showing the highest concentrations of radioactivity, excluding the GI tract, included liver, adrenal, kidney, and pituitary. The tissues with the lowest C_{max} values were eye, spinal cord, and brain, bone, and secondary sex organs. Low levels of radioactivity in the brain, spinal cord, and testes suggest minimal transport across the blood:brain and blood:testes barriers. Compared to albino rats, the pigmented rats showed very similar patterns of distribution of radioactivity, but with higher concentrations in the uveal tract of the eye. There were also higher concentrations of radioactivity in pigmented skin compared to nonpigmented skin, suggesting that COBI was associated with melanin. Tissue concentrations of radioactivity declined largely in parallel with those in plasma. In pigmented animals there was more prolonged retention of radioactivity in melanin-containing tissues, but dosimetry analysis confirmed that concentrations were declining and association with the tissues was reversible.

3.4.2.3. FTC

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

3.4.2.4. TAF and TFV

Following oral administration of [^{14}C]TAF to mouse (m2.6.5, Section 5.1, AD-120-2011), rat (m2.6.5, Section 5.2, AD-120-2020), and dog (m2.6.5, Section 5.3, AD-120-2009 and m2.6.5, Section 5.4, D990173-BP), [^{14}C]TAF-derived radioactivity was widely distributed to most of the

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

Following single intravenous administration of [^{14}C]TFV in male rats, the highest concentrations of radioactivity were found in the kidney, liver, urine, and large intestine and trace amounts were observed in bone or bone marrow (m1.4.4, 96-DDM-1278-002).

3.4.2.5. E/C/F/TAF

Drug interactions, within the 4-drug combination, that affect distribution would not be expected from the data available. An in vivo study with [^{14}C]EVG and co-dosed RTV (m2.6.4, Section 4.4) revealed no change in the tissue distribution of EVG, and notably no increase in CNS penetration of EVG (m1.4.4, 60N-0518). Because COBI displays transporter inhibition potencies similar to, or weaker than, RTV (m2.6.4, Section 7.2.4), COBI would not be expected to affect the tissue distribution of the other agents. Since COBI has similar or lower potency than RTV as an inhibitor of transporters (Section 3.7.3.2), no changes in distribution of E/C/F/TAF administered in combination would be expected.

3.4.3. Distribution in Pregnant Animals

Pharmacokinetics parameters for EVG were determined after single and multiple doses in pregnant rats (m1.4.4, JTK303-TX-020, TX-183-2008, respectively) and rabbits (m1.4.4, TX-183-2001, TX-183-2002). Exposure in pregnant rats was generally similar to that in nonpregnant animals.

Pharmacokinetic parameters for COBI were determined in pregnant rats (m1.4.4, TX-216-2018, TX-216-2020) and rabbits (m1.4.4, TX-216-2019, TX-216-2021). Exposure in pregnant rats was generally similar to that in nonpregnant animals.

Pharmacokinetic parameters for FTC in pregnant animals appeared to be generally similar to those reported for nonpregnant animals.

While accumulation of TFV was observed after multiple dosing of TAF as GS-7340-02 up to 200 mg/kg/day in pregnant rats in the range-finding study (m2.6.7, Section 11, TX-120-2001), no accumulation of TAF and TFV was observed up to 250 mg/kg/day in the embryo-fetal development study (m2.6.7, Section 13.1, TX-120-2002). No accumulation occurred in pregnant rabbits in the range-finding study (m2.6.7, Section 11, TX-120-2004) or the embryo-fetal development study (m2.6.7, Section 13.2, TX-120-2005).

Placental transfer studies were conducted for TFV (rhesus monkeys) and FTC (mice and rabbits). Both drugs are transferred across the placenta, but did not concentrate in fetal tissues. Fetal/maternal exposure ratios, determined on appropriate gestation days (GDs) by the concentrations of TFV in serum and FTC in plasma and umbilical cord blood, were ≤ 0.5 (see Section 3.2 and m1.4.4, 96-DDM-1278-005; TOX103; TOX038).

3.5. Metabolism

3.5.1. Intracellular Metabolism

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

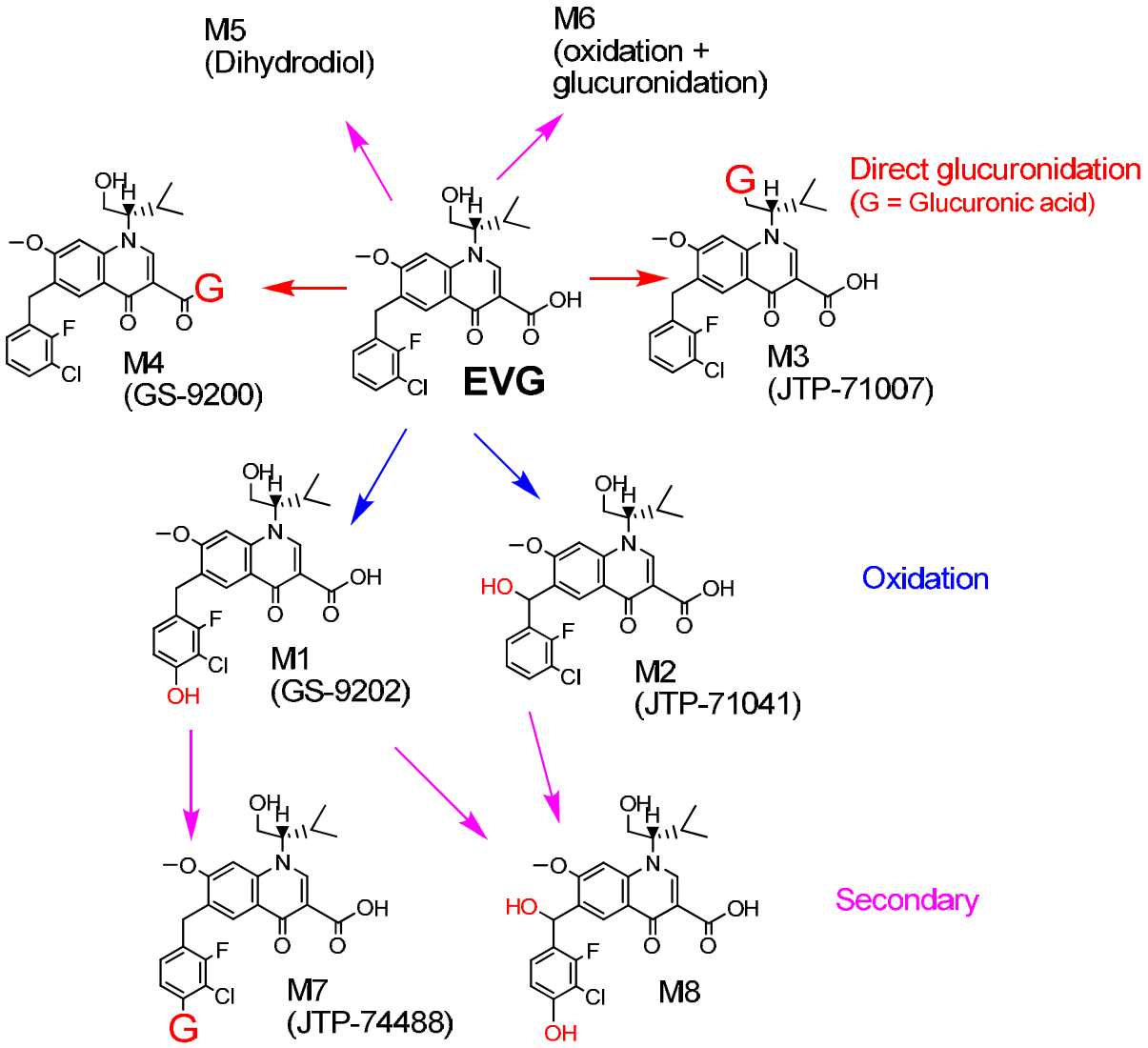
Emtricitabine and TFV are analogues of 2 different nucleosides, cytosine and adenosine, respectively, and do not share a common intracellular metabolism pathway. In experiments where both FTC and TFV were incubated together at concentrations higher than achieved in the plasma (10 μM), the intracellular phosphorylation of FTC and TFV to their active intracellular anabolites was not affected (m1.4.4, PC-164-2002).

3.5.2. Routes of Metabolism

3.5.2.1. EVG

The primary metabolic pathways for EVG are illustrated in Figure 1 and are hydroxylation to M1 (GS-9202), catalyzed by CYP3A (m1.4.4, JTK303-AD-017), and glucuronidation at the carboxylic acid moiety, catalyzed by UGT1A1 and UGT1A3 in humans (m1.4.4, AD-183-2034), yielding M4 (GS-9200). Minor pathways detected include benzylic hydroxylation (M2), generation of the direct ether glucuronide (M3) and combinations of the primary pathways (M7 and M8).

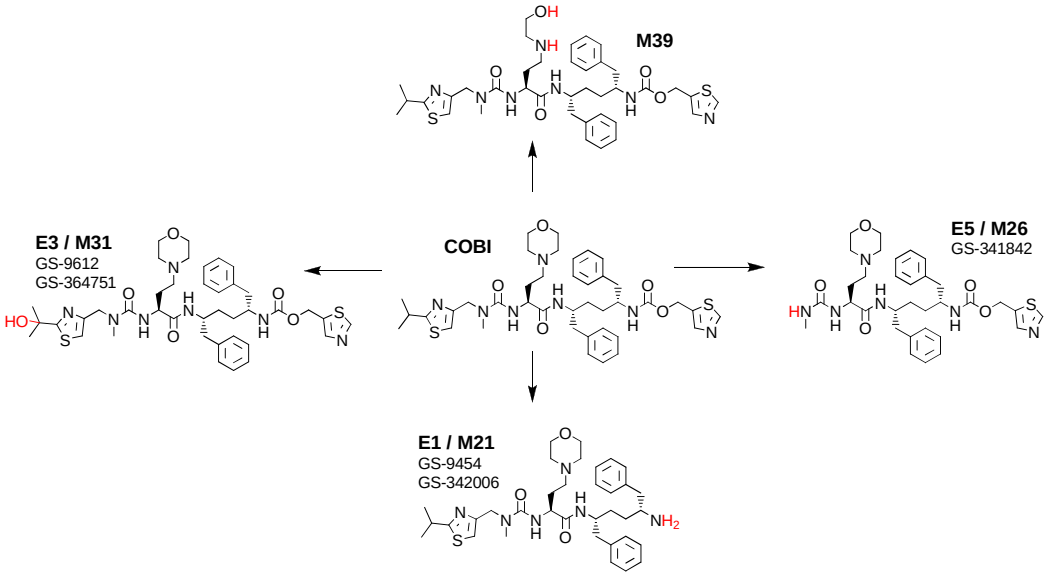
Figure 1. Proposed Metabolic Pathway of EVG



3.5.2.2. COBI

The primary metabolic pathways for COBI are illustrated in Figure 2 and are methine oxidation of the isopropyl moiety (M31, GS-9612), cleavage adjacent to the methylurea (M26, GS-341842), cleavage of the carbamate (M21, GS-9454), and cleavage and deethylation of the morpholine (M39). Combinations of these routes and other routes of oxidative metabolism were also detected. Oxidation is primarily catalyzed by CYP3A, which can generate all metabolites, with a minor role for CYP2D6 (which contributes to the generation of M31) (m1.4.4, AD-216-2025). In vitro metabolism in nonclinical species was relatively rapid but rates of metabolism by human hepatic microsomal fractions, human hepatocytes, and recombinant CYP3A4 were relatively slow due to concurrent CYP3A inactivation (m1.4.4, AD-216-2024; AD-216-2074; AD-216-2025).

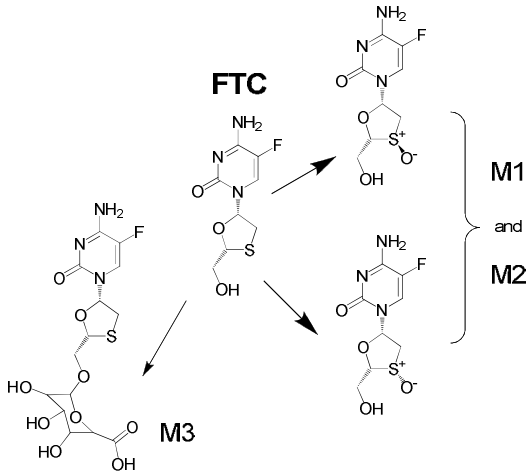
Figure 2. Proposed Primary Metabolic Pathway of COBI



3.5.2.3. FTC

Emtricitabine is not subject to significant metabolism by CYP enzymes. Generation of a minor (~1%) sulfoxide metabolite (M1 and/or M2) was catalyzed by CYP3A4, and inhibitor studies suggested that at least one other enzyme, possibly flavin-containing monooxygenase, may play a role (m1.4.4, 15396 v1). A minor direct glucuronide metabolite, M3, was also detected (Figure 3) {4570}.

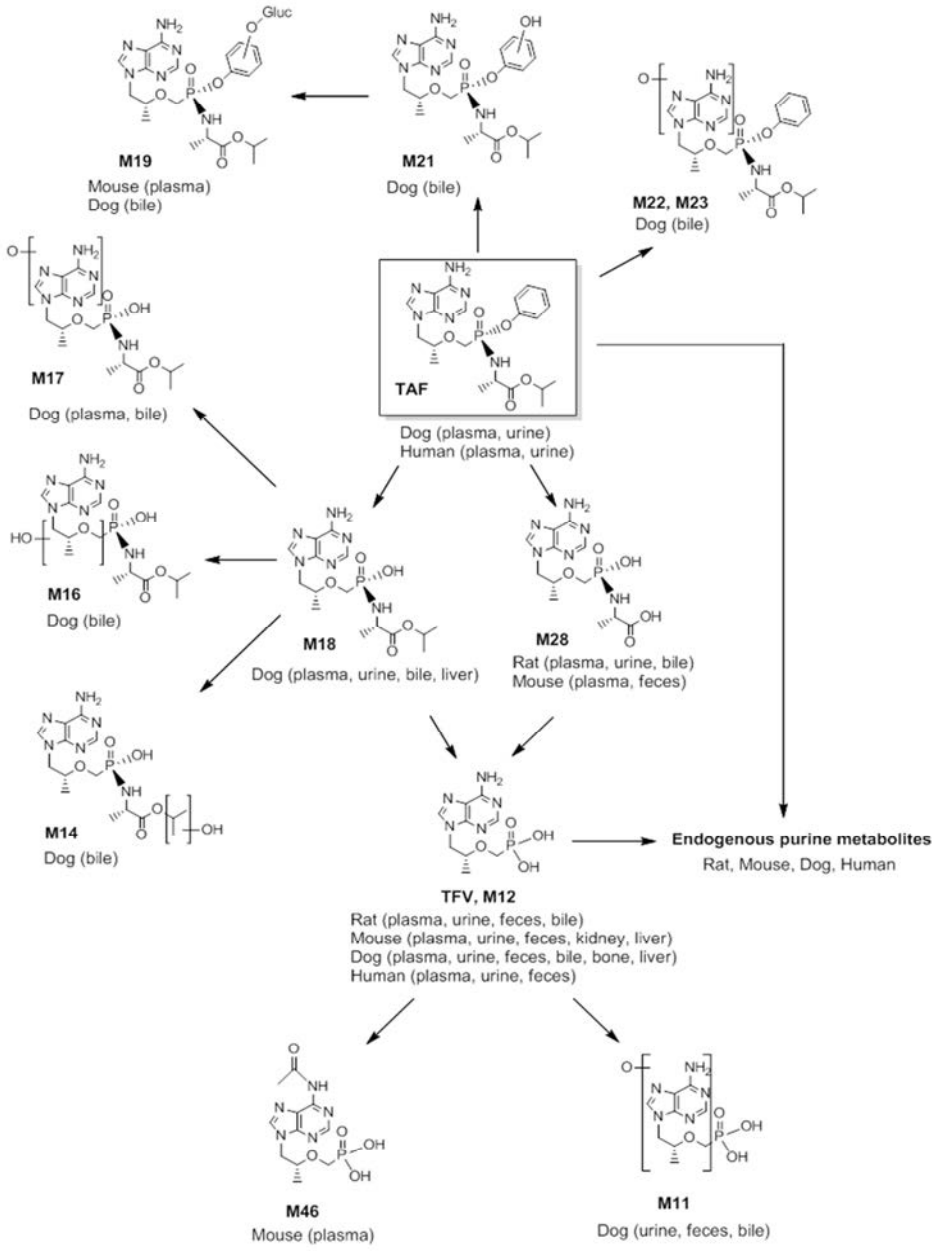
Figure 3. Oxidative Metabolism of FTC



3.5.2.4. 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, TFV-MP and TFV-DP, with TFV-DP being the pharmacologically active form.

Figure 4. Metabolites of TAF



3.5.2.5. E/C/F/TAF

In vitro antiviral studies showed no antagonism of the action of FTC or TFV by EVG or by COBI, suggesting no interference with the intracellular activation of the nucleoside analogs (m2.7.2, Section 4.2). Drug metabolizing enzymes do not contribute significantly to the elimination of FTC or TFV, so no interactions with the disposition of EVG or COBI are anticipated. Cobicistat is a proven inhibitor of the oxidative metabolism of EVG and this is its intended pharmacological effect.

3.5.3. In Vitro Metabolism

3.5.3.1. EVG

Studies with recombinant human CYPs and enzyme-selective inhibitors revealed that CYP3A enzymes (primarily CYP3A4) are responsible for the oxidative metabolism of EVG and yield a metabolite profile similar to that generated by human hepatic microsomal fraction.

3.5.3.2. COBI

The rates of metabolism of COBI and RTV were determined by incubating the compounds with complimentary deoxyribonucleic acid (cDNA) expressed human CYP450 enzyme preparations co-expressed with human NADPH cytochrome P450 reductase (m1.4.4, AD-216-2025; AD-216-2108). Cobicistat was a substrate for CYP2D6 and CYP3A4, but there was no significant metabolism by the other 5 enzymes tested. Ritonavir was also metabolized by CYP2D6 and CYP3A4 and there was also detectable metabolism by CYP2C19. The apparent slow rates of metabolism of COBI and RTV by CYP3A4 are likely due to self-limiting inhibition during the incubation.

3.5.3.3. FTC

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

3.5.3.4. TAF

The potential for CYP enzymes to metabolize TAF was assessed (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.

3.5.4. In Vivo Metabolism

3.5.4.1. EVG

After oral or intravenous administration of [^{14}C]EVG to rats (m1.4.4, JTK303-AD-019) and dogs (m1.4.4, JTK303-AD-020), parent EVG was the most abundant analyte in plasma, with the glucuronide, M4, being the most abundant circulating metabolite. The glucuronides, M4 and M7, were the most abundant analytes in rat bile, but in feces these were apparently subject to

deconjugation as EVG and M1 were the most abundant analytes. However, as described below (Section 3.6), significant enterohepatic recirculation is unlikely. A similar pattern was seen in dog feces with EVG and oxidative metabolites being the most abundant analytes.

3.5.4.2. COBI

After oral administration of [^{14}C]COBI to mice (m1.4.4, AD-216-2073), rats (m1.4.4, AD-216-2082), and dogs (m1.4.4, AD-216-2101), COBI was the most abundant analyte in plasma. Parent COBI, M21, and M31 were the most abundant analytes in feces, with M39 also being significant in dog feces. Profiles in bile from rats and dogs were complex, with many small peaks being detected (each accounting for ≤ 5.3% of the dose).

3.5.4.3. FTC

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

3.5.4.4. TAF

Based on the studies from mouse, rat, dog and human (m2.6.5, Section 10.3, AD-120-2008; m2.6.5, Section 10.1, AD-120-2012; m2.6.5, Section 10.2, AD-120-2021; m2.7.2, Appendix 6.1.3, GS-US-120-0109), endogenous purine metabolites including hypoxanthine, xanthine, allantoin, and uric acid were observed in all species. Tenofovir accounted for a majority of drug related material in plasma, urine, and feces from all species except for human plasma, in which uric acid was the predominant metabolite accounting for 73.9% of the total AUC over 96 hours. M18 was the major metabolite in rat bile accounted for 63% of total radioactivity recovered in bile. M18 and its oxidized metabolite, M16 were the major metabolites in dog bile accounted for 29 and 38% of total radioactivity recovered in bile. Various oxidative metabolites were found in dog bile. No metabolites unique to human were observed.

Tenofovir alafenamide-related metabolites were also monitored in kidney, liver, and nasal turbinate from mice (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}. M18 was detected as a minor metabolite in plasma,

urine, and liver. Relatively high level of M18 was observed in bile. Low levels of M28 were observed in rat and mouse plasma and relatively high levels were in rat bile.

3.5.4.5. E/C/F/TAF

As discussed in the previous section (Section 3.5.2), the available data indicate no significant potential for metabolic interaction among the individual components based on metabolism, apart from the intended inhibition of the metabolism of EVG by COBI. Consequently, no additional studies with the combination product were conducted. Also, due to the lack of mechanism-based inhibition of CYP3A by COBI in nonclinical species, in vivo studies are not appropriate to study metabolic drug interactions.

3.6. Excretion

3.6.1. Recovery in Excreta

3.6.1.1. EVG

After IV or oral administration of [^{14}C]EVG to rats (m1.4.4, JTK303-AD-005) and dogs (m1.4.4, JTK303-AD-006) recovery of radioactivity was high (≥ 97.7% in all groups), with the majority being found in feces (≤ 1.0% in urine after intravenous administration). Recovery was largely complete by 48 hours postdose. After oral administration of [^{14}C]EVG to BDC rats, an average of 25.0% of radioactivity was recovered in bile and 69.2% in feces (m1.4.4, JTK303-AD-005). Intraduodenal administration of collected bile to naive BDC rats led to only 6.0% of radioactivity being recovered in bile and urine (92.5% in feces and intestinal contents), suggesting low potential for enterohepatic recirculation.

3.6.1.2. COBI

After oral administration of [^{14}C]COBI to mice (m1.4.4, AD-216-2073), rats (m1.4.4, AD-216-2034), and dogs (m1.4.4, AD-216-2067), recovery of radioactivity was high (≥ 86.1% in all groups) with the majority being found in feces (≤ 2.06% in urine). Recovery was largely complete by 48 hours postdose. After oral administration of [^{14}C]COBI to BDC animals, an average of 69.3% and 63.9% of dosed radioactivity was recovered in bile in rats (m1.4.4, AD-216-2034) and dogs (m1.4.4, AD-216-2068), respectively.

3.6.1.3. FTC

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

3.6.1.4. TAF and TFV

Following oral dosing of mice, rats, and dogs with [^{14}C]TAF, the majority of radiolabel is recovered in the feces or urine in all species. (m2.6.5, Section 5.1, AD-120-2011; m2.6.5, Section 5.2, AD-120-2020; m2.6.5, Section 13.1, AD-120-2007). The excretion of [^{14}C]TAF was determined after administration of a single 5-mg/kg oral dose of [^{14}C]TAF to bile duct-intact and BDC male Sprague-Dawley rats (m2.6.5, Section 5.2, AD-120-2020). In BDC rats, means of 72.6%, 23.2%, and 2.11% of the administered radioactivity were excreted in feces, urine, and bile, respectively, by 168 hours postdose. Recoveries of radioactivity in bile and urine from BDC rats indicated that at least 25% of the dose was absorbed. The mean overall recovery of radioactivity after oral dosing to BDC rats was 99.9%. The excretion of [^{14}C]TAF was determined after administration of a single 15-mg/kg oral dose of ^{14}C-TAF to bile duct-intact and BDC male dogs (m2.6.5, Section 13.1, AD-120-2007). In BDC dogs, means of 42.7%, 26.5%, and 14.0% of the administered radioactivity were excreted in feces, urine, and bile, respectively, through 168 hours postdose. The elimination of a large amount of radioactivity in bile of BDC dogs indicates that biliary excretion is a major route of elimination of [^{14}C]TAF-derived radioactivity. The overall recovery of radioactivity in BDC dogs was 86.2%.

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

3.6.1.5. E/C/F/TAF

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 multidrug resistance associated protein 4 (MRP4) (m1.4.4, AD-216-2094, AD-216-2105), the transporters responsible for renal excretion of TFV.

3.6.2. Excretion into Breast Milk

Excretion of EVG in rat milk was studied as part of a prenatal and postnatal developmental toxicology study in rats (m1.4.4, TX-183-2006). The concentration of EVG in milk on lactation day (LD) 14 increased in a dose-related manner to the dams; 30 minutes after administration to the dams, the EVG milk to plasma ratio was 0.1, indicating limited, but detectable, distribution from the plasma into milk.

Excretion of COBI in rat milk was studied as part of a prenatal and postnatal developmental toxicology study in rats (m1.4.4, TX-216-2033). Cobicistat was present in milk samples 2 hours post dose on LD 10 with milk to plasma ratios ranging from 1.3 to 1.9.

Excretion into milk has not been evaluated for FTC.

Tenofovir was excreted into the breast milk of lactating rats and rhesus monkeys (m2.6.7, Section 18.3, R990202; m1.4,4, P2000116). The TFV milk to plasma ratios ranged from 0.11 to 0.24 in rats and 0.19 to 0.22 in rhesus monkeys.

3.7. Pharmacokinetic Drug Interactions

To aid in the interpretation of the data presented below and allow a quantitative estimate of the potential drug interaction liability from the IC_{50} values in the section below, the key human pharmacokinetic data from multiple clinical studies with the E/C/F/TAF FDC (m2.7.2, Appendix 6.1.1) are summarized in Table 1.

Table 1. Steady State Pharmacokinetic Parameters for FDC Components

Parameter	EVG	COBI	FTC	TAF, TFV
Dose (mg)	150	150	200	10[d]
C_{max} ($[I]_1$) (μM)	3.8	1.4	7.7	1.6[c]
$C_{max,u}$ (μM)[a]	0.03	0.09		
$[I]_2$ (μM)[b]	1340	770	3236	4176[d]

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

a Steady state C_{max} × in vitro plasma fraction unbound (f_u) (f_u = 0.7% (EVG); 6.3% (COBI); ~ 100% (FTC and TFV)

b Dose / 250 mL

c Value for TFV in plasma

d Value for TAF dose

3.7.1. Metabolic Drug Interactions

3.7.1.1. EVG

Elvitegravir showed no detectable inhibition of activities catalyzed by CYP1A2, 2A6, 2C9, 2C19, 2D6, or 2E1 in human hepatic microsomal fraction (m1.4.4, JTK303-AD-027). Elvitegravir is not a clinically meaningful inhibitor of UGT 1A1, UGT1A3, and UGT2B7 while it was a weak inhibitor of UGT1A1 and UGT1A3 in vitro (m1.4.4, AD-183-2036, AD-183-2037, and AD-183-2038).

3.7.1.2. COBI

The intended pharmacological action of COBI is inhibition of human CYP3A enzymes. This ability was confirmed in vitro using multiple activities catalyzed by human hepatic microsomal fractions and also by clinical studies. Cobicistat is a potent mechanism-based inhibitor of human CYP3A with inactivation kinetics (k_{inact} 0.47 min^{-1}, K_I 1.1 μM), similar to those of RTV (m1.4.4, AD-216-2028). Inhibition of CYP3A is relatively specific as COBI does not inhibit human CYP1A2, CYP2C9, or CYP2C19, is a very weak inhibitor of CYP2C8 (IC_{50} 30.1 μM), a weak inhibitor of CYP2D6 (IC_{50} 9.2 μM), and a modest inhibitor of CYP2B6 (IC_{50} 2.8 μM) (m1.4.4, AD 216-2029; AD-216-2070). This is in contrast to RTV, which is a more potent inhibitor of CYP2D6 (IC_{50} 3.4 μM), CYP2C9 (IC_{50} 3.9 μM), and CYP2C8 (IC_{50} 5.5 μM). This higher specificity for COBI has been confirmed in clinical drug interaction studies in which COBI had

no effect on the pharmacokinetics of EFV (CYP2B6) and little effect on the pharmacokinetics of desipramine (CYP2D6) (m2.7.2, Appendix 6.1.5 [GS-US-216-0112]). Cobicistat is a weak inhibitor of human hepatic microsomal UGT1A1 activity (IC_{50} 16.3 μM), being less potent than RTV (IC_{50} 4.7 μM) and ATV (IC_{50} 0.8 μM) (m1.4.4, AD-216-2075).

3.7.1.3. FTC

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

3.7.1.4. TAF and TFV

The potential for TAF and TFV to inhibit human CYP-mediated drug metabolism was examined in vitro using hepatic microsomal fractions and enzyme-selective activities (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. The TAF-mediated CYP3A inhibition is unlikely to play any role since COBI is a specific and potent inhibitor of CYP3A. Tenofovir at 100 μM did not inhibit CYP1A2, CYP2C9, CYP2D6, CYP2E1, and CYP3A.

The potential for TAF to be a mechanism-based inhibitor of the human CYP enzymes, CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19 and CYP2D6 was assessed at TAF concentration at 50 μM (m2.6.5, Section 11.2, AD-120-2040). There was no evidence for time- or cofactor-dependent inhibition of any enzyme by TAF, with the maximum change in activity of 17.4% with CYP2C8 relative to control.

3.7.1.5. E/C/F/TAF

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

The clinical drug-drug interaction studies are described in detail in the Summary of Clinical Pharmacology Studies (m2.7.2, Section 4.1.3).

3.7.2. Induction Liability

3.7.2.1. EVG

In studies in human hepatocytes EVG showed no significant ability to activate human aryl hydrocarbon receptor (AhR) at concentrations up to 10 µg/mL (22 µM) (< 2-fold increase in CYP1A2 activity), but showed more liability to induce enzymes and transporters controlled by PXR, with an average increase of CYP3A activity of 18.9% and 46.8% of the positive control at 1 and 10 µg/mL (2.2 and 22 µM), respectively (m1.4.4, JTK303-AD-023). In the context of the E/C/F/TAF FDC, any increases in CYP3A activity due to induction would be masked by the potent inhibition by COBI and not clinically significant.

3.7.2.2. COBI

In xenobiotic receptor transactivation studies, COBI showed no ability to activate human AhR and was a very weak activator of human PXR (2.2-fold activation at 10 µM, compared to 10-fold activation by 10 µM RTV; m1.4.4, AD-216-2027). This was confirmed in human hepatocyte studies where COBI, at concentrations up to 30 µM, increased CYP1A2 activity and mRNA and protein by < 2% of the positive control and increased CYP3A4 mRNA expression by an average of 27.4% (m1.4.4, AD-216-2071). CYP3A activity was below that of the vehicle control, due to mechanism-based inhibition by COBI, but a slight increase in immunodetectable CYP3A was detected. Other targets for induction (uridine diphosphate glucuronosyltransferase 1A1 [UGT1A1] mRNA, P-gp mRNA, and CYP2B6 mRNA and protein) were all unaffected or weakly affected by COBI treatment. Ritonavir is a potent inducer in human hepatocytes {18809} and cell lines {18812} and is known to cause clinical drug interactions through induction of multiple phase I and phase II enzymes ({18810}, {11017}, {3355}, {11026}, {11031}, {11950}, {18808}, {18811}).

In contrast to its lack of effect on human PXR, COBI activates rodent PXR and increases the expression of proteins regulated by this receptor, such as rat CYP3A, UGT1A1, and presumably OATP2 (see Section 4.2.2 and m1.4.4, AD-216-2039).

3.7.2.3. FTC

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

3.7.2.4. TAF

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

induction at clinically relevant concentration (1 μM). No significant induction of P-gp and UGT1A1 mRNA was observed (less than 2-fold).

The potential for TAF to induce human drug metabolizing enzymes and drug transporters through the activation of human AhR or human PXR was further evaluated in cell-based systems (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.

3.7.3. Transporter Drug Interactions

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

Table 2. Transporter Substrate Assessment of E/C/F/TAF Components

Transporter	Substrate Assessment (y/n)					Module, Section, Report
	EVG	COBI	FTC	TAF	TFV	
P-gp	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, AD-236-2011 m1.4.4, PC-103-2001
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; MRP1 2, 3, or 4 = multidrug resistance associated protein 1, 2, or 4; n = no; ND = not determined; OAT1 or 3 = organic anion transporter 1 or 3; OATP1B1 or B3 = organic anion transporting polypeptide 1B1 or B3; OCT1 or 2 = Organic cation transporter 1; P-gp = permeability glycoprotein; y = yes

Table 3. Transporter Inhibition Assessment of E/C/F/TAF Components

Transporter	IC_{50} (µM)					Module, Section, Report
	EVG	COBI	FTC	TAF	TFV	
P-gp	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; MRP1 2, 3, or 4 = multidrug resistance associated protein 1, 2, or 4; ND = not determined; OAT1 or 3 = organic anion transporter 1 or 3; OATP1B1 or B3 = organic anion transporting polypeptide 1B1 or B3; OCT1 or 2 = Organic cation transporter 1; OCTN1 = organic cation transporter novel, type 1; P-gp = permeability glycoprotein

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

b Imaoka et al 2007

3.7.3.1. EVG

As described above (Section 3.2.1), transport of EVG can be detected in cells overexpressing human P-pg but there is no evidence for efflux-limitation of absorption in vivo. With digoxin as the substrate, EVG affected human P-gp-dependent transport only at a concentration (30 µM) above its aqueous solubility (m1.4.4, JTK303-AD-026). Elvitegravir was a moderate inhibitor of human organic anion transporting polypeptide 1 (OATP1) (< 40% inhibition at 2 µM), but a

more potent inhibitor of human OATP1B3 (IC_{50} 0.44 μM; m1.4.4, AD-183-2030). Inhibition of OATP transporters is consistent with a clinical drug interaction study in which there was a modest increase in exposure of co-dosed rosuvastatin after dosing with 150 mg EVG and 150 mg COBI (m2.7.2, Appendix 6.1.6 [GS-US-216-0123]).

3.7.3.2. COBI

At systemic concentrations achieved in plasma at the 150 mg COBI dose, COBI would not inhibit the drug transporters P-gp, MRP1, MRP2, breast cancer resistance protein (BCRP), organic anion transporter 1 (OAT1), or OAT3 ($[I]_1/IC_{50} < 0.1$). However, at concentrations achievable briefly in the intestinal lumen during absorption ($[I]_2$ = 770 μM) COBI can inhibit intestinal efflux transporters such as P-gp and BCRP ($[I]_2/IC_{50} > 10$).

With respect to hepatic uptake transporters, COBI is a moderate inhibitor of OATP1B1 and OATP1B3 ($[I]_1/IC_{50}$ 0.4 and 0.8, respectively).

With respect to renal transporters, COBI is a weak inhibitor of MRP4, multidrug and toxin extrusion protein 2-K (MATE2-K), and organic cation transporter 2 (OCT2), and a more potent inhibitor of MATE1 and organic cation transporter novel, type 1 (OCTN1), with similar potencies to RTV. Since OCT2 and MATE1 transporters appear to play a role in the active tubular secretion of creatinine by the kidney ({15340}, {18805}, {18806}), inhibition of these transporters by COBI provides a plausible explanation for the clinical finding of a reduction in renal creatinine clearance without a change in glomerular filtration rate, ie, COBI effects the active secretion of creatinine, but not passive filtration (m2.7.2, Appendix 6.1.5, GS-US-216-0121). This phenomenon has been reported for a variety of other compounds including cimetidine {11773}, trimethoprim {18742}, pyrimethamine {17898}, amiodarone {18743}, ranolazine {18322}, dronedarone {18745}, rilpivirine {17726}, dolutegravir {18741}, the antitubercular agent PA-824 {18739}, the fluoroquinolone DX-619 {18740}, and the thrombin inhibitor AZD0837 {18744}.

3.7.3.3. FTC

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

3.7.3.4. TAF

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

Tenofovir alafenamide is a substrate for intestinal efflux transporters, P-gp and BCRP. An increase in TAF absorption was observed in the presence of efflux transport inhibitors, CsA or COBI in vitro (m2.6.5, Section 3.1, AD-120-2037; 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). Following oral administration of TAF at 2 mg/kg to untreated or

pretreated dogs with 75 mg CsA, the CsA pretreatment increased the plasma exposure to TAF and oral bioavailability by approximately 10-fold, while the PK profile of TFV was not significantly affected by CsA. Consistent with the increased TAF plasma exposure, the exposure to TFV-DP in PBMCs isolated from the CsA pretreated dogs was approximately 2-fold higher than that in cells from untreated animals. These results suggest that coadministration of efflux inhibitors increases TAF absorption and may potentiate the antiviral effect by increasing the TFV-DP levels in PBMCs.

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

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

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

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

3.7.3.5. E/C/F/TAF

Renal excretion of TFV is facilitated by basolateral uptake by OAT1 and OAT3 and apical efflux by the MRP4 efflux transporter. There is no evidence for inhibition of TFV renal excretion by FTC, EVG, or COBI as they show weak or undetectable inhibition of OAT1, OAT3, and MRP4 in vitro. Consistently, transport of TFV by OAT1, OAT3, 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 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 by E/C/F/TAF. 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.

As described in Section 3.2.5, 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 co-dosed with both EVG and COBI (m2.7.2, Appendix 6.1.6 [GS-US-216-0123].

3.8. Summary of Pharmacokinetics

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

Based on the data supporting the individual components, adverse pharmacokinetic interactions that would negatively affect pharmacological efficacy are not anticipated. This assumption is based on the well-characterized routes of absorption and elimination demonstrated for each compound and the differences in physicochemical properties between the compounds which influence drug distribution.

Elvitegravir is largely eliminated by oxidative metabolism by CYP3A (the major route) and by glucuronidation (minor route) by UGT1A1 and 1A3. When administered with a CYP3A inhibitor, such as COBI, oxidative metabolism is blocked and the resulting bioavailability and half-life of EVG are compatible with once-daily dosing.

Elvitegravir is metabolized via a combination of oxidation and glucuronidation in all toxicity species, including humans (m2.7.2, Appendix 6.1.4). All observed metabolites, including several minor metabolites, constitute < 10% relative systemic exposure (AUC_{tau}) to parent drug in humans. The M1 (acyl glucuronide, GS-9200) and M4 (p-hydroxylated, GS-9202) metabolites are markedly less potent than parent drug, and they are not considered to contribute to the antiviral activity of EVG (m2.7.2, Section 4.2). The most abundant metabolites, M4 and M1, were quantified in mouse, rat, and dog repeat-dose toxicity studies; further details are provided in m2.6.6, Section 3.

Cobicistat is a potent mechanism-based inhibitor of human CYP3A enzymes, in contrast to its effect in nonclinical species. The proposed Prescribing Information for the E/C/F/TAF FDC highlights the potential for CYP3A associated drug-drug interactions (see Section 5.2). The

liability for other drug interactions is low as, from in vitro and clinical data, COBI is a relatively selective inhibitor and shows low potential to be an inducer. Although both EVG and COBI are inhibitors of OATP1B1 and OATP1B3 in vitro, when the 2 agents were codosed with the OATP substrate, rosuvastatin, only a modest increase in rosuvastatin exposure was observed, indicating no combinatorial inhibitory effects.

Cobicistat is extensively metabolized in all species examined, including humans. There are no unique or major (> 10%) human metabolites. The metabolites of COBI are weaker inhibitors of CYP3A compared to COBI (m1.4.4, AD-216-2041; AD-216-2107), and due to their low systemic concentrations should not contribute to the primary pharmacodynamic effect of CYP3A inhibition. The most abundant metabolite, GS-9612 (oxidation of isopropylthiazole, M31, E3) was quantified in mouse, rat, and dog repeat-dose toxicity studies; further details are provided in m2.6.6, Section 3.2.

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

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

Tenofovir alafenamide is not an inhibitor or an inducer UGT1A1 or most CYP enzymes known to metabolize xenobiotics; 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 by inducers of the intestinal efflux transporters and inhibitors or genetic polymorphisms of OATPs. Tenofovir alafenamide was not a substrate for renal transporters OAT1 and OAT3.

Based on the clinical data with EVG, COBI, FTC, and TAF in Phase 2 and 3 studies, adverse PK 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 co-dosed 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 PK profile of EVG.

No additional nonclinical pharmacokinetic studies are considered warranted with the E/C/F/TAF FDC in view of the results of extensive nonclinical and clinical pharmacokinetic studies of the individual components and the clinical studies with the FDC.

4. TOXICOLOGY

Comprehensive nonclinical programs with EVG, COBI, FTC, and TAF have been completed. These studies have characterized the single and repeat dose toxicity, mutagenicity, carcinogenicity (TDF studies in place of TAF), and reproductive toxicity of each the individual agents, and the toxicity of EVG/COBI and FTC/TDF combinations. The nonclinical toxicology studies discussed in this section provide an adequate basis to evaluate potential toxicities of the individual components and the 4-drug combination, and for comparing and interpreting results from clinical studies.

4.1. Single Dose Toxicity

Elvitegravir has demonstrated minimal acute toxicity after oral dosing to rats and dogs (lethal dose > 2000 mg/kg and > 1000 mg/kg in rats and dogs, respectively; m1.4.4 JTK303-TX-001, JTK303-TX-002).

The single dose toxicity of COBI was low; the maximum tolerated dose (MTD) was 100 mg/kg in mice (moribund euthanasia occurred at 300 mg/kg), and the NOAEL was 500 mg/kg in rats (m1.4.4, PC-216-2013, TX-216-2003).

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

The single dose NOAEL for a single oral dose TAF as GS-7340-02 in the rat was determined to be > 1000 mg/kg (m2.6.7, Section 5, R990185). The no observed effect level (NOEL) in dogs administered a single dose of TAF was 30 mg/kg (treatment-related clinical signs, renal lesions in the kidneys at 90 and 270 mg/kg (m2.6.7, Section 5, D990181).

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

4.2. Repeat Dose Toxicity

4.2.1. EVG

A series of GLP oral repeat-dose toxicity studies were conducted with EVG in mice (m1.4.4, TX-183-2004]; rats (4 weeks [m1.4.4, JTK303-TX-003], 13 weeks [m1.4.4, JTK303-TX-021], and 26 weeks [m1.4.4, JTK303-TX-022]), and dogs (4 weeks [m1.4.4, JTK303-TX-004], and 39 weeks [m1.4.4, JTK303-TX-023]). In addition, two 13-week combination toxicity studies were conducted with EVG in combination with COBI (m1.4.4, TX-236-2001) or in combination with RTV (m1.4.4, TX-183-2007).

There were no significant adverse effects in mice treated with EVG for 13 weeks at doses up to 2000 mg/kg/day administered by oral gavage (m1.4.4, TX-183-2004). Two nonadverse findings, not considered clinically relevant, were observed in rats and dogs.

In the 4-, 13-, and 26-week repeat oral dose studies in rats, medium-to-large lipid-like vacuoles were observed (at doses ≥ 100 mg/kg/day) in the lamina propria, mainly in the upper small intestine (duodenum and/or jejunum), but there were no toxic or reactive changes associated with these vacuoles (m1.4.4, JTK303-TX-003, JTK303-TX-021, and JTK303-TX-022, respectively). The severity of this finding did not increase with duration of dosing. A similar finding was noted in the 39-week dog study (m1.4.4, JTK303-TX-023), but was not seen in the 4-week dog study (m1.4.4, JTK303-TX-004). In a series of mechanistic studies, the vacuoles were confirmed to be lipid vacuoles containing mainly triglycerides, and the findings were shown to be slowly reversible. Lipid vacuoles were decreased when food was not present, and the incidence and severity of vacuoles was shown to be related to the local concentration of EVG.

Observations of increased cecal weight and dilatation with whitish loose contents were noted in the repeat-dose rat studies at doses ≥ 300 mg/kg/day. In the juvenile toxicity portion of the perinatal/postnatal study in rats, increased cecum weights were observed following doses of 2000 mg/kg/day in males and doses of 1000 and 2000 mg/kg/day in females (m1.4.4, TX-183-2006). These changes were not accompanied by histopathologic changes or adverse clinical observations. Similar changes in the cecum have been reported with antibacterial quinolones {18406}. Elvitegravir has a quinolone moiety in its structure and was confirmed to have some antibacterial activity in a bacterial reverse mutation test. Although the antibacterial activity was much lower than that of the antibacterial quinolones, these changes in the cecum were considered to be due to the antibacterial activity of EVG.

There were no significant adverse effects observed in a 13-week combination toxicity study conducted in rats with EVG alone, COBI alone, or the combination of EVG and COBI (m1.4.4, TX-236-2001). The NOAELs were 1000 mg/kg/day EVG and 30 mg/kg/day COBI, either alone or in combination. Similarly, there were no significant adverse effects observed in a 13-week combination toxicity study conducted in rats with EVG alone, RTV alone, or the combination of EVG and RTV (m1.4.4, TX-183-2007). The NOAELs were 1000 mg/kg/day EVG and 10 mg/kg/day RTV, either alone or in combination.

The NOAELs for EVG are considered to be 2000 mg/kg/day for mice and rats, and 100 mg/kg/day for dogs – the highest doses evaluated in the 13-week, 26-week, and 39-week repeat-dose studies in mice, rats, and dogs, respectively. The exposures based on plasma AUC values at the NOAEL doses in the animals were approximately 2- to 3-fold (mice), 20- to 36-fold (rats), and 2- to 3-fold (dogs) higher than the AUC in patients treated once daily with EVG at 150 mg in the EVG/COBI/FTC/TDF FDC.

4.2.2. COBI

A series of GLP oral repeat-dose toxicity studies were conducted with COBI in mice (2 weeks [m1.4.4, TX-216-2032], 4 weeks [m1.4.4, TX-216-2041], and 13 weeks [m1.4.4, TX-216-2026]; rats (4 weeks [m1.4.4, TX-216-2004], and 26 weeks [m1.4.4, TX-216-2017]), and dogs (4 weeks [m1.4.4, TX-216-2005], and 39 weeks [m1.4.4,TX-216-2016]). In addition, two 13-week combination toxicity studies were conducted with COBI in combination with EVG (m1.4.4, TX-236-2001) or in combination with ATV (m1.4.4, TX-216-2024).

In repeat-dose studies (up to 13 weeks in mice, up to 26 weeks in rats; up to 39 weeks in dogs), target organs identified were liver (mouse, rat, and dog) and thyroid (rat). Slight hematological changes were noted in rats; clinical chemistry changes were observed in mice, rats, and dogs; and urinalysis/urine chemistry changes were noted in rats and dogs.

In the 13-week mouse study, mild-to-marked elevations in alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were noted in males at 15 and 50 mg/kg/day (m1.4.4, TX-216-2026). These changes were associated with microsomal enzyme induction, increases in liver weight and minimal hepatocellular hypertrophy at 50 mg/kg/day. Female mice were notably less sensitive; a marked elevation in ALT and AST was noted in only one high-dose female (50 mg/kg/day). The NOAELs are considered to be 5 mg/kg/day in males and 50 mg/kg/day in females. In a 4-week non-pivotal toxicity study using wild type mice, that was conducted to assess the feasibility of the transgenic CB6F1-non Tg(HRAS) strain for a possible 6-month transgenic carcinogenicity study, the NOAEL is considered to be 100 mg/kg/day (m1.4.4, TX-216-2041). Mild increases (2 to 3-fold) in ALT and AST correlated with increased liver weights at 100 mg/kg/day in both sexes, and with minimal to slight hepatocellular hypertrophy in males at 100 mg/kg/day.

In the 4-week and 26-week oral dose rat studies (m1.4.4, TX-216-2004, TX-216-2017), increases in liver and thyroid weights were associated with CYP3A enzyme induction, hepatocellular hypertrophy, thyroid hormone changes (decreased thyroxine [T4]; increased thyroid stimulating hormone [TSH]) and thyroid follicular cell hyperplasia/hypertrophy (see Section 4.9.1.2.2 for additional details). These findings were reversible, and were not considered adverse. However, one high-dose male animal had a follicular cell carcinoma in the thyroid in the 26-week study. The liver and thyroid effects are considered adaptive changes, are commonly seen in rodents with microsomal enzyme inducers, and are considered secondary to microsomal enzyme induction and thyroid hormone imbalance (decreases in T4 and increases in TSH), respectively {11923}, {11925}, {11927}, {11931}, {11926}, {11933}, {17078}, {18407}. Hematological and clinical chemistry changes were not considered adverse. Hematological changes (not exceeding 10% versus controls) included slightly lower mean values for erythrocyte count, hemoglobin, hematocrit, mean corpuscular volume, and mean corpuscular hemoglobin, and slightly higher mean platelet counts. Serum chemistry changes observed after 13 and/or 26 weeks of dosing included slightly higher mean gamma glutamyltransferase (GGT), cholesterol, total protein, albumin, globulin, and calcium. After a 13-week recovery period, cholesterol and total protein values remained slightly higher in high-dose females, whereas other values were generally comparable to control values, indicating reversibility (TX-216-2017). The NOAEL for COBI in the 26-week rat study is considered to be 30 mg/kg/day, based on significant decreases in body weight and food consumption, slight changes in hematological parameters, and increases in urine volume at 100 mg/kg/day.

In dogs, apart from salivation and vomiting associated with dosing, treatment with COBI was well tolerated at doses up to 15 mg/kg/day in the 4-week study (m1.4.4, TX-216-2005), and up to 10 mg/kg/day in the 39-week study (m1.4.4, TX-216-2016). Changes in the thymus and adrenal gland in dogs observed in high-dose animals after 13 weeks of dosing were absent after 39 weeks of dosing, and were considered stress-related, and not a direct effect of COBI. In dogs administered 20 mg/kg/day for 39 weeks, clinical signs (salivation, emesis, fecal changes),

decreases in body weight and food consumption, nonadverse changes in clinical pathology parameters, and minimal, adaptive changes in the liver (increased weights, hypertrophy) were noted. After 39 weeks of dosing at 10 mg/kg/day, effects were limited to minimal hepatocellular hypertrophy in males, and slightly increased liver weights in females. Clinical pathology changes in the 28-day study included minimal-to-mild increases in bilirubin, ALT, and alkaline phosphatase activities. In the 39-week study, slightly higher platelets counts, slightly higher alkaline phosphatase, and slightly lower total protein and albumin were observed; these changes were reversible following cessation of dosing. Based on these findings, the NOAEL for COBI when administered daily by oral gavage to dogs for up to 39 weeks is 10 mg/kg/day.

Urinalysis and urine chemistry changes, noted primarily in high-dose rats at 100 mg/kg/day and in female dogs at 20 mg/kg/day, included slightly higher electrolyte excretion and slightly lower electrolyte concentrations, consistent with findings of lower urine osmolality, higher urine volume and/or pH. These changes showed no progression after long term dosing, were reversible, were not associated with remarkable clinical chemistry changes, including serum creatinine and blood urea nitrogen (BUN), and were without histopathological correlates in the kidney. In dogs, a greater incidence of bilirubinuria was noted in males at 20 mg/kg/day during the 39-week study; no changes were observed at recovery.

There were no significant adverse effects and no evidence of additive or synergistic effects in 90-day rat toxicity studies conducted with COBI in combination with EVG (m1.4.4, TX-236-2001) or with ATV (m1.4.4, TX-216-2024). The NOAELs were 30 mg/kg/day COBI and 1000 mg/kg/day EVG, either alone or in combination, and 30 mg/kg/day COBI and 50 mg/kg/day ATV, either alone or in combination.

The exposures based on plasma AUC values at the NOAEL doses in the longest duration studies were approximately 0.1- to 7.2-fold (mice), 1.2- to 1.6-fold (rats), and 2.1 to 2.4-fold (dogs) higher than the AUC in patients treated once daily with COBI at 150 mg in the EVG/COBI/FTC/TDF FDC.

4.2.3. EVG/COBI

Two GLP oral dose toxicology studies in rats have been conducted with the combination of EVG and COBI. As briefly noted above, a 13-week oral combination toxicity study was conducted in with EVG and COBI (m1.4.4, TX-236-2001), and a 4-week impurity qualification study was conducted with the EVG/COBI layer of the EVG/COBI/FTC/TDF tablet (m1.4.4, TX-236-2002).

The potential for additive or unexpected toxicities with COBI and EVG administered in combination was evaluated in the 13-week combination toxicity study (TX-236-2001). Male and female rats were administered COBI at 0 (vehicle I) and 30 mg/kg/day, EVG at 0 (vehicle II), and 1000 mg/kg/day, and EVG and COBI in combination at 0 (vehicle I and vehicle II), 100/30 and 1000/30 mg/kg/day EVG/COBI. No adverse effects were observed with EVG or COBI alone, or with the EVG/COBI combination. Clinical chemistry changes were of small magnitude, similar to those observed in previous rat studies with COBI, and were not considered adverse. There were no treatment-related macroscopic or microscopic findings. Increased liver weights in animals administered COBI alone, and in combination with EVG at both dose levels were

considered adaptive changes secondary to microsomal enzyme induction by COBI (up to 2.2-fold increases in CYP3A activity). Coadministration of EVG with COBI generally resulted in similar COBI exposures, and EVG exposures were similar (in females) or slightly increased (in males) compared to when EVG was administered alone. After multiple dosing in combination with COBI, exposures to EVG generally decreased compared to Day 1 values likely due to CYP3A induction by COBI. The NOAEL for males and females rats is considered to be 1000 mg/kg/day EVG and 30 mg/kg/day COBI, either alone or in combination.

In the 4-week impurity qualification study with nondegraded and degraded crushed tablets of the EVG/COBI layer of the bilayer EVG/COBI/FTC/TDF tablet (m1.4.4, TX-236-2002) in female rats, the NOAEL was the highest dose tested, 50/50 mg/kg/day EVG/COBI for both nondegraded and degraded tablets.

4.2.4. FTC

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

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

4.2.5. TAF

The repeat-dose oral toxicity of TAF has been studied in mice, (2 weeks [m2.6.7, Section 6, TX-120-2006] and 13 weeks [m2.6.7, Section 7.1, TX-120-2007]), rats (6-7 days [m2.6.7, Section 17, R2000044; m2.6.7, Section 6, R000139], 4 weeks [m2.6.7, Section 7.2, R990182] and 26 weeks [m2.6.7, Section 7.3, TOX-120-001]), dogs (4-weeks [m2.6.7, Section 7.4, D990175] and 39 weeks [m2.6.7, Section 7.5, TOX-120-002]) and monkeys (4 weeks [m2.6.7, Section 7.6, P2000114]). In chronic studies, kidneys (karyomegaly, tubular degeneration), and bone (atrophy of metaphyseal cancellous bone) were the primary target organs. TAF also appeared to increase biochemical markers of bone turnover and decrease serum 1, 25-dihydroxy- and 25-hydroxyvitamin D_3 at doses in rats and dogs.

4.2.5.1. Kidney

Renal tubular karyomegaly was observed in rats and dogs orally administered TAF. Focal areas of minimal renal cortical tubular basophilia and associated minimal nuclear karyomegaly were present in rats administered 400 mg/kg/day for 4 weeks and 100 mg/kg/day for 26 weeks. Renal

tubular karyomegaly and/or basophilia were observed in dogs administered 3 and 10 mg/kg/day for 4 weeks and dogs administered 6 or 18/12 mg/kg/day for at least 13 weeks.

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

4.2.5.2. Bone

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

4.2.5.3. Other

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

TAF had no discernible electrocardiographic effect at the low dose of 2 mg/kg/day. There was some evidence at 6 and 18/12 mg/kg/day for an effect to slightly prolong PR intervals (~13-24%) which was associated with significant decreases in T3 {29101}, {29104}. After the 13-week recovery period, serum T3 values returned to levels similar to the control group animals at the end of the study. No PR prolongation or any change in ECG results occurred in the safety pharmacology study that evaluated a TAF dose up to 100 mg/kg (m2.6.3, Section 4.2, D2000006) or in the thorough QT study (m2.7.2, Appendix 6.1.3 [GS-US-120-0107]).

At 18/12 mg/kg/day in dogs, the highest dose tested, a minimal infiltration of histiocytes was present in some organs (eye [choroid plexus, ciliary body], lung, and spleen) in some animals. In-life ophthalmologic examinations were normal. The histiocytic infiltration observed in multiple organs was most likely an indirect drug effect due to general debilitation and was not observed in other repeat dose toxicity studies. There were no drug-related effects on ophthalmic exams or microscopic exams of ocular tissue observed in repeat-dose toxicity studies in mice (up to 13 weeks), rats (up to 26 weeks), nonhuman primates (4 weeks) or in the 4-week dog toxicology study. The minimal to slight infiltration of mononuclear cells in the ocular posterior

uvea occurred only in dogs administered the highest dose of TAF where compound-related toxicities occurred on clinical condition, weight gain, serum chemistry, bone mineral density, and other organ histopathology. Distribution of [^{14}C]TAF to eyes has been assessed in mice, rats, and dogs (AD-120-2011, AD-120-2020, and D990173-BP). Melanin binding has specifically been assessed by comparing distribution in pigmented and non-pigmented mice (C57 black and CD-1, respectively) and rats (Long Evans and Sprague-Dawley, respectively). [^{14}C]TAF-related radioactivity distributed poorly to the eyes of rats and dogs (C_{max} in eyes < 8% that observed in plasma). Transient exposure to low levels of [^{14}C]TAF-related radioactivity was observed in the eyes of rats decreasing to undetectable levels at 8 hours postdose. No difference in distribution was observed between Sprague-Dawley and Long Evans rats, including in the skin and eyes, suggesting no binding to melanin. The distribution of [^{14}C]TAF to eyes in mice was higher than other species studied (C_{max} in eyes 15%-20% that observed in plasma). More persistent exposures in eye lens, eye uveal tract, and eyes were observed in C57 black mice compared to CD-1 mice. However, no difference in distribution between pigmented and nonpigmented skin was observed illustrating that [^{14}C]TAF-related radioactivity was not selectively associated with melanin-containing tissues. The posterior uveitis in dogs administered the highest dose of TAF occurred at 3.7- and 17-fold higher exposure to TAF and TFV, respectively, than that observed in human subjects administered a 25-mg dose of TAF and does not correlate with the tissue distribution where TAF was found to poorly penetrate across the blood brain and blood retinal barrier in dogs. Because TAF has poor penetration across the blood brain and blood retinal barrier in dogs, it is unlikely that TAF directly caused the observed histiocytic infiltration in the posterior uvea. Based on the evidence from tissue distribution and toxicology studies, Gilead concludes that the risk of posterior uveitis in humans is very low.

TAF is unlikely to cause mitochondrial toxicity. TAF did not affect the amount of mitochondrial DNA levels up to 1 μM (approximately 2-fold higher than C_{max} after a 25 mg TAF dose), the highest concentration tested, in HepG2 cells in a 10-day assay (m2.6.3, Section 1.2, PC-120-2006). The active metabolite of TAF, tenofovir diphosphate, is highly discriminated as a substrate by mitochondrial DNA polymerase γ relative to the natural substrate, adenosine triphosphate (ATP) (> 10,000 fold) {4923}. Therefore, TAF is unlikely to inhibit mitochondrial DNA polymerase γ under clinical relevant conditions.

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

4.2.6. FTC/TDF

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

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

4.2.7. E/C/F/TAF

The 4 drugs, EVG, COBI, FTC, and TAF, exhibit different patterns of target organ toxicity. No adverse, target organ toxicity was observed with EVG. Target organs identified for COBI were liver (mouse, rat and dog) and thyroid (rat). Liver effects were qualitatively similar across species, and considered adaptive, non-adverse changes {17078}, {18407}. Similarly the thyroid changes are considered adaptive changes, secondary to hepatic microsomal enzyme induction and thyroid hormone imbalance. These thyroid changes are considered rodent specific, and predispose rats, but not humans, to thyroid neoplasms, and it is unlikely that COBI presents a risk to the human thyroid {11923}, {11925}, {11927}, {11931}, {11926}, {11933}. The only significant effect of FTC identified at dose levels constituting large clinical multiples was a minor anemia. The principal target organs of toxicity following oral administration of TAF were the kidney (karyomegaly, tubular degeneration) and bone.

Cobicistat was associated with urinalysis and urine chemistry changes (increased urine volume; decreased urine specific gravity; increased electrolyte excretions) at high doses in rats and dogs. These changes were reversible, were not associated with remarkable clinical chemistry changes, including serum creatinine and BUN, and were without morphological evidence of kidney damage. COBI is a weak inhibitor of human renal transporters OCT2, MRP4, and MATE2-K, and is a more potent inhibitor of OCTN1 and MATE1, with similar potencies being found for RTV (Section 3.7.3). These data, along with clinical data (m2.7.4), suggest that COBI reversibly blocks secretion of creatinine in humans most likely via MATE1 inhibition. Because there is no apparent pathological change in the kidney due to COBI and that the routes of excretion differ for TFV and COBI, it is not anticipated that the combination of E/C/F/TAF would exacerbate the renal toxicity of TAF. The other potential drug interactions among the 4 components include inhibition of intestinal efflux of TAF by COBI and 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. In a Phase 1 clinical study, no statistically significant difference in FTC and TAF exposures were observed following multiple-dose administration of E/C/F/TAF and FTC/TAF 10 mg (GS-US-292-0103). Cobicistat does not inhibit OAT1 or MRP4, the transporters responsible for the renal excretion of TFV and so will not interfere with the elimination of TFV

Administration of EVG and COBI in combination is unlikely to exacerbate known toxicities of the individual agents. This was confirmed by the absence of any new or more marked toxicities in a 13-week rat combination toxicology study with EVG and COBI (m1.4.4, TX-236-2001). Based upon the differences in routes of elimination, EVG and COBI are unlikely to affect the pharmacokinetic profiles of FTC or TFV adversely.

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

The only toxicity observed in chronic animal studies with FTC was mild, reversible anemia in mice and minor decreases in erythrocyte counts/increases in mean corpuscular hemoglobin in monkeys at large multiples of clinical exposure (110-fold in mice; 21-fold in monkeys). These hematological findings are not considered relevant to clinical use, and should not cause an overlapping toxicity with COBI which produced minimal, reversible decreases (< 10%) in RBC parameters in rats at 5- to 8-fold multiples of clinical exposure. Cobicistat, EVG, and FTC have not shown any potential for bone toxicity in chronic rat and dog toxicity studies; thus, exacerbation of any TAF effects on bone is not expected.

The ample nonclinical safety database on these drugs, including combination toxicity studies with EVG and COBI, and with FTC and TDF, indicates further toxicological investigations are unlikely to yield new data relevant to humans.

4.3. Genotoxicity

4.3.1. EVG

Elvitegravir was not genotoxic in the reverse mutation bacterial test (Ames test) (m1.4.4, JTK303-TX-005). In a chromosome aberration test, EVG showed a weak or equivocal potential to induce chromosomal aberrations with a 6-hour treatment without S9 in Chinese Hamster Lung (CHL) cells, but did not show any evidence of genotoxic activity after 24-hour treatment without S9 or in the presence of S9 (m1.4.4, JTK303-TX-006). In 2 micronucleus assays in rats, EVG showed no genotoxic activity at dose levels up to 1000 mg/kg/day (conducted as part of a non-GLP 2-week oral gavage study [m1.4.4, JTK303-TX-012], or after a single oral dose of 2000 mg/kg [m1.4.4, JTK303-TX-007]).

4.3.2. COBI

Cobicistat was not genotoxic in the reverse mutation bacterial test (Ames test) (m1.4.4, TX-216-2010), mouse lymphoma (m1.4.4, TX-216-2011), or rat micronucleus assays (m1.4.4, TX-216-2012).

4.3.3. FTC

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

4.3.4. TAF

Tenofovir alafenamide was not genotoxic in a battery of in vitro and in vivo assays. The in vitro assays included gene mutation assays with bacterial strains (*Salmonella typhimurium*, *Escherichia coli;* m2.6.7, Section 8.1, V990212), and a L5178Y gene mutation assay in mouse lymphoma cells (m2.6.7, Section 8.2, V990213). The in vivo evaluation consisted of a mouse bone marrow micronucleus study at oral doses of 500, 1000, and 2000 mg/kg (m2.6.7, Section 9, M2000113).

4.3.5. FTC/TDF

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

4.3.6. E/C/F/TAF

Cobicistat, FTC, and TAF were negative in genotoxicity studies. Elvitegravir showed an equivocal effect in an in vitro chromosome aberration study, was negative in the reverse mutation assay and in 2 in vivo micronucleus studies, and is thus unlikely to have genotoxic potential in vivo. The combination of the 4 components is therefore not expected to have an altered genotoxicity profile as compared with that of the individual agents.

4.4. Carcinogenicity

Per separate agreements with the FDA (Reference ID: 3161161) and with the EMA (EMA/CHMP/SAWP/629722/2012, FAL 2410-1-2012), carcinogenicity studies are not required for TAF registration due to the lack of TAF exposure in rats and TgRasH2 mice and lower TFV exposure in rats and mice compared to TDF. Summaries of the carcinogenicity studies with TDF are provided below.

4.4.1. EVG

In long-term carcinogenicity studies of EVG, no drug-related increases in tumor incidence were found in mice at doses up to 2000 mg/kg/day (2.4- to 3.8-fold the human systemic exposure at the therapeutic dose of 150 mg/day; m1.4.4, TX-183-2011) or in rats at doses up to 2000 mg/day/day (12- to 27-fold the human systemic exposure at the therapeutic dose; m1.4.4, TX-183-2012). In the mouse study, high-dose EVG (2000 mg/kg/day) was also dosed in combination with RTV (25 mg/kg/day) as it was observed previously that the addition of RTV, a CYP3A inhibitor substantially increased the exposure of EVG in mice. No drug-related increases in tumor incidence were noted in these animals at exposures approximately 14-fold the human systemic exposure at the therapeutic EVG dose.

4.4.2. COBI

In a long-term carcinogenicity study with cobicistat in mice (m1.4.4, TX-216-2030), no drug-related increases in tumor incidence were observed at doses up to 50 and 100 mg/kg/day (males and females, respectively). Cobicistat exposures at these doses were approximately 7 (male) and 16 (females) times, respectively, the human systemic exposure at the therapeutic daily dose. In a long-term carcinogenicity study of cobicistat in rats (m1.4.4, TX-216-2031), an increased incidence of follicular cell adenomas and/or carcinomas in the thyroid gland was observed at doses of 25 and 50 mg/kg/day in males, and at 30 mg/kg/day in females. The follicular cell findings are considered to be rat-specific, secondary to hepatic microsomal enzyme induction and thyroid hormone imbalance, and are not relevant for humans. At the highest doses tested in the rat carcinogenicity study, systemic exposures were approximately 2 times the human systemic exposure at the therapeutic daily dose.

4.4.3. FTC

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

4.4.4. TAF

Per separate agreements with the FDA (Reference ID: 3161161) and with the EMA (EMA/CHMP/SAWP/629722/2012, FAL 2410-1-2012), carcinogenicity studies are not required for TAF registration due to the lack of TAF exposure in rats and TgRasH2 mice and lower TFV exposure in rats and mice administered TAF than after administration of TDF.

Long-term oral carcinogenicity studies of TDF in mice and rats were carried out at exposures up to approximately 10 times (mice) and 4 times (rats) those observed in humans at the 300 mg therapeutic dose in the EVG/COBI/FTC/TDF FDC (m2.6.7, Section 18.1, M990205; m2.6.7, Section 18.2, R990204). Female mice showed a low incidence of liver adenomas at the highest dose of 600 mg/kg/day. Rats did not show any carcinogenic potential in the long-term study.

4.4.5. E/C/F/TAF

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

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

4.5. Reproductive Toxicity

4.5.1. EVG

There were no significant adverse effects observed in fertility studies in female and male rats (m1.4.4, JTK303-TX-019, TX-183-2003), in embryo-fetal development studies in rats and rabbits (m1.4.4, JTK303-TX-020, TX-183-2008,TX-183-2002), or in a prenatal/postnatal study in rats (m1.4.4, TX-183-2006). The NOEL for reproductive parameters in the fertility studies was 2000 mg/kg/day at exposures approximately 16- to 30-fold higher than human therapeutic exposures. The developmental NOAEL/NOEL for EVG was 2000 mg/kg/day for rats and 450 mg/kg/day for rabbits, the highest doses tested, at exposures approximately 23- and 0.2-fold higher, respectively, than human therapeutic exposure. In the pre/postnatal study, the maternal NOEL for general toxicity and the NOEL for reproduction in the dams and viability and growth of the offspring were 2000 mg/kg/day (exposures on lactation Day 14 were approximately 18-fold higher than human therapeutic exposures). Elvitegravir was secreted in the milk of nursing rats in the pre/postnatal study, and at the NOEL of 2000 mg/kg/day, the EVG milk:plasma ratio was 0.1. In a combination embryo-fetal development study with EVG and RTV, the NOELs were 10 mg/kg/day RTV and 1000 mg/kg/day EVG when administered separately or in combination (m1.4.4, TX-183-2008).

4.5.2. COBI

No adverse effects were observed in a rat fertility study; the NOEL for reproductive parameters was 100 mg/kg/day at exposures approximately 4-fold higher than human therapeutic exposures (m1.4.4, TX-216-2023). No teratogenic effects were observed in rat and rabbit developmental toxicity studies (m1.4.4, TX-216-2020, TX-216-2021). In rats at 125 mg/kg/day, increases in postimplantation loss and decreased fetal weights were associated with significant maternal toxicity (adverse clinical signs, decreased body weight and food consumption). The NOEL/NOAELs in the rat and rabbit studies were 50 and 100 mg/kg/day, respectively, where exposures were approximately 1.8- and 4.3-fold higher, respectively, than human therapeutic exposures. In the pre/postnatal study (m1.4.4, TX-216-2033), the maternal NOAEL for general toxicity was 30 mg/kg/day, and the NOAEL for reproduction in the dams and viability and growth of the offspring was 75 mg/kg/day, the highest dose tested (exposures on lactation Day 10 were 1.2-fold higher than human therapeutic exposures). Cobicistat was secreted in the milk of nursing rats in the pre/postnatal study, with COBI milk:plasma ratios of 1.3 to 1.9.

4.5.3. FTC

Emtricitabine did not affect fertility in male rats at approximately 140-fold or in male and female mice at approximately 60-fold higher exposures than in humans administered the recommended 200 mg daily dose (m1.4.4, TTEP/95/0028, TOX036). There were no adverse effects in embryo-fetal development studies in mice at exposures approximately 60-fold higher and in rabbits at exposures approximately 120-fold higher than human exposures (m1.4.4, TOX037, TOX038). In the pre/postnatal study in mice, F_1 dams at 1000 mg/kg/day had slightly longer estrous cycles than controls, but fertility was normal in the offspring exposed daily from before birth (in utero) through sexual maturity at daily exposures of approximately 60-fold higher than human exposures at the recommended 200 mg daily dose (m1.4.4, TOX039).

4.5.4. TAF

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

The TAF fumarate (GS-7340-03) oral rat fertility study (m2.6.7, Section 12, TX-120-2012) data indicate dose related decreases in body weight gain in males and females, but no drug related changes occurred in male or female fertility endpoints including mating index, fertility index, sperm motility, sperm concentration, number of corpora lutea, implantation sites, early and late resorptions and live fetuses at doses up to 160 mg free base equivalents/kg/day. The TAF NOAEL for reproductive and early embryonic toxicity was 160 mg/kg/day.

There was no effect on fetal viability or fetal development in pregnant rats administered doses of GS-7340-02 up to 250 mg/kg/day (m2.6.7, Section 13.1, TX-120-2002), or in pregnant rabbits administered GS-7340-02 up to 100 mg/kg/day (m2.6.7, Section 13.2, TX-120-2005). The highest doses were maternally toxic. In the rat, decreased fetal body weight associated with some minor transitory delays in the rate of ossification was observed at 250 mg/kg/day, a maternally toxic dose. At the NOAEL for embryo-fetal development of approximately 100 mg/kg/day in rats, AUC_{tau}values for TAF and TFV on Day 17 were 0.2 and 17.4 µg·h/kg, respectively. At the NOAEL for embryo-fetal development of 100 mg/kg/day in rabbits, AUC_{tau} values for TAF and TFV on Day 20 were 11.0 and 27.3 µg·h/kg, respectively.

Tenofovir DF, but not TAF, has been tested in a perinatal/postnatal study. Per separate agreements with the FDA (Reference ID: 3231054) and with the EMA (EMA/CHMP/SAWP/214541/2013, FAL_2410-1-FU-1-2013) a perinatal/postnatal study in rats is not required for TAF registration due to the lack of TAF exposure in rats and lower TFV exposure after TAF administration compared to TDF administration. There was an alteration of the estrous cycle in female rats in the perinatal study in rats (m2.6.7, Section 18.3, R990202). The NOEL for behavioral, reproductive, and development toxicity was 150 mg/kg/day. Maternally toxic doses (≥ 450 mg/kg/day) had effects on pup survival, pup body weights, and sexual maturation.

4.5.5. E/C/F/TAF

4.5.5.1. Fertility and Early Embryonic Development

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

4.5.5.2. Embryo-Fetal Development

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

4.5.5.3. Pre- and Postnatal Development

Per separate agreements with the FDA (Reference ID: 3231054) and with the EMEA (EMA/CHMP/SAWP/214541/2013, FAL_2410-1-FU-1-2013) a perinatal/postnatal study in rats is not required for TAF registration due to the lack of TAF exposure in rats and lower TFV exposure compared to TDF. The perinatal/postnatal study conducted with TDF is provided below.

Slightly longer estrous cycles were observed in first (F_1) generation rats after exposure to high doses of FTC and a delay in sexual maturation was observed in F_1 generation rats after exposure to high (maternally toxic) doses of TDF. No significant effects were noted for EVG, or COBI. For all 4 individual agents, NOELs/NOAELs were at exposures above human exposures. As with other reproductive toxicity tests, a repeat of this test with the E/C/F/TAF FDC is unlikely to add any new information.

4.6. Juvenile Toxicity

4.6.1. EVG

In the juvenile toxicity evaluation portion of the pre/postnatal study in rats (m1.4.4, TX-183-2006), daily oral gavage administration of EVG to F_1 generation pups from postnatal day (PND) 22 to 49 was well tolerated at doses up to 2000 mg/kg/day. The only test article-related observation was increased cecum weights at 1000 and 2000 mg/kg/day, consistent with findings in other rat studies. The NOAEL for toxicity of EVG is 2000 mg/kg/day for juvenile rats where exposures were 7-fold higher than therapeutic human exposures at the 150 mg dose.

4.6.2. COBI

In the juvenile toxicity phase of the pre/postnatal study in rats (m1.4.4, TX-216-2033), daily oral gavage administration of COBI to F_1 generation pups from PND 22 to 49 was well tolerated at doses up to 75 mg/kg/day, with adaptive liver and thyroid changes observed at similar dose levels and exposures to adult animals. The NOAEL for toxicity of COBI is 75 mg/kg/day for

juvenile rats where exposures were 2.5-fold higher than therapeutic human exposures at the 150 mg dose.

4.6.3. FTC

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

4.6.4. TAF/TFV

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

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

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

4.6.5. E/C/F/TAF

There were no notable findings in the juvenile toxicity studies with EVG or with COBI. Although TDF and COBI have produced effects in reproductive toxicity studies, effects on rat fetuses have only been observed at dose levels associated with significant maternal toxicity. Neither agent has shown an effect on rabbit fetuses. For all four agents, EVG, COBI, FTC and

TDF, NOELs and NOAELs have been clearly identified and were at exposures above human exposures. No additive effects are anticipated with the four drug combination.

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

The Agreed Initial Pediatric Study Request (iPSP) for E/C/F/TAF, detailing the age groups and rationale for the use of E/C/F/TAF as an effective treatment for pediatric patients from ≥ 6 years of age through < 18 years of age, was submitted to IND 111007 on [redacted] 20[redacted], SN[redacted], and is included in m1.9.6 of this NDA. In addition, the Agency letter dated [redacted] 20[redacted] acknowledging the Agreed PSP is also included in m1.9.6 ([redacted]).

Further, requests for waiver and deferral of pediatric studies under the Pediatric Research Equity Act are provided in m1.9.1 and m1.9.2, respectively.

4.7. Local Tolerance

Elvitegravir was not irritating to skin (m1.4.4, TX-183-2020), not a severe irritant to eyes (m1.4.4, TX-183-2021), and showed no potential for phototoxicity (m1.4.4, JTK303-TX-010).

Cobicistat was mildly irritating to skin (m1.4.4, TX-216-2044), not a severe irritant to eyes (m1.4.4, TX-216-2043), and showed no potential for phototoxicity.

No local tolerance studies have been conducted with FTC.

Local toxicity studies concluded that TAF was predicted to be a noncorrosive/nonsevere eye irritant (m2.6.7, Section 16, TX-120-2013), and nonirriating/noncorrosive to rabbit skin under semi-occluded conditions (m2.6.7, Section 16, TX-120-2011).

The EVG/COBI/FTC/TAF tablet is intended for oral use. No local tolerance studies were conducted for the EVG/COBI/FTC/TAF combination. Evaluation of local tolerance to the GI tract has been conducted during the repeat-dose oral toxicity studies with each of the individual agents. Although EVG and COBI have shown changes in the stomach, they have occurred at high dose levels associated with high local concentrations, or have been isolated occurrences that have not been observed in other long term studies and are not considered clinically relevant to the EVG/COBI/FTC/TAF FDC. In the pivotal registration clinical studies (m2.5), ECF/TAF was generally well tolerated; the incidences of GI disorders were consistent with those expected in the subject population and the known safety profiles of the study drugs.

4.8. Other Toxicity Studies

4.8.1. Antigenicity

Elvitegravir, COBI and TAF showed no potential for sensitization (m1.4.4, TX-183-2022, TX-216-2042; m2.6.7, Section 17, TX-120-2014, respectively).

4.8.2. Immunotoxicity

The immunotoxicity of EVG was evaluated in a 28-day study in rats at doses up to 1000 mg/kg/day (m1.4.4, JTK303-TX-011). There were no adverse effects of EVG during the dosing period and EVG did not affect the antibody titers to sheep RBCs at any of the doses administered. EVG was not considered immunotoxic at doses up to 2000 mg/kg/day.

The immunotoxicity of COBI was evaluated in a 28-day TDAR study in rats at doses up to 150 mg/kg/day (m1.4.4, TX-216-2022). Immunosuppressive effects were noted in females at 50 and 150 mg/kg/day, based on a decreased response to keyhole limpet hemocyanin (KLH) immunization (lower anti-KLH immunoglobulin G [IgG] antibody titers). Decreased anti-KLH IgG responses in males did not reach statistical significance at 150 mg/kg/day. No COBI-related changes in the anti-KLH IgM response in males and females were noted. In addition, clinical signs, decreases in body weight gain and/or food consumption, increases in liver and thyroid weights, and lymphoid depletion of germinal centers in the spleen were observed at 50 and/or 150 mg/kg/day. The NOEL for the TDAR is considered to be 20 mg/kg/day in females, and 50 mg/kg/day in males, and the NOAEL was 20 mg/kg/day in both sexes. Additional immunohistochemical (IHC) analysis of spleens from all animals was conducted, as described in the ICH S8 Guideline, Immunotoxicity Studies for Human Pharmaceuticals {12999}. Formalin-fixed, paraffin-embedded tissues (spleen) were evaluated for detection of B cells (KiB1R-positive), T cells (CD3-positive), and germinal centers (peptide nucleic acid (PNA)-positive). Immunohistochemical findings observed in COBI-treated animals did not correlate with the decreases noted in anti-KLH IgG levels, as greater decreases in anti-KLH IgG levels were observed in females versus males, but IHC trends were noted only in males. In females, there was a notable lack of any dose-response with respect to the IHC changes.

To further assess the potential for immunotoxicity associated with COBI, immune tissues (spleen, thymus, lymph nodes, and Peyers patches) from rats administered COBI in the 4-week and 26-week toxicity studies (m1.4.4, TX-216-2004, TX-216-2017) were subjected to a pathology peer-review. Evaluation was conducted according to recommendations for rat lymphoid tissue evaluation and included an estimate of the numbers of germinal centers in the spleen for each animal {13001}, {14240}, {14242}. The Peer Reviewing Pathologist was in agreement with the overall interpretations and conclusion of the histopathology diagnoses and conclusions of the studies regarding the lack of treatment-related effects on rat lymphoid tissues. The NOAELs for COBI in the 4-week and 26-week studies were 50 and 30 mg/kg/day, respectively.

In the 26-week rat study with COBI (m1.4.4, TX-216-2017), peripheral blood immunophenotyping was conducted during Week 26 and prior to recovery sacrifice (Recovery Week 13). Total T cells, helper T cells, cytotoxic T cells, B cells and natural killer (NK) cells

were quantified using flow cytometry. No adverse effects on mean immunophenotyping values were noted.

In the 39-week dog study with COBI (m1.4., TX-216-2016), peripheral blood immunophenotyping (total T cells, helper T cells, cytotoxic T cells, and B cells) conducted during Week 26 revealed no treatment-related changes. Histopathological changes in immune system tissues were limited to minimal to moderate thymic involution in high dose (20 mg/kg/day) males at the 13-week interim sacrifice. The thymic changes after 13 weeks of dosing were attributed to stress (thin appearance, decreased body weight and food consumption, excessive salivation, emesis and abnormal feces) and not considered direct COBI-related effects. Further, these changes were no longer apparent in terminal sacrifice or recovery sacrifice animals suggesting tolerance to these stress-related changes. The NOAEL after 39 weeks daily oral gavage dosing to dogs is considered to be 10 mg/kg/day.

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

In a 4-week study in dogs where animals received FTC, TDF, or the combination of FTC and TDF, no remarkable changes were observed for immunophenotyping or NK cell assay values (m1.4.4, TX-164-2004).

Data from repeat-dose toxicity studies with EVG, FTC, or TAF (hematology, lymphoid organ weights, microscopy of lymphoid tissues, bone marrow cellularity) and immunotoxicity studies with EVG and FTC did not suggest immunotoxic potential for any of these agents. There were no notable effects of the combination of FTC/TDF on immune cells or NK cell assay values in a 4-week dog study (m1.4.4, TX-164-2004). For COBI, decreased IgG levels were noted in female rats at dose levels (≥ 50 mg/kg/day) above the NOAEL for systemic toxicity in long term studies (30 mg/kg/day). However, in standard toxicity studies with COBI in mice (up to 13 weeks dosing; m1.4.4, TX-216-2026), rats (up to 26 weeks dosing; m1.4.4, TX-216-2017) and dogs (up to 39 weeks dosing; m1.4.4, TX-216-2016), and at higher dose levels and exposures, no signs of immune function changes have been observed. No further studies were deemed necessary with the E/C/F/TAF FDC.

4.8.3. Toxicological Findings of FTC/TDF in SIV Efficacy Studies

Findings from efficacy studies of TFV in SIV-infected and non-infected rhesus monkeys are summarized in Section 2.1 and Section 4.6.4.

4.8.4. Impurities/Degradation Products

4.8.4.1. EVG

Two 4-week studies were conducted in rats to determine if there were unexpected toxicologic effects of EVG spiked with up to 3.8% impurities (m1.4.4, TX-183-2010, TX-183-2023). No adverse treatment-related findings were observed following at least 28 days of oral gavage

dosing of either EVG or EVG lots spiked with impurities at doses up to 2000 mg/kg/day. No differences between treatment with either EVG or EVG spiked with impurities were noted.

In silico evaluation of several process intermediates and potential impurities in EVG for potential mutagenicity, chromosome damage, genotoxicity, and carcinogenicity revealed no unique structural alerts (m1.4.4, TX-183-2024). As the structural alert (quinoline-3-carboxylic acid scaffold) identified in several impurities is shared with EVG, these impurities are not considered genotoxic based on the weight of evidence that EVG is not genotoxic or carcinogenic.

Based on their impurity profiles, the multiple GLP batches of EVG tested in the toxicology program are considered, in composite, to be representative of the Good Manufacturing Practice (GMP) material and support the specified limits of impurities proposed for commercial in the EVG drug substance specification. No EVG-related degradation products have been observed in E/C/F/TAF tablets and therefore no impurity is specified in E/C/F/TAF drug product specification.

4.8.4.2. COBI

A 4-week study was conducted in rats to determine if there were unexpected toxicologic effects of COBI spiked with up to 4.9% impurities (m1.4.4, TX-216-2045). No adverse treatment-related findings were observed following at least 28 days of oral gavage dosing of either COBI or COBI lots spiked with impurities at doses up to 100 mg/kg/day to rats. No differences between treatment with either COBI or COBI spiked with impurities were noted.

In silico evaluation of several process intermediates and potential impurities in COBI for potential mutagenicity, chromosome damage, genotoxicity and carcinogenicity revealed no structural alerts (m1.4.4, TX-216-2046).

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

4.8.4.3. FTC

The process impurities and degradation products of FTC have been qualified in animal studies. The major degradation product, *related substance D, was qualified in 2 genotoxicity studies (m1.4.4, TOX151; TOX152) using a batch of FTC that contained 1% (w/w) of the *related substance D degradant. Both studies were negative for genotoxicity. In addition, there was no toxicity in a 28-day mouse study at doses (FTC/*related substance D) of 50/1 mg/kg/day, 150/3 mg/kg/day, and 450/9 mg/kg/day (m1.4.4, TOX153).

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

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

4.8.4.4. TAF

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

Two 1-month impurity qualification studies were conducted in rats to evaluate potential drug substance impurities. Administration of 97.7% pure and 83.1% pure GS-7340-02 by oral gavage for 14 days to male rats was well tolerated at dose levels of 5 and 50 mg/kg/day (m2.6.7, Section 17, TX-120-2008). No test article-related findings were noted, and no differences were found between the 2 lots tested. The NOAEL for both lots is 50 mg kg/day (40 mg f.b.e./kg/day).

The second impurity qualification study evaluated daily administration of GS-7340-03 via oral gavage to male and female rats for at least 28 days (m2.6.7, Section 17, TX-120-2021). Three lots of GS-7340-03 were each administered at 25 and 50 mg/kg/day (free base equivalents). Test article 1 was 99.3% pure GS-7340-03. Test article 2 was 98% pure GS-7340-03 containing 11 spiked known and potential impurities. Test article 3 was 97.8% pure GS-7340-03 containing 4 spiked potential impurities. Control animals were administered the vehicle control article. Administration of GS-7340-03 drug substance with 3 different impurity profiles by oral gavage for 28 days to rats was well tolerated at dose levels of 25 and 50 mg/kg/day. There were no significant clinical or histopathological differences between the 3 lots tested. The NOAEL for all 3 lots is 50 mg f.b.e./kg/day.

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

4.8.4.5. FTC/TDF

Four degradation products not present in the individual drug substances have been observed in FTC/TDF tablets placed on accelerated stability at high temperature. The trivial name for these degradation products are *related substance F, *related substance G, *related substance H and *related substance I. The adducts of FTC/TDF form when [redacted], formed by the [redacted] of TDF (*related substance J), reacts with one molecule of each TDF and FTC to form an adduct. FTC may undergo [redacted] and may additionally [redacted] group of the [redacted], thereby creating *related substance K. While *related substance K

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has the potential to exist as 4 diastereomers, only 2 of these diastereomers have been observed in FTC/TDF containing products. The 2 observed diastereomers of *related substance K are *related substance H and *related substance I .

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

The qualification of these 4 degradation products is summarized in m3.2.P.5.6, Justification of Specifications [E/C/F/TAF Tablets].

The impurities and degradation products in the 2 active ingredients, FTC and TDF, as well as the tableted drug product have been identified and qualified in toxicology studies. The safety margins support the specified limits proposed for these impurities and degradation products.

4.8.4.6. E/C/F/TAF

The E/C/F/TAF FDC is a monolayer tablet. No degradation products related to EVG have been identified in batches of E/C/F/TAF tablets. Several degradation products related to COBI and FTC have been identified in batches of E/C/F/TAF tablets and all of them have been observed in batches of EVG/COBI/FTC/TDF tablets. Degradation products were evaluated in a 28-day toxicity study in rats by comparing nondegraded and degraded EVG/COBI tablets (m1.4.4, TX-236-2002). The impurity profiles for batches used in the toxicology studies are provided in m2.6.7, Section 4. The degradation products of TAF observed in the E/C/F/TAF tablets are consistent with those in the TAF drug substance. There are no unique impurities or degradants in the E/C/F/TAF tablets.

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

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4.9. Summary of Toxicology and Target Organ Effects

4.9.1. Target Organ Effects

4.9.1.1. EVG

No clinically relevant adverse effects were observed in the safety pharmacology, general toxicity, genotoxicity, carcinogenicity, reproductive, juvenile toxicity, local tolerance, and immunotoxicity studies, or in special mechanistic studies to investigate potential quinolone-related toxicity.

No adverse target-organ toxicity was observed in single- or repeat-dose nonclinical studies with EVG. Two nonadverse findings, not considered relevant to clinical use, were observed in rats and dogs.

In rats, cecal weight and/or its contents were increased at doses ≥ 300 mg/kg/day, with dilatation of the cecum observed at ≥ 1000 mg/kg/day. These observations were not accompanied by any effects on the GI or histological changes in the cecum. Similar changes in the cecum have been reported with antibacterial quinolones which affect the GI microflora {18406}. Elvitegravir has a quinolone moiety and was confirmed to have antibacterial activity in the reverse mutation assay (23.4 μg/plate or higher; m1.4.4, JTK303-TX-005). Although the activity was much weaker than that of the antibacterial quinolones, the changes in the cecum were considered to be due to the antibacterial activity of high local concentrations of EVG in the GI tract.

Lipid-like vacuoles were observed in the lamina propria in the upper small intestine (duodenum and/or jejunum) in rats, with increased incidence and severity at doses ≥ 1000 mg/kg/day. The incidence and severity did not increase with long-term dosing, and there was no evidence of toxicity or any adverse tissue reactions associated with these vacuoles. The cause of the vacuolization is considered related to the high local EVG concentrations to which the GI epithelium was exposed. In a series of mechanistic studies, the vacuoles were shown to contain mainly triglycerides, tended to disappear slowly after withdrawal of treatment with EVG, and may be related to the lipid absorption process although there were no changes in plasma lipid parameters or adverse clinical observations. In the 2-year rat carcinogenicity study, there were no notable findings in the upper small intestine, further suggesting that the presence of the vacuoles was not adverse (m1.4.4, TX-183-2012). Considering the totality of the data, the vacuoles were not considered to be toxicologically significant.

In dogs, dilatation of the cecum was observed in males at 100 mg/kg/day in the 4-week repeat-dose study (m1.4.4, JTK303-TX-004). Lipid vacuoles containing mainly triglycerides were observed in the upper small intestinal lamina propria in both sexes at doses ≥ 30 mg/kg/day in the 39-week dog study (m1.4.4, JTK303-TX-023). Similar to rats, these observations were also not accompanied by any GI adverse events or histological changes in the cecum and the small intestines, and they were not considered adverse.

4.9.1.2. COBI

No clinically relevant adverse effects were observed in the safety pharmacology, genotoxicity, reproductive, juvenile toxicity, and local tolerance studies with COBI.

4.9.1.2.1. Liver

Liver effects in mice, rats, and dogs were qualitatively similar. In rodents, the predominant effects were increased weights, microsomal enzyme induction, and hepatocellular hypertrophy. In dogs, increased liver weights and hepatocellular hypertrophy were similarly observed. Elevations in liver enzyme levels (ALT and AST) were most prominent in mice, not notable in rats, and only observed in high dose animals in the 4-week dog study (accompanied by hepatocyte vacuolation).

In the 13-week mouse study, mild to marked elevations in ALT and AST were noted in males at 15 and 50 mg/kg/day, respectively (m1.4.4, TX-216-2026). These changes were associated with increases in liver weight, microsomal enzyme induction, and minimal hepatocellular hypertrophy at 50 mg/kg/day. Female mice were notably less sensitive; a marked elevation in ALT and AST was noted in only one high-dose female (50 mg/kg/day). Similar liver findings were noted in the 4-week toxicity in HRAS wild type mice (m1.4.4, TX-216-2041). In rats, increases in liver weights were associated with hepatocellular hypertrophy. These findings were reversible and were not considered adverse. The liver effects are considered adaptive changes, are commonly seen in rodents with microsomal enzyme inducers, and are considered secondary to microsomal enzyme induction {17078}, {18407}. Cobicistat induces hepatic CYP3A activity in mice and in rats likely due to a species-specific activation of rodent PXR (Section 3.7.2.2).

In dogs, minimal-to-mild increases in bilirubin levels, ALT, and alkaline phosphatase activities, increased liver weights, and hepatocyte vacuolation were noted after 4 weeks dosing at 45 or 45/30 mg/kg/day (m1.4.4, TX-216-2005). In the 39-week study, there were no notable serum chemistry changes; however, minimal liver changes (hepatocellular hypertrophy) were observed in males at 10 mg/kg/day, and in both sexes at 20 mg/kg/day (m1.4.4, TX-216-2016). These hepatic changes observed in the 39-week study are considered an adaptive response, and not adverse based on their minimal severity, the absence of degeneration, and their reversibility after cessation of dosing {17078}, {18407}.

The nature and degree of the observed effects in serum chemistry, liver histopathology, and liver enzyme induction, as well as the absence of significant bioaccumulation, generation of reactive metabolites, and immune-related hepatic effects support the conclusion that COBI has a low potential for inducing hepatotoxicity (Draft Non-Clinical Guideline for Drug-Induced Hepatotoxicity. European Medicines Agency. CHMP. Doc. Ref. EMEA/CHMP/SWP/150115/2006. London, 24 January 2008). Phase 2 and 3 clinical safety data with the E/C/F/TAF FDC do not indicate an adverse effect on the liver (m2.7.4, Section 3.3).

4.9.1.2.2. Thyroid

Effects on the thyroid glands in rats in the 26-week study were characterized by decreases in T4 in males at 100 mg/kg/day, increases in TSH in 10 mg/kg/day females and in both sexes at 30

and 100 mg/kg/day, increased thyroid weights at 30 and 100 mg/kg/day, and thyroid follicular cell hypertrophy (in one female at 10 and 30 mg/kg/day, and in most male and female animals at 100 mg/kg/day). These findings were reversible and were not considered adverse. However, one high dose 100 mg/kg/day male in the 26-week study also had a follicular cell carcinoma in the thyroid. An increased incidence of follicular cell adenomas and/or carcinomas in the thyroid gland was observed in the rat carcinogenicity study.

Thyroid effects were slightly more pronounced in females possibly due to the higher exposures achieved in this sex. There are no indications that COBI has a direct effect on the thyroid gland, or a particular affinity for thyroid tissue (m2.6.4, Section 4.2.2). These clinical and anatomic pathology changes are considered adaptive changes, secondary to hepatic microsomal enzyme induction (Section 3.3.2) and thyroid hormone imbalance. The thyroid effects are considered rodent specific, and predispose rats, but not humans, to thyroid neoplasms. It is unlikely that COBI presents a risk to the human thyroid {11923}, {11925}, {11927}, {11931}, {11926}, {11933}. No clinically relevant adverse effects on thyroid function have been observed in clinical studies conducted to date with COBI, or with the E/C/F/TAF FDC (m2.7.4, Section 2.1).

4.9.1.2.3. Urinalysis

Urinalysis and urine chemistry changes, noted primarily in high-dose rats (100 mg/kg/day) and in dogs (≥ 30 mg/kg/day), included higher urine volume, lower urine specific gravity, and increases in electrolyte excretion. These changes showed no progression after long term dosing, were not associated with remarkable serum clinical chemistry changes, including serum electrolytes, serum creatinine and BUN, were without histopathological correlates, and were reversible. Although the mechanism associated with these urinalysis changes is not understood (there were no treatment-related changes in serum vasopressin or aldosterone levels in the 26-week rat study), similar findings have been reported with other structurally-related agents, including ATV {13588}, DRV and RTV {18580}. In the 13-week combination toxicity study in rats with COBI and ATV (m1.4.4, TX-216-2024), increases in urine volume were noted in groups administered COBI and ATV alone, and in combination. However, these effects were slight, not additive when COBI and ATV were administered in combination, nor associated with microscopic correlates and were reversible; therefore these changes were not considered adverse.

4.9.1.2.4. Hematology, Coagulation and Clinical Chemistry

Red blood cells

Minimal changes in RBC parameters (not exceeding 10%) were noted in high-dose rats administered 100 mg/kg/day in the 26-week study (m1.4.4, TX-216-2017). There were no correlative effects (eg, symptoms of anemia, bone marrow suppression) and similar effects were not seen in mice dosed for 13 weeks (m1.4.4, TX-216-2026) or in dogs dosed for 39 weeks (m1.4.4, TX-216-2016). Due to the minimal change and reversibility, these effects were not considered adverse.

Coagulation

In rats, increases (≤ 38%) in mean platelet counts were noted in the 4-week study at doses ≥ 50 mg/kg/day (m1.4.4, TX-216-2004), and in the 26-week study in males at ≥ 30 mg/kg/day and in females at 100 mg/kg/day (m1.4.4, TX-216-2017). In dogs, increases in platelet counts (up to 43%) were noted after 13, 26 and 39 weeks dosing in high dose (20 mg/kg/day) females, and in 10 mg/kg/day females at Week 26 only (m1.4.4, TX-216-2016). Further, in dogs, minimal decreases (up to 15%) in APTT were noted in 10 mg/kg/day females and in both sexes at 20 mg/kg/day. Similar changes were not observed in the 4-week dog study at doses up to 45/30 mg/kg/day. In all cases, there were no associated effects on bleeding, the changes were reversible, and they were not considered adverse. Since these changes only occurred at high doses, the relevance of these limited changes is questionable.

Clinical Chemistry

Slight but statistically significant increases in cholesterol were observed in the 13-week mouse study (up to 46% increase at dose levels ≥ 15 mg/kg/day; m1.4.4, TX-216-2026) and in female rats in the 26-week study (up to 35% increase at dose levels ≥ 30 mg/kg/day; m1.4.4, TX-216-2017). Cobicistat is a rat PXR inducer (m1.4.4, AD-216-2039), with activation similar to known PXR inducers (RTV and miconazole). Induction of PXR can reduce CYP7A1 transcription and cholesterol 7a-hydroxylase activity, which are involved in the conversion of cholesterol to bile acids {17604}. Down-regulation of Cyp7a1 results in less cholesterol being converted to bile acids and consequently more free cholesterol. An approximate 6-fold induction of rat PXR was observed with COBI at 10 μM. In the 13-week mouse and 26-week rat toxicity studies, COBI C_{max} values associated with cholesterol changes were 2.7 to 11.4 μg/mL (3.5 to 14.7 μM), suggesting adequate PXR activation to explain the slight increase in cholesterol levels observed with COBI. At clinically-relevant concentrations, COBI would not activate human PXR (m2.6.4, Section 7.2.3.1).

Minor nonadverse changes in total protein, globulin, and albumin were observed in rats and dogs. In rats, increases in mean total protein were less than 10% in high dose males and females, with similar changes in albumin and globulin values. As expected, increases in serum calcium correlated with the increases in serum albumin. In rats, these changes can be considered secondary to the effects on the liver (increased weights). In dogs, decreases in total protein (less than 13%), albumin and globulin were observed in high dose animals in the 39-week study; these

changes may have been secondary to decreases in food consumption in high dose animals. The relevance of these limited effects in high dose animals is questionable.

4.9.1.2.5. Immune System

Results from a rat immunotoxicity study showed lower anti-KLH IgG antibody titers in females at ≥ 50 mg/kg/day (m1.4.4, TX-216-2022). There were no COBI-related changes in the anti-KLH IgM antibody response at any dose in either sex. The NOEL for the T-cell dependent antibody response is considered to be 20 mg/kg/day in females and 50 mg/kg/day in males. However, in standard 13-week mouse, 26-week rat and 39-week dog toxicity studies at doses up to 50, 100 and 20 mg/kg/day in mouse, rat and dog, respectively, microscopic changes suggestive of immunotoxicity were not been observed in lymphoid organs (m1.4.4, TX-216-2026, TX-216-2017 and TX-216-2016, respectively). Further, immunophenotyping of peripheral blood cells evaluated in the chronic rat and dog toxicity studies did not reveal any adverse effects, and there were no signs of potential immunosuppression as assessed by animal health status (ie, no signs of opportunistic infections) and clinical chemistry and hematological analyses. The clinical significance of the decrease in anti-KLH IgG levels in a single study in female rats is unclear considering that no adverse effects on hematological parameters, IgG levels, or rate of infections considered related to study drug that could be suggestive of immunosuppression have been observed in clinical studies conducted with COBI, or with the E/C/F/TAF FDC (m2.7.4).

4.9.1.3. FTC

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

4.9.1.4. TAF

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

4.9.1.4.1. Kidney

Renal tubular karyomegaly was observed in rats (m2.6.7, Section 7.2, R990182, and Section 7.3, TOX-120-001) and dogs (m2.6.7, Section 7.4, D990175, and Section 7.5, TOX-120-002) orally administered TAF. Focal areas of minimal renal cortical tubular basophilia and associated minimal nuclear karyomegaly were present in rats administered 400 mg/kg/day for 4 weeks and 100 mg/kg/day for 26 weeks. Renal tubular karyomegaly and/or basophilia were observed in dogs administered 3 and 10 mg/kg/day for 4 weeks and dogs administered 6 or 18/12 mg/kg/day for at least 13 weeks.

Renal cortical tubular degeneration/regeneration findings were limited to animals administered 6 or 18/12 mg/kg/day for at least 13 weeks in the 39-week dog toxicity study. Similar findings of renal cortical tubular degeneration/regeneration and karyomegaly were present in dogs administered either 6 or 18/12 mg/kg/day for 39 weeks. These changes were minimal to slight in

affected males and females at 6 mg/kg/day. In high-dose males (18/12 mg/kg/day) the severity ranged from mild to moderate. Similar lesions (karyomegaly and tubular degeneration) but of only minimal severity were also present in 2 males administered 2 mg/kg/day of TAF for 39 weeks. After a 13-week recovery period, treatment-related histology changes were still observed in the kidney but were of reduced incidence and severity.

4.9.1.5. Bone

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

4.9.1.6. Other

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

TAF had no discernible electrocardiographic effect at the low dose of 2 mg/kg/day. There was some evidence at 6 and 18/12 mg/kg/day for an effect to slightly prolong PR intervals (~13-24%) which was associated with significant decreases in T_3 {29101}, {29104}. After the 13-week recovery period, serum T_3 values returned to levels similar to the control group animals at the end of the study. No PR prolongation or any change in ECG results occurred in the safety pharmacology study that evaluated a TAF dose up to 100 mg/kg (m2.6.3, Section 4.2, D2000006) or in the thorough QT study (m2.7.2, Appendix 6.1.3 [GS-US-120-0107])

At 18/12 mg/kg/day in dogs, the highest dose tested, a minimal infiltration of histiocytes was present in some organs (eye (choroid plexus, ciliary body), lung, and spleen) in some animals. In-life ophthalmologic examinations were normal. The histiocytic infiltration observed in multiple organs was most likely an indirect drug effect due to general debilitation and was not observed in other repeat dose toxicity studies. There were no drug-related effects on ophthalmic exams or microscopic exams of ocular tissue observed in repeat dose toxicity studies in mice (up to 13 weeks), rats (up to 26 weeks), nonhuman primates (4 weeks) or in the 4-week dog toxicology study. The minimal to slight infiltration of mononuclear cells in the ocular posterior uvea occurred only in dogs administered the highest dose of TAF where compound-related toxicities occurred on clinical condition, weight gain, serum chemistry, BMD, and other organ histopathology. Distribution of ^{14}C-TAF to eyes has been assessed in mice, rats, and dogs (m2.6.5, Section 5.1, AD-120-2011; m2.6.5, Section 5.2, AD-120-2020; and m2.6.5, Section 5.4, D990173-BP). Melanin binding has specifically been assessed by comparing distribution in pigmented and non-pigmented mice (C57 black and CD-1, respectively) and rats (Long Evans and Sprague-Dawley, respectively). [^{14}C]-TAF-related radioactivity distributed poorly to the

eyes of rats and dogs (C_{max} in eyes < 8% that observed in plasma). Transient exposure to low levels of [^{14}C]-TAF-related radioactivity was observed in the eyes of rats decreasing to undetectable levels at 8 hours postdose. No difference in distribution was observed between Sprague-Dawley and Long Evans rats, including in the skin and eyes, suggesting no binding to melanin. The distribution of [^{14}C]-TAF to eyes in mice was higher than other species studied (C_{max} in eyes 15% -20 % that observed in plasma). More persistent exposures in eye lens, eye uveal tract, and eyes were observed in C57 black mice compared to CD-1 mice. However, no difference in distribution between pigmented and nonpigmented skin was observed illustrating that [^{14}C]-TAF-related radioactivity was not selectively associated with melanin-containing tissues. The posterior uveitis in dogs administered the highest dose of TAF occurred at 3.7- and 17-fold higher exposure to TAF and TFV, respectively, than that observed in human subjects administered a 25-mg dose of TAF and does not correlate with the tissue distribution where TAF was found to poorly penetrate across the blood brain and blood retinal barrier in dogs. Because TAF has poor penetration across the blood brain and blood retinal barrier in dogs it is unlikely that TAF directly caused the observed histiocytic infiltration in the posterior uvea. Based on the evidence from tissue distribution and toxicology studies, Gilead concludes that the risk of posterior uveitis in humans is very low.

TAF is unlikely to cause mitochondrial toxicity. TAF did not affect the amount of mtDNA levels up to 1 μM (approximately 2-fold higher than C_{max} after a 25 mg TAF dose), the highest concentration tested, in HepG2 cells in a 10-day assay (m2.6.3, Section 1.2, PC-120-2006). The active metabolite of TAF, tenofovir diphosphate is highly discriminated as a substrate by mitochondrial DNA polymerase γ relative to the natural substrate, ATP (> 10,000 fold) {4923}. No toxicity indicative of mitochondrial toxicity was observed in nonclinical or clinical studies. Therefore, TAF is unlikely to inhibit mitochondrial DNA polymerase γ under clinical relevant conditions.

4.9.1.7. E/C/F/TAF

Administration of TAF in combination with EVG, COBI and FTC is unlikely to exacerbate known toxicities of the individual agents. The 4 drugs, EVG, COBI, FTC, and TAF, exhibit different patterns of target organ toxicity. For EVG, increases in cecal weights and/or its contents, likely due to weak antibacterial activity, and lipid-like vacuoles in the upper small intestine were associated with high local concentrations and are not considered relevant to clinical use. Although EVG and COBI have shown changes in the stomach, they have occurred at high dose levels associated with high local concentrations, or have been isolated occurrences that have not been observed in other long term studies and are not considered clinically relevant to the E/C/F/TAF FDC. In the pivotal registration clinical studies (m2.5), ECF/TAF was generally well tolerated; the incidences of GI disorders were consistent with those expected in the subject population and the known safety profiles of the study drugs.

The only toxicity observed in chronic animal studies with FTC was mild, reversible anemia in mice and minor decreases in erythrocyte counts/increases in mean corpuscular hemoglobin in monkeys at large multiples of clinical exposure (137-fold in mice; 20-fold in monkeys); therefore, these hematological findings are not considered relevant to clinical use.

Cobicistat has the potential to prolong the PR interval; a modest, dosing-related increase in PR interval observed in the thorough QT study was not considered to be clinically significant (m2.7.2, Section 3.3.2.3, GS-US-216-0107). Although TAF showed some potential to prolong the PR interval (~13-24%) in the 39-week dog study at 18/12 mg/kg/day, the slight change was associated with bone and renal toxicity and included significant decreases in T3. No PR prolongation or any change in ECG results occurred in the safety pharmacology study that evaluated a TAF dose up to 100 mg/kg (m2.6.3, Section 4.2, D2000006) or in the thorough QT study (m2.7.2, Appendix 6.1.3 [GS-US-120-0107]).

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

From in vitro data and clinical experience (m2.7.2), the anticipated drug-drug interaction upon administration of the 4-drug combination is 1) the intended inhibition of CYP3A activity by COBI and the consequent increase in EVG exposure and 2) the inhibition of P-gp-mediated intestinal secretion by TAF which increases the bioavailability of TAF. The inhibition of P-gp has minimal impact because of the comparatively low plasma concentrations of TAF and TFV.

4.9.2. Safety Margins

Safety margins remain the same for EVG, COBI, FTC as in Stribild since no additional exposure data was collected.

4.9.2.1. EVG

No clinically-relevant target-organ toxicity was observed in single- or repeat-dose nonclinical studies with EVG. The NOAELs are considered to be 2000 mg/kg/day for mice and rats, and 100 mg/kg/day for dogs—the highest doses evaluated in the 13-week, 26-week, and 39-week repeat-dose studies in mice, rats, and dogs, respectively. The combination of 1000 mg/kg/day EVG with 30 mg/kg/day COBI or with 10 mg/kg/day RTV, dosed to rats for 90 days, did not result in any notable toxicity findings.

Estimated safety margins (Table 4) were calculated based on exposure after repeat dosing (AUC_{tau}) from the 13-week mouse, 6-month rat, and 9-month dog studies with EVG, as well as exposure to EVG when administered with COBI or with RTV in rats. Calculations of the safety margins are based on a human AUC_{tau} value of 23 μg•h/mL following administration of 150 mg EVG as part of the EVG/COBI/FTC/TDF FDC in HIV-1 infected subjects (m2.7.2). While the margin of safety was approximately 2-fold in the 13-week mouse study, in the mouse carcinogenicity study (in animals dosed with EVG and RTV in combination) no adverse effects were noted at an exposure margin of approximately 14-fold. Elvitegravir exposure in the chronic toxicity studies (26-week rat and 39-week dog) exceeds the estimated exposure at the efficacious dose in humans.

Table 4. Estimated Safety Margins for EVG 150 mg Based on Exposure (AUC) at Animal No-Adverse-Effect-Level (NOAEL)

Species Gender	Study Type	NOAEL Dose (mg/kg/day)	AUC_{0-t} (µg•h/mL)	Safety Margin[a] (Male-Female range)
Mouse				
Male - Female	13-week Toxicity	2000	44 - 59	1.9 – 2.6 X
Rat				
Male - Female	26-week Toxicity	2000	460 - 836	20 – 36 X
Rat + 10 mg/kg RTV				
Male - Female	13-week Combination Toxicity	1000	140 - 167	6.1 – 7.3 X
Rat + 30 mg/kg COBI				
Male - Female	13-week Combination Toxicity	1000	183 - 201	8.0 – 8.7 X
Dog				
Male - Female	39-week Toxicity	100	54 - 66	2.3 – 2.9 X

COBI, cobicistat; EVG, elvitegravir; NOAEL, no observed adverse effect level; RTV, ritonavir
a Human AUC_{tau} 23 µg•h/mL (m2.7.2, Table 28)

4.9.2.2. COBI

A comprehensive program of nonclinical studies with COBI has been conducted. In repeat-dose studies (up to 13 weeks in mice, up to 26 weeks in rats; up to 39 weeks in dogs), target organs identified were liver (mouse, rat, and dog) and thyroid (rat). The liver effects in mice and rats are considered adaptive changes, are commonly seen in rodents with microsomal enzyme inducers, and are considered secondary to microsomal enzyme induction {17078}, {18407}. In dogs, the hepatic changes observed in the 39-week study are considered an adaptive response, and not adverse based on their minimal severity, the absence of degeneration, and their reversibility after cessation of dosing {17078}, {18407}. The thyroid changes in rats are considered adaptive changes, secondary to hepatic microsomal enzyme induction and thyroid hormone imbalance {11923}, {11925}, {11927}, {11931}, {11926}, {11933}. The thyroid effects are considered rodent specific and predispose rats, but not humans, to thyroid neoplasms. The clinical relevance of the liver and thyroid effects were discussed in Section 4.9.1.2.

Combination toxicity studies indicate that administration of COBI with EVG or with ATV is unlikely to exacerbate the known toxicities of the individual agents, or lead to unexpected toxicities.

Estimated COBI safety margins (Table 5) were calculated based on exposure after repeated dosing from the 13-week mouse, 26-week rat, and 39-week dog toxicity studies, as well as exposure to COBI when administered with EVG or with ATV in rats. Calculations of the safety margin are based on a human AUC_{tau} value of 8.3 µg•h/mL following administration of 150 mg COBI as part of the EVG/COBI/FTC/TDF FDC in HIV-1 infected subjects (m2.7.2).

Table 5. Estimated Safety Margins for COBI 150 mg Based on Exposure (AUC) at Animal No-Adverse-Effect-Level (NOAEL)

Species Gender	Study Type	NOAEL Dose (mg/kg/day)	AUC_{0-t} (µg•h/mL)	Safety Margin[a]
Mouse				
Male - Female	13-week Toxicity	5 - 50	0.93 – 60.1	0.1 – 7.2 X
Rat				
Male - Female	26-week Toxicity	30	9.9 – 13.3	1.2 – 1.6 X
Rat + 1000 mg/kg EVG				
Male - Female	13-week Combination Toxicity	30	5.2 – 7.5	0.6 – 0.9 X
Rat + 50 mg/kg ATV				
Male - Female	13-week Combination Toxicity	30	6.1 – 6.3	0.7 – 0.8 X
Dog				
Male - Female	39-week Toxicity	10	19.6 – 16.8	2.4 – 2.1 X

ATV, atazanavir; COBI, cobicistat; EVG, elvitegravir; NOAEL, no observed adverse effect level
a Human AUC_{tau} 8.3 µg•h/mL (m2.7.2, Table 31)

While the safety margins are not large, effects above the NOAELs were minimal and some effects were species-specific. At doses above the NOAEL in male mice, liver changes (transaminase elevations and minimal hepatocellular hypertrophy) were observed; female mice were notably less sensitive. In rats, notable effects were limited to decreased body weight gain and food consumption, with slight changes in hematology, clinical chemistry, and urinalysis parameters, and adaptive liver and thyroid changes. In dogs, salivation and emesis, decreased body weight gain and food consumption, slight changes in some clinical chemistry parameters, and minimal adaptive changes in the liver were noted above the NOAEL.

4.9.2.3. FTC

Cross-species comparisons of FTC exposure (expressed based on AUC_{ss} levels) for the major target organs are shown in Table 6.

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

Table 6. Estimated Safety Margins of Emtricitabine Based on AUCss When Comparing Animal No-Effect-Level (NOEL)

Target Organ Effect	Species	Study Duration	NOEL (mg/kg/day)	AUC_{ss} (μg·h/mL) NOEL	Margin Relative to Human AUC_{ss}
			FTC		
Anemia	Mouse	6 months	500	350	34 X
	Rat	3 months	600	346	33 X
	Monkey	1 year	200	98	10 X

Human AUC_{tau} (13 μg·h/mL) following a 200 mg/day dose of FTC (m2.7.2, Table 34).

4.9.2.4. TAF

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

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

Table 7. Estimated Safety Margins of TAF Based on AUCss When Comparing Animal No-Adverse-Effect-Level (NOAEL)

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

NA = not applicable; NC = insufficient data to calculate

a Predicted safety margin for TFV human exposure is based on pooled PK data from E/C/F/TAF Phase 3 pivotal studies GS-US-292-104 and GS-US-292-111 where the mean TFV AUC_{ss} = 0.293 µg.h/mL; m5.3.3.5

b Predicted safety margin for TAF human exposure is based on pooled PK data from E/C/F/TAF Phase 3 pivotal studies GS-US-292-104 and GS-US-292-111 where the mean TAF AUC_{ss} = 0.206 µg.h/mL; m5.3.3.5

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

5. INTEGRATED OVERVIEW AND CONCLUSIONS

5.1. Correlation of Nonclinical and Clinical Findings

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

5.2. Justification for Text in Labeling

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

Based on findings in the nonclinical studies, the key safety points for consideration that are related to EVG, COBI, FTC, or TAF include: (1) decreases in estimated creatinine clearance due to COBI, (2) use in patients with severe hepatic impairment, (3) the potential for CYP3A associated drug interactions, (4) use during pregnancy and lactation, (5) potential for carcinogenicity, and (6) potential for PR interval prolongation and decreased LV function due to COBI.

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

1) In clinical studies, COBI has been shown to decrease estimated creatinine clearance due to inhibition of tubular secretion of creatinine without affecting glomerular function. Cobicistat is a weak to moderate inhibitor of OCT2, MATE2-K, and OCTN1, while COBI and EVG are more potent inhibitors of MATE1. However, TAF is not an inhibitor of any of these transporters; therefore, there is low potential to further decrease the activity of the transporters relative to STB. The small increase in serum creatinine levels may be associated with inhibition of MATE1-mediated creatinine secretion by COBI {28345}. In fact, the E/C/F/TAF FDC produces a smaller increase in serum creatinine than the EVG/COBI/FTC/TDF FDC. Clinical results with the E/C/F/TAF FDC in HIV-infected patients with mild to moderate renal impairment demonstrated that no dose adjustment of the E/C/F/TAF FDC is warranted in patients with estimated creatinine clearance ≥ 30 mL/min. (m2.5 and m2.7.2).

2) The potential for hepatotoxicity appears to be low. Emtricitabine and TFV are not metabolized, do not interact significantly with P450 enzymes and are not excreted to any significant extent by the liver. In addition, there was no substantive hepatotoxicity identified in the nonclinical studies with FTC and TAF, nor with EVG. Although adaptive liver changes were observed in nonclinical species with COBI, the potential for hepatotoxicity is considered low. Based on available pharmacokinetic data in patients with mild or moderate hepatic impairment, no dose adjustment is required in patients with mild or moderate hepatic impairment (m2.5 and m2.7.2).

3) Cobicistat is a selective, mechanism-based CYP3A inhibitor. EVG and COBI are CYP3A substrates. Cobicistat may increase the plasma concentration of drugs metabolized by

CYP3A, and coadministration of drugs that inhibit or induce CYP3A may alter the clearance of EVG and COBI.

4) Animal data indicate that EVG, COBI, FTC, and TAF do not cause reproductive or embryo-fetal toxicity. Emtricitabine has been shown to cross the placenta and the ratio of FTC concentrations in plasma in pregnant mice and rabbits as compared to their fetuses was approximately 0.4. Elvitegravir and COBI have both been shown to be secreted in rat milk with a milk-to-plasma ratio of 0.1 for EVG, and up to 1.9 for COBI. It is unknown if TAF is secreted in rat milk.

5) In long-term carcinogenicity studies of EVG, and FTC, no drug-related increases in tumor incidence were found in mice or in rats. In a long-term carcinogenicity study with cobicistat in mice, no drug-related increases in tumor incidence were observed at doses up to 50 and 100 mg/kg/day (males and females, respectively). Cobicistat exposures at these doses were approximately 7 (male) and 16 (females) times, respectively, the human systemic exposure at the therapeutic daily dose. In a long-term carcinogenicity study of cobicistat in rats, an increased incidence of follicular cell adenomas and/or carcinomas in the thyroid gland was observed at doses of 25 and 50 mg/kg/day in males, and at 30 mg/kg/day in females. The follicular cell findings are considered to be rat-specific, secondary to hepatic microsomal enzyme induction and thyroid hormone imbalance, and are not relevant for humans. At the highest doses tested in the rat carcinogenicity study, systemic exposures were approximately 2 times the human systemic exposure at the therapeutic daily dose. Tenofovir DF was negative in the rat carcinogenicity assay, but weakly positive at the highest dose in the mouse carcinogenicity assay (liver adenomas) at exposures 10 times those in humans. While the mechanism of this tumor formation is uncertain, the findings are unlikely to be of relevance to humans. Appropriate information regarding the results of the carcinogenicity studies is included in the 'Nonclinical Toxicology' section of the proposed Prescribing Information.

6) Cobicistat showed the potential to prolong the PR interval and decrease LV function in isolated rabbit hearts. Electrocardiograms and echocardiograms conducted during clinical development with COBI and electrocardiograms with the E/C/F/TAF FDC did not reveal clinically-significant changes in these parameters (m2.7.4, Section 4.1.1; m2.7.2, Section 2.2.1).

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

5.3. Overall Conclusions

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

The pharmacokinetic and toxicologic profiles of EVG, COBI, FTC, TAF, and TFV are well characterized in multiple animal species and the findings are pertinent in consideration of the use of these agents in combination. Data from clinical studies of the E/C/F/TAF FDC demonstrated acceptable tolerability and safety profiles to support use in patients ≥ 12 years old.

The overall program including the data from the combination and individual agent studies is considered adequate to support the efficacy and safety of E/C/F/TAF FDC tablet based on the following considerations.

The HIV-1 INSTI EVG, and the NRTIs FTC and TFV have potent antiretroviral activity against wild-type and many drug-resistant strains of HIV-1 in vitro and in vivo. The combination of EVG, FTC, and TFV in 3-drug combination experiments showed additive to synergistic anti-HIV-1 activity, and synergistic anti-HIV-1 activity in 4-drug combination experiments with COBI (m2.7.2, Section 4.2).

Although COBI showed the potential to decrease LV function and prolong the PR interval in isolated rabbit hearts, the thorough QT prolongation study (m2.7.2, Section 3.3.2.3, GS-US-216-0107), and ECGs and echocardiograms conducted during clinical development did not reveal clinically-significant changes in these parameters. The potential for cardiovascular effects with the E/C/F/TAF FDC tablet is considered low.

The E/C/F/TAF FDC is not anticipated to produce any new human metabolites. Because significant pharmacokinetic interactions are unlikely (other than the intended pharmacokinetic boosting of EVG by COBI) and that the target organ profiles are different, administration of the combination product is unlikely to exacerbate known toxicities of the individual agents. 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 toxicity profiles of the 4 agents differ substantially with no clinically significant overlapping toxicity. Nonclinical studies with EVG have not identified any specific target organ toxicities or cause for concern. Potential toxicities related to COBI observed in nonclinical toxicology studies (hematology, clinical chemistry, and urinalysis changes; lower IgG antibody titers; and adaptive liver and thyroid changes) have not been observed in clinical studies with the E/C/F/TAF FDC tablet (m2.7.4). The only toxicity observed in chronic animal studies with FTC was mild, reversible anemia at large multiples of clinical exposure; therefore, these hematological findings are not considered relevant to clinical use. Emtricitabine has an established clinical safety profile with no significant toxicities observed. The principal target organs of toxicity in animals following oral administration of TAF were the kidney (karyomegaly, tubular degeneration) and bone.

Although COBI was associated with urinalysis and urine chemistry changes at high doses in rats and dogs, these changes were reversible, were not associated with remarkable clinical chemistry changes, including serum creatinine and BUN, and were without morphological evidence of kidney damage. Cobicistat is a weak inhibitor of human renal transporters OCT2, MRP4, and MATE2-K, and is a more potent inhibitor of OCTN1 and MATE1, with similar potencies being found for RTV (m2.6.4, Section 7.2.4). These data, along with clinical data (m2.7.4), suggest that COBI reversibly blocks secretion of creatinine in humans. With no apparent pathological change in the kidney due to COBI, the routes of excretion differ for TFV and COBI, and that

COBI would not be expected to inhibit the major renal transporters of TFV at clinically relevant concentrations, it is not anticipated that the combination of E/C/F/TAF could exacerbate the renal toxicity of TAF. The small increase in serum creatinine levels may be associated with inhibition of MATE1 mediated creatinine secretion by COBI {28345}. In fact, in the Phase 3 pivotal trials, the E/C/F/TAF FDC produces a smaller increase in serum creatinine than the EVG/COBI/FTC/TDF FDC.

The mild hematological changes with FTC should not cause an overlapping toxicity with COBI which was associated with minimal decreases in RBC parameters in rats at exposures 5- to 8-fold higher than clinical exposures.

Cobicistat, EVG, and FTC have not shown any potential for bone toxicity in chronic rat and dog toxicity studies; thus, exacerbation of any TAF effects on bone is not expected.

None of the 4 compounds had positive findings in genotoxicity studies. Although EVG showed an equivocal effect in one in vitro study, it was negative in 2 in vivo studies and is unlikely to have the potential to induce chromosome aberrations in vivo. The combination of FTC and TDF in a mouse lymphoma cell assay did not exacerbate the genotoxic potential of TDF. The E/C/F/TAF FDC is not anticipated to alter the genotoxicity profiles of the individual agents.

Elvitegravir, COBI, FTC, and TDF/TFV have all demonstrated low carcinogenic potential in conventional 2-year bioassays. Combination dosing would not be expected to change these profiles, and no exacerbation of toxicity is expected.

Elvitegravir, COBI, FTC, and TAF have not shown significant adverse effects in reproductive and developmental toxicity studies, and the combination of the 4 components is not expected to have an altered reproductive toxicity profile compared with that of the individual agents.

Identified impurities and degradants have been assessed as part of the routine toxicology or qualification studies with the individual agents and with the EVG/COBI and FTC/TDF combinations.

The absence of nonclinical safety studies with the E/C/F/TAF combination is in accordance with the FDA Guidance for Industry, Nonclinical Safety Evaluation of Drug or Biologic Combinations, March 2006 and the CHMP Guideline on the Non-Clinical Development of Fixed Combinations of Medicinal Products (EMEA/CHMP/SWP/258498/2005, January 2008). There are no anticipated clinically relevant pharmacokinetic or toxicological interactions expected in the E/C/F/TAF FDC beyond the anticipated pharmacokinetic boosting of EVG by COBI. Further, extensive clinical safety data are available for the approved drugs FTC, TDF, the FTC/TDF FDC product, Truvada, and the EVG/COBI/FTC/TDF FDC product, Stribild, and support the overall risk/benefit of this new E/C/F/TAF FDC product for the treatment of HIV-1 infection.

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