SECTION 2.4
NONCLINICAL OVERVIEW

ELVITEGRAVIR/COBICISTAT/EMTRICITABINE/ TENOFOVIR DISOPROXIL FUMARATE SINGLE TABLET REGIMEN (EVG/COBI/FTC/TDF, QUAD STR)

NDA 203-100

Gilead Sciences

[redacted] 20[redacted]

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GLOSSARY OF ABBREVIATIONS AND DEFINITION OF TERMS

AAG	α1 acid glycoprotein
AhR	aryl hydrocarbon receptor (AHR gene product)
ALP	alkaline phosphatase
ALT	alanine aminotransferase
APD	action potential duration
APTT	activated partial thromboplastin time
ARV	antiretroviral
AST	aspartate aminotransferase
ATV	atazanavir (Reyataz®, Bristol-Myers Squibb)
AUC	area under the curve
AUC_{ss}	area under the plasma concentration curve at steady state
BCRP	breast cancer resistance protein (ABCG2)
BMD	bone mineral density
BUN	blood urea nitrogen
Caco-2	human colon carcinoma cell line
CC_{50}	drug concentration that results in a 50% reduction in cell viability
CHL	Chinese hamster lung
CHMP	Committee for Medicinal Products for Human Use
CHO	Chinese hamster ovary (cell line)
C_{max}	maximum observed concentration of drug in serum, plasma, or peripheral blood mononuclear cells
CNS	central nervous system
COBI	cobicistat (GS-9350)
COX II	cytochrome c oxidase II
CYP	cytochrome P450
dATP	deoxyadenosine triphosphate
dCTP	deoxycytidine triphosphate
DEXA	dual-emission X-ray absorptiometry
DHEA	dehydroepiandrosterone
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DRV	darunavir (Prezista®, Tibotec)
EAD	early after depolarization
EC_{50}	concentration of compound resulting in 50% of maximum effect
EC_{95}	concentration of compound resulting in 95% of maximum effect
ECG	electrocardiograph, electrocardiogram
EFV	efavirenz (Sustiva®, Bristol-Myers Squibb)
EFV/FTC/TDF	efavirenz/emtricitabine/ tenofovir DF (Atripla®, Gilead)

GLOSSARY OF ABBREVIATIONS AND DEFINITION OF TERMS (CONTINUED)

EMA	European Medicines Agency
EtOH	ethanol
EVG	elvitegravir
EVG/COBI/FTC/TDF	elvitegravir/cobicistat/emtricitabine/tenofovir DF (coformulated); QUAD
FDC	fixed-dose combination
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
GSI	Gilead Sciences, Inc.
HBV	hepatitis B virus
HCl	hydrochloric acid
HEK	human embryonic kidney (cell line)
hERG	human ether-à-go-go related gene
HIV-1	human immunodeficiency virus type 1
HS	human serum
HSA	human serum albumin
IC_{50}	concentration resulting in 50% of maximum inhibition
ICH	International Conference on Harmonization (of Technical Requirements for Registration of Pharmaceuticals for Human Use)
IN	integrase
INSTI	integrase strand transfer inhibitor
K_I	affinity constant for enzyme inactivation
k_{inact}	theoretical maximum enzyme inactivation rate
LC/MS/MS	liquid chromatography with tandem mass spectrometry
LD	lactation day
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)
MC	methylcellulose
MDCK	Madin-Darby canine kidney (cell line)
MDR1	multidrug resistance protein 1 (P-glycoprotein, Pgp)
MEL	minimal effect level
MRP1, 2, or 4	multidrug resistance associated protein 1, 2, or 4
MTD	maximal tolerated dose

GLOSSARY OF ABBREVIATIONS AND DEFINITION OF TERMS (CONTINUED)

mtDNA	mitochondrial DNA
NDA	new drug application
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)
OCT2	organic cation transporter 2 (SLC22A2)
OCTN1	organic cation transporter novel, type 1 (SLC22A4)
PBMC	peripheral blood mononuclear cell
PG	propylene glycol
PI	protease inhibitor
PMPA	tenofovir, TFV
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
RTV	ritonavir (Norvir®, Abbott)
S9	tissue post-mitochondrial (9,000 x g) supernatant
SEDDS	Self-Emulsifying Drug Delivery System
SIV	simian immunodeficiency virus
STR	single-tablet regimen
T4	thyroxine
TDF	tenofovir disoproxil fumarate, tenofovir DF (Viread®, Gilead)
TdP	Torsades de Pointes
TFV	tenofovir, PMPA
TFVpp	tenofovir diphosphate
TSH	thyroid stimulating hormone
TQT	thorough QT prolongation study according to ICH E14
TVD	emtricitabine/tenofovir DF, FTC/TDF (Truvada®, Gilead)
UGT	uridine diphosphate glucuronosyltransferase
US	United States
ZDV	Zidovudine, AZT (Retrovir®, GlaxoSmithKline)

NOTE TO REVIEWER

This application is being submitted in support of a new drug application (NDA) for a single tablet regimen (STR) that contains the active substances elvitegravir (EVG), cobicistat (COBI), emtricitabine (FTC), and tenofovir disoproxil fumarate (TDF). The STR is referred to as elvitegravir/cobicistat/emtricitabine/tenofovir disoproxil fumarate (EVG/COBI/FTC/TDF) STR throughout this document.

As the EVG and COBI components are new chemical entities, [redacted]. Per the agreement reached at the [redacted] 20[redacted] ([redacted]) meeting between Gilead Sciences, Inc. (Gilead) and the Food and Drug Administration (FDA; refer to the Agency's comments, dated [redacted] 20[redacted] in Module 1.6.3), this NDA is supported [redacted].

The efficacy and safety of FTC, TDF, and TVD as part of a regimen for the treatment of HIV-1 infection has been established through comprehensive programs of clinical and nonclinical studies with these medicinal products as submitted in the original NDAs for Emtriva 200-mg hard capsules (approved on 02 July 2003) and Emtriva 10-mg/mL oral solution (approved on 28 September 2005); Viread 300-mg film-coated tablets (approved on 26 October 2001); and Truvada film-coated tablets (approved on 02 August 2004). In addition, a variety of labeling and safety supplements have been submitted postapproval to update the prescribing information with emergent data. Periodic safety update reports (PSURs) and annual reports have been submitted in accordance with local requirements. Supplementary information either in accordance with specific postmarketing commitments or based on request by Health Authorities has been provided as appropriate.

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, TDF, FTC/TDF combination, and EVG/COBI/FTC/TDF combination studies.

The following conversions are provided to aid the reviewer:

- EVG 1 μM = 0.448 μg/mL
- COBI 1 μM = 0.776 μg/mL
- FTC 1 μM = 0.247 μg/mL
- TDF 1 μM = 0.636 μg/mL (fumarate salt); tenofovir (TFV) 1 μM = 0.287 μg/mL

1. NONCLINICAL OVERVIEW

This application is being submitted in support of a new drug application (NDA) for a single tablet regimen (STR) that contains a fixed-dose combination of elvitegravir (EVG), cobicistat (COBI), emtricitabine (FTC, Emtriva®), and tenofovir disoproxil fumarate (TDF, Viread®): the EVG/COBI/FTC/TDF (QUAD, 150/150/200/300 mg) tablet. The proposed indication for the EVG/COBI/FTC/TDF (QUAD) tablet is for use once daily as a complete regimen for the treatment of human immunodeficiency virus type 1 (HIV-1) infection in adults aged 18 years and over who are antiretroviral treatment naive or have no known substitutions associated with resistance to the individual components.

Elvitegravir is a new chemical entity that belongs to the new class of HIV-1 integrase strand-transfer inhibitors (INSTI) that prevent integration of HIV-1 genetic material into the host-cell genome. Cobicistat is a new chemical entity and structural analogue of ritonavir (RTV, r) with no antiretroviral activity. It is a more specific, mechanism-based cytochrome P450 3A (CYP3A) inhibitor than RTV that enhances or "boosts" the exposure of CYP3A substrates, including EVG. Gilead Sciences, Inc. (Gilead) has developed EVG and COBI for use within a new 4-drug fixed-dose combination tablet that also contains the current standard-of-care dual nucleoside/nucleotide reverse transcriptase inhibitor (N[t]RTI) backbone emtricitabine/tenofovir disoproxil fumarate (FTC/TDF, TVD, Truvada®).

The EVG/COBI/FTC/TDF (QUAD) tablet contains the same dosages of FTC and TDF that are currently approved within Viread, Emtriva, and Truvada for use in adults (200 mg of FTC and 300 mg of TDF). The dose of EVG (150 mg) was selected based on results from a Phase 1/2 pharmacokinetic/pharmacodynamic study (GS-US-183-0101), a Phase 2 study in heavily treatment-experienced HIV-1 infected subjects (GS-US-183-0105), and a Phase 1 biopharmaceutics/formulation study (GS-US-183-0140). The dose of COBI (150 mg) was selected based on the results from 2 studies in healthy volunteers (GS-US-216-0101 and GS-US-236-0101).

The EVG/COBI/FTC/TDF STR provides clinically equivalent exposures of EVG, COBI, FTC, and tenofovir (TFV) in comparison to the individual components, and its long-term safety and efficacy have been demonstrated in Phase 2 and Phase 3 clinical trials.

Individual presentations of FTC (Emtriva) and TDF (Viread) are currently approved for the treatment of HIV-1 in the United States (US), the European Union, and other countries worldwide for use in adults. In some regions, Emtriva and Viread are approved for use in adolescents; Emtriva, which is also available as an oral solution formulation, may be administered to children as young as 4 months of age. Emtricitabine and TDF are listed as preferred agents in US and international treatment guidelines {18889}, {18917}, {15964}, {14065}. It is proposed that EVG/COBI/FTC/TDF STR be indicated for the treatment of HIV-1 infection in adults and taken orally once daily with a meal.

Comprehensive programs of nonclinical studies with EVG, COBI, FTC, and TDF/TFV have been conducted. Information from all nonclinical studies with EVG, COBI, FTC, and TDF/TFV should be considered in the context of the substantial clinical experience with FTC and TDF within antiretroviral combination therapy for the treatment of HIV-1 infection, the Phase 2 clinical experience with EVG and COBI, and the Phase 2 and 3 clinical experience with EVG and COBI administered in combination with FTC and TDF as the EVG/COBI/FTC/TDF STR.

To facilitate the evaluation of the EVG/COBI/FTC/TDF STR, nonclinical virology studies of EVG, COBI, FTC, and TFV/TDF are described in detail in the virology summary contained in Module 2.7.2, Section 4.1, together with the clinical virology data, and discussed in Module 2.5, Section 4.

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, TDF, FTC/TDF combination, and EVG/COBI/FTC/TDF combination studies.

Nonclinical data for EVG and COBI are provided in Module 2.6; and nonclinical study reports are provided in Module 4. Results of FTC, TDF, and FTC/TDF studies are incorporated in Module 2.6 when needed to describe the presence or absence of overlapping findings; a listing of all the previously conducted and submitted FTC, TDF, and FTC/TDF studies are detailed in the cross-reference tables in Module 1.4.4. Safety pharmacology and toxicology studies conducted during the early development program for Gilead's investigational HIV-1 protease inhibitor (PI), GS-8374, that utilized COBI as a pharmacoenhancer, are briefly summarized in Module 2.6; however, as there were no new or unexpected findings in these studies, they are not discussed in Module 2.4.

The nonclinical data discussed within this document support the favorable benefit/risk profile for the proposed use of EVG/COBI/FTC/TDF STR for the treatment of HIV-1 infection in adults. 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 EVG/COBI/FTC/TDF STR. The overview is structured as a logical 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 EVG/COBI/FTC/TDF for the treatment of HIV-1 infected treatment-naive adults is included in the 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, TDF, 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.

A comprehensive nonclinical pharmacology/virology, pharmacokinetic, and toxicology program was undertaken in support of the registration of each of the individual agents. The results of the evaluations for FTC and TDF were presented in detail in the original new drug application and subsequent submissions for Emtriva and Viread, respectively. A small number of key studies were conducted using the combination of FTC and TDF; these studies were presented in detail in the original licensing application and subsequent submissions for Truvada.

1.1.1. EVG

Elvitegravir (EVG; 3-quinolinecarboxylic acid, 6-[(3-chloro-2-fluorophenyl)-methyl]-1,4-dihydro-1-[(1*S*)-1-(hydroxymethyl)-2-methylpropyl]-7-methoxy-4-oxo-) is a low molecular weight, human HIV-1 INSTI that has shown potent activity against laboratory viral strains and clinical isolates of HIV-1, HIV-2, and against HIV-1 isolates with resistance to NRTIs, nonnucleoside reverse transcriptase inhibitor (NNRTIs), and PIs, both in vitro and in vivo. Elvitegravir (once daily) is being evaluated in clinical studies in HIV-1 infected, treatment-experienced adults as an individual agent in combination with a background regimen, and in antiretroviral (ARV) treatment-naive adults as part of the EVG/COBI/FTC/TDF STR.

To facilitate the evaluation of the EVG/COBI/FTC/TDF STR, nonclinical virology studies of EVG are described in detail in the integrated virology summary contained in Module 2.7.2, Section 4.1, 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 novel antiretroviral 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, pre- 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 humans and the ability to achieve high systemic exposure. The nonclinical toxicity studies demonstrate that EVG is well tolerated for up to

6 months in the rat and 9 months 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 (COBI; 1,3-thiazol-5-ylmethyl [(*2R,5R*)-5-{[(*2S*)-2-[(methyl{[2-(propan-2-yl)-1,3-thiazol-4-yl]methyl}carbamoyl)amino]-4-(morpholin-4-yl)butanoyl]amino}-1,6-diphenylhexan-2-yl]carbamate) is a potent mechanism-based inhibitor of human CYP3A enzymes that can increase the bioavailability and decrease the clearance of other agents metabolized by CYP3A. Cobicistat is structurally related to RTV, but has no antiviral activity. Cobicistat is being developed as a pharmacoenhancer ("booster") to increase the systemic levels of coadministered agents metabolized by CYP3A enzymes, including EVG and the HIV PIs, atazanavir (ATV) and darunavir (DRV).

For COBI, aside from the ongoing mouse and rat carcinogenicity studies, 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; chronic toxicity studies in rats and dogs; genotoxicity; assessment of fertility, early embryonic development, pre- 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. Notwithstanding 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. Inhibition of CYP3A enzymes is briefly discussed in the primary pharmacodynamics section (Section 2.1), with more specific details provided in the drug interaction section (Section 3.8.1.2) and in Module 2.7.2, Summary of Clinical Pharmacology Studies, Section 4.1 to facilitate the presentation and interpretation of data relevant to the overall drug-drug interaction profile.

1.1.3. FTC

Emtricitabine (FTC; 5-fluoro-1-(2*R*,5*S*)-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine) 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 cellular

enzymes to form emtricitabine triphosphate (FTC-TP), the active metabolite. Emtricitabine is a nucleoside reverse transcriptase inhibitor (NRTI) that has activity against HIV and hepatitis B virus (HBV), and is indicated for use in combination with other antiretroviral agents in the treatment of HIV-1 infection.

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

1.1.4. TDF

Tenofovir disoproxil fumarate, (TDF; 9-[(R)-2-[[bis[[(isopropoxycarbonyl) oxy]methoxy]phosphinyl] methoxy]propyl] adenine fumarate (1:1)), is an oral prodrug of TFV (PMPA). After absorption, TDF is rapidly converted to TFV, which is metabolized intracellularly to the active metabolite, tenofovir diphosphate (TFVpp). Tenofovir is a nucleotide reverse transcriptase inhibitor (NtRTI) that has activity against retroviruses and hepadnaviruses, and is indicated for use in combination with other antiretroviral agents in the treatment of HIV-1 infection, and for the treatment of chronic HBV infection.

A comprehensive nonclinical pharmacology/virology, pharmacokinetic, and toxicology program was undertaken in support of the registration of TDF. The results of this evaluation were presented in detail in the original NDA and subsequent submissions for Viread.

1.1.5. EVG/COBI/FTC/TDF

As noted above, comprehensive nonclinical pharmacology/virology, pharmacokinetic, and toxicology programs were undertaken in support of the registration of FTC and TDF. Similarly for EVG and COBI, comprehensive nonclinical packages are included in this new drug application. A small number of key studies were conducted using the combination of EVG and COBI, FTC and TDF, and EVG/COBI/FTC/TDF. The overall program, including the data from the combination and individual agent studies, is considered sufficient to support the safety of the EVG/COBI/FTC/TDF combination tablets.

The absence of nonclinical safety studies with the EVG/COBI/FTC/TDF 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 relevant pharmacokinetic or toxicological interactions expected in the EVG/COBI/FTC/TDF combination beyond the anticipated pharmacokinetic boosting of EVG by COBI.

The well-characterized toxicity profiles of EVG, COBI, FTC, and TDF; the low potential for toxicologic interaction noted in the combination toxicology studies with EVG+COBI and FTC+TDF; along with the clinical safety data available for the approved agents FTC, TDF, the FTC/TDF FDC product Truvada; and the clinical data from Phase 1, 2, and 3 studies conducted with the EVG/COBI/FTC/TDF STR jointly support a favorable benefit/risk profile

for the proposed use of this new INSTI-based STR for the treatment of HIV-1 infection in adults.

2. PHARMACOLOGY/VIROLOGY

2.1. Primary Pharmacodynamics

The EVG/COBI/FTC/TDF STR contains 3 active antiviral agents (the standard of care N(t)RTI FTC/TDF backbone, and the INSTI, EVG) and COBI, a pharmacoenhancer that lacks antiviral activity. Nonclinical virology studies of EVG, COBI, 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 Module 2.7.2, Section 4.1, together with the clinical virology data. The primary pharmacodynamics of COBI and the secondary pharmacodynamics (excluding all virology data) and safety pharmacology of EVG, COBI, FTC, and TFV/TDF are summarized below.

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 also showed activity against HIV-2. 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 (see Module 2.7.2, Section 4.1).

Cobicistat is an efficient mechanism-based inhibitor of human CYP3A enzyme activity that lacks HIV-1 activity. Studies confirmed the specificity of CYP3A inhibition (Tabulated Summary 2.6.5.12.6, AD-216-2029 and AD-216-2070), the mechanism of inhibition, and also determined the enzyme inactivation parameters of COBI (Tabulated Summary 2.6.3.1.2, 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; Tabulated Summary 2.6.3.1.2, AD-216-2028). In the context of the STR, 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 (Tabulated Summary 2.6.5.10.4, JTK303-AD-017). Further information is provided in the drug-drug interaction section below (Section 3.8). More specific details are provided in Module 2.6.4, Pharmacokinetics Written Summary, Section 7 and in Module 2.7.2, Summary of Clinical Pharmacology Studies, Section 4.1 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 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}.

Tenofovir disoproxil fumarate, the oral prodrug of TFV, is a NtRTI. Tenofovir DF is converted to TFV by enterocytes and serum esterases. Intracellularly, TFV is then converted through 2 phosphorylation reactions to its active phosphorylated anabolite, TFVpp {1574}. Tenofovir diphosphate inhibits viral polymerases by direct binding competition with the natural deoxyribonucleotide substrate (deoxyadenosine triphosphate; dATP) and after incorporation into DNA, by DNA chain termination {1131}. The EC_{50} of TFV against wild-type HIV-1_{IIIB} is 1 to 6 μM in T lymphoblastoid cell lines and 0.2 to 0.4 μM in PBMCs {1574}, {39}. With clinical isolates of HIV-1, EC_{50} values ranged from 0.4 to 2.2 μM {5044}.

Tenofovir and FTC are analogues of 2 different nucleosides, adenosine and cytidine, respectively, and do not share a common intracellular metabolism pathway (see Section 3.6.1). Therefore, there should be no competition between TFV and FTC. In vitro analysis of intracellular phosphorylation demonstrated that activation of TFV to TFVpp was not influenced by FTC, and the activation of FTC to FTC-TP was not affected by the presence of TFV (Module 1.4.4, PC-164-2001).

The nonclinical virology studies of the EVG/FTC/TFV 3-drug combination, and the EVG/COBI/FTC/TFV 4-drug combination, are presented in detail in Module 2.7.2, Section 4.1.

In summary, EVG, FTC, and 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 TFV are phosphorylated intracellularly through nonoverlapping pathways, and in combination show no antagonism for the formation of their active anabolites. 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 the Module 2.7.2, Section 4.1.

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 to primary PBMCs (drug concentration that results in a 50% reduction in cell viability [CC_{50}] > 100 μM) (Tabulated Summary 2.6.3.1.4; JTK303-PH-010), primary T-lymphocytes (CC_{50} 40 μM), primary monocytes/macrophages (CC_{50} > 500 μM), and macrophages (CC_{50} 26 μM) (Tabulated Summary 2.6.3.1.4, 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 (selectivity index [SI] of > 48,000) in the absence of HS and 170 μM in the presence of 50% HS (SI of > 100,000; Tabulated Summary 2.6.3.1.4, 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; Tabulated Summary 2.6.3.1.4, 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 (Tabulated Summary 2.6.3.1.5, 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, and quiescent renal tubular epithelial cells (Module 1.4.4, P4331-00038). In addition, TFV showed no toxicity to myeloid and erythroid hematopoietic progenitor cells in vitro {4077}.

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 (Module 1.4.4, 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 (Module 1.4.4, TX-104-2001).

Collectively, these data demonstrate that EVG, COBI, FTC, and TFV all have low cytotoxicity and a large selectivity index 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 (Tabulated Summary 2.6.3.1.4; 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 ability of FTC, or TFV alone 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; Module 1.4.4, 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 mitochondrial DNA (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 + zidovudine (ZDV), with or without TFV, appeared to have greater cytotoxicity than the agents alone, but showed no increase in mitochondrial effects.

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

Specialized parameters such as serum lactate, assessment of mtDNA content, or ultrastructural analysis of target tissues were added to several nonclinical studies with FTC or TDF (including the woodchuck model, an established model for detecting mitochondrial toxicity of nucleosides [FTC Reports: Module 1.4.4; TOX628, TOX600, and TOX627; TDF Reports: Module 1.4.4, 97-TOX-4331-002, 97-TOX4331-001, R2000096, W2000042, and P2000078]). There was no evidence of mitochondrial injury in these studies.

Based on the experimental data and the clinical experience with the FTC/TDF regimen, the potential for mitochondrial toxicity is considered to be low. Further, as EVG and COBI are not anticipated to significantly increase the exposure of FTC or TFV, the potential for mitochondrial toxicity is low with the EVG/COBI/FTC/TDF STR. No additional nonclinical studies are therefore considered warranted with the combination of EVG, COBI, FTC, and TDF.

2.2.3. Off Target Activity

Elvitegravir showed no significant inhibition or stimulation of 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), IL-2 secretion, and mixed lymphocyte reaction (splenic lymphocytes; Tabulated Summary 2.6.3.1.4; 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 (Tabulated Summary 2.6.3.1.4; 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 (Tabulated Summary 2.6.7.17.1; 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 (Tabulated Summary 2.6.7.7.1; TX-183-2006, TX-183-2004, JTK303-TX-022, JTK303-TX-023). Further, 2-year carcinogenicity studies in mice and rats (Tabulated Summary 2.6.7.10; TX-183-2011 and TX-183-2012, respectively) 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 (Tabulated Summary 2.6.3.1.5; TX-168-2007 and TX-168-2011). At 10 μM, COBI demonstrated significant binding at calcium, potassium, and sodium ion channels. Additional electrophysiology studies conducted to evaluate the effects of COBI on the steady-state block of cardiac ion channels (potassium, calcium, and sodium channels) using patch clamp techniques are summarized in Section 2.3.2.

Emtricitabine had no pharmacologically significant binding affinity at 19 different receptors (Module 1.4.4; 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 (Module 1.4.4; TPZZ/92/0055).

Tenofovir and TDF showed no significant inhibition or stimulation of binding to a series of 111 protein targets (neuroreceptors, ion channels, transporters, and nuclear receptors) (Module 1.4.4; 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 (hyperlipidemia), 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 (Tabulated Summary 2.6.3.1.5; 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 (Tabulated Summary 2.6.3.1.5; PC-216-2004). The noted effects of COBI are unlikely to be significant at a maximal unbound clinical exposure of 0.09 μM (Module 2.7.2, Appendix 5.5, Table 2.2).

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) (Tabulated Summary 2.6.3.4.2; JTK303-SP-001), intestinal transport (Tabulated Summary 2.6.3.4.2; JTK303-SP-006), and renal/urinary systems (Tabulated Summary 2.6.3.4.2; 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 (Tabulated Summary 2.6.3.4.2; 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 (Tabulated Summary 2.6.3.4.1; 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 (Tabulated Summary 2.6.3.4.1; 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 (Tabulated Summary 2.6.3.4.4; 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) (Tabulated Summary 2.6.3.4.4; 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) (Tabulated Summary 2.6.3.4.3; 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 (Tabulated Summary 2.6.3.4.3; 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 (Tabulated Summary 2.6.3.4.4; 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 atazanavir (ATV), on QRS and QT intervals, monophasic action potential duration (MAPD), or triangulation; and there were no EADs (Tabulated Summary 2.6.3.4.3; 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 (Tabulated Summary 2.6.3.4.4; 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 Cmax at the 150 mg dose (Module 2.7.2, Appendix 5.5, Table 2.2). 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 (Module 2.6.6, Section 3.2, [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 (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 Cmax at the 150 mg dose (Module 2.7.2, Appendix 5.5, Table 2.2). In a thorough QT clinical study (Module 2.7.2, Section 2.3.2.2.1 [Study 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 (Module 2.7.2, Section 2.3.2.2.2).

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) (Module 1.4.4; 477, TPZZ/92/0056, TPZZ/93/0001, TPZZ/93/0119, and TPZZ/92/0057). No effects on the cardiovascular system were reported in anesthetized dogs given a cumulative dose of 38.5 mg/kg of FTC intravenously over a 1-hour period (Module 1.4.4, 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 given the 200 mg dose (Module 1.4.4; TOX600, TOX627, and TOX032).

2.3.4. TDF

Tenofovir DF was evaluated in safety pharmacology studies of the CNS, cardiovascular system, GI system, and renal system. There were no adverse effects detected in the CNS in rats dosed at 500 mg/kg (Module 1.4.4, R990152), or of the cardiovascular system of dogs dosed at 30 mg/kg (Module 1.4.4, D990155). There was reduced gastric emptying in rats dosed at 500 mg/kg, but not at 50 mg/kg (Module 1.4.4, R990153). There was increased urinary electrolyte excretion and urine volume in rats dosed at 500 mg/kg, but not at 50 mg/kg (Module 1.4.4, R990154).

2.3.5. EVG/COBI/FTC/TDF

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. Neither EVG, FTC, nor TDF had significant unwanted pharmacologic activity as determined in a variety of in vitro and in vivo 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, or within the EVG/COBI/FTC/TDF STR (Module 2.7.4, Section 4.2).

Given the lack of effects for EVG, FTC, and TDF on the cardiovascular system, no additional studies on the cardiovascular system with the combination of EVG/COBI/FTC/TDF are considered warranted.

2.4. Pharmacodynamic Drug Interactions

The potential for pharmacodynamic drug interactions for EVG, COBI, FTC, TDF, FTC/TDF, and EVG/COBI/FTC/TDF are presented in detail in the nonclinical virology summary contained in Module 2.7.2, Section 4.1.

2.5. Summary of Pharmacology

The HIV-1 INSTI, EVG, and the 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, and TFV/TDF are summarized and described in detail in the virology summary contained in Module 2.7.2, 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 TDF have low in vitro cytotoxicity in a variety of human cell types.

Nucleoside reverse transcriptase inhibitors carry a class labeling for mitochondrial toxicity; however, both FTC and TDF 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 exposure of FTC and TDF, the potential for mitochondrial toxicity with the EVG/COBI/FTC/TDF combination is low.

Elvitegravir, FTC, and TDF 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 STR (assuming 6.3% fraction unbound from equilibrium dialysis experiments; Module 2.6.4, Section 4.1.3 [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 (Module 2.7.2, Section 2.4.1.1 and 2.4.1.2 [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 expected to be low in HIV-1 infected patients. Given the favorable safety pharmacology profiles of EVG, FTC, and TDF, combination of these 3 agents with COBI is not expected to exacerbate the potential for minor cardiovascular effects with COBI. Thus, additional safety pharmacology studies on the EVG/COBI/FTC/TDF combination are considered unwarranted.

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

3. PHARMACOKINETICS

The absorption, distribution, metabolism, and excretion of EVG, COBI, FTC, and TFV/TDF were evaluated in vitro and in nonclinical species in vivo. 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), all bioanalytical methods for toxicokinetic analysis supporting GLP safety studies were validated. All other bioanalytical methods were conducted using appropriate protocols and documentation to assure data integrity (Module 2.6.4, Pharmacokinetics Written Summary, Section 2).

3.2. 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 (Tabulated Summary 2.6.5.3.1, JTK303-AD-026). While polarized transport of EVG could be demonstrated in MDR1-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 (Tabulated Summary 2.6.5.3.2, 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%; Tabulated Summary 2.6.5.3.3, 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 (Tabulated Summary 2.6.5.3.4, JTK303-AD-010 and JTK303-AD-012). As with the rat, absorption after oral dosing was rapid, and bioavailability was moderate (26%–33%) and similar to that for total radioactivity (41%; Tabulated Summary 2.6.5.3.5, JTK303-AD-006 and JTK303-AD-008).

3.2.2. COBI

In Caco-2 cell monolayers, COBI showed high forward permeability and no evidence for efflux (Tabulated Summary 2.6.5.3.7, AD-216-2023). Single-dose pharmacokinetics were studied in rats (Tabulated Summary 2.6.5.3.9, AD-216-2020), dogs (Tabulated Summary 2.6.5.3.10, AD-216-2021), and monkeys (Tabulated Summary 2.6.5.3.12, 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 (Module 1.4.4; TEIN/93/0003, TEIN/93/0004, and IUW00101, respectively), rats {4570}, and cynomolgus monkeys (Module 1.4.4, 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. TDF

In Caco-2 cell monolayers, TDF showed concentration-dependent permeability, with efflux saturated at high concentrations likely achieved in the intestinal lumen, and TFV showed modest permeability with low efflux (Module 1.4.4, AD-104-2010). Single-dose pharmacokinetics of TFV following oral administration of TDF have been examined in mice (Module 1.4.4, M990203-PK and 97-TOX-4331-008-PK, respectively), rats (Module 1.4.4, 96-TOX-4331-003-PK; R2000036-PK; 97-TOX-4331-002-PK; and R990204, respectively), woodchucks (Module 1.4.4, W2000108), dogs (Module 1.4.4, D2000076; 96-TOX-4331-004-PK; 98-TOX-4331-003-PK; and 97-TOX-4331-001-PK, respectively), and monkeys (Module 1.4.4, P2000031). Following oral administration of TDF, absorption and conversion to TFV was rapid, with maximal concentrations of TFV in plasma reached between 0.25 to 1.5 hours postdosing in all species, and declined in a biphasic manner. The observed terminal half life values were approximately 7, 9, and 60 hours in rats, monkeys, and dogs, respectively. The oral bioavailability of TFV following oral administration of TDF ranged from 20% to 46% in these species.

3.2.5. EVG/COBI/FTC/TDF

Although formal nonclinical studies of the absorption kinetics of the EVG/COBI/FTC/TDF combination have not been conducted, a single-dose study of the exposure after oral administration of a prototype 4-drug STR demonstrated generally similar systemic exposure to all agents in comparison to that seen after coadministration of the individual agents (Tabulated Summary 2.6.5.3.13, AD-216-2061). Comprehensive pharmacokinetic clinical studies with the EVG/COBI/FTC/TDF combination have been performed (Module 2.7.2).

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 (Tabulated Summary 2.6.5.4.1, 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; Tabulated Summaries 2.6.7.7.1.1 and 2.6.7.10.1; TX-183-2004 and TX-183-2011, respectively), rats (100 to 2000 mg/kg/day; Tabulated Summaries 2.6.7.7.1.2, 2.6.7.7.1.3, 2.6.7.7.1.4 and

2.6.7.10.2; JTK303-TX-003, JTK303-TX-021, JTK303-TX-022 and TX-183-2012, respectively), and dogs (10 to 100 mg/kg/day; Tabulated Summaries 2.6.7.7.1.6 and 2.6.7.7.1.7; JTK303-TX-004 and JTK303-TX-023, respectively) 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 (Tabulated Summary 2.6.7.10.1, TX-183-2011) and rats (Tabulated Summary 2.6.7.7.1.5, TX-183-2007) and in combination with COBI in rats (Tabulated Summaries 2.6.7.7.3.1 and 2.6.7.17.3; TX-236-2001 and TX-236-2002, respectively). EVG exposures were modestly higher when co-dosed with RTV or COBI.

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; Tabulated Summaries 2.6.7.6.2, 2.6.7.6.2 and 2.6.7.7.2.1; TX-216-2032, TX-216-2041 and TX-216-2026, respectively), rats (10 to 100 mg/kg/day; Tabulated Summaries 2.6.7.7.2.2, and 2.6.7.7.2.3; TX-216-2004 and TX-216-2017, respectively), and dogs (5 to 45 mg/kg/day; Tabulated Summaries 2.6.7.7.2.5 and 2.6.7.7.2.6; TX-216-2005 and TX-216-2016, respectively) dosed for periods of 4 weeks to 39 weeks. Toxicokinetic parameters are presented in Module 2.6.6.

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 (Tabulated Summary 2.6.5.12.11, AD-216-2039) but did not activate human PXR or induce human drug metabolizing enzymes or multidrug resistance protein 1 (MDR1) in hepatocytes (Tabulated Summaries 2.6.5.12.9 and 2.6.5.12.10; AD-216-2027 and AD-216-2071).

Multiple dose toxicology studies in rats were also performed with COBI in combination with EVG (Tabulated Summaries 2.6.7.7.3.1 and 2.6.7.17.3; TX-236-2001 and TX-236-2002, respectively) and ATV (Tabulated Summary 2.6.7.7.2.4, 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; Module 1.4.4, TOX109; IUW00701, TOX599, and TOX628), rats (60 to 3000 mg/kg/day; Module 1.4.4, TOX108; TOX097), and monkeys (40 to 2000 mg/kg/day; Module 1.4.4, TOX600, TOX627 and TOX032 respectively) 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. TDF

The multiple-dose pharmacokinetics of TFV were characterized in toxicokinetic studies following oral administration of TDF in mice (Module 1.4.4, M990203-PK), rats (Module 1.4.4; 96-TOX-4331-003-PK, R2000036-PK, 97-TOX-4331-002-PK, and R990204), dogs, (Module 1.4.4; 96-TOX-4331-004-PK, 98-TOX-4331-003-PK, 97-TOX-4331-001-PK, and 96-TOX-4331-001-PK), and monkeys (Module 1.4.4, P2000078-PK). No apparent changes in exposure were observed after repeat dosing in mice (100 to 1000 mg/kg/day), rats (30 to 1000 mg/kg/day), or monkeys (30 to 600 mg/kg/day) dosed for periods of 56 days to 104 weeks. In contrast, dogs showed a 2- to 3-fold increase in exposure over time and generally reached steady-state by Day 28, with daily TDF doses of 10 or 30 mg/kg/day.

3.3.5. EVG/COBI/FTC/TDF

Comprehensive pharmacokinetic analysis after multiple dosing of the 4-drug STR has been performed in clinical studies (Module 2.7.2).

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 (Tabulated Summary 2.6.5.6.2, 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 (Module 2.7.2, Sections 2.4.1.1 and 2.4.1.2, respectively [GS-US-183-0133 and GS-US-216-0124, respectively]). Elvitegravir does not distribute well into the cellular fraction of blood from rat, dog, monkey, or human in vitro (Tabulated Summary 2.6.5.8.1, JTK303-AD-013). The whole blood/plasma ratio for human blood was 0.7 and this value was confirmed in an in vivo study (Module 2.7.2, Section 2.2.1.3 [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 (Tabulated Summary 2.6.5.6.3, 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 (Module 2.7.2, Sections 2.4.1.1 and 2.4.1.2, respectively [GS-US-183-0133 and GS-US-216-0124, respectively]). 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 (Module 1.4.4, TBZZ/93/0025).

3.4.1.4. TFV

The protein binding of TFV was very low (< 10 %) in the plasma and serum of humans and all other species examined (Module 1.4.4, P0504-00039).

3.4.1.5. EVG/COBI/FTC/TDF

Although plasma protein (primarily to albumin) binding of EVG is high, and moderate for COBI, 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 EVG/COBI/FTC/TDF combination 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 (Tabulated Summary 2.6.5.5.1, 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; Tabulated Summary 2.6.5.5.2, 60N-0518).

3.4.2.2. COBI

After oral administration of [^{14}C]COBI to albino and pigmented rats (Tabulated Summaries 2.6.5.5.5 and 2.6.5.5.6, 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 (Module 1.4.4, 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 central nervous system tissues were 2% to 10% of the concentration in plasma.

3.4.2.4. TDF and TFV

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 (Module 1.4.4, Report 95-DDM-1278-002). One hour following an oral dose of [^{14}C]TDF (10 mg/kg) in dogs, radioactivity was detected in all tissues, except brain. The majority of the radioactivity was present in the contents of the GI tract, jejunal tissue, and liver (> 66%). Concentrations of radioactivity were highest in bile, kidney, liver, and jejunum. Tissue concentrations of radioactivity declined largely in parallel with those in plasma. At 24 hours postdose, the last time point sampled, the highest concentrations of radioactivity were in kidney, liver, and the intestinal contents (Module 1.4.4, 97-DDM-4331-001).

3.4.2.5. EVG/COBI/FTC/TDF

A study comparing the tissue distribution of [^{14}C]EVG in rats in the absence or presence of RTV pretreatment revealed no qualitative differences (including no penetration of EVG to the CNS) (Tabulated Summary 2.6.5.5.2, 60N-0518). Since COBI has similar or lower

potency than RTV as an inhibitor of transporters (Section 3.8.2.2), no changes in distribution of EVG/COBI/FTC/TDF given in combination would be expected.

3.5. Distribution in Pregnant or Nursing Animals

3.5.1. Distribution in Pregnant Animals

Pharmacokinetics parameters for EVG were determined after single and multiple doses in pregnant rats (Tabulated Summaries 2.6.7.13.1 and 2.6.7.13.2; JTK303-TX-020, TX-183-2008, respectively) and rabbits (Tabulated Summaries 2.6.7.11.1 and 2.6.7.13.3; TX-183-2001, TX-183-2002, respectively). Exposure in pregnant rats was generally similar to that in nonpregnant animals.

Pharmacokinetic parameters for COBI were determined in pregnant rats (Tabulated Summaries 2.6.7.11.2 and 2.6.7.13.4; TX-216-2018 and TX-216-2020, respectively) and rabbits (Tabulated Summaries 2.6.7.11.2 and 2.6.7.13.5; TX-216-2019 and TX-216-2021, respectively). Exposure in pregnant rats was generally similar to that in nonpregnant animals.

Pharmacokinetic parameters for TDF and FTC in pregnant animals appeared to be generally similar to those reported for nonpregnant animals. 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 Module 1.4.4, 96-DDM-1278-005, TOX103, and TOX038, respectively).

3.6. Metabolism

3.6.1. Intracellular Metabolism

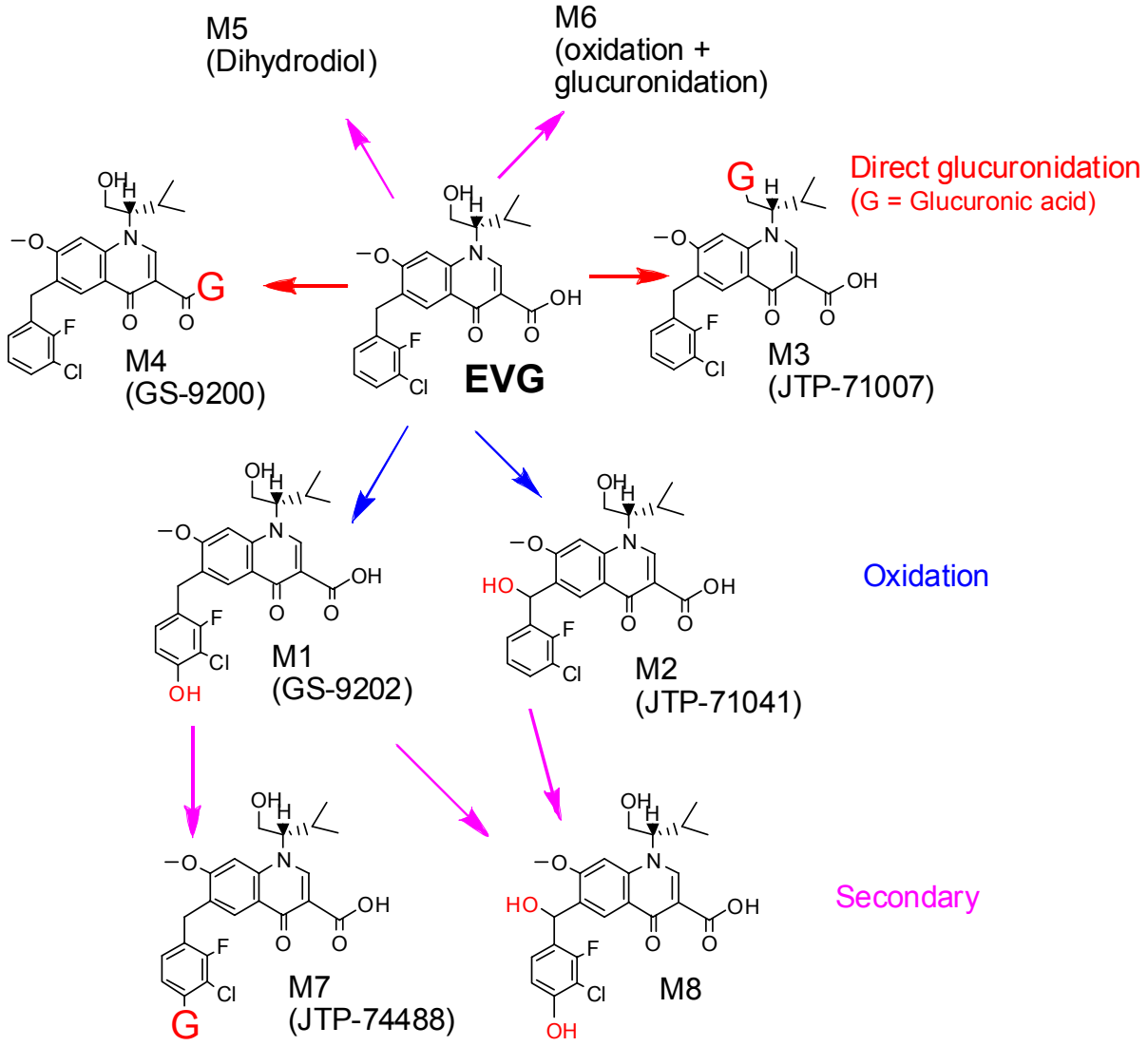
Emtricitabine and TFV are analogues of 2 different nucleosides, cytosine and adenosine, respectively, and do not share a common intracellular metabolism pathway. In experiments where both drugs were incubated together at concentrations higher than achieved in the plasma (10 μM), the intracellular phosphorylation of FTC and TFV to their active intracellular anabolites was not affected (Module 1.4.4, PC-164-2001).

3.6.2. Routes of Metabolism

3.6.2.1. EVG

The primary metabolic pathways for EVG are illustrated in Figure 1 and are hydroxylation to M1 (GS-9202), catalyzed by CYP3A (Tabulated Summary 2.6.5.10.4, JTK303-AD-017), and glucuronidation at the carboxylic acid moiety, catalyzed by UGT1A1 and UGT1A3 in humans (Tabulated Summary 2.6.5.10.6, 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

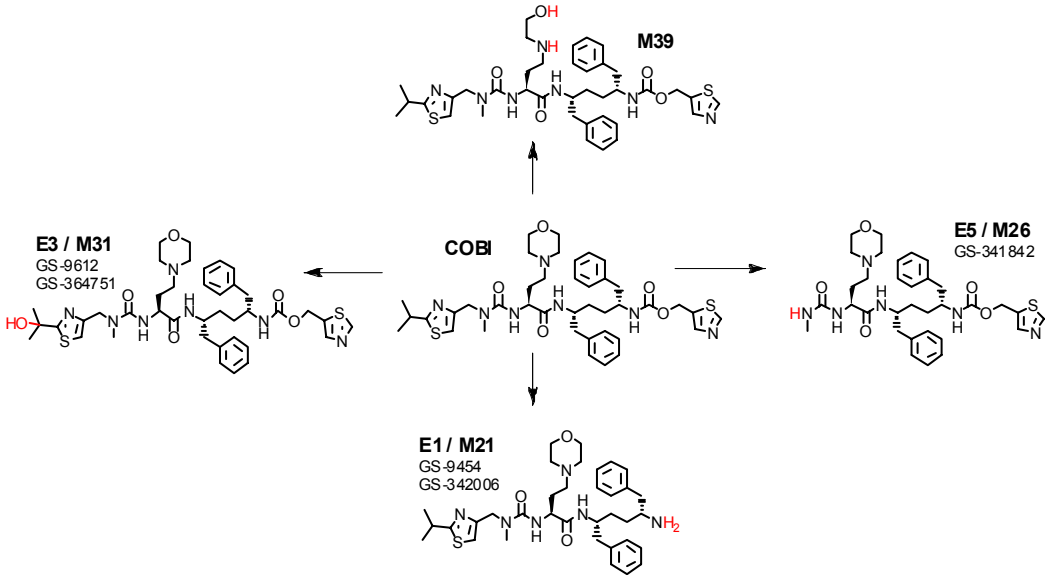


3.6.2.2. COBI

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) (Tabulated Summary 2.6.5.10.8, 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 (Tabulated Summaries 2.6.5.10.7 and 2.6.5.10.8, AD-216-2024, AD-216-2074, and AD-216-2025, respectively).

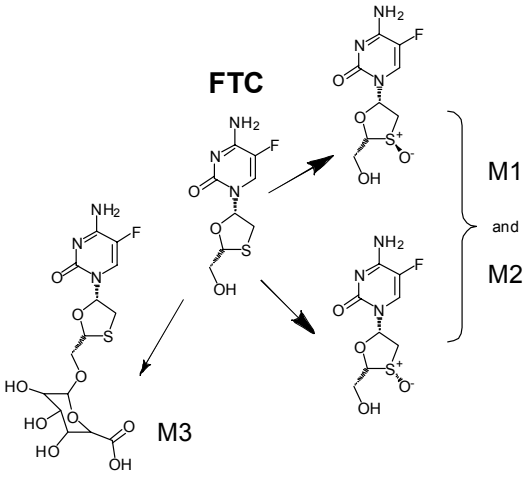
Figure 2. Proposed Primary Metabolic Pathway of COBI



3.6.2.3. FTC

Emtricitabine was 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 (Module 1.4.4; 15396 v1). A minor direct glucuronide metabolite, M3, was also detected (Figure 3) {4570}.

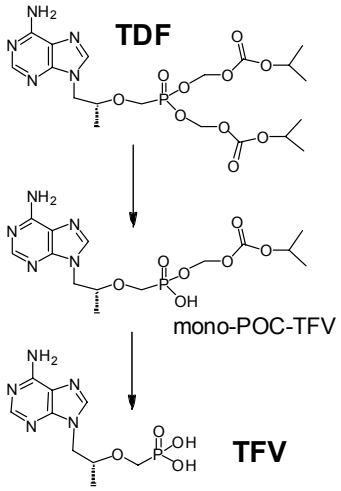
Figure 3. Oxidative Metabolism of FTC



3.6.2.4. TDF and TFV

There was no detectable oxidation or conjugation of TDF or TFV. The only metabolic pathway was deesterification of TDF to TFV (Figure 4) (Module 1.4.4; P4331-00015.1).

Figure 4. Metabolites of TDF



3.6.2.5. EVG/COBI/FTC/TDF

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 (Module 2.7.2, Section 4.1). 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.

3.6.3. In Vivo Metabolism

3.6.3.1. EVG

After oral or intravenous administration of [^{14}C]EVG to rats (Tabulated Summary 2.6.5.9.1, JTK303-AD-019) and dogs (Tabulated Summary 2.6.5.9.2, 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 3.7), significant enterohepatic recirculation is unlikely. A similar pattern was seen in dog feces with EVG and oxidative metabolites being the most abundant analytes.

3.6.3.2. COBI

After oral administration of [^{14}C]COBI to mice (Tabulated Summary 2.6.5.9.3, AD-216-2073), rats (Tabulated Summary 2.6.5.9.4, AD-216-2082), and dogs (Tabulated Summary 2.6.5.9.5, 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).

3.6.3.3. 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} (Module 1.4.4, TEIN/93/0015, TEIN/93/0016, and TOX063, respectively). 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.

3.6.3.4. TDF and TFV

Tenofovir was primarily eliminated by renal excretion of unchanged parent drug and was not extensively metabolized in rats. Following oral administration of TDF in rats, tenofovir soproxil accounted for 37% of the radioactivity in the 0.5- to 2.2-hour bile fraction, but was not detected at later time points. The total percent of the dose excreted in bile was 0.12%, which was consistent with findings in other studies that biliary excretion is a minor route of elimination (Module 1.4.4, 96-DDM-1278-001 and 97-DDM-4331-003).

3.6.3.5. EVG/COBI/FTC/TDF

As discussed in the previous section (Section 3.6.2), 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.

3.7. Excretion

3.7.1. Recovery in Excreta

3.7.1.1. EVG

After intravenous or oral administration of [^{14}C]EVG to rats (Tabulated Summary 2.6.5.13.1, JTK303-AD-005) and dogs (Tabulated Summary 2.6.5.13.3, 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 bile duct-cannulated rats, an average of 25.0% of radioactivity was recovered in bile and 69.2% in feces (Tabulated Summary 2.6.5.14.1, JTK303-AD-005). Intraduodenal administration of collected bile to naive bile duct-cannulated 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.

3.7.1.2. COBI

After oral administration of [^{14}C]COBI to mice (Tabulated Summary 2.6.5.13.4, AD-216-2073), rats (Tabulated Summary 2.6.5.13.5, AD-216-2034), and dogs (Tabulated Summary 2.6.5.13.6, 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 bile duct-cannulated animals, an average of 69.3% and 63.9% of dosed radioactivity was recovered in bile in rats (Tabulated Summary 2.6.5.13.5, AD-216-2034) and dogs (Tabulated Summary 2.6.5.14.3, AD-216-2068), respectively.

3.7.1.3. FTC

The primary route of elimination of [^{3}H]FTC and [^{14}C]FTC was via renal excretion of parent drug after oral and intravenous administration in mice, rats, and cynomolgus monkeys {4570}, (Module 1.4.4, 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.

3.7.1.4. TDF and TFV

Renal excretion is the primary systemic route of elimination of TFV in all preclinical species tested. The majority of radioactivity was recovered in the urine in rats and dogs (Module 1.4.4, 96-DDM-1278-001 and 97-DDM-4331-001, respectively) following intravenous administration of [^{14}C]TFV. Less than 0.3% of radioactivity was recovered in bile from bile duct-cannulated animals following oral administration of [^{14}C]TDF to rats and intravenous administration of [^{14}C]TFV to dogs. The radioactivity recovered in the feces following oral administration of [^{14}C]TDF represents primarily unabsorbed drug.

3.7.1.5. EVG/COBI/FTC/TDF

Given that the excretion routes of the individual components have been well characterized, EVG and COBI and their metabolites in bile and FTC and TFV in urine, interactions between the compounds during excretion are unlikely. The potential for transporter-dependent interactions is explored and discussed in Section 3.8.2.

3.7.2. Excretion into Breast Milk

Excretion of EVG in rat milk was studied as part of a prenatal and postnatal developmental toxicology study in rats (Tabulated Summary 2.6.7.14.1, 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 (Tabulated Summary 2.6.7.14.2, 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 (Module 1.4.4; R990202-PK; P2000116). The TFV milk to plasma ratios ranged from 0.11 to 0.24 in rats and 0.19 to 0.22 in rhesus monkeys.

The proposed Prescribing Information for the QUAD STR indicates that because of both the potential for HIV transmission and the potential for serious adverse reactions in nursing infants, mothers should be instructed not to breastfeed if they are receiving the QUAD STR (see Section 5.2).

3.8. 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 EVG/COBI/FTC/TDF STR (Module 2.7.2, Appendix 5.5) are summarized in Table 1.

Table 1. Steady State Pharmacokinetic Parameters for STR Components

Parameter	EVG	COBI	FTC	TDF, TFV
Dose (mg)	150	150	200	300[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 TDF dose

3.8.1. Metabolic Drug Interactions

3.8.1.1. EVG

Elvitegravir showed no detectable inhibition of activities catalyzed by CYP1A2, 2A6, 2C9, 2C19, 2D6, or 2E1 in human hepatic microsomal fraction (Tabulated Summary 2.6.5.12.1, JTK303-AD-027). Inhibition of CYP3A activity was very weak (IC_{50} 28.3 μM). The K_M for the metabolism of EVG to M4, the acyl glucuronide metabolite, was 21 μM, suggesting low potential for inhibition of UGT1A1 or 1A3, the catalysts of this reaction (Tabulated Summary 2.6.5.15.3, AD-183-2028).

3.8.1.2. 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 (Tabulated Summary 2.6.5.12.4, 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) (Tabulated Summary 2.6.5.12.6, AD 216-2029 and 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 efavirenz (CYP2B6) and little effect on the pharmacokinetics of desipramine (CYP2D6) (Module 2.7.2, Section 2.3.2.2.3 [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) (Tabulated Summary 2.6.5.12.8, AD-216-2075).

3.8.1.3. 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 (Module 1.4.4, 15247).

3.8.1.4. TDF and TFV

At concentrations substantially higher (~ 300-fold) than those observed in vivo, TFV or TDF did not significantly inhibit CYP1A2, 2C9, 2D6, 2E1, or 3A in human hepatic microsomal fractions (Module 1.4.4, V990172-104).

3.8.1.5. EVG/COBI/FTC/TDF

Because of their lack of potency for CYP inhibition, FTC, TDF, and TFV should not affect the metabolism of EVG or COBI. 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 (Module 2.7.2, Section 4.1). The clinical drug-drug interaction studies are described in detail in the Summary of Clinical Pharmacology Studies (Module 2.7.2, Section 3.4).

3.8.2. Transporter Drug Interactions

3.8.2.1. EVG

As described above (Section 3.2.1), transport of EVG can be detected in cells overexpressing human MDR1 but there is no evidence for efflux-limitation of absorption in vivo. With digoxin as the substrate, EVG affected human MDR1-dependent transport only at a concentration (30 μM) above its aqueous solubility (Tabulated Summary 2.6.5.15.4, JTK303-AD-026). Elvitegravir was a moderate inhibitor of human OATP1 (< 40% inhibition at 2 μM), but a more potent inhibitor of human OATP1B3 (IC_{50} 0.44 μM; Tabulated Summary 2.6.5.15.5, 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 (Module 2.7.2, Section 2.5.2.2.3 [GS-US-216-0123]).

3.8.2.2. COBI

The effects of COBI and RTV on the activities of human transporters are summarized in Table 2.

Table 2. Effects of COBI and RTV on the Activities of Human Transporters

Transporter	Cell line	Substrate (concentration)	IC_{50} (µM)		Tabulated Summary (Report)
			COBI	RTV	
MDR1	MDCK II	calcein AM (10 µM)	22.5 – 45.0[a]	10.0 – 20.0[a]	2.6.5.15.9 (AD-216-2030)
MRP1	MDCK II	calcein AM (10 µM)	45.0 – 90.0[a]	10.0 – 20.0[a]	
MRP2	MDCK II	calcein[b]	45.0 – 90.0[a]	> 20[d]	
MRP4	LLC-PK1[c]	DHEAS (0.02 µM)	20.7	> 20[d]	2.6.5.15.16 (AD-216-2105)
BCRP	MDCK II	Hoechst 33342 (10 µM)	59.0	> 20[d]	2.6.5.15.10 (AD-216-2099)
OAT1	CHO	p-aminohippurate (5 µM)	> 100[d]	> 20[d]	2.6.5.15.16 (AD-216-2105)
OAT3	HEK293	estrone 3-sulfate (0.2 µM)	> 100[d]	8.46	
OCT2	CHO	metformin (2 µM)	8.24	22.6	2.6.5.15.12 (AD-216-2093)
OCTN1	S_2	tetraethylammonium (5 µM)	2.49	2.08	2.6.5.15.14 (AD-216-2098)
MATE1	HEK293	tetraethylammonium (5 µM)	1.87	1.34	2.6.5.15.13 (AD-216-2094)
MATE2-K	HEK293	tetraethylammonium (5 µM)	33.5	100	
OATP1B1	CHO	Fluo 3 (2 µM)	3.50	2.05	2.6.5.15.11 (AD-216-2100)
OATP1B3	CHO	Fluo 3 (2 µM)	1.88	1.83	

AM = acetomethoxy ester; BCRP = breast cancer resistance protein; COBI = cobicistat; DHEAS: 5-dehydroepiandrosterone sulfate; MATE1 = multidrug and toxin extrusion protein 1 (SLC47A1); MATE2-K = multidrug and toxin extrusion protein 2-K (SLC47A2); MDR1 = P-glycoprotein (multidrug resistance protein 1); MRP = multi-drug resistance-associated protein; OAT = organic anion transporter; OATP = organic anion transporting polypeptide; OCT2 = organic cation transporter 2; OCTN1 = organic cation transporter N1; RTV = ritonavir

a Range of tested concentrations bracketing 50% inhibition (IC_{50} not calculated)

b Generated from 10 µM calcein AM

c Study performed with vesicles derived from the cell line

d Maximum concentration tested

At systemic concentrations achieved in plasma at the 150 mg COBI dose, COBI would not inhibit the drug transporters MDR1, MRP1, MRP2, BCRP, 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 MDR1 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, MATE2-K and OCT2, and a more potent inhibitor of MATE1 and 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 (Module 2.7.2, Section 2.3.2.2.4, 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}.

3.8.2.3. FTC, TDF and TFV

There is no clinical evidence for FTC interacting with drug transporters, as a substrate or inhibitor, so in vitro transport studies have not been performed. In vitro studies have shown that the intestinal absorption of TDF is affected by MDR1-dependent efflux transport and esterase degradation {5939}. Clinical data suggest that HIV PIs can affect the absorption of TDF and studies in human intestinal S9 fractions, the Caco-2 cell line, and cells overexpressing human MDR1 suggest that the relative ability of the PIs to inhibit esterase activity and inhibit or induce intestinal P-gp may account for the modest changes in plasma TFV levels when TDF is coadministered with some PIs (Module 1.4.4, AD-104-2010), {11255}. The route of elimination of TFV is renal excretion by a combination of glomerular filtration and tubular secretion. 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 (Module 1.4.4, PC-103-2001, AD-104-2001, AD-104-2002), {2520}, {7299}, {8418}, {9318}, {10260}, {11309}. Human OAT3 may play a secondary role in the tubular uptake of TFV. Neither MDR1 nor MRP2 appear to be involved in the tubular efflux of TFV. A number of other renally secreted therapeutics, including antibiotics, anti-inflammatory agents, and other antivirals (including PIs) did not inhibit OAT1-mediated transport of TFV, indicating a low potential for renal interactions with TFV (Module 1.4.4, PC-104-2010 and PC-104-2011, respectively), {9863}. Further, the PIs ATV, lopinavir, and RTV did not inhibit transport of TFV mediated by MRP4 {8418}. A lack of interaction between COBI and OAT1 or MRP4 has been described above (Section 3.8.2.2).

3.8.2.4. EVG/COBI/FTC/TDF

As discussed above (Section 3.8.2.2), due to the noted inhibition by COBI of MDR1 and/or BCRP, a modest increase in TFV exposure due to COBI inhibition of the intestinal efflux of TDF by MDR1 and/or BCRP is predicted, and has been observed in clinical studies. The increase in exposure is of similar magnitude to that seen when TDF is co-dosed with RTV-boosted HIV PIs {11255}. Since, as described above, COBI is a weak inhibitor of OAT1 and MRP4, the transporters responsible for the renal transport of TFV, no effects on urinary excretion of TFV are expected. Both EVG and COBI are inhibitors of OATP1B1 and

OATP1B3, but there was only a modest increase in the exposure of the OATP substrate, rosuvastatin, when it was co-dosed with the 2 agents (Module 2.7.2, Section 2.5.2.2.3 [GS-US-216-0123]) suggesting no synergistic interaction.

While COBI is a weak inhibitor of OCT2 and a more potent inhibitor of MATE1, TFV is not an inhibitor of either transporter (Module 1.4.4, AD-104-2012) so there should be no further decrease in activity of these transporters when the 2 drugs are combined.

3.8.3. Induction Liability

3.8.3.1. 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 (Tabulated Summary 2.6.5.12.2, JTK303-AD-023). In the context of the STR, any increases in CYP3A activity due to induction would be masked by the potent inhibition by COBI and not clinically significant.

3.8.3.2. 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 10.1-fold activation by 10 µM RTV; Tabulated Summary 2.6.5.12.9, 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% (Tabulated Summary 2.6.5.12.10, 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, MDR1 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 Tabulated Summary 2.6.5.12.11, AD-216-2039).

3.8.3.3. FTC and TDF

Emtricitabine did not activate human AhR or PXR at concentrations up to 50 µM (Module 1.4.4, AD-162-2005). There is no clinical evidence for TDF acting as an inducer so this property was not examined in vitro.

3.9. Summary of Pharmacokinetics

A comprehensive nonclinical program defining the absorption, disposition, metabolism, and drug interaction potential of EVG, COBI, FTC, and TFV/TDF 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 and the FTC/TDF combination, 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 (Module 2.7.2, Section 2.2.1.3). 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 (Module 2.7.2, Section 4.1). The most abundant metabolites, M4 and M1, were quantified in mouse, rat, and dog repeat-dose toxicity studies; further details are provided in Module 2.6.6, Section 3.1.

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 QUAD STR 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 two 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 (Module 2.6.4, Section 7.2.1.3), 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 Module 2.6.6, 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.

Neither FTC nor TDF interact with drug metabolizing enzymes as substrates, inhibitors, or inducers, so metabolic drug interactions between these agents and EVG or COBI are very unlikely. The intended pharmacokinetic drug interaction of inhibition of the CYP3A-dependent metabolism of EVG by COBI has been studied extensively in vitro and in humans in vivo (Module 2.7.2).

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 weak inhibition of MRP4, the transporters responsible for renal excretion of TFV and should not affect the renal elimination of TFV. While COBI is a weak inhibitor of OCT2 and a more potent inhibitor of MATE1, TFV is not an inhibitor of either transporter so there should be no further decrease in activity of the transporters when the 2 drugs are combined. Cobicistat is a weak inhibitor of intestinal efflux transporters, but high concentrations of COBI in the intestinal lumen, achievable briefly during absorption, may inhibit MDR1 and result in a modest increase in TFV exposure (as seen with HIV-PIs {11255}).

Cobicistat is an in vitro inhibitor of the renal transporters, OCT2 and MATE1, which have been shown to transport creatinine and are thought to play a role in the active secretion of creatinine by the kidney (in addition to the majority of creatinine which is renally excreted by passive glomerular filtration). Inhibition of OCT2 and/or MATE1, and thus inhibition of active secretion of creatinine by the kidney, provides a plausible mechanistic explanation for the reduction in creatinine clearance seen during COBI dosing, in the absence of changes of true GFR. This phenomenon has previously been described for a variety of other agents.

In conclusion, based on the data supporting the individual components, the extensive clinical data with the FTC/TDF combination within HIV-1 therapy, and the clinical data with the EVG/COBI/FTC/TDF STR in Phase 1, 2 and 3 studies, adverse pharmacokinetic interactions that would negatively affect safety or pharmacological efficacy have not been observed (see Modules 2.7.2 and 2.7.4). 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 TFV exposure, due to inhibition of intestinal MDR1 by COBI, is predicted in vitro and observed in vivo, and the magnitude is similar to that observed when TDF is co-dosed with RTV-boosted HIV PIs {11255}. Single-dose pharmacokinetic studies in dogs demonstrate that comparable exposures for each component can be achieved through coformulation of the 4 agents relative to coadministration of the individual clinical formulations. Pharmacological activation of FTC and TFV is by enzymes with highly restricted substrate specificities, so inhibition by EVG or COBI is very unlikely. This is supported in antiviral assays, where no

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

No additional nonclinical pharmacokinetic studies are considered warranted with the EVG/COBI/FTC/TDF combination in view of the results of extensive nonclinical and clinical pharmacokinetic studies of the individual components and the clinical studies with the STR.

4. TOXICOLOGY

Comprehensive nonclinical programs with EVG, COBI, FTC, and TDF have been completed. These studies have characterized the acute toxicity, subchronic/chronic toxicity, mutagenicity, carcinogenicity (2-year carcinogenicity studies with COBI are ongoing), and reproductive toxicity of each the individual agents, and the toxicity of EVG/COBI and FTC/TDF combinations. Studies with EVG and COBI are described in detail in Module 2.6.6 (Toxicology Written Summary), and are listed in Tabulated Summary 2.6.7.1. 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. Acute 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; Tabulated Summary 2.6.7.5.1, JTK303-TX-001 and 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 no observed adverse effect level (NOAEL) was 500 mg/kg in rats (Tabulated Summary 2.6.7.5.2, PC-216-2013 and TX-216-2003).

Emtricitabine has demonstrated minimal acute toxicity in rodents (oral LD_{50} > 4000 mg/kg and intravenous LD_{50} > 200 mg/kg; Module 1.4.4, TTEP/93/0020, TTEP/93/0023, TTEP/93/0021, and TTEP/93/0024).

The single dose NOAEL of TDF in rats was 1500 mg/kg (Module 1.4.4, R990200). The no observed effect level (NOEL) in dogs given a single dose of TDF was 30 mg/kg (treatment-related lesions in the kidneys were observed at 90 and 270 mg/kg [Module 1.4.4, D990201]).

No single-dose studies have been performed with the combination of EVG, COBI, FTC, and TDF. Single-dose toxicity studies indicated that EVG, FTC and TDF had low acute toxicity. The MTD for COBI was 100 mg/kg in mice, and the NOAEL was 500 mg/kg in rats. With no overlapping toxicities, coadministration is unlikely to change the acute toxicity profile.

4.2. Subchronic and Chronic Toxicity

4.2.1. EVG

A series of GLP oral repeat-dose toxicity studies were conducted with EVG in mice (13 weeks [Tabulated Summary 2.6.7.7.1.1, TX-183-2004]; rats (4 weeks [Tabulated Summary 2.6.7.7.1.2, JTK303-TX-003], 13 weeks [Tabulated Summary 2.6.7.7.1.3, JTK303-TX-021] , and 26 weeks [Tabulated Summary 2.6.7.7.1.4, JTK303-TX-022]), and dogs (4 weeks [Tabulated Summary 2.6.7.7.1.6, JTK303-TX-004], and 39 weeks [Tabulated

Summary 2.6.7.7.1.7, JTK303-TX-023]). In addition, two 13-week combination toxicity studies were conducted with EVG in combination with COBI (Tabulated Summary 2.6.7.7.3.1, TX-236-2001) or in combination with RTV (Tabulated Summary 2.6.7.7.1.5, 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 (Tabulated Summary 2.6.7.7.1.1; 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 (Tabulated Summaries 2.6.7.7.1.2, 2.6.7.7.1.3 and 2.6.7.7.1.4; 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 (Tabulated Summary 2.6.7.7.1.7; JTK303-TX-023), but was not seen in the 4-week dog study (Tabulated Summary 2.6.7.7.1.6; JTK303-TX-004). In a series of mechanistic studies (Module 2.6.6, Section 8.3.1), 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 (Tabulated Summary 2.6.7.14.1; 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 (Tabulated Summary 2.6.7.7.3; 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 (Tabulated Summary 2.6.7.7.1.5; 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 STR.

4.2.2. COBI

A series of GLP oral repeat-dose toxicity studies were conducted with COBI in mice (2 weeks [Tabulated Summary 2.6.7.6.2, TX-216-2032], 4 weeks [Tabulated Summary 2.6.7.6.2, TX-216-2041], and 13 weeks [Tabulated Summary 2.6.7.7.2.1, TX-216-2026]; rats (4 weeks [Tabulated Summary 2.6.7.7.2.2, TX-216-2004], and 26 weeks [Tabulated Summary 2.6.7.7.2.3, TX-216-2017]), and dogs (4 weeks [Tabulated Summary 2.6.7.7.2.5, TX-216-2005], and 39 weeks [Tabulated Summary 2.6.7.7.2.6, TX-216-2016]). In addition, two 13 week combination toxicity studies were conducted with COBI in combination with EVG (Tabulated Summary 2.6.7.7.3.1, TX-236-2001) or in combination with ATV (Tabulated Summary 2.6.7.7.2.4, 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 (Tabulated Summary 2.6.7.7.2.1, 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 (Tabulated Summary 2.6.7.6.2, TX-216-2041). Mild increases (2-3-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 (Tabulated Summary 2.6.7.7.2.2, TX-216-2004 and Tabulated Summary 2.6.7.7.2.3, 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 (Tabulated Summary 2.6.7.7.2.5, TX-216-2005), and up to 10 mg/kg/day in the 39-week study (Tabulated Summary 2.6.7.7.2.6, 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 (Tabulated Summary 2.6.7.7.3.1, TX-236-2001) or with ATV (Tabulated Summary 2.6.7.7.2.4, 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 STR.

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 (Tabulated Summary 2.6.7.7.3.1, TX-236-2001), and a 4-week impurity qualification study was conducted with the EVG/COBI layer of the EVG/COBI/FTC/TDF tablet (Tabulated Summary 2.6.7.17.3, 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 given 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 (Tabulated Summary 2.6.7.17.3, 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 [Module 1.4.4, TOX599 and TOX599 addendum]; TOX118] and 26 weeks [Module 1.4.4, TOX022 and TOX628]), rats (13 weeks [Module 1.4.4, TOX097]), and cynomolgus monkeys (4 weeks [Module 1.4.4, TOX600 and TOX600 addendum], 13 weeks [Module 1.4.4, TOX627], and 52 weeks [Module 1.4.4, 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 1-year 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 (1 year). 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 STR.

4.2.5. TDF

A series of GLP oral repeat-dose studies were conducted in mice (13 weeks; [Module 1.4.4, M990203]), rats (28 day and 13/42 weeks; [Module 1.4.4, 96-TOX-4331-003; 97-TOX-4331-002]), dogs (28 day and 13/42 weeks; [Module 1.4.4, 96-TOX-4331-004; 98-TOX-4331-003; 97-TOX-4331-001]) and monkeys (8 weeks; [Module 1.4.4, P2000078]).

Test article-related renal karyomegaly was observed in all species at most dose levels. This finding was considered a morphologic change without pathological or toxicological consequences.

Treatment-related findings in the 13-week mouse study (doses from 100 to 1000/600 mg/kg/day) included tubular karyomegaly in the kidneys and epithelial hypertrophy in the duodenum. The NOAEL for this study was 100 mg/kg/day (Module 1.4.4, M990203), a dose equivalent to approximately 5-fold the human exposure (Module 1.4.4, M990203-PK).

In a 13/42-week rat study (doses ranging from 30 to 1000 mg/kg/day), reversible changes in serum chemistry parameters included dose-related decreases in cholesterol and triglycerides; and increases in ALT, AST, and creatinine (Module 1.4.4, 97-TOX-4331-002). Duodenal mucosal hyperplasia was observed in the ≥ 300 mg/kg/day groups, and duodenal epithelial hypertrophy occurred at doses ≥ 100 mg/kg/day. Renal tubular epithelial karyomegaly and pigment accumulation occurred at doses ≥ 30 mg/kg/day. Decreases in bone mineral content and bone mineral density were observed at 1000 mg/kg/day after 13 or 42 weeks of treatment, with evidence for a similar effect in males at 300 mg/kg/day. There were no test article-related effects on bone parameters at the 30 or 100 mg/kg/day dose levels, and no gross or histopathological changes were observed in the bone at any dose. The renal, GI, and bone changes were partially reversible. The NOAEL was considered to be 30 mg/kg/day. Exposure at this dose is approximately equivalent to human exposure (Module 1.4.4, 97-TOX-4331-002-PK).

In a 13/42 week dog study (doses of 3, 10, and 30 mg/kg/day), changes in clinical biochemistry parameters were slight to moderate, dose-related, and reversible (Module 1.4.4, 97-TOX-4331-001). Bone resorption markers generally were increased in the 30 mg/kg/day dose group at all time points evaluated. Dose-related histopathological changes included slight to mild renal tubular dilatation or degeneration/regeneration and interstitial nephritis in

animals in the 10 and/or 30 mg/kg/day dose groups. Slight to moderate renal tubular karyomegaly was observed in the 3, 10, and 30 mg/kg/day dose groups. Slight decreases in bone mineral content and bone mineral density of the distal femur of treated animals were observed at 30 mg/kg/day. There was no consistent evidence of recovery in bone parameters at Week 55. The NOAEL for this study was considered to be 3 mg/kg/day, a dose with exposure approximately 0.5-fold the AUC in patients treated with TDF at 300 mg in the EVG/COBI/FTC/TDF STR (Module 1.4.4, P4331-00006).

The toxicity profile in rhesus monkeys administered doses of 30, 250, or 600 mg/kg/day TDF for 56 days was similar to that reported in other animal models (Module 1.4.4, P2000078). Renal toxicity was dose limiting (cell degeneration observed at ≥ 250 mg/kg) and there were some indications of secondary alterations in the liver and thymus in the 600 mg/kg/day group. A dose-dependent reduction in mean serum phosphorus concentrations was observed through the first 4 weeks of study. Following oral phosphate supplementation (beginning Day 29), the serum phosphate concentrations normalized. There were no consistent changes in biochemical markers of bone remodeling and no alterations in bone morphology at any dose. Biochemical analysis (cytochrome c oxidase and citrate synthase activities, mitochondrial DNA content), serum lactate levels, and electron microscopic examination of sections of the kidney, liver, cardiac muscle, and skeletal muscle in animals in the 30 and 250 mg/kg/day TDF groups showed no evidence of drug-related mitochondrial injury after 56 days of treatment. Due to serum phosphate changes noted at 30 mg/kg/day, a NOAEL was not established for this study.

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 (Module 1.4.4, 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. Further details on these studies are provided in Section 4.8.5.

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 (Module 1.4.4, 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 given 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. EVG/COBI/FTC/TDF

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 (Tabulated Summary 2.6.7.7.3.1, TX-236-2001.

Administration of FTC and TDF 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 two 14-day rat toxicology studies and a 4-week dog study with the combination (Module 1.4.4, TX-164-2001, TX-164-2005, and TX-164-2004).

The 4 drugs, EVG, COBI, FTC, and TDF, 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 TDF were the kidney (karyomegaly, tubular degeneration), bone, and GI tract (in rodents).

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 3.8.2). These data, along with Phase 1, 2 and 3 clinical data (Module 2.7.4), suggest that COBI reversibly blocks secretion of creatinine in humans most likely via MATE1 inhibition. Given that 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 EVG/COBI/FTC/TDF would exacerbate the renal toxicity of TDF.

The only toxicity observed in chronic animal studies with FTC was mild, reversible anemia in mice and minor decreases in erythrocyte counts/increases in MCH 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 red blood cell 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 TDF effects on bone is not expected.

Gastrointestinal toxicity is dose limiting in rodents for TDF, and was due to high local concentrations. This TDF-related toxicity is not considered relevant for humans and should not cause an overlapping toxicity with COBI which caused emesis and salivation in dogs.

From in vitro data and clinical experience (Module 2.7.2), the only major anticipated drug-drug interaction upon administration of the 4-drug combination is the intended inhibition of CYP3A activity by COBI and the consequent increase in EVG exposure. 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) (Tabulated Summary 2.6.7.8.1, 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 (Tabulated Summary 2.6.7.8.2; 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 [Tabulated Summary 2.6.7.6.1, JTK303-TX-012], or after a single oral dose of 2000 mg/kg (Tabulated Summary 2.6.7.9.1, JTK303-TX-007).

4.3.2. COBI

Cobicistat was not genotoxic in the reverse mutation bacterial test (Ames test) (Tabulated Summary 2.6.7.8.3, TX-216-2010), mouse lymphoma (Tabulated Summary 2.6.7.8.4, TX-216-2011), or rat micronucleus assays (Tabulated Summary 2.6.7.9.2, TX-216-2012).

4.3.3. FTC

Emtricitabine was not genotoxic in the reverse mutation bacterial test (Ames test) (Module 1.4.4, 18637-0-409R, MUT203, and K01-3154, respectively), mouse lymphoma (Module 1.4.4, TOX012), or mouse micronucleus assays (Module 1.4.4, TOX011).

4.3.4. TDF

Tenofovir DF was mutagenic in the in vitro mouse lymphoma assay (Module 1.4.4, 97-TOX-4331-007), weakly positive in an unscheduled DNA synthesis test (Module 1.4.4, R2002104), and generally negative in in vitro bacterial mutagenicity tests (Ames test)

(Module 1.4.4, 96-TOX-4331-005, 97-TOX-1278-003, and K01-3037, respectively). In an in vivo mouse micronucleus assay, TDF was negative when administered to male mice (Module 1.4.4, 97-TOX-4331-008).

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 (Module 1.4.4, TX-164-2002 and TX-164-2003, respectively).

4.3.6. EVG/COBI/FTC/TDF

Cobicistat and FTC 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 unlikely to have genotoxic potential in vivo. Tenofovir DF had positive findings in genotoxicity studies (mouse lymphoma cell assay and UDS assay), but was negative in a micronucleus test in mice. The combination of FTC and TDF in a mouse lymphoma cell assay did not worsen the genotoxic potential of TDF. 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

104-week oral carcinogenicity studies in mice and rats have been conducted with EVG, FTC, and TDF. 104-week carcinogenicity studies in mice and rats are ongoing with COBI.

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; Tabulated Summary 2.6.7.10.1, 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; Tabulated Summary 2.6.7.10.2, 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

104-week oral gavage carcinogenicity studies with COBI in mice (TX-216-2030) and rats (TX-216-2031) are ongoing.

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; Module 1.4.4, TOX109) or in rats at doses up to 600 mg/day/day (28-fold the human systemic exposure at the therapeutic dose; Module 1.4.4, TOX108).

4.4.4. 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 STR (Module 1.4.4, M990205 and 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. EVG/COBI/FTC/TDF

Elvitegravir, FTC and TDF have all demonstrated low carcinogenic potential in conventional 2-year bioassays at exposures that exceeded (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.

Carcinogenicity studies with COBI are ongoing. Cobicistat was negative in a battery of genotoxicity studies (Section 4.3.2; Ames test, mouse lymphoma assay and micronucleus assay in rats).

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

Given the outcomes of the genotoxicity studies, the 2-year carcinogenicity studies with the individual agents, the difficulty of extrapolating rodent results to humans and the large number of animals required to carry out these studies, the conduct of carcinogenicity studies with the EVG/COBI/FTC/TDF combination is considered unjustifiable.

4.5. Reproductive Toxicity

4.5.1. EVG

There were no significant adverse effects observed in fertility studies in female and male rats (Tabulated Summaries 2.6.7.12.1 and 2.6.7.12.2, JTK303-TX-019 and TX-183-2003), in embryo-fetal development studies in rats and rabbits (Tabulated Summaries 2.6.7.13.1, 2.6.7.13.2 and 2.6.7.13.3, JTK303-TX-020, TX-183-2008 and TX-183-2002, respectively), or in a pre/postnatal study in rats (Tabulated Summary 2.6.7.14.1, 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 (Tabulated Summary 2.6.7.13.2, 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 (Tabulated Summary 2.6.7.12.3, TX-216-2023). No teratogenic effects were observed in rat and rabbit developmental toxicity studies (Tabulated Summaries 2.6.7.13.4 and 2.6.7.13.5, TX-216-2020 and TX-216-2021, respectively). 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 (Tabulated Summary 2.6.7.14.2, 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 given the recommended 200 mg daily dose (Module 1.4.4, TTEP/95/0028 and TOX036, respectively). 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 (Module 1.4.4, TOX037 and TOX038, respectively). 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 (Module 1.4.4, TOX039).

4.5.4. TDF

There were no effects on fertility, mating performance or early embryonic development when TDF was administered to male rats at a dose equivalent to 10 times the human dose based on body surface area comparisons (Module 1.4.4, 98-TOX-4331-006). Embryo-fetal toxicity studies have been performed in rats (Module 1.4.4, 97-TOX-4331-004) and rabbits (Module 1.4.4, 98-TOX-4331-005) at doses up to 14 and 19 times the human dose based on body surface area and revealed no evidence of harm to the fetus due to TDF. In a pre/postnatal study in rats (Module 1.4.4, R990202), the NOEL for behavioral, reproductive, and development toxicity was 150 mg/kg/day (exposure approximately 4-fold the human exposure). Maternally toxic doses (≥ 450 mg/kg/day) had effects on pup survival, pup body weights, and sexual maturation.

4.5.5. EVG/COBI/FTC/TDF

4.5.5.1. Fertility and Early Embryonic Development

The reproductive and developmental NOELs/NOAELs for the individual agents were generally at exposure levels above human exposures. With no expected toxicologic interactions with the EVG/COBI/FTC/TDF combination, further studies with EVG/COBI/FTC/TDF combination 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 TDF were tested individually, and no effects when EVG was dosed in combination with RTV. No cause for concern has been identified and studies with the EVG/COBI/FTC/TDF combination are unlikely to show new effects.

4.5.5.3. Pre- and Postnatal Development

Slightly longer estrous cycles were observed in 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 EVG/COBI/FTC/TDF combination 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 (Tabulated Summary 2.6.7.14.1; TX-183-2006), daily oral gavage administration of EVG to F_1 generation pups from post natal 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 (Tabulated Summary 2.6.7.14.2; 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. TDF/TFV

Although no specific juvenile toxicity studies have been conducted with 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 [BMD], 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 (AUC 150 μg·h/mL) at this dose were more than 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) 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}. The exposure (AUC) in rhesus monkeys at this dosage is more than 30-fold that in humans following the oral administration of 300 mg/day of TDF. 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 (DEXA) scan {7311} findings of an adverse effect on bone. The AUC associated with this dosage are 3- to 18-fold greater than the human AUC_{ss} following a 300 mg/day dose of TDF.

4.6.5. EVG/COBI/FTC/TDF

There were no notable findings in the juvenile toxicity studies with EVG or with COBI. No specific studies were conducted with the EVG/COBI/FTC/TDF combination. This new drug application proposes that the EVG/COBI/FTC/TDF STR be initially be registered for adults only. A Proposed Pediatric Study Request (PPSR) under the Best Pharmaceuticals for Children Act (BPCA) was submitted to IND 101,283 on [redacted] 20[redacted] (Serial No. [redacted]) with a view to extending the use of the STR to adolescents and children > 6 years of age. Requests for waiver and deferral of pediatric studies under the Pediatric Research Equity Act (PREA) are provided in Module 1.9.1 and 1.9.2, respectively, together with a pediatric plan in Module 1.9.6.

4.7. Local Tolerance

Elvitegravir was not irritating to skin (Tabulated Summary 2.6.7.16.1, TX-183-2020), not a severe irritant to eyes (Tabulated Summary 2.6.7.16.1, TX-183-2021), and showed no potential for phototoxicity (Tabulated Summary 2.6.7.16.1, JTK303-TX-010).

Cobicistat was mildly irritating to skin (Tabulated Summary 2.6.7.16.2, TX-216-2044), not a severe irritant to eyes (Tabulated Summary 2.6.7.16.2, TX-216-2043), and showed no potential for phototoxicity (Written Summary 2.6.6., Section 7.2.3).

No local tolerance studies have been conducted with FTC.

Tenofovir DF is considered to be a very severe irritant to rabbit ocular tissue (Module 1.4.4, B990165), and a slight irritant to rabbit skin (Module 1.4.4, B990166).

The EVG/COBI/FTC/TDF tablet is intended for oral use. No local tolerance studies were conducted for the EVG/COBI/FTC/TDF combination.

4.8. Other Toxicity Studies

4.8.1. Antigenicity

Elvitegravir, COBI and TDF showed no potential for sensitization (Tabulated Summaries, 2.6.7.17.1 and 2.6.7.17.2, TX-183-2022 and TX-216-2042; and Module 1.4.4, G990167, 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 (Tabulated Summary 2.6.7.17.1, 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 T-cell dependent antibody response (TDAR) study in rats at doses up to 150 mg/kg/day (Tabulated Summary 2.6.7.17.2, 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 T-cell dependent antibody response (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 (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 (Tabulated Summaries 2.6.7.7.2.2 and 2.6.7.7.2.3, TX-216-2004 and TX-216-2017, respectively) 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, 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 NK cells were quantified using flow cytometry. No adverse effects on mean immunophenotyping values were noted.

In the 39-week dog study with COBI (Tabulated Summary 2.6.7.7.2.6, 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 (Module 1.4.4, TOX146). There were no adverse effects of FTC during the dosing period and FTC did not affect the 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 natural killer cell assay values (Module 1.4.4, TX-164-2004).

Data from repeat-dose toxicity studies with EVG, FTC, or TDF (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 natural killer (NK) cell assay values in a 4-week dog study (Module 1.4.4, 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; Tabulated Summary 2.6.7.7.2.1, TX-216-2026), rats (up to 26 weeks dosing; Tabulated Summary 2.6.7.7.2.3, TX-216-2017) and dogs (up to 39 weeks dosing; Tabulated Summary 2.6.7.7.2.6, 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 EVG/COBI/FTC/TDF combination.

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 4.6.4.

4.8.4. Mitochondrial Toxicity

An overview of investigations into the potential of EVG, COBI, FTC and TDF to mediate mitochondrial toxicity is provided in Section 2.2.2.

4.8.5. Impurities/Degradation Products

4.8.5.1. EVG

Over 18 impurities and degradation products related to EVG have been identified in batches of the active pharmaceutical ingredient (API) or drug product. The impurity profiles for batches of API or drug product used in nonclinical toxicology studies are provided in Tabulated Summary 2.6.7.4.1.

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 (Tabulated Summary 2.6.7.17.1, TX-183-2010 and Tabulated Summary 2.6.7.17.1, 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 (Tabulated Summary 2.6.7.17.1, 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 GMP material and support the specified limits of impurities proposed for commercial production (Module 3.2.P.5.5, Characterization of Impurities [EVG/COBI/FTC/TDF Tablets] and Module 3.2.P.5.6, Justification of Specifications [EVG/COBI/FTC/TDF Tablets]).

4.8.5.2. COBI

Over 15 impurities and degradation products related to COBI have been identified in batches of the API or drug product. The impurity profiles for batches of API or drug product used in nonclinical toxicology studies are provided in Tabulated Summary 2.6.7.4.2.

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 (Tabulated Summary 2.6.7.17.2, 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 (Tabulated Summary 2.6.7.17.2, 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 proposed for commercial production (Module 3.2.P.5.5, Characterization of Impurities [EVG/COBI/FTC/TDF Tablets] and Module 3.2.P.5.6, Justification of Specifications [EVG/COBI/FTC/TDF Tablets]).

4.8.5.3. FTC

The process impurities and degradation products of FTC have been qualified in animal studies. The major degradation product, related substance A*, was qualified in 2 genotoxicity studies (Module 1.4.4, TOX151 and TOX152) using a batch of FTC that contained 1% (w/w) of the related substance A* degradant. Both studies were negative for genotoxicity. In addition, there was no toxicity in a 28-day mouse study at doses (FTC/related substance A*) of 50/1 mg/kg/day, 150/3 mg/kg/day, and 450/9 mg/kg/day (Module 1.4.4, TOX153).

A 28-day mouse bridging study (Module 1.4.4, TX-162-2001) was performed to qualify impurities in FTC (specifically related substance B*). There was no toxicity of FTC at doses of 50, 150, and 450 mg/kg/day.

Based on their impurity profiles, the multiple GLP batches of FTC tested in the toxicology program are considered, in composite, to be representative of the GMP material and support the specified limits of impurities proposed for commercial production (Module 3.2.P.5.5, Characterization of Impurities [EVG/COBI/FTC/TDF Tablets] and Module 3.2.P.5.6, Justification of Specifications [EVG/COBI/FTC/TDF Tablets]).

4.8.5.4. TDF

At least 17 impurities, which are related substances to TDF, have been identified in batches of the API produced under both GLP and GMP conditions. All of the impurities derived from the chemical synthesis or formed as degradation products were present in the test material used in nonclinical toxicity studies; hence their toxicity was adequately assessed.

A 14-day study was conducted to determine if there were unexpected toxicologic effects of degraded products using TDF tablets that had been degraded under accelerated conditions (Module 1.4.4, R2000081). The study was conducted in Sprague-Dawley rats dosed via oral gavage with either nondegraded TDF or degraded TDF for 14 consecutive days. No treatment-related findings were observed following 14 days of oral gavage dosing of either TDF or degraded TDF tablets at doses up to 300 mg/kg/day to rats. No differences between treatment with either TDF or degraded TDF were noted.

Based on their impurity profiles, the multiple GLP batches of TDF tested in the toxicology program are considered, in composite, to be representative of the GMP material and support the specified limits of impurities proposed for commercial production (Module 3.2.P.5.5, Characterization of Impurities [EVG/COBI/FTC/TDF Tablets] and Module 3.2.P.5.6, Justification of Specifications [EVG/COBI/FTC/TDF Tablets]).

* 情報公表時に置き換えた。

4.8.5.5. 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 C* , related substance D* , related substance E* and related substance F* . The adducts of FTC/TDF form when [redacted], formed by the [redacted] of TDF (related substance G*), reacts with one molecule of each TDF and FTC to form an adduct. FTC may undergo [redacted] and may additionally [redacted] group of the [redacted], thereby creating related substance H* . While related substance H* has the potential to exist as 4 diastereomers, only 2 of these diastereomers have been observed in FTC/TDF containing products. The 2 observed diastereomers of related substance H* are related substance E* and related substance F* .

Two 14-day GLP oral toxicity studies have been conducted in rats to qualify impurities and degradants in the FTC/TDF tablets (Module 1.4.4, TX-164-2001, TX-164-2005). The second qualification study (TX-164-2005) was conducted to verify the qualification of related substance E* and related substance F* as these degradants were identified later in development by virtue of a new analytical assay. In these studies, rats were given formulations prepared from crushed tablets that were experimentally degraded by humidity and high temperatures or formulations prepared from crushed tablets that were not degraded. The doses were 0/0, 20/30, 67/100 and 200/300 mg/kg/day FTC/TDF in both studies. Although there were slight differences in the findings from both studies, there were no new toxicities or exacerbation of previously defined toxicities, and there was no difference in toxicity between non-degraded and degraded material. The NOAEL in the initial study (TX-164-2001) was considered to be 67/100 mg/kg/day, and 200/300 mg/kg/day FTC/TDF in the second study (TX-164-2005).

The qualification of these 4 degradation products is summarized in Module 3.2.P.5.5, Characterization of Impurities [EVG/COBI/FTC/TDF Tablets] and Module 3.2.P.5.6, Justification of Specifications [EVG/COBI/FTC/TDF 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.

4.8.5.6. EVG/COBI/FTC/TDF

EVG/COBI/FTC/TDF tablets are bilayer tablets with EVG and COBI in one layer referred to as the EVG/COBI layer; and FTC and TDF in the other layer, referred to as the FTC/TDF layer. No degradation products related to EVG have been identified in batches of EVG/COBI/FTC/TDF tablets. Several degradation products related to COBI have been identified in batches of EVG/COBI/FTC/TDF tablets. These products were tested in a 28-day toxicity study in rats using non-degraded and degraded EVG/COBI tablets (Tabulated Summary 2.6.7.17.3, TX-236-2002). Both non-degraded and degraded EVG/COBI lots were

* 情報公表時に置き換えた。

well-tolerated in this study, with no new toxicities identified and with findings similar to those previously observed with COBI administered alone. The NOAEL was 50/50 mg/kg/day EVG/COBI for both non-degraded and degraded tablets. The impurity profile for the batches used in the toxicology study is provided in Tabulated Summary 2.6.7.4.6. The degradation of each layer of the tablets is consistent with the degradation products observed in FTC/TDF tablets or in the 4 individual drug substances. There are no unique impurities or degradants in the FTC/TDF portion of the EVG/COBI/FTC/TDF tablets.

The impurities and degradation products present in EVG, COBI, FTC, and TDF, and in EVG/COBI, FTC/TDF and EVG/COBI/FTC/TDF tablets have been qualified through toxicology studies which employed drug substance from normal productions batches, laboratory scale batches with enhanced levels of impurities, and samples subjected to forced degradation conditions (high heat and humidity). There are no unique impurities or degradation products present in the EVG/COBI/FTC/TDF tablets that require qualification (Module 3.2.P.5.5, Characterization of Impurities [EVG/COBI/FTC/TDF Tablets] and Module 3.2.P.5.6, Justification of Specifications [EVG/COBI/FTC/TDF Tablets]).

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 non-adverse findings, not considered relevant to clinical use, were observed in rats and dogs.

In rats, cecal weights 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 histological changes in the cecum or GI adverse events. 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; Tabulated Summary 2.6.7.8.1; 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 (Toxicology Written Summary 2.6.6, Section 8.3.1). 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 (Tabulated Summary 2.6.7.10.2, 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 (Tabulated Summary 2.6.7.7.1.6, 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 (Tabulated Summary 2.6.7.7.1.7, 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 (Tabulated Summary 2.6.7.7.2.1, 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 (Tabulated Summary 2.6.7.6.2, 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 3.8.3.2).

In dogs, minimal-to-mild increases in bilirubin, ALT, and ALP activities, increased liver weights, and hepatocyte vacuolation were noted after 4 weeks dosing at 45 or 45/30 mg/kg/day (Tabulated Summary 2.6.7.7.2.5, 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 (Tabulated Summary 2.6.7.7.2.6, 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 safety data with the EVG/COBI/FTC/TDF STR do not indicate an adverse effect on the liver (Module 2.7.4, 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.

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 (Module 2.6.4, 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 EVG/COBI/FTC/TDF single tablet regimen (Module 2.7.4, Section 3.5).

4.9.1.2.3. Urinalysis

Urinalysis and urine chemistry changes, noted primarily in high-dose rats (at 100 mg/kg/day) and in dogs (at ≥ 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 (Section 4.2.2, Tabulated Summary 2.6.7.7.2.4, TX-216-2024), increases in urine volume were noted in groups given 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 red blood cell parameters (not exceeding 10%) were noted in high-dose rats given 100 mg/kg/day in the 26-week study (Tabulated Summary 2.6.7.7.2.3, 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 (Tabulated Summary 2.6.7.7.2.1, TX-216-2026) or in dogs dosed for 39 weeks (Tabulated Summary 2.6.7.7.2.6, TX-216-2016). Due to the minimal change and reversibility, these effects were not considered adverse.

Coagulation

In rats, increases (up to 38%) in mean platelet counts were noted in the 4-week study at doses ≥ 50 mg/kg/day (Tabulated Summary 2.6.7.7.2.2, TX-216-2004), and in the 26-week study in males at ≥ 30 mg/kg/day and in females at 100 mg/kg/day (Tabulated Summary 2.6.7.7.2.3, 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 (Tabulated Summary 2.6.7.7.2.6, 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; Tabulated Summary 2.6.7.7.2.1, TX-216-2026) and in female rats in the 26-week study (up to 35% increase at dose levels ≥ 30 mg/kg/day; Tabulated Summary 2.6.7.7.2.3, TX-216-2017). Cobicistat is a rat PXR (rPXR) inducer (Tabulated Summary 2.6.5.12.11, 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 rPXR 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 (Module 2.6.4, Section 7.2.3.1). In Phase 3 studies with the FTC/COBI/FTC/TDF STR, mean values for fasting glucose and lipid parameters remained in the normal range (Module 2.7.4, Section 3.4).

Minor nonadverse changes in total protein, globulin, 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 (Tabulated Summary 2.6.7.17.2, 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 (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 EVG/COBI/FTC/TDF STR (Module 2.7.4, Section 3.5).

4.9.1.3. FTC and TDF

No specific cause for concern were identified in the safety pharmacology, genotoxicity, carcinogenicity and reproductive toxicity studies with FTC or TDF.

Emtricitabine and TDF exhibit different patterns of target organ toxicity. Specifically, the only significant effect of FTC identified at dose levels constituting large clinical multiples was a minor anemia. In contrast, extensive nonclinical investigations of the toxicity of TDF have shown that the bone marrow is not a target for this agent, and that the target organs for TDF are distinctly different (GI, bone, and kidney). While the toxicity profile for TDF is more diverse than that of FTC, the ample nonclinical safety database on both drugs strongly indicates that no potential for drug interaction exists and further toxicological investigations are unlikely to yield new data relevant to humans.

In a variety of in vitro and in vivo studies, FTC and TDF alone or in combination, have shown a low potential for mitochondrial toxicity (Section 2.2.2).

4.9.1.3.1. Bone Toxicity, Renal Toxicity, and Gastrointestinal Tract

Nonclinical studies of TDF conducted in rats (Module 1.4.4, 97-TOX-4331-002), dogs (Module 1.4.4, 97-TOX-4331-001), and monkeys {7311} (Module 1.4.4, P2000078) revealed target organ effects in the GI tract (rodents only), kidney, bone, and a decrease in serum phosphate concentration. Gastrointestinal toxicity was dose limiting in rodents with TDF, and was due to high local concentrations. Kidney changes (karyomegaly, tubular degeneration) have been observed in all species tested. Bone toxicity was diagnosed as osteomalacia in monkeys and reduced BMD in rats and dogs. Findings in the rat and monkey studies indicated that there was a substance-related decrease in intestinal absorption of phosphate with potential secondary reduction in BMD. The mechanisms of these toxicities are not completely understood.

4.9.1.4. EVG/COBI/FTC/TDF

The 4 drugs, EVG, COBI, FTC, and TDF, 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); these adaptive effects are considered secondary to hepatic microsomal enzyme induction and thyroid hormone imbalance. The only notable effect of FTC was a minor anemia identified at dose levels constituting large clinical multiples. Extensive nonclinical investigations have shown that the target organs for TDF are distinctly different (GI, bone, and kidney).

Administration of EVG and COBI, and FTC and TDF in combination did not exacerbate the toxicities of the individual agents.

No specific cause for concern has been identified in genotoxicity, carcinogenicity, and reproductive toxicity studies with the individual agents.

4.9.2. Safety Margins

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, 6-month, and 9-month 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 3) were calculated based on exposure after repeat dosing (AUC_{0-t}) 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 STR in HIV-1 infected subjects (Module 2.7.2, Appendix 5.5). 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) exceed the estimated exposure at the efficacious dose in humans.

Table 3. 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]
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 (Module 2.7.2, Appendix 5.5, Table 1)

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 4) 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 STR in HIV-1 infected subjects (Module 2.7.2, Appendix 5.5).

Table 4. 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 (Module 2.7.2, Appendix 5.5, Table 2.2)

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 and TDF

Cross-species comparisons of exposure (expressed based on AUC at steady state [AUC_{ss}]) for the major target organs are shown in Table 5. The margin relative to the human AUC_{tau} is given for the NOAEL and the minimal-effect-level (MEL) for TDF, where the MEL was available from the same study in which the NOEL was determined.

For FTC, the NOELs obtained in the toxicity studies represent systemic exposures in animals well in excess of those expected in humans given the daily recommended dose of 200 mg.

Although the GI toxicity in the rat following TDF administration appears to have a very low margin of safety based on the systemic exposure levels, this effect is not related to systemic exposure, but to local GI exposure, which is 6- to 20-fold higher than the human exposure on a direct dosage basis.

Dogs were the most sensitive species to renal effects of TDF. The NOAEL was based on morphologic lesions (karyomegaly) that were not associated with renal functional or biochemical changes. The NOEL for renal effects in monkeys is likely above 30 mg/kg/day, but no interim doses were tested between 30 and 250 mg/kg/day, where mild effects were noted. Monkeys were quite sensitive to the TDF effects related to decreased serum phosphorus. In the 56-day monkey study (Module 1.4.4, P2000078), there was not a significant dose response for serum phosphate changes between 30 and 250 mg/kg/day, and a NOEL for this effect has not been established. All species tested have shown some loss of bone mineral density at relatively high doses (≥ 4 × human exposure); however, clinically evident osteomalacic lesions occurred only in juvenile monkeys (treated subcutaneously daily with TFV) whose TFV AUC_{ss} levels were > 30 fold those of humans receiving the 300 mg/day dose. Therefore, the effect on serum phosphate levels at lower doses in monkeys appears to have no toxicologic consequence.

Table 5. Estimated Safety Margins of FTC and TDF Based on AUC When Comparing Animal No-Adverse-Effect-Level (NOAEL) or Minimal-Effect-Level (MEL)

Target Organ Effect	Species	Study Duration	NOAEL/MEL (mg/kg/day)	AUC_{ss} (µg·h/mL) NOAEL/MEL	Margin Relative to Human AUC_{ss}
			FTC		
Anemia	Mouse	6 months	500[a]	350	27 X
	Rat	3 months	600[a]	346	27 X
	Monkey	1 year	200[a]	98	7.5 X
			TDF		
GI Toxicity	Rat	42 weeks	30/100	4/8	1/1.8 X
	Dog	42 weeks	30	30	7 X
	Monkey	56 days	250	20	4.5 X
Renal Toxicity	Rat	42 weeks	300/1000	18/65	4/15 X
	Dog	42 weeks	3/10	2/7	0.5/1.6 X
	Monkey	56 days	30	4	1 X
Reduced Serum Phosphate	Rat	42 weeks	1000	65	15 X
	Dog	42 weeks	30	30	7 X
	Monkey	56 days	< 30	4	< 1 X
Bone Mineral Loss	Rat	42 weeks	100/300	8/18	2/4 X
	Dog	42 weeks	10/30	7/30	1.6/7 X
	Monkey	56 days	ND	ND	ND

ND = Not Determined

a NOEL

Human AUC_{tau} (13 µg·h/mL) following a 200 mg/day dose of FTC (Module 2.7.2, Appendix 5.5, Table 2.3).

Human AUC_{tau} (4.4 µg·h/mL) following a 300 mg/day dose of TDF (Module 2.7.2, Appendix 5.5, Table 2.4).

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 EVG/COBI/FTC/TDF STR 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 TDF include: (1) potential for bone loss upon chronic dosing due to TDF, (2) potential for renal toxicity due to TDF, especially related to use with other drugs that have been shown to cause renal toxicity and in patients with renal impairment (3) decreases in estimated creatinine clearance due to COBI, (4) use in patients with hepatic impairment, (5) the potential for CYP3A associated drug interactions, (6) use during pregnancy and lactation, (7) potential for mitochondrial toxicity, (8) potential for carcinogenicity, and (9) potential for PR interval prolongation and decreased LV function due to COBI.

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

1. A reduction in bone mineral density has been observed in nonclinical and clinical studies with TDF and EVG/COBI/FTC/TDF (QUAD) STR (Module 2.7.4, Section 4.1). It is therefore considered prudent to include a warning within the 'Warnings and Precautions' section of the proposed Prescribing Information to highlight the small decreases in bone mineral density that have been observed in clinical studies. A statement has also been included in the section in the 'Animal Toxicology and/or Pharmacology' section to highlight that preclinical studies of TDF revealed effects on bone and that the mechanisms are not completely understood.

2. As nephrotoxicity has been seen nonclinically with TDF, and there have been reports of renal toxicity with TDF and the QUAD STR, warnings regarding these reports and appropriate monitoring guidance is included in the proposed Prescribing Information (Module 2.7.4, Section 2.1).

3. In clinical studies, COBI has been shown to decrease estimated creatinine clearance due to inhibition of tubular secretion of creatinine without affecting glomerular function. These changes are consistent with nonclinical findings of COBI inhibition of the renal transporters, OCT2 and MATE1. Results from pharmacokinetic studies with EVG and COBI demonstrated that no dose adjustment of the QUAD STR is warranted in patients with renal impairment (Module 2.7.2, Section 3.4.8.8). However, due in part to the effect of COBI on serum creatinine, and the requirement for dose-interval adjustment for FTC and TDF in patients with creatinine clearance (CL_{cr}) < 50 mL/min, it is recommended

that creatinine clearance be estimated prior to initiating therapy, and that treatment with the QUAD STR should not be initiated in patients with estimated creatinine clearance below 70 mL/minute.

4. 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 TDF, nor with EVG. Although adaptive liver changes were observed in nonclinical species with COBI, the potential for hepatotoxicity is considered low. Results from pharmacokinetic studies demonstrated that no dose adjustment of the QUAD STR is required in patients with mild or moderate hepatic impairment (Module 2.7.2, Section 3.4.8.7).

5. 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. The potential for CYP3A associated drug-drug interactions is described in the proposed Prescribing Information.

6. Animal data indicate that neither EVG, COBI, FTC, nor TDF, cause reproductive or fetal toxicity, and therefore it is proposed that QUAD STR be classified as Pregnancy Category B in the Prescribing Information. Tenofovir has been shown to cross the placenta in monkeys and to be excreted in milk. Emtricitabine has also 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.

7. Elvitegravir, COBI, FTC, and TDF appear to have a low potential for mitochondrial toxicity, as demonstrated by enzyme and cell analyses in vitro and by markers of mitochondrial injury in vivo. Ongoing assessment of clinical safety data from company-sponsored clinical studies and postmarketing experience has shown that the risk of mitochondrial toxicity with FTC and TDF is low. Nonetheless, since the risk cannot be excluded, a boxed warning regarding lactic acidosis/severe hepatomegaly with steatosis is included in the proposed Prescribing Information.

8. In long-term carcinogenicity studies of EVG and FTC, no drug-related increases in tumor incidence were found in mice or in rats. Long-term carcinogenicity studies of COBI are ongoing. 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.

9. 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 with the EVG/COBI/FTC/TDF STR did not reveal clinically-significant changes in these parameters (Module 2.7.4, Section 4.2; Module 2.7.2, Section 2.3.2). Appropriate information regarding the results of the studies is included in the 'Nonclinical Toxicology' section of the proposed Prescribing Information.

In addition to the items addressed above, which are product specific, other appropriate warnings have been included in the proposed Prescribing Information. These include warnings or precautions regarding use in patients with HIV and HBV coinfection, and lipodystrophy and metabolic abnormalities, and immune reactivation syndrome.

The toxicities of potential concern for FTC and TDF 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 adults 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 EVG/COBI/FTC/TDF (QUAD) STR tablet for the treatment of HIV infection is scientifically sound given 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, 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 QUAD STR demonstrated acceptable tolerability and safety profiles to support use in the adult HIV-1 infected population. Further, extensive postmarketing experience with the FTC and TDF individual components of the combination, and also the Truvada combination tablet, supports the proposed use of the QUAD STR tablet for the treatment of HIV-1 infection in adults.

The overall program including the data from the combination and individual agent studies is considered adequate to support the efficacy and safety of the QUAD STR 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 (Module 2.7.2, Section 4.1).

NRTIs carry a class labeling for mitochondrial toxicity; however, both FTC and TDF have shown a low potential for mitochondrial toxicity in in vitro studies and long-term toxicity

studies. In an in vitro study, EVG showed no inhibitory effects on mitochondrial DNA suggesting a low potential for mitochondrial toxicity. As EVG and COBI are not anticipated to significantly increase the exposure of FTC or TFV, the potential for exacerbating mitochondrial toxicity is low.

Although COBI showed the potential to decrease LV function and prolong the PR interval in isolated rabbit hearts, the TQT study (GS-US-216-0107), and ECGs and echocardiograms conducted during clinical development did not reveal clinically-significant changes in these parameters. Given the lack of effects for EVG, FTC and TDF on the cardiovascular system, the potential for cardiovascular effects with the QUAD STR is considered low.

The EVG/COBI/FTC/TDF combination is not anticipated to produce any new human metabolites. Given that 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 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 QUAD STR (Module 2.7.4). 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 TDF were the kidney (karyomegaly, tubular degeneration), bone, and GI tract (in rodents). These findings correlate with the known clinical toxicities for TDF (renal and bone toxicity).

Combination toxicity studies with EVG and COBI, and FTC and TDF did not reveal any new or additive toxicities.

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 (Module 2.6.4, Section 7.2.4). These data, along with Phase 1 and 2 clinical data (Module 2.7.4), suggest that COBI reversibly blocks secretion of creatinine in humans. Further, given that there is 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 EVG/COBI/FTC/TDF could exacerbate the renal toxicity of TDF.

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

Gastrointestinal toxicity is dose limiting in rodents for TDF, and was due to high local concentrations. For EVG, changes in the cecum and upper small intestine in rats and dogs were due to high local concentrations and were not considered adverse. These effects are not considered relevant for humans and should not cause an overlapping toxicity with COBI which caused emesis and salivation in dogs.

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

Of the 4 compounds, only TDF 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 EVG/COBI/FTC/TDF combination is not anticipated to alter the genotoxicity profiles of the individual agents.

Elvitegravir, FTC, and TDF have all demonstrated low carcinogenic potential in conventional 2-year bioassays. Although carcinogenicity studies with COBI are ongoing, it is considered unlikely that combination dosing would change these profiles, and no exacerbation of toxicity is expected.

Elvitegravir, COBI, FTC, and TDF 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, with the FTC/TDF combination, and with the EVG/COBI layer of the bilayer EVG/COBI/FTC/TDF tablet.

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

The lack of overlapping toxicity in animals, along with clinical data with EVG and COBI, and the QUAD STR support the overall risk/benefit of this INSTI-based STR for HIV-1 infection.

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